Charles University, Faculty of Science

Univerzita Karlova, Přírodovědecká fakulta

Study program: Zoology

Studijní program: Zoologie



Mgr. Eva Štefková Kašparová

The effect of climatic changes on genetic build-up of populations and their role in promoting adaptive radiations

Efekt klimatických změn na genetickou strukturu populací a jejich úloha při adaptivních radiacích

Ph.D. thesis/ Disertační práce

Školitel/ Supervisor: Mgr. Karel Janko, Ph.D.

Praha 2021

Prohlášení autora

Prohlašuji, že jsem tuto disertační práci vypracovala samostatně, pod vedením školitele Mgr. Karla Janka, Ph.D. a že jsem všechny použité prameny řádně citovala. Disertační práce je založena na vlastních manuscriptech publikovaných ve vědeckých časopisech a na bázi shrnujícího manuskriptu, toho času v přípravě. Přiložená práce, ani žádná její část, nebyla použita k dosažení jiného akademického titulu. Text práce je originální.

V Praze

Mgr. Eva Štefková Kašparová

Prohlášení školitele

Prohlašuji, že data prezentovaná v této disertační práci jsou výsledkem vědecké činnosti Mgr. Evy Štefkové Kašparové a její spolupráce se spoluautory přiložených publikací. Její zapojení a podíl byly významné a jsou jasně uvedeny v textu (viz str. 30-33).

V Praze

.....

Mgr. Karel Janko, Ph.D.

Poděkování

Děkuji svému školiteli Mgr. Karlu Jankovi, Ph.D. za jeho přednášku na Ostravské univerzitě v době, kdy jsem si vybírala téma diplomové práce, čímž mě přivedl do jeho domovské Laboratoře genetiky ryb UŽFG AV ČR v Liběchově. Zde jsem získala přátelské a intelektuální zázemí po celou dlouhou dobu doktorského studia. Doufám, že spolupráce, kterou jsme navázali, brzy neskončí. Děkuji prof. Ing. Petrovi Rábovi,DrSc., dr.h.c., který laskavě vede laboratoř, Mgr. Vendule Bohlen Šlechtové, Ph.D., se kterou jsme vedly diskuse o molekulární genetice, RNDr. Vlastimilu Šlechtovi, CSc., se kterým jsme řešili chemickou podstatu dějů v genetice a také všem ostatním členům týmu za velkou podporu jak technickou tak vědomostní. Děkuji Mgr. Michailovi T. Rovatsos, Ph. D., za psychickou, ale i odbornou podporu v průběhu sepisování prvoautorského článku a za dodání motivace k sepsání disertace v období, kdy bylo času méně díky dvěma dětem. Děkuji Mgr. Janu Pačesovi, Ph.D., který se mi snažil předat vhled do úpravy NGS dat a taktéž jeho týmu na UMG AV ČR, tj. Mgr. Jakubovi Rídlovi Ph.D. a Ing. Miluši Hradilové Ph.D. Děkuji také prof. Ing. Ivě Langrové, CSc., která mi umožnila dokončit disertační práci v rámci pracovního poměru na ČZU v Praze. Děkuji také kamarádce Martě Špakulové, RNDr., DrSc. za několikeré přečtení práce a dodání sil k dokončování práce.

Děkuji ze srdce mým drahým rodičům, kteří mě všestranně podporovali, ale hlavně s láskou vedli od nejútlejšího dětství k poznávání světa a nejvíce přírodních zákonitostí. Je mi opravdu líto, že ač si to moc přáli, nedočkali se mého akademického růstu.

Abstract

The Southern Ocean and its enormously cold, remote and yet the wildest continent, the Antarctic, have experienced numerous rapid climatic changes. It used to be viewed as a barren wasteland with limited knowledge of species diversity or phylogeography. Recently, all aforementioned premises are being increasingly questioned, and this thesis aims to shed light on them.

Firstly, multi-locus genetic markers were used to evaluate if it is the lifestyle, benthic or pelagic, including the duration of the pelagic larval period that determines the population connectivity of particular notothenioid species. It was the adult's fish lifestyle along with the oceanographic factors that are responsible for the gene flow between localities. Secondly, is lifestyle a factor determining the extent to which Pleistocene growing grounded ice sheet affected species past population dynamics? The attention was paid to Notothenioidea fish, then to Echinoidea sea urchins, and lastly to NCBI database available antarctic marine shelf and deep-sea fauna. Our findings suggest that the last glacial maximum affected more profoundly the population size of benthic shelf species. At the same time, those living in the water column or inhabiting deep slopes had more ancient population size changes.

The Antarctic terrestrial evolution was studied on the ubiquitous soil invertebrate bdelloid rotifers (Rotifera, Bdelloidea), the area of which was described as extremely patchy and isolated with intraregional radiation, thus denying the pan-Antarctic distribution. Antarctic bdelloids have the highest endemicity on species level and are significantly younger, probably due to the long term isolation of the continent and harsh environmental conditions causing extinctions of ancient lineages.

It follows that the contemporary Antarctic organisms had to persist in situ even admittedly harmed and selected by glaciation conditions. It was shown that marine benthic shelf communities had been more profoundly affected than the pelagic and eurybathic organisms.

Keywords: Bdelloidea, Notothenioidea, gene flow, population expansion, filestyle, phylogenetic radiation, endemicity

Abstrakt

Jižní Oceán a jeho enormně chladný a křehký kontinet Antarktida si doposud prošly řadou zásadních klimatických změn. Antarktida byla tradičně nazírána jako pustá krajina a mnoho se také nevědělo o druhové diverzitě organizmů nebo fylogeografii. Tato práce si klade za cíl osvětlit a upřesnit zásadní hypotézy týkající se zmíněných biologických vlastností živočišných společenstev jak v šelfových vodách, tak i v suchozemském prostředí Antarktidy.
Pomocí multilokusové analýzy bylo zjišťováno, jak souvisí životní styl - bentický nebo pelagický, se zřetelem na délku pelagického larválního stádia notothenioidních ryb, s konektivitou mezi jejich populacemi. Životní styl, spolu s oceánografickými faktory, je vhodným vodítkem k odhadu genového toku mezi populacemi. Je však životní styl faktorem ovlivňujícím populační dynamiku v dobách glaciace? Tuto otázku jsme aplikovali jak u fylogeneticky blízce příbuzných skupin ryb Notothenioidea nebo ježovek Echinoidea, tak také u fylogeneticky nepříbuzných druhů shelfových a hlubokomořských antarktických Invertebrata, dostupných v NCBI databázi. Ukazuje se, že populace bentických druhů prošly recentnější expanzí, než populace hlubokomořských nebo pelagických orgnismů.

Studium evoluce mořského života jsme vyvážili studiem evoluce terestrických ekosystémů. Zde jsme se věnovali populační genetice, fylogeografii a hodnocení endemicity a stáří nejčastějších antarktických linií pijavenek - bdeloidních vířníků (Rotifera, Bdelloidea). Antarktické druhy jsou vývojově mladší než mimoantarktické pijavenky a mají také nižší genetickou diverzitu, ale naopak drží přední místo v počtu endemitů; patrně díky dlouhé evoluci v extrémních podmínkách. Popíráme hypotézu pan-antartického rozšíření pijavenek. Areál jejich druhů je naopak fragmentovaný a izolovaný s intaregionální radiací.

Nynější antarktická fauna tedy musela vytrvat in situ, i když byla jistě ovlivněna a selektována proměnami glaciálních podmínek. Větší dopad mělo glaciální maximum na bentické šelfové organismy, než na pelagické nebo eurybatické druhy.
Klíčová slova: Bdelloidea, Notothenioidea, genetický tok, populační expanze, životní styl, fylogenetická radiace, endemicita

Table of content

Prohlášení autora 1
Prohlášení školitele 1
Poděkování 2
Abstract 3
Abstrakt 4
1. Introduction 7
2. Literature rewiew 10
2.1 Antarctica: The origin of the frozen continent 10
2.2 The Antarctic Cicumpolar Current 12
2.3 Antarctic biogeography 14
2.4 Are there Northern invaders crossing the circum-Antarctic divergence and could they settle the viable population? 16
2.5 The Antarctic continental shelf and terrestrial regions as a contemporary, indigenous centrum of diversity 18
2.5.1 The Antarctic marine fauna 18
2.5.2 Antarctic terrestrial fauna 21
3. Aims of the thesis 25
4. The list of publications on which the thesis is based 27
5. Outcomes of the publications on which the thesis is based 28
6. Publications on which the thesis relies 32
Publication I: Comparative phylogeography of three trematomid fishes reveals contrasting genetic structure patterns in benthic and pelagic species..... 33
Publication II: Preliminary report on fish diversity at the Prince Gustav Channel, the northern part of the James Ross Island, Antarctica..... 45
Publication III: Otostephanos (Rotifera, Bdelloidea, Habrotrochidae) with the description of two new species..... 56
Publication IV: Lifestyle and Ice: The Relationship between Ecological Specialization and Response to Pleistocene Climate Change..... 74
Publication V: Antarctic bdelloid rotifers: diversity, endemism and evolution..... 95
Publication VI: Comparative phylogeography reveals consistently shallow genetic diversity in a mitochondrial marker in Antarctic bdelloid rotifers. 134
7. Publication in preparation, bearing preliminary title: Southern Ocean: home for brave; Pelagic and deep-sea communities have had bigger population size throw time. 147
7.1 Materials and methods: 149
7.1.1 Sampling, data mining and species categorisation 149
7.1.2 DNA extraction, PCR amplification 151
7.1.3 Genetic diversity and population structure 151
7.1.4 Demographic inference 154
7.2 Results 156
7.2.1 Genetic diversity 156

7.2.2 Demographic inference 157
7.3 Conclussion: 172
7.4 References belonging to the table 3: 175
8. Summary and discusin 180
8.1 The terrestrial Antarctic 180
8.2 The marine Antarctic 182
9. Conclusion 184
10. References 185

1. Introduction

Environmental problems, particularly the issue of climate change, have become of great concern even to the general public. The study of climate change consequences belongs to the main interest of conservation biologists. In particular, it is to protect the environment and seek for the most threatened populations both by climate change and also by still rising needs of humans. Exposure (rate and magnitude of climate change), sensitivity (ability to mitigate fitness declines via genetic adaptation) and range shift potential (ability to move to new locations or disperse genes through fragmented landscapes) are considered to be the main estimations of vulnerability and species resilience as reviewed by Hoban (2018).

A promising approach to understanding the climate change consequences to contemporary biota is to investigate how contemporary species would react to climate change and to describe them. The Earth indeed went through plenty of different extents glacial cycles (e.g. (Kopp *et al.*, 2005)), allowing us to learn how organisms have withstood the extreme conditions, how life has evolved on Earth from its really beginning and deduce how they may react to another“ multifactorial” (anthropogenic and environmental) climate change. Hereby we aimed to study how species populations responded to such changes in history, which may help derive their future reacti**o**n to ongoing climate change.

Although paleoecology can help to understand species population history and phylogeography, their data are often scarce. How will species react in the future is now a tempting question. The power of population genetics stems from its ability to link past demographic events in studied species to known past climate oscillations. Population dynamic detection and dating of such events as a bottleneck or contrary population expansions likewise speciation/extinction are tools to describe species history.

Antarctica is yet a continent the least impacted by humans, the most isolated and hence the most stable. Moreover, Antarctica is surrounded by the coldest, most temperature stable Ocean in terms of annual temperature and salinity range. These characteristics nominated the Antarctic continent and the Southern Ocean to become our“ natural laboratory” for studying the contemporary terrestrial and marine life and the last glacial maximum impact to local populations“ in situ**”.**

Besides the aforementioned superlatives, the Antarctic is in essential interest for global climate stability. Above all, the Southern Ocean covering 8% of the global marine realm, have a far-reaching impact on the global ocean currents circulation and deep water formation (Rintoul and Sokolov, 2001). It is the highest, driest, windiest and coldest continent on Earth and has the biggest reservoir of freshwater (70% in Antarctic Ice Sheet). Even though glaciers and ice sheets cover approximately 10% of the global surface, the retention of" cold white islands", above all, at the Poles and therefore in Antarctica, is essential for global climate stability.

Even though the Antarctic continent possess just 0.3% free of ice land (Convey and Stevens, 2007a), there is a long-range terrestrial life existence. It was historically thought that oscillating ice sheets and glaciers virtually sterilized the continent during ice ages. Hence there were extinctions both on the continent and high Antarctic marine shelf (Linse *et al.*, 2006). Today, the insight has changed, and studies found both terrestrial and continental shelf harbouring high diversity. The Antarctic communities adapted to freezing marine and continental conditions and survived in situ for long periods (Convey, 1996; Dömel *et al.*, 2019).

Researches can trace the Last glacial maximum impact of the extended continental shelf. Growing grounded ice sheet covered and sterilized the vast majority of the shelf communities and besides hindered the light penetration and as a consequence limited the primary production. Yet, since Antarctica's ice sheet has not advanced and retreated synchronously along the whole continent, Antarctic fauna have survived indeed, presumably in shelf refugia or refugia like nunatacts, coastal capes and bays or dry valleys. Nowadays, there is no more the question that Antarctic fauna has persisted during the last glacial maximum (LGM) in situ (Pugh and Convey, 2008; González-Wevvar, David and Poulin, 2011; Halanych and Mahon, 2018; McGaughran *et al.*, 2019; Lau *et al.*, 2020; González-Wevar *et al.*, 2021). Consequently, we can ask more specifically about the population's stability during LGM. Why have some populations flourished, and some have undergone bottlenecks during LGM? Is valid the hypothesis that everything is everywhere (Pugh and Convey, 2008) or are there any gene flow barriers? These issues are explained in the thesis publications and outcomes.

2. Literature rewiew

2.1 Antarctica: The origin of the frozen continent

The existence of the Antarctic continent is linked back to the super-continent Gondwana. During the Triassic, Antarctica formed „the core" of the Gondwana continent which was inhabited by a warm-temperate biota. The tectonic reorganisation of the super-continent Gondwana between Antarctica and Australia with New Zealand, connected in an area of cool temperate shallow seas known as the Weddellian province, started in Mesozoic, Cretaceous (90 Mya**);** moreover, the East and West Antarctica were separated apart (Pugh and Convey, 2008; Near *et al.*, 2015). In the Cenozoic era after the warm early Eocene, the so-called greenhouse world, the global temperatures started to cool down. The climatic deterioration of the middle Eocene formed at the highest latitudes caused that warm-temperature waters were forced away from the poles and therefore warm water surface dwellers were rather eliminated (Briggs, 2003). During the Eocene-Oligocene (E/O) transition, spanning from the late middle Eocene to early Oligocene, global temperatures cooled more than any time since the Mesozoic leading to the formation of the first Antarctic ice sheets and the sea surface temperature started to cool down up to today's -2°C (Figures 1, 2).

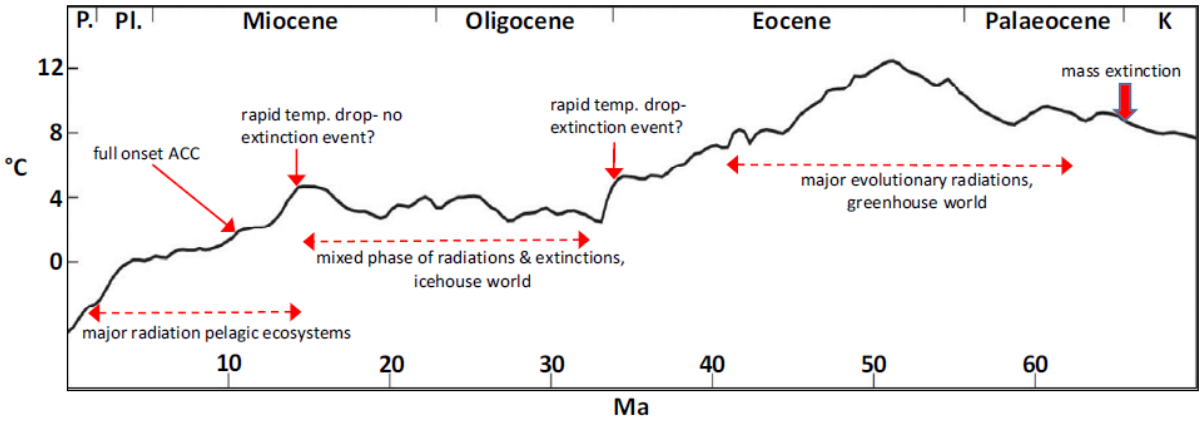


Figure 1: Climate temperature fluctuations in the Antarctic evolution with highlighted most pronounced cooling undertaken from (Crame, 2018)

The separation of Southern America from Antarctica started in Eocene (~ 42 Mya). The complete separation of the Southern America of the Antarctic continent was in the Eocene-Oligocene transition (~ 28 Mya), which was followed by the development of deep water circulation in the Drake Passage. The Tasman Gateway opening, which began (~ 90 Mya) and finished ~ 38 Mya developed unrestricted Antarctic Circumpolar Current (ACC) (the initial formation of the Antarctic Circumpolar Current (41 to 23 Mya)) and the oceans changed from a thermospheric to thermohaline circulation as a result of Antarctic glaciation. This tectonic activity positioned Antarctica at the Southern Pole, where it was completely isolated from lower-latitude warm ocean currents(Reguero *et al.*, 2014; Near *et al.*, 2015; Halanych and Mahon, 2018). The E/O transition belongs to the three major mass extinction events during the past 65 Mya. Rich Eocene fauna had disappeared and E/O transition survivors bore a surprisingly close resemblance to Antarctic today's living taxa (Molina, Gonzalvo and Keller, 1993; Barnes and Conlan, 2007; Clarke and Crame, 2010). The Oligocene cooling (34- 23 million years ago; the full continental glaciation initiation) and increased volcanic activity and plate tectonic movement resulted in large-scale glaciation in Antarctica which culminated in the Pleistocene Ice Age (Near *et al.*, 2015).

The massive expansion of the Antarctic ice sheet congruently with the formation of the ACC led to the freezing water flows down to the abyssal and upwelling of colder bottom water. Major waves of extinctions among terrestrial and marine organisms are associated with these climatic and oceanic circulation changes (Crame, 2018). It was the sharp climate cooling in mid-Miocene that caused the re-establishment of a permanent continental ice sheet (the major massive continental ice sheet growth (~ 14 Mya)) in East (10 Ma) and West (5 Ma) Antarctica. A large ice mass covered the East Antarctic land and a smaller one the West Antarctic mountainous (Colleoni *et al.*, 2018). On the other hand, in the age of the cooling and intensification of the ACC, it was promoted speciation by the opening of the new niche which is the most known in Notothenioid fish (Thornhill *et al.*, 2008; Near *et al.*, 2012). The ice shelf collapse (1.1 Mya) coincided with warmer temperatures and ever since the shelf has been re-established (Halanych and Mahon, 2018); see Figure 1 for timescale.

2.2 The Antarctic Cicumpolar Current

The Antarctic region, comprising an area of about 35 million km2, is constantly encircled by strong western winds (clockwise) moving the water masses and thus forming the Antarctic Circumpolar Current (ACC).

Low temperature the Polar Front and the Southern ACC front are separated by higher temperature Sub-Antarctic front. The Antarctic Convergence is the place of nutrients upwelling. The Polar Front, besides cool Sub- Antarctic water flow, is the most intense of a series of eastward-flowing jets of the Antarctic Circumpolar Current (ACC) and represents a physical barrier to free North/ South exchange of water and organisms.

Despite low velocity, the ACC transports the volume of 1000 times higher than the Amazon River (in the Drake Passage), is 200- 1000 km wide in various sectors and reaches the seafloor up to 3. 500 m depth (Eastman, 1993; Aronson *et al.*, 2007). The ACC poses strong horizontal gradients in density, temperature, salinity and air-sea fluxes (Cristini *et al.*, 2012). Opposite direction blowing Antarctic Ice Sheet freezing katabatic winds create sea ice from the Antarctic surface well-oxygenated freezing water which becomes more saline and dense. This super-cooled water flows down as Antarctic Bottom Water, ventilating the deep ocean. The cold water continues to flow northward to fill all the oceans basins to 50 °N in the Pacific and 45°N in the Atlantic Ocean. This mechanism cooled down the deep water from Cenozoic 14°C to today's -2°C (Figure 2). This super-cooled water is created by sudden pulses mainly in Weddell and Ross Sea. The other nutrient important phenomenon, the Antarctic Divergence is formed by the pushes of deep water and divergently blowing winds (North of 65°S move the water out of the continent whereas South of the 65°S the katabatic winds moves water toward the continent). It is the place of upwelling of deep 0°C water coming from the North. The Antarctic deep water brings nutrients necessary for biological productivity. The Antarctic Divergence is the place of massive primary production and krill stocks (Eu*phausia superba*) occurrence. The East wind drift is blocked by the Antarctic Peninsula and Victoria Land and creates the Weddell Sea, the Bellingshausen Sea and Roos Sea gyre. All of them are nutrient-rich bays (Eastman, 1993; Griffiths, 2010); see Figure 3 for Weddell Sea bathymetry.

The horizontal advection, the upper layer water movement, has a broad effect on both dispersals of organisms and nutrient supply. Antarctic polar water is nutrient-rich especially near the shore (nitrates, silicate, and phosphates) but is iron-limited (Hodson et al., 2017). Iron is necessary for chlorophyll synthesis and so hamper primary production which is the base of the trophic structure. Surface phytoplankton sinks and is broken down by microbes. The productivity is high only in coastal regions in ice edge zones at sub-Antarctic islands or the continental shelf (Eastman, 1993).

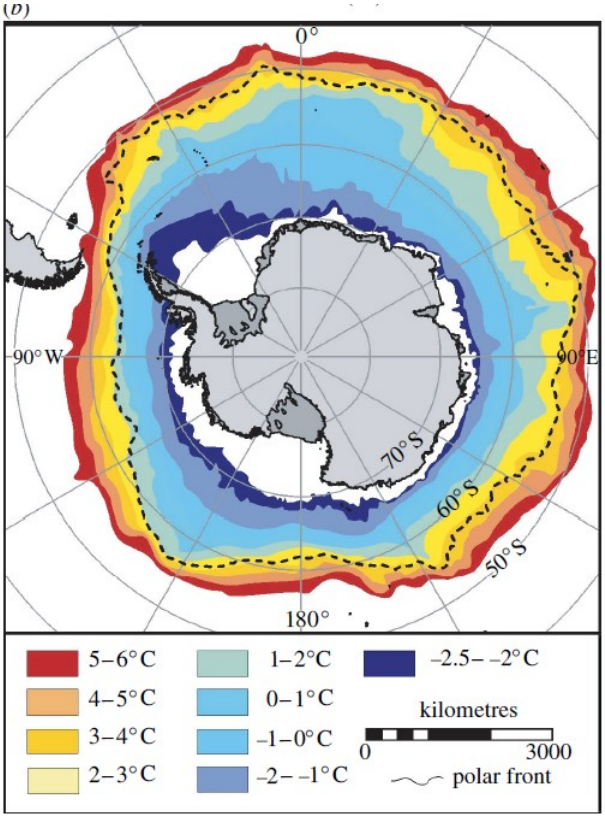


Figure 2: Sea temperatures around Antarctica from remote sensed data undertaken from. (Barnes and Conlan, 2007)

2.3 Antarctic biogeography

The antarctic region is very complex due to its large area and geographical origins. Originally, biogeography was assessed by the percentage of endemism. At least a 10% endemism nested within biogeographic regions that covered large geographic areas with contrasting biotic characteristics were considered as a province. Nowadays, attention is paid to phylogeography results showing genetic subdivisions and taxonomic partitions (Briggs and Bowen, 2012). Barnes and Griffiths (2008) state for bryozoans" Antarctic genetic endemism is double the level previously calculated and regional faunal similarities are much higher than around other continents – both reflecting long-term isolation."

Today, biogeography is conventionally defined by consistencies in climate and biology (Rivas-Martínez *et al.*, 2021). Briggs and Bowen (2012) divided the Antarctic territory into six regions. Firstly, there is the entire cold Antarctic region: Antarctic continent with the Scotia Arch islands: South Shetland, South Orkney and the South Sandwich Islands. Secondly, there is South Georgia and Bouvet Island on their own. Lastly, there are the sub-Antarctic oceanic islands divided into provinces: the Kerguelen Province includes the McDonald as well as Heard Island and the Prince Edward province including Marion Island. Crozet Province and Macquarie Island Province belongs to the other sub-Antarctic provinces.

Similarly, Convey and McInnes (2005) defined three geographic regions by climate and biology. (1**)** It was the sub-Antarctic, a ring of isolated oceanic islands surrounding the continent at relatively high latitude in the Southern Ocean. (2**)** It was the maritime Antarctic, consisting of the western coast of the Antarctic Peninsula to 72° S and associated island archipelagos (the South Shetland, South Orkney, and the South Sandwich Islands, and Bouvetøya) and (3) the continental Antarctic which includes the bulk of the Antarctic continent and the eastern coast of the Antarctic Peninsula, which does not correspond to geological separation of the continent into East (continental) and West (peninsular) Antarctic.

South Georgia (SG), Island with the warmest water within the Southern Ocean, served as an example for Barnes et al. (2009) to show the meeting of the Southernmost and contrary Northernmost limit of the species distribution. See Figure 3 for Weddell Sea topography.

SG was evaluated as the Island of the highest endemicity compared with other Antarctic areas (for bivalves and gastropods) (Linse *et al.*, 2006). Weddell Sea was a region rich in endemic gastropods. Apparently, it was as a result of the Weddell Sea gyre; the genetic exchange physical barrier (Mercado Casares, Soto Àngel and Peña Cantero, 2017). Congruently Pierrat et al. (2013) described the Weddell Sea and Dronning Maud Land dispersal discontinuity by the ACC. Those regions were never connected to each other. Interestingly, to Linse and Douglas regions delimitation (2006), the existence of unnamed gyre showed the linkage of Weddell Sea gyre under the Balleny Island to the Ross Sea gyre, explaining the environmental similarities (Mercado Casares, Soto Àngel and Peña Cantero, 2017). Another explanation of Weddell and Ross sea similarity links to the persistence of the species diversity from the direct connection in the Pleistocene via trans- Antarctic seaway as a result of West Antarctic Ice sheet collapse suggested as well by Strugnell (Strugnell *et al.*, 2012).

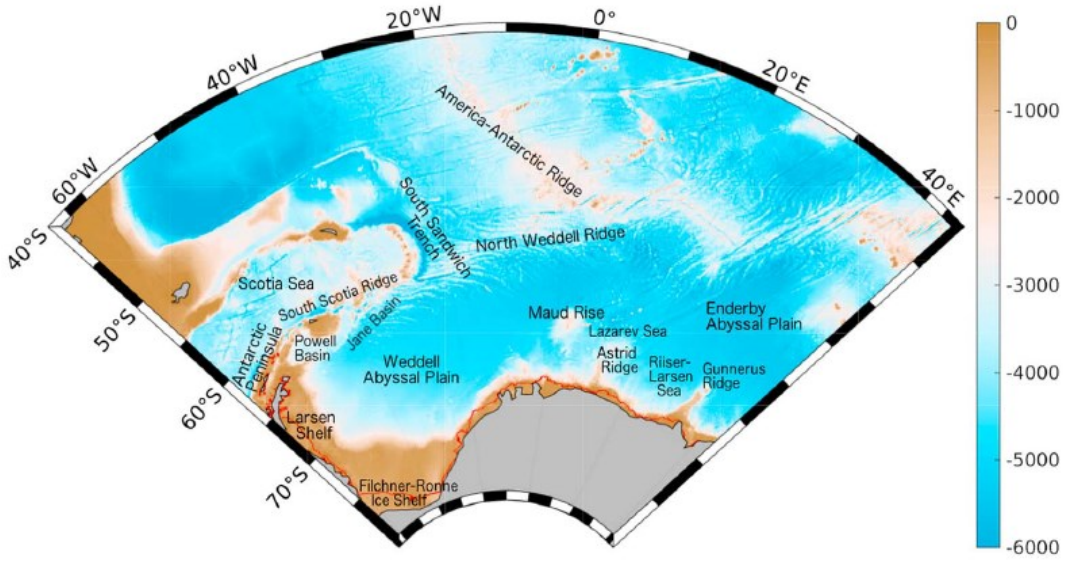


Figure 3: Antarctic Weddell Gyre topography (Vernet *et al.*, 2019)

At the Antarctic Peninsula, there is a continental ancient biogeographic boundary, the Gressitt line (Chown and Convey, 2007; McGaughran *et al.*, 2010) which has been detected for nematodes, springtails and mites.

2.4 Are there Northern invaders crossing the circum-Antarctic divergence and could they settle the viable population?

The ice-bound Antarctic continent isolated by vastness of the Southern Ocean is approximately 1000 km far from Drake Passage, 4.000–5.000 km from Australia/New Zealand and South Africa. Isolation of terrestrial biota is caused by remoteness of other continents, temperature drop and as well by wind currents defending the north south dispersal (Sabovljević and Frahm, 2011). However, it appears that neither the ACC nor the Polar Front had been an absolute physical barrier against invaders from the North (Aronson *et al.*, 2007).

Nowadays, the rising sea temperature changes the ocean currents positions and the ACC is being penetrated by the natural currents bearing floating organisms on material such as kelps, or anthropogenic plastic flotsam. Organisms are transmited by both the fishery and especially traffic ships transporting the settled benthic community“ ship hulls” from one coast to another (Lewis, Riddle and Smith, 2005; Deppeler and Davidson, 2017). Besides, the rising sea temperature permits Northern invaders to survive beyond the ACC as in the case of the Arctic Ocean origin *Hyas araneus* (Crustacea: Decapoda: Majidae) or copepod from Acartia genus seen by both the zoeal stages and adults (Tavares and Melo, 2004). The most serious would be the introduction of durofagous predators having a profound potential to alter the Antarctic benthic ecosystem food web and community structure (Aronson *et al.*, 2007; Clarke and Crame, 2010).

Even though McCarthy (2019) claims that there is no settled population of invaders like *Ulva intestinalis* (cryptogenic, grass kelp) , *Ciona intestinalis* (vase tunicate) or *Ectopleura crocea* (pinkmouth hydroid) in lithodids, believed to be sub-Antarctic, is the situation more crucial.

Lithodids, the durofagous (skeleton crushing) predators, were environmentally detached along with crabs, lobsters, cartilaginous and non-notothenioid fishes. Those before mentioned taxonomical groups comprised common communities in non-Antarctic sector despite they were known from the Antarctic fossil record until the Eocene (approximately 40 Mya). The antarctic benthinc species are nowadays the suspension feeder's epifaunal communities resembling the Paleozoic or modern deep-sea. The invasion of lithodids would have far-reaching impact on the archaic food-web structure (Aronson *et al.*, 2007). Lithodids adults have slow metabolism, slow grow and long life and ability to regulate magnesium salt concentration. High concentration of magnesium ions with combination of low temperature paralysis or in other word have „ narcoticum" effect and kills most of reptant decapods (Frederich, Sartoris and Pörtner, 2001). Lithodids progeny have lecitothrophic eggs and two years of embryonic development (Lovrich&Vinuesa 1999). Are thouse above mentioned features key preadaptations to facilitate the survival in the Southern Ocean low tempratures?

The APF forms a major faunal boundary; e.g. south of the Polar front diatoms with silica shells are abundant contrary to the North, where carbonate shells are dominant. The reason is the diffrences in concentration of nutrients (low CO_2 in Antarctic waters).

Put simply, there are occurrence occasions but not settle populations of *Hyas araneus* population, the great spider crab, in deep water continental slope of Antarctic Peninsula (Thatje and Arntz, 2004; Thatje and Lörz, 2005). There are individuals of *Halicarcinus planatus* seen in geothermal Deception Island (Aronson *et al.*, 2007), which are able to reproduce in summer and even in winter and whom juveniles tolerate the cooler temperatures (Diez and Lovrich, 2010). There are also *Lithodes murrayi* seen in the Bellingshausen Sea (Klages *et al.*, 1994). As a terrestrial fauna invader, the *Macrotrachela ioannae* comming from the South America was confirmed (Iakovenko *et al.*, 2015).

Even though prevention of non-native introduction is nowadays the conservation priority, **terrestrial organisms** transfer has been detected in Antarctic Peninsula and Antarctic islands (e.g. seeds on visitors (Lee and Chown, 2011) or flies (Potocka and Krzemińska, 2018)) . However, little is monitored the impact of homogenisation or biogeographic reorganisation of indigenous species by human activity (Hughes *et al.*, 2019).

On the other hand the anemochory is traditionally proposed as a mean of transport. This transport mechanism is documented e.g. by the trapping of alive *Cryptopygus antarcticus* springtail into the water trap (Hawes *et al.*, 2007).

2.5 The Antarctic continental shelf and terrestrial regions as a contemporary, indigenous centrum of diversity

While the North Pacific Ocean and terrestrial fauna have been traditionally looked at as a biota able to transgress biogeographic boundaries during climatic shifts (Briggs, 2003), the Antarctic appears to be an isolated continent with" in situ" evolution.

2.5.1 The Antarctic marine fauna

The Antarctic marine fauna evolution can be described from Devonian. Devonian fauna was lacking tropical groups, but Warm temperate Weddellian province (South America, Australia and New Zealand, Antarctica) in Cretaceous to Oligocene harboured rich fauna of fish, penguins, whales and marsupials documented from Seymour Island, La Meseta Formation. The middle Eocene global climatic deterioration (Eocene-Oligocene boundary) cooled down the higher latitude seas from 12 to -2 °C. The Tertiary cooling caused the continual sinking of cold, saline water adjacent to the Antarctic continent and its subsequent movement northward at abyssal depths from the late Eocene through the Oligocene (Keller, Macleod and Barrera, 1992; Briggs, 2003).

The oceanic cooling during the Cenozoic resulted in the onset of continental glaciations. Glaciation eradicated many shallow-water and terrestrial habitats and the cooling has led to widespread extinction in some groups.

New suitable habitats were populated by cold-acclimated organisms from the Antarctic, playing a role as a centre of origin for the abyssal and hadal zones. The dispersal was facilitated throw the cold abyssal waters in a converse direction either. From the North to the refugee in the Antarctic (e.g. Liparidae, Zoarcidae, Rajidae, Notothenioidae and Mollusca). Significant changes occurred in the composition of molluscan assemblages

(Aronson *et al.*, 2007; Clarke and Crame, 2010). Notothenioidae and Rajidae have been recognised as ancestral fossils from the late Eocene of Seymour Island (Briggs, 2003). Apparently, the Eocene was a time of rapid diversification and the cladogenesis of the teleost fishes, Notothenioidea (Eastman, 1993). While environmental conditions at that glacial maxima would have been very different from those existing today, fossil evidence indicates that some lineages extend back well into the Cenozoic (Aronson and Blake, 2001).

According to a rising amount of molecular data (Stevens *et al.*, 2006), today's fauna is regarded as indigenous, unique to the continent, consisting of species surviving repeated glaciations confined to ice-free paleo-refugial mountains, nunataks and coastal exposures; parochial terrestrial pre-LGM and maritime refugia or shelf refugia (Raupach *et al.*, 2010; Halanych and Mahon, 2018). It follows that the Last glacial maximum extensive grounded ice sheet did not extend to all parts all in once and asynchronously grew and retreated between different regions. Presumably, even though such ephemeral ice-free regions were likely covered by floating ice shelves on the surface, the biota could have survived LGM if found suitable habitat in refugia (Lau *et al.*, 2020). Pre-LGM maritime refugia were found e.g. in Alexander Island and Thurston Island, then in Dronning Maud Land, Prydz Bay, parts of the Ross Sea, George V Land, Victoria Land, Granite Harbour, Shackleton Range, Ellsworth Mountains as reviewed Convey (Convey *et al.*, 2008; Clarke and Crame, 2010). Maritime Islands and Antarctic Peninsula were probably covered by the extensive ice sheet and permitted the evolution of contemporary population, but younger than 10 Kya (Pugh and Convey, 2008) or thorough glacial periods travelling from refugees e.g. by wind (ALLEGRUCCI *et al.*, 2012; McGaughran *et al.*, 2019). Moreover, species population dynamics and phylogeographic changes are in line with the northward moving of marine fronts (Convey *et al.*, 2008; Thatje *et al.*, 2008; Raupach *et al.*, 2010; Allcock and Strugnell, 2012).

Despite all mentioned contstrains and the fact that the Antarctic continental shelf is unusually deep and narrow, contrary to other continents, it boasts the highest productivity region and is clearly a centrum of diversity (Eastman, 1993). Since the seafloor South from the Polar Front consists of silica accumulation muds; the primary producers, sponges, bryozoans, sea spiders, echinoderms, amphipods and isopods are abounding and diverse

here, it indicates that the continent's isolation and climatic changes have not impeded their success (Clarke and Crame, 2010; Crame, 2018).

Even though the West Antarctic shelf is broad, up to 200 km, a considerable portion is occupied by the shelf ice reducing the available habitat. The shelf ice extent is supported by the largest embayment the Ross and Weddell Seas and to a lesser extent the Amundsen and Bellingshausen seas. Conversely, the East Antarctic shelf is narrow and sharply sloping down, which is the effect of isostatic depression (the enormous pressure of the ice sheet), ice sheet advances and shelf scouring. Obviously, the depth of habitats on the shelf can have a profound effect on the level of disturbance (Griffiths, 2010). No less important is to acknowledge that overall Antarctica has 2.3% of the world's coastline, but only less than one-seventh of this coastline is ice-free in summer, and there is no ice-free coastline in winter (Crame, 2018; Peck, 2018).

Today's subtidal and intertidal zones, exposed to iceberg scour or fast ice formation etc., starts their production with diatoms and ephemeral algae first colonisers then polychaetes, ascidians and amphipods. Shrimps like *Gondogeneia antarctica* and *Paramoera edouardi*, or snail *Laevilitorina antarctica*, the limpet *Nacella concinna*, the sea urchin *Sterechinus neumayeri* and the sea star *Odontaster validus* immigrate from the subtidal zone to graze on the diatoms carpet and the diatoms move deeper. Then, the notothenioid fish *Pagothenia borchgrevinki* hides under the stones to consume crustaceans, zooplankton and small fishes. As winter approaches, the diatom carpet rapidly disappears and most of the grazers move back into the subtidal zone. When *H. balfourensis* (sea sponge) invades during low-ice years, it`s sea star predators *Odontaster meridionalis*, *O. validus* and *P. f. antarcticus* follows (Dayton, 1989). *Odontaster validus, S. neumayeri*, the nemertean *Parborlasia corrugatus*, isopod *Glyptonotus antarcticus*, pycnogonid *Colossendeis* spp. and juvenile notothenioid *P. borchgrevinki* all inhabit the anchor ice zone during high-ice years. Some may graze diatoms off the ice surface. The notothenioid fish *Trematomus eulepidotus, T. lepidorhinus and T. scotti* dominate undisturbed areas in the eastern Weddell Sea, and *T. nicolai and T. pennellii* dominate areas disturbed by ice scour (Brenner *et al.*, 2001).

2.5.2 Antarctic terrestrial fauna

Antarctic terrestrial fauna evolution is documented even from pre-Gondwana break-up (e. g., chironomid midges from Antarctic Peninsula). It thus appears that substantial elements of the contemporary Antarctic terrestrial biota have been continuously isolated in situ (Dietz *et al.*, 2015a) on a multi-million-year timescale. Mites survived in nunataks, springtail, mites, nematodes and tardigrades in the Transantarctic Mountains since the Gondwana break-up (McMurdo, Dry Valleys). On the other hand, cladocerans, rotifers and diatoms have been present in lakes since 130 Kya. Animals have survived in ice-free regions such as refuges, nunataks, as documented from Antarctic peninsula nematodes (Convey and Stevens, 2007b).

In accordance with the low availability of the marine shelf, even though the Antarctic continent spans 13 million km^2, it offers less than 0.3 % of ice-free habitats, or in other words, almost 99.7 % is covered by permanent ice. Main drivers of population structuring of Antarctic terrestrial biota is the patchiness of snow and ice-free regions and length of the growing season (McGaughran *et al.*, 2010).

It follows that the soil in Antarctica is scarce but still comprise the unique communities within the most extreme terrestrial habitat in the World. The severe limitations comprise low temperatures, aridity (water is a biological driver of diversity), high level of solar radiation and lack of ice-free terrestrial habitat. That is to say, coasts are more diverse and better-vegetated thanks to the liquid water availability, which is higher in coastal regions, mainly in East Antarctica and in the Antarctic Peninsula (Smykla *et al.*, 2018). Such as the coastal soil, mosses and lichens or moraines but also nunataks and mountain ranges are just seasonally accessible (Kagoshima *et al.*, 2019; McGaughran *et al.*, 2019) and resemble islands in the ice isolated by frozen water. Antarctic terrestrial ecosystems and food chains are relatively simple, with fauna almost entirely composed of soil invertebrates comprising microarthropods, nematodes, tardigrades, rotifers, and protozoans. The terrestrial flora is made up of bryophytes, lichens, algae, and cyanobacteria (Convey and McInnes, 2005). The most common diverse and abundant animals comprise rotifers, tardigrades and nematodes. Rotifers and tardigrades are found in cryptogamic vegetated

moist soils in nunataks, even in Dry Valleys (Victoria Land), contrary to everywhere present nematodes (Smykla *et al.*, 2018). Concerning rotifers, it seems that bdelloids dominate harsh habitats and their diversity increases along with rising latitude, contrary to monogononts. It might be due to the low predation, parasite and competitor pressure. Monogononts are found in the freshwater lagoons and lakes, whereas bdelloids are limno-terrestrial, preferring moisture soil and moss or lichens of coastal bays or maritime Antarctic Islands (Fontaneto, Iakovenko and De Smet, 2015). Fontaneto (2015) also summarises the presence of Rotifers at 67% of investigated moss, soil or lichens sampled at nunataks which can be described as mountain peaks bare of ice. Rotifers can be putatively present even in the Lake Vostok borehole in the vicinity of the embayment (Shtarkman *et al.*, 2013; Griffiths *et al.*, 2021). Here is appropriate to mention that the Antarctic explorer Shackleton states" the Rotifers will triumph over the scientist in those conditions" (O.B.E, 1999).

What allows rotifers and tardigrades to boast as the most universe effect studied species are their natural abilities. Tardigrades, the "iron ladies", multicellular organisms showing extreme reparation mechanisms of fragmented DNA by X-ray and so survive open space without any protection (Hashimoto *et al.*, 2016; Carrero *et al.*, 2019; Hespeels *et al.*, 2020). Moreover, Rotifers bear a more honoured position of evolutionary scandals. They have evolved asexually, lacking normal meiosis for approximately 60 Mya, which may help them to establish a population in newly colonized areas more easily. Rotifers origin has been congruently conveyed by genomic sequencing of the bdelloid *Adineta vaga*, showing the avoidance of homologous chromosome pairing and therefore excluding the possibility of standard meiosis (Flot *et al.*, 2013). Bdelloids are degenerate tetraploids with highly diverged homeologs gene copies rather than homologs (Signorovitch *et al.*, 2015). Even though bdelloids consisted of 500 lacking males and missing meiosis species, the uncertainty of the bdelloid asexuality has been erased by (Eyres *et al.*, 2015; Signorovitch *et al.*, 2015), who more precisely expressed that asexuality, along with reproducing without meiosis, lasts for millions of years (60 Mya), and final confirmation of asexuality but with some exceptions has been made by (Flot *et al.*, 2013, 2016; Nowell *et al.*, 2018). Moreover, they are famous for common horizontal gene transfer not just from the environment but also from the congener. Interestingly, Rotifers can cope even with radiation-induced DNA double-strand breaks

(Debortoli, Laender and Doninck, 2018). Rotifers' ancient origin is indicated by the considerable number of synonymous sequences between families and by finding bdelloid remains in 35 to 40 million-year-old amber (Welch, Ricci and Meselson, 2009). Nevertheless, Eyres et al. (2015) specified the bdelloids divergence to 60Mya.

To conclude, both the Southern Ocean shelf and terrestrial Antarctica were apparently inhabited by species able to withstand chiefly extreme low temperature and the most intense seasonality by phenotypic plasticity (DeVries and Wohlschlag, 1969; Barnes and Peck, 2008; Hawes, Marshall and Wharton, 2011; Clark *et al.*, 2018). There is an intense debate about the steepness of diversity and abundance decrease with increasing latitude, which needs to consider habitat type, continent and, importantly, stress on the taxonomic group (Hillebrand, 2004). It follows that the extinction rate differed in a marine and terrestrial ecosystem with respect to acquired adaptations of particular taxa.
The most pronounced pre-adaptation, the freeze avoidance, is acquiring ample Antifreeze glycoproteins (AFGPs). AFGPs surface binds the ice crystals preventing their growth (Patarnello *et al.*, 2011). Important to highlight, they are not the sole trigger of Notothenioids adaptive radiation since the origin of occurrence is dated to the Circum-Antarctic current creation (42 Mya). However, their ecological and morphological radiation started in the Middle Miocene climate transition, the second cooling period (14 Mya). It was the time of creating a marine physical barrier, the geographical isolation consequently the time of species radiation throw extinction of potential competitors and subsequent opening of the new niche (Matschiner, Hanel and Salzburger, 2011; Near *et al.*, 2012).
Subsequently, there is often studied and often refuted phenomenon the Thorson's Rule presuming the high proportion of brooders in polar regions (Jossart, Sands and Sewell, 2019; Moreau *et al.*, 2019). Other physiological reproduction adaptations include a slower evolution rate, prolonged periods spent in the inactive cryptobiotic state, longer generation time, or the parthenogenesis occurrence (Kagoshima *et al.*, 2019). Mechanisms to decrease freezing point, thermal hysteresis, or tolerance to freezing of body water, DNA repair, and telomere maintenance belongs to thermal stress adaptations (Convey, 1996; Hengherr *et al.*, 2009; Hawes, Marshall and Wharton, 2011; Carrero *et al.*, 2019; Kamilari *et al.*, 2019).

Terrestrial adaptation of the freezing avoidance in springtails is achieved by supercoiling, depression of body fluids, desiccation, gut evacuation and the synthesis of low molecular-weight polyols that colligatively depress the freezing point (Hawes, Marshall and Wharton, 2011). To mitigating stress factors in freezing temperatures belong genes up-regulation, or duplication (gene expansion), of, e.g. protein biosynthesis, anti-oxidation, anti-apoptosis (Chen *et al.*, 2008). There are also epigenetic studies suggesting physiological flexibility and plasticity, associated with changing environmental niche (Clark *et al.*, 2018; Hawes *et al.*, 2018). On the contrary, loss of heat shock proteins makes limitations, and organisms become more stenothermal (Hofmann *et al.*, 2000).

3. Aims of the thesis

The motivation for the present thesis stems from the questions related to evolution in a harsh, cold and isolated environment, particularly to the impact of glacial cycles on southern polar biota, both marine and terrestrial. My thesis aims are centred on two sets of major tasks that might respond to some emerging patterns of the polar organism's persistence and evolution.

1, Given the broad spectrum of population genetic patterns revealed among Antarctic organisms, we expanded on Thatje et al. premises (2005) explaining: "Differences in early life-history patterns are key to the resilience potential of species in response to climate changes", where he explains further that:" Benthic organisms, if they are to survive, must either find refuges on the shelf and slope or migrate further into the deep sea, if they are able to tolerate such conditions" . We tried to identify the common life-history traits underlying the variety of observed patterns and asked if the lifestyle, namely where the particular species live (adult and its eggs and juvenile stages), is the key trait to predict the species population vulnerability to climate change. To achieve this aim, we initially focused on Notothenioidea, a case of adaptive radiation in the marine realm (Near *et al.*, 2012), which is particularly suited for comparative purposes composed of phylogenetically closely related, yet ecologically widely differing species. Specifically, the following questions were addressed in a series of comparative phylogeographic studies:

- Are populations of marine Notothenioidiea fishes panmictic with unlimited gene flow on the pan-Antarctic scale? Are there any intrinsic traits, namely duration of pelagic larval period and the tendency towards pelagic lifestyle in adulthood, explaining the population connectivity? **(Publication I)**
- As a specific answer to Thatje *et al.* (2005)**,** we tested how the tendency to benthic lifestyle shapes the species and population response to glacial cycles and depletion of the marine environment by expanding ice during the Ice ages. What is the intrinsic factor explaining population size through time, and what aspect explains who

surpassed LGM condition's less affected than the other? Plays the phylogenetic relationship a role? **(Publication IV, Publication VII in prep.)**

- **Publication II** describes the Notothenioidea species diversity at the Prince Gustave Channel.

2, In the second section, we focused on the evolution of terrestrial ecosystems dominated by microinvertebrates, mainly bdelloid rotifers. Specifically, using a robust combination of morphological and DNA taxonomy as well as comparative phylogeography. **Publications III, V and VI** address the following questions:

- Is the hypothesis that everything is everywhere valid? What is the species diversity within the terrestrial Antarctic bdelloid Rotifera? Is the hypothesis of the high endemicity rate steaming from the Antarctic isolation correct?
- Do Antarctic rotifers systematically differ from non-Antarctic ones in an evolutionary sense? To what extent do the harsh Antarctic climate shape the Rotifera evolution and today's diversity compared to species from mild climates?

4. The list of publications on which the thesis is based

Materials & Methods used in the thesis are included in seven publications listed below.

I. Van de Putte, A.P., Janko, K., **Kasparova, E**., Maes, G.E., Rock, J., Koubbi, P., Volckaert, F.A.M., Choleva, L., Fraser, K.P.P., Smykla, J., Van Houdt, J.K.J., Marshall, C., **2012.** Comparative phylogeography of three trematomid fishes reveals contrasting genetic structure patterns in benthic and pelagic species. *Marine Genomics*, 8, pp. 23–34. doi:10.1016/j.margen.2012.05.002

II. Mašová, Š., and **Kašparová, E.** (**2012**). Preliminary report on fish diversity at the Prince Gustav Channel, the northern part of the James Ross Island, Antarctica *Czech Polar Reports*, 2(2), pp. 92–102. doi:10.5817/CPR2012-2-9.

III. Iakovenko, N.S., **Kašparová, E.,** Plewka, M., Janko, K., **2013**. Otostephanos (Rotifera, Bdelloidea, Habrotrochidae) with the description of two new species. *Systematics and Biodiversity*, 11(4), pp. 477–494. doi:10.1080/14772000.2013.857737

IV. **Kašparová, E.,** Putte, A.P.V. de, Marshall, C., Janko, K., **2015.** Lifestyle and Ice: The Relationship between Ecological Specialization and Response to Pleistocene Climate Change. PLOS ONE 10, e0138766.

V. Iakovenko, N.S., Smykla, J., Convey, P., **Kašparová, E**., Kozeretska, I.A., Trokhymets, V., Dykyy, I., Plewka, M., Devetter, M., Duriš, Z., Janko, K., 2015. Antarctic bdelloid rotifers: diversity, endemism and evolution. *Hydrobiologia*, 761(1), pp. 5–43. doi:10.1007/s10750-015-2463-2.

VI. Cakil, Z.V., Garlasché, G., Iakovenko, N., Cesare, A.D., Eckert, E.M., Guidetti, R., Hamdan, L., Janko, K., Lukashanets, D., Rebecchi, L., Schiaparelli, S., Sforzi, T., **Štefková Kašparová**, **E.,** Velasco-Castrillón, A., Walsh, E.J., Fontaneto D (2021). Comparative phylogeography reveals consistently shallow genetic diversity in a mitochondrial marker in Antarctic bdelloid rotifers. *Journal of Biogeography*, 48(7), pp. 1797–1809. doi:10.1111/jbi.14116.

VII. Š**tefková Kašparová, E.** *et al***.**, (in preparation): Southern Ocean: home for brave; Pelagic and deep-sea communities have had bigger population size throwgh time.

5. Outcomes of the publications on which the thesis is based

Comments of the publications results and statement of my personal contribution to the particular publication –*in italics*).

Publication I: Comparative phylogeography of three trematomid fishes reveals contrasting genetic structure patterns in benthic and pelagic species (Van de Putte *et al*., 2012)

As for Perciformes, the Antarctic shelf is inhabited dominantly by the Notothenioid fish, which are the most abundant both by the species number and by biomass (Near *et al.*, 2012). There was thought to be the virtual fish panmixia in the Southern Ocean shelf seas comprising the High Antarctic and Antarctic Islands. Later published papers indicated that Nototenioid population structure range from population panmixia to relatively strong population structuration or even cryptic speciation (Wilson *et al.*, 2007; Damerau *et al.*, 2012). In this paper, we tested whether the connectivity between populations in the Antarctic marine shelf fish species could be positively correlated to a combination of life-history traits, namely duration of pelagic larval period and the tendency towards pelagic lifestyle in adulthood. Species genetic gene flow was investigated on three closely related Trematomus fish species using six microsatellite markers and one mitochondrial cytochrome *b* gene. The pelagic *Trematomus newnesi* with a shorter pelagic larval period had lower genetic differentiation suggesting higher population connectivity than two benthic species *Trematomus hansoni* and *Trematomus bernacchii*; both with pelagic larvae but *T. hansoni* with a long period of them in water column. The *T. newnesi* population from Adélie Land had no differences in gene flow pattern on a time scale; the only sampled locality in different periods**.** The survey did not confirm the overall hypothesis of passive larval dispersal as a major driver of genetic connectivity between populations**;** population structuration was significant for both benthic species**.** On the other hand, interestingly**,** it appeared that the adult lifestyle, namely the pelagic strategy, plays a prominent role in maintaining cohesiveness of studied populations.

My contribution: Performing Trematomus hansoni cyt b sequencing; mitochondrial data analysis along with its interpretation and writing the respective parts of the manuscript.

Publication II: Preliminary report on fish diversity at the Prince Gustav Channel, the northern part of the James Ross Island, Antarctica (Mašová and Kašparová, 2012).

This publication describes the ichthyofauna of James Ross Island caught in 2012. Until this report, there was no fish species monitoring in this region.

My contribution: species identification and writing respective parts of the manuscript.

Publication III: **Otostephanos (Rotifera, Bdelloidea, Habrotrochidae) with the description of two new species.** (Iakovenko *et al.*, 2013)

This publication served as a proof of concept for our ability to study and robustly describe invertebrate taxa found in the Antarctic. In particular, it is devoted to morphology, ecology and taxonomy revision of the genus *Otostephanos,* the European Rotifera. For me, this paper served as a starting point for micro-fauna research and species delimitation methods extended in polar related topics mentioned above**.**

My contribution: DNA laboratory work, data processing, writing of the appropriate part of the manuscript.

IV: Lifestyle and Ice: The relationship between ecological specialization and response to Pleistocene climate change (Kašparová *et al.*, 2015)**.**

The antarctic shelf, the centrum of therein diversity, was repeatedly scoured by a grounded ice sheet during the ice ages (Thatje, Hillenbrand and Larter, 2005). Nevertheless, most species survived the last glaciations „in situ"(Janko *et al.*, 2011). Here we asked: Which species were less affected by glaciation and why? Previous publication **(Publication I)** virtually raised the question of intrinsic factors like length of the larval period, ecology of adult stages and eggs contributing to interspecific differences in population connectivity among notothenioid fish.

Here, we took advantage of a comprehensive sample collection of 13 Notothenioid species and categorized them according to differences in lifestyle (benthic vs. pelagic), and we also took into account the addiction of their developmental stages to the seafloor, such as, e.g. guarding and incubation time of benthic eggs (Kock, Pshenichnov and Devries, 2006). We found that fish dependent on the shelf benthos were more prone to population bottlenecks putatively driven by growing grounded ice sheet and falling debris than those having all developmental stages in the water column.

My contribution: Performing laboratory work; data analysis along with its interpretation and writing the majority of the manuscript text.

Publication V: Antarctic bdelloid rotifers: diversity, endemism and evolution (Iakovenko *et al.*, 2015)**.**

The paper describes both morphologically (detailed morphological morphometrics and SEM) and molecularly new bdelloid species to science along with cryptic species in the Antarctic. The Antarctic is the continent suspected for high endemicity, which was firstly mentioned by Velasco-Castrillón et al. (2014) and hereby secondly detected and specified as a continent with the largest number of endemic rotifers. The Antarctic Rotifers comprise genera Adineta, Habrotrocha, Philodina and Macrotrachela. Adineta genus shows a high endemicity rate even in different continents contrary to Philodina; two of which are cosmopolitan.

The paper outcomes called into question the validity of the well-known hypothesis of " everything is everywhere" in Williams (2011) by particular species distinctive pattern of distribution.

My contribution: Laboratory work, data processing, writing the appropriate part of the manuscript text.

Publication VI: **Comparative phylogeography reveals consistently shallow genetic diversity in a mitochondrial marker in Antarctic bdelloid rotifers** (Cakil *et al.*, 2021)

This paper aimed the search at the genetic diversity pattern of Antarctic rotifers, particularly at phylogeography and phylogeny, in contrast with non- Antarctic species. We highlighted the high endemicity in the Antarctic and emphasized that neither non-Antarctic bdelloid rotifer is endemic to the one continent only. Antarctic endemic bdelloid species belonging to the same cosmopolitan genera and families (Donner, 1965; Fontaneto, Iakovenko and De Smet, 2015) possess overall shallower genetic diversity (and less cryptic species) and younger Most Recent Common Ancestor (MRCA) than non-Antarctic bdelloids. The approximate absolute dating gives the Antarctic MRCA to 2 Mya (The ice shelf collapse (1.1 Mya) coincided with warmer temperatures, ever since, the shelf has been re-established (Halanych and Mahon, 2018) or 1-24 Mya (Eocene climate transition)) contrary to non-Antarctic MRCA 50 Mya. Corcondantly, the geographical and genetic distances was significant and positive for both Antarctic and non- Antarctic species.

My contribution: Laboratory work, data processing

Publication VII: Southern Ocean: home for brave; Pelagic and deep-sea communities have had bigger population size throw time (in preparation)

The manuscript in preparation is a part of this dissertation in the chapter.7, pages 149- 179.

My contribution: Laboratory work, data processing, manuscript writing

6. Publications on which the thesis relies

ARTICLE IN PRESS
MARGEN-00138; No of Pages 12
Marine Genomics xxx (2012) xxx–xxx
Contents lists available at SciVerse ScienceDirect
Marine Genomics
journal homepage: www.elsevier.com/locate/margen

Comparative phylogeography of three trematomid fishes reveals contrasting genetic structure patterns in benthic and pelagic species

Anton P. Van de Putte [a,b,*,1], Karel Janko [c,1], Eva Kasparova [c], Gregory E. Maes [a], Jennifer Rock [d], Philippe Koubbi [e], Filip A.M. Volckaert [a], Lukáš Choleva [c], Keiron P.P. Fraser [f], Jerzy Smykla [g,h], Jeroen K.J. Van Houdt [i], Craig Marshall [j]

[a] Laboratory of Biodiversity and Evolutionary Genomics, KU Leuven, Ch. Deberiotstraat 32, B-3000 Leuven, Belgium
[b] Royal Belgian Institute for Natural Sciences, Vautierstraat 29, B-1000 Brussels, Belgium
[c] Laboratory of Fish Genetics, Institute of Animal Physiology and Genetics, Academy of Sciences of the Czech Republic, Rumburská 89, 27721 Liběchov, Czech Republic
[d] Department of Zoology, University of Otago, Dunedin, 9054, New Zealand
[e] UPMC Université Paris 06, UMR 7093, Laboratoire d'Océanographie de Villefranche, BP28, 06234 Villefranche-sur-Mer, France
[f] Government of South Georgia and the South Sandwich Islands, Government House, Stanley, Falkland Islands
[g] Department of Biodiversity, Institute of Nature Conservation, Polish Academy of Sciences, Mickiewicza 33, 31–120 Kraków, Poland
[h] Department of Biology and Marine Biology, University of North Carolina Wilmington, 601 S. College Rd., Wilmington, NC 28403–5915, USA
[i] Laboratory for Cytogenetics and Genome Research, KU Leuven, O&N I Herestraat 49 - box 602 , B-3000 Leuven, Belgium
[j] Department of Biochemistry and Genetics Otago, University of Otago, PO Box 56, Dunedin 9054, New Zealand

ARTICLE INFO

Article history:
Received 2 February 2012
Received in revised form 18 May 2012
Accepted 22 May 2012
Available online xxxx

Keywords:
Microsatellite markers
Mitochondrial
Larval duration
Glaciations
Notothenioidei
Life history

ABSTRACT

Population genetics patterns of marine fish in general and of Southern Ocean fish in particular range from virtual panmixia over ocean-wide scale to deeply fragmented populations. However the causes underlying these different patterns are not properly understood. In this paper, we tested the hypotheses that population connectivity is positively related to a combination of life history traits, namely duration of pelagic larval period and the tendency towards pelagic life style in the adulthood. To do so, we analysed the variability of six microsatellite and one mitochondrial marker (cytochrome *b*) in three Southern Ocean fish species (*Trematomus newnesi*, *Trematomus hansoni* and *Trematomus bernacchii*). They share a recent common ancestor but notably differ in their duration of pelagic larval period as well as pelagic versus benthic lifestyle. We sampled over a range of more than 5000 km for all three species and used a number of population genetics tools to investigate past and contemporary levels of connectivity. All species experienced population fluctuations, but coalescent simulations suggested that contemporary populations are in migration-drift equilibrium. Although global F_{ST} values were rather low, a significant population structure separated the High-Antarctic from the Peninsular regions in all species. The level of genetic differentiation was much lower in the pelagic *versus* benthic species. Present data suggest that past and present genetic structuring in the Southern Ocean are indeed related with the ecological traits of Antarctic fish, however the relative importance of individual factors remains unclear.

© 2012 Elsevier B.V. All rights reserved.

1. Introduction

Many marine species disperse primarily during the larval phase, while adults show limited movements (Bonhomme and Planes, 2000; Thorrold et al., 2007). Indeed, seventy percent of marine organisms have a planktonic stage during which larvae may disperse (Thorson, 1950). In general, fishes with longer larval duration display less genetic differentiation than those with shorter larval duration (Waples, 1987; Bay et al., 2006; Bradbury et al., 2008; Hauser and Carvalho, 2008). However, a growing body of evidence suggests the importance of other factors, such as currents and larval retention (Carreras-Carbonell et al., 2006), that may cause strong differentiation even in species with a long larval phase (Taylor and Hellberg, 2003; Planes et al., 1998). Many larvae are also capable of active vertical migration, which in combination with vertically stratified flows may allow them to avoid advection. In many cases, particularly in later stages of their pelagic developmental phase, larvae may be able to swim even faster than ambient currents (Leis, 2006).

Direct measurements of larval/individual dispersal of small organisms are difficult to obtain in any marine environment (Bay et al., 2006; Hedgecock et al., 2007; and references therein) but population genetics studies, although not without limitations, provide some of the most valuable tools for estimating such parameters (Hedgecock et al., 2007; Selkoe et al., 2008; Skillings et al., 2011). Populations evolve through changes in allele frequencies, which in turn change through

* Corresponding author at: Royal Belgian Institute for Natural Sciences, Vautierstraat 29, B-1000 Brussels, Belgium. Tel.: +32 2 6294318.
E-mail address: anton.vandeputte@naturalsciences.be (A.P. Van de Putte).
[1] Made an equal contribution.

1874-7787/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.margen.2012.05.002

Please cite this article as: Van de Putte, A.P., et al., Comparative phylogeography of three trematomid fishes reveals contrasting genetic structure patterns in benthic and pelagic species, Mar. Genomics (2012), doi:10.1016/j.margen.2012.05.002

evolutionary forces such as mutation, random genetic drift, gene flow and selection. In the absence of environmental change and over prolonged periods of time, a population will reach a genetic equilibrium determined by the opposing evolutionary forces of drift and migration. Historical factors such as climate associated extinctions and recolonisations or changes in the direction of currents also play an important role in shaping the population structure of marine species (Maggs et al., 2008). Genetic methods for estimating effective dispersal and connectivity are becoming increasingly effective. However publications often focus on low latitude reef species and general conclusions concerning the processes driving differentiation within species remain hard to draw (Bohonak, 1999; Bay et al., 2006).

The Southern Ocean, in spite of obvious challenges for sampling, offers a unique environment to study the effect of biological and environmental forces on patterns of dispersal and evolution in marine organisms. Characterised by two major currents that promote dispersal on a circumpolar scale, the majority of the surface flow moves in a clockwise (eastward) motion around Antarctica forming the Antarctic Circumpolar Current (ACC). Along the margins of the continent, however, there is a westward current, the Antarctic Slope Current (ASC) (Orsi et al., 1995). Application of genetic tools has revealed that patterns of population differentiation in Antarctic/Southern Ocean organisms range from virtual panmixia on a circum-Antarctic scale to isolated populations or even crypic species (for an overview see Rogers, 2007, 2012; Volckaert et al., 2012). The causes for such differences are not fully clear but the ultimate outcome likely depends on the interplay of several factors. Some studies emphasize the dominant roles of major climatic and geological events affecting southern polar organisms more or less simultaneously (Thatje et al., 2008). Other studies suggest that conditions for survival and dispersal differ across Antarctic biogeographic regions and hence the genetic structure of given species depends on its distribution range and the local conditions, especially temperature (McGaughran et al., 2010; Patarnello et al., 2011).

Yet other studies highlight the dominant role of intrinsic factors such as habitat preference in shaping the genetic structure of Antarctic species. Comparative analysis of four trematomids, *Trematomus bernacchi*, *Trematomus pennelli*, *Pagothenia borchgrevinki* and *Trematomus newnesi* showed a qualitatively different response of benthic and pelagic species to the Pleistocene glaciations (Janko et al., 2007). Benthic species in particular are expected to express a more pronounced genetic structure than pelagic ones (Rogers, 2007, 2012), which may either reflect lower dispersal at larval and adult stages or a higher complexity of the benthic environment, promoting habitat specialization. Such a situation may become extreme when populations have been historically separated during cycles of glaciation, suggesting that life style may decisively affect the evolution and adaptability of the species. Although Matschiner et al. (2009) recently showed that passive larval dispersal within Antarctic currents may be sufficient to homogenise populations of benthic fish species at a scale of hundreds of kilometres, one may expect that the length of pelagic larval phase in combination with specialisation to a benthic or pelagic life history in adulthood may have a prominent effect on the genetic build-up of a species' genepool (Purcell et al., 2006; Bradbury et al., 2008; Papetti et al., 2012).

The strength of a comparative approach lies in the possibility to disentangle the major determinants of population structure of Southern Ocean organisms and to broaden our scope of understanding of marine ecological speciation in general. Here we focus on the Trematominae, an endemic tribe of notothenioid teleosts from the Southern Ocean widely used as a model of cold adaptation (Patarnello et al., 2011). The group forms a monophyletic species flock strikingly diverse in morphology and demonstrates significant evolutionary versatility with a variety of ecological forms including species with benthic, pelagic or cryopelagic ecology (Janko et al., 2011; Rutschmann et al., 2011).

This study aims at comparing genetic diversity, genetic differentiation and environmental and life-history (traits) between three fish species with distinct characteristics living over the same environmental gradient. For this the nuclear and mitochondrial genetic structure and demographic history of *T. bernacchii*, *T. hansoni* and *T. newnesi* was compared. The former two are bottom-feeding fish that lay demersal eggs (with *T. bernacchii* even laying eggs in sponges and displaying nest guarding behaviour) (DeWitt et al., 1990; Kock et al., 2006; La Mesa et al., 2006). In contrast, *T. newnesi* prefers a semi-pelagic or even cryopelagic habitat. The larval phase of all three species is pelagic but differs in duration; *T. hansoni* has a long larval phase spanning over the winter, while the pelagic larval phase of *T. newnesi* is considerably shorter and accomplished during the first fall (Duhamel et al., 1993; detailed information on larval ecology of *T. bernacchii* remains undetermined). Adult individuals may differ substantially in dispersal ecology, since *T. newnesi* populations are known to largely vary in local abundance, indicative of seasonal migrations, while *T. bernacchii* is a rather sedentary fish (as an extreme example of this behaviour, we noted the re-capture of a specimen at the Ross Sea region, which had been tagged two years ago in the very same spot (J. MacDonald pers. Comm..). We hypothesize that life history traits such as larval duration and adult ecology affect genetic structure and diversity.

2. Material and methods

2.1. Sampling, DNA purification, DNA amplification and species validation

Samples of *T. newnesi* Boulenger, 1902, *T. hansoni* Boulenger, 1902 and *T. bernacchii* Boulenger, 1902 of the family Nototheniidae were collected using land-based sampling in Adelie Land and the Ross Sea and by bottom trawls during various scientific expeditions (Fig. 1, Table 1). For *T. newnesi*, two temporal samples were available from the same site at Adelie Land. Specimens were morphologically identified on site and muscle or fin tissue was preserved in 100% ethanol.

Total genomic DNA was extracted using the Nucleospin Extraction kit (Macherey-Nagel GmBH) following the manufacturer's specifications. Amplification of a set of six microsatellites followed the methods described in Van Houdt et al. (2006). PCR products were visualised on an ABI 3130 Genetic Analyser (Applied Biosystems). Allele size was determined by means of an internal Genescan 500-LIZ size standard and genotypes were obtained using GENEMAPPER v.3.7 (Applied Biosystems). Mitochondrial sequences of the cytochrome *b* gene (*cyt b*) for a subset of individuals were obtained according to the protocol of Janko et al. (2007) using the primers: CytBU15786-Tremato (5′TGAGGkGGrTTTTCGGTAGATA-3′) and CytB-L16317-Tremato (5′-GATrTAnGGrTCCTCAaCGGG-3′). Haplotypes were compared to those of Janko et al. (2007). Novel sequences were submitted to genbank JX138967-JX138996. For an overview of the accession number see Table A4–6. Sequences were aligned and analyses were performed in BioEdit v.7.0.9. Morphological identification was validated genetically either using the method described in Van de Putte et al. (2009) or by blasting the *cyt b* sequences. The former method assigns specimens to an *a priori* defined baseline group of eight trematomid species based on samples for which there was a congruent morphological and *cyt b* identification. Analyses were performed in STRUCTURE v.2.3 (Pritchard et al., 2000) using the non-admixture model in combination with the non-correlated allele frequency model. A burn in period of 10^5 steps and 10^6 Markov Chain Monte Carlo simulations was used. A total of 5 runs were carried out to check the repeatability of the results. Assignments scores (q) were plotted and the proportion of correctly assigned individuals ($q > 0.8$) was calculated.

2.2. Microsatellites

2.2.1. Genetic diversity

Genotype and allele frequencies of the microsatellite loci were used to obtain standard estimates of genetic diversity within and between sample sites. Tests for null alleles and other potential technical artefacts, such as stuttering and large allele dropout, were performed

Please cite this article as: Van de Putte, A.P., et al., Comparative phylogeography of three trematomid fishes reveals contrasting genetic structure patterns in benthic and pelagic species, Mar. Genomics (2012), doi:10.1016/j.margen.2012.05.002

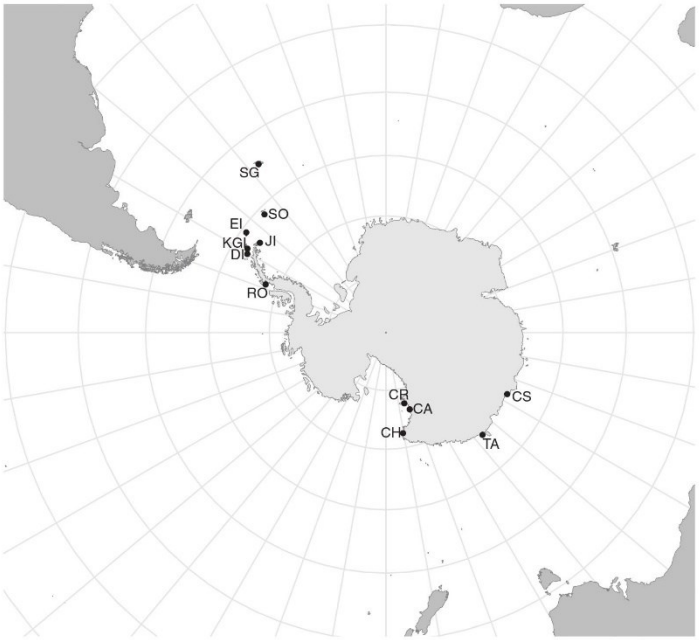


Fig. 1. Geographical distribution of the sampling sites. Sample codes as in Table 1.

using the software package MICRO-CHECKER v.2.2.1 (Van Oosterhout et al., 2004). Genetic variation in each population was measured by calculating the mean numbers of alleles per locus, the observed (H_O) and unbiased expected (H_E) heterozygosities and the F_{IS}. Deviations from Hardy-Weinberg equilibrium (HWE) were assessed with GENETIX v.4.05 (Belkhir et al., 2002). Allelic richness was determined using FSTAT v.2.9.3.2 (Goudet, 2001). Exact tests of linkage disequilibrium between pairs of loci were calculated at each location using GENEPOP v.3.4 (Rousset, 2008). To assess the statistical power of our microsatellite markers compared to the observed levels of differentiation (F_{ST}), we used a modified version of the program POWSIM (Ryman and Palm, 2006) tailored to incorporate a higher numbers of alleles. This program allows to estimate the probability of type I and type II errors for a given set of loci.

2.2.2. Population subdivision

Six complementary methods were used to determine the population structure of *T. newnesi*, *T. hansoni* and *T. bernacchii*. A factorial correspondence analysis (FCA) on multilocus genotypes was performed using GENETIX v.4.05 (Belkhir et al., 2002). Population differentiation was quantified in FSTAT v.2.9.3.2 (Goudet, 2001) using the standard allelic variation F_{ST} estimated as θ (Weir and Cockerham, 1984). To calculate the relationship between geographical distance (coastline distances among sampling sites) and genetic distance (population pairwise F_{ST}) we performed a mantel test in ARLEQUIN v.3.5 (Excoffier and Lischer, 2010). We also plotted a linear regression between geographic distance (LN(distance)) and genetic distance ($F_{ST}/(1-F_{ST})$) for a visual guidance. Bayesian clustering was performed using STRUCTURE v.2.3.2 to determine the number of populations (K) with the highest posterior probability and to estimate admixture proportions. Simulations were performed using an admixture model with correlated allele frequencies between populations and using sampling location information (MCMC consisted of 20^4 burn in iterations followed by 10^5 sampled iterations). The range of possible tested K was one to ten and five trial runs were carried out for each putative K. K was estimated using the procedure of Evanno et al. (2005). We performed an hierarchical analysis of genetic structure through an Analysis of Molecular Variance (AMOVA) (Excoffier et al., 1992) implemented in ARLEQUIN v3.5 (Excoffier and Lischer, 2010) to test for geographical structure. Using F_{ST} measures of genetic distance, we estimated the level of genetic structuration within populations, between populations within a region, and between regions (which were defined according to the results of STRUCTURE).

In order to detect putative deviations from migration-drift, we applied a Bayesian approach developed by Ciofi et al. (1999) and implemented in the program 2MOD by M.A. Beaumont (http://www.rubic.rdg.ac.uk/~mab/software.html). Two models were evaluated: (1) the gene flow model, which assumes that gene frequencies within populations are caused by a balance between genetic drift and gene flow, and (2) the drift model, in which allele frequencies within populations are evolving purely through drift and differentiation among populations was caused by splitting of ancestral population with no subsequent migration. Both models assume negligible effect of mutations such that alleles are identical by descent, and the gene flow model assumes that the mutation rate is much smaller than the immigration rate (Ciofi et al., 1999). For each population, the program also calculates F (an analogue of F_{ST}), which is the probability of coalescence among randomly chosen chromosomes within the same population. For each species, we analysed two types of dataset: a) treating each sampling site as a separate population and b) pooling the sites according to the STRUCTURE results. The Markov Chain Monte Carlo search was carried out twice on each dataset using 10^5 iterations. The first 10% of the runs were discarded to remove effects of initial starting parameters. In each run, the probability of given model was estimated from the number of times it appeared during the simulation.

Please cite this article as: Van de Putte, A.P., et al., Comparative phylogeography of three trematomid fishes reveals contrasting genetic structure patterns in benthic and pelagic species, Mar. Genomics (2012), doi:10.1016/j.margen.2012.05.002

Table 1
Description of sampling sites for all species.

Trematomus species	Location	Location code	Sampling year	Expedition name	N_m	N_s
T. newnesi	Cape Hallett	CH	2004	ANZ K066	30	31
	Joinville Island	JI	2007	RV Polarstern ANTXIII/8	49	15
	King George Island	KGI	2007	RV Polarstern ANTXIII/8	15	3
	South Orkneys	SO	2006	RV James Clark Ross AFI 6/16	43	6
	Adelie Land I	TA1	2003	land based / ICOTA	89	0
	Adelie Land II	TA2	2005	land based / ICOTA	57	20
T. hansoni	Cape Hallett	CH	2004	ANZ K066	33	27
	Cape Roberts	CR	2002	ANZ K066	9	7
	Joinville Island	JI	2007	RV Polarstern ANTXIII/8	15	22
	South Georgia	SG	2007	S. Georgia Groundfish Survey SG07	21	10
	South Orkneys	SO	2006	RV James Clark Ross AFI 6/16	5	4
	Adelie Land	TA	2003	land based / ICOTA	19	3
T. bernacchii	Cape Armitage	CA	2002	ANZ K066	42	11
	Cape Roberts	CR	2002	ANZ K066	28	10
	Casey	CS	2008	Australian Antarctic Division	43	17
	Deception Island	DI	2006	RV James Clark Ross AFI 6/16	7	2
	Elephant Island	EI	2007	RV Polarstern ANTXIII/8	5	5
	Joinville Island	JI	2007	RV Polarstern ANTXIII/8	5	5
	South Orkneys	SO	2006	RV James Clark Ross AFI 6/16	5	5
	Adelie Land	TA	2003	land based / ICOTA	12	11
	Rothera	RO	2006	BAS	12	8

N_m: sampling size used for microsatellite analysis, N_s: sampling size used for *cytochrome b* sequence analysis.

2.2.3. Inferences of past population size changes

To detect deviations from mutation-drift equilibrium indicating recent population oscillations from microsatellite markers, the mode-shift indicator test was employed to detect genetic bottlenecks using BOTTLENECK v.1.2.02 (Cornuet and Luikart, 1997). The test assumes that a population under mutation-drift equilibrium is expected to have a larger proportion of alleles with low frequencies than an expanding population. Due to limited sample size at some sampling sites, the test was performed for the genetically homogenous population clusters as indicated by STRUCTURE.

2.3. Mitochondrial DNA analysis

2.3.1. Genetic diversity

The optimal model for sequence evolution for each dataset of *cyt b* was assessed through jModeltest (Posada, 2008) and used to measure the Φ_{ST} over all populations in ARLEQUIN v.3.5, which is analogous to Wright's F_{ST}, but takes into account the divergence between haplotypes. We tested whether the geographic partitioning observed from nuclear markers also explains a significant portion of mtDNA variability. To do so, we applied AMOVA in ARLEQUIN to estimate the level of genetic structure within populations, between populations within a region, and between regions, defined according to STRUCTURE (see below). Appropriate values of F statistic components were calculated from Φ values (Excoffier et al., 1992).

The relative likelihoods of a model of immigration-drift equilibrium versus drift was determined using 2MOD (see above). We recoded the presence/absence of haplotypes into a haplotype per population matrix and analysed two types of datasets for each species; a) treating each sampling site as a separate population and b) pooling the sites according to design by the FCA/STRUCTURE analysis of nuclear markers. The Markov chain Monte Carlo search was carried out as described above.

2.3.2. Inferences of past population size changes

Two neutrality tests were employed to detect traces of historical demographic changes. These tests, originally developed to test the neutral hypothesis under an infinite-site model with constant population size scenario, are known to be sensitive to past population size changes causing deviation from the mutation-drift equilibrium. The Tajima's D test of neutrality (Tajima, 1989) evaluates the significance of two estimators of population parameter θ, one estimated from the number of segregating sites, the other from π. D is expected to be negative if the population has experienced an expansion. Fu's Fs test (Fu, 1997), evaluates the probability of sampling the same or lower amount of alleles as in a sample from neutral population assuming the same number of pairwise differences as in the studied sample. It is expected to produce negative values in expanded populations and is considered as one of the strongest methods for detecting traces of simulated logistic as well as stepwise expansion models (Ramos-Onsins and Rozas, 2002). Both tests were performed in DnaSP v.5.0 (Librado and Rozas, 2009) and their significance was estimated via 1000 implemented coalescent simulations. Given value was considered as significant if the probability of its occurrence in neutral population of stable size was lower than 0.05.

Finally, we used a Markov Chain Monte Carlo (MCMC) approach implemented in FLUCTUATE v.1.4 (Kuhner et al., 1998) to estimate the Maximum Likelihood of the exponential growth parameter g ($\theta_t = \theta_{initial}{}^{-gt}$) for our datasets. The significance of ML estimate can be judged from likelihood surface provided by Fluctuate (Kuhner, 2003) The log likelihood output table shows an approximate 95% confidence interval consisting of all values of and g that lay within the 2 units of the maximum likelihood estimate. If $g = 0$ occurs within that interval of values of the best fitting , than is the population plausibly not expanding. Furthermore, the approximate of standard deviation gives an idea of expected error around the estimate. We conservatively considered that a given dataset bears a signal of significant population growth if the ML estimate of g was greater than three standard deviations (Lessa et al., 2003).

3. Results

3.1. Microsatellites

3.1.1. Suitability of the data to evaluate population structure

The power to detect population differentiation given the observed global F_{ST}-s (see below) and the sampling scheme for each species was assessed with the software package POWSIM. Estimates of α-error with the current sampling design were low (<5%) and the power approached unity in all species suggesting that our sampling scheme was adequate to detect deviations from panmixia given the observed variability of markers.

Three loci, Trne035F22A, Trne055F55A and Trne066F58A showed significant heterozygote deficiency after Bonferroni correction in some species-locality combinations (see below and Tables A1–A3) but no

Please cite this article as: Van de Putte, A.P., et al., Comparative phylogeography of three trematomid fishes reveals contrasting genetic structure patterns in benthic and pelagic species, Mar. Genomics (2012), doi:10.1016/j.margen.2012.05.002

Table 2
Estimates of genetic diversity based on microsatellite and mtDNA genotypes of *T. newnesi*, *T. hansoni* and *T. bernacchii*.

Species/ population	microsatellites				mtDNA		
	$H_{E\ n.b.}$	H_O	F_{IS}	R_A	alleles	Hd	π
T. newnesi					Tamura and Nei + Γ (Γ = 0.011)[a]		
CH	0.831	0.856	−0.030	9.430	10	0.809	0.0617
JI	0.868	0.847	0.025	10.930	10	0.895	0.0768
KGI	0.841	0.724	**0.143**	9.154	2	1	0.0032
SO	0.846	0.783	**0.076**	10.437	3	0.600	0.0930
TA1	0.849	0.823	0.031	9.768	11	0.926	0.0474
TA2	0.856	0.860	−0.004	9.780			
T. hansoni					Kimura 2 parameter[a]		
CH	0.716	0.703	0.018	4.55	7	0.655	0.0046
CR	0.766	0.722	0.060	4.68	2	0.571	0.0029
JI	0.740	0.789	−0.068	4.63	9	0.797	0.0038
SG	0.660	0.531	0.199	4.18	4	0.733	0.0034
SO	0.730	0.567	0.244	5.00	3	0.833	0.0038
TA	0.696	0.743	−0.070	4.30	2	0.667	0.0017
T. bernacchii					Tajima and Nei + Γ (Γ = 0.1278)[a]		
CA	0.725	0.725	0	4.63	2	0.182	0.0004
CR	0.746	0.774	−0.039	4.77	4	0.533	0.0021
CS	0.821	0.764	**0.071**	5.03	5	0.574	0.0018
DI	0.705	0.762	−0.088	4.43	1	0	0
EI	0.611	0.567	0.081	3.52	1	0	0
JI	0.726	0.667	0.091	4.77	1	0	0
RO	0.792	0.792	0.001	4.98	3	0.464	0.0026
SO	0.631	0.708	−0.146	4.00	1	0	0
TA	0.721	0.706	0.023	4.60	2	0.182	0.0004

$H_{E\ n.b.}$: expected heterozygosity, H_O: observed heterozygosity, F_{IS}: Fixation index according to Weir and Cockerham (1984), R_A: allelic richness, alleles: number of alleles, Hd: haplotype diversity, π: mean pairwise divergence for each population.
[a] The best-fit model of sequence evolution indicated for each species by jModeltest for the mtDNA dataset. See Table 1 for sample codes. Bold values: significant F_{IS} values after Bonferroni correction.

marker pointed to a consistent pattern of heterozygote deficiency in more than 2 localities. MICROCHECKER analysis indicated that null alleles may be present at these loci. Calculations on the original data were replicated using corrected allele frequencies according to Brookfield (1996). Replicates using the corrected allele frequencies did not change the results and were not retained for further analyses. Pairwise comparisons between loci revealed no significant linkage disequilibrium after Bonferroni corrections.

In each nuclear dataset, the 2MOD analysis suggested that the model assuming migration-drift equilibrium is more likely (probabilities > 0.99) than the non-equilibrium model. Hence, measures of genetic differentiation do reflect ongoing gene flow among populations. In the *T. bernacchii* dataset, modal F values ranged between 0.0047 (JI) to 0.0880 (DI) with the exception of EI sampling site (F = 0.1950). In the *T. hansoni* dataset, the F values were similar, ranging between 0.0014 (CR) and 0.0727 (SO) except for the SG population (F = 0.1325). In the *T. newnesi* total dataset, the F values ranged between 0.0063 (JI) to 0.0350 (KGI).

3.1.2. Genetic diversity

Mean allelic richness corrected for sample size was highest for *T. newnesi* (9.15–10.93) while *T. hansoni* (4.18–5.00) and *T. bernacchii* (3.52–5.03) were characterised by similar levels of allelic richness. *T. newnesi* displayed relatively uniform range in mean expected heterozygosity among sampling locations (0.81–0.86) which was generally higher than the wider ranges of *T. hansoni* (0.64–0.72) and *T. bernacchii* (0.55 – 0.81). Significant departures from Hardy–Weinberg equilibrium after Bonferroni correction were observed in two populations of *T. newnesi* (KGI and SO), one population of *T. bernacchii* (CS) and none of the *T. hansoni* populations (Table 2, Tables A1–A3).

3.1.3. Population structure

Testing of the stepwise clustering procedure performed in STRUCTURE for *T. newnesi* resulted in the separation of two hypothetical clusters (highest Δk for k = 2). There was a distinct separation between the cluster of samples from the High Antarctic composed of the Ross Sea and Adelie Land area (CH, TA1, TA2,) and the cluster of samples from the Peninsula region (KGI, JI, SO). *T. hansoni* and *T. bernacchii* each showed three clusters (highest Δk for k = 3). Specifically, *T. hansoni* showed a separation between the High Antarctic (TA, CH, CR) and the peninsula region (JI, SO) similar to *T. newnesi* but with an additional cluster for the more northern sample at South Georgia. For *T. bernacchii*

Table 3
Summary of the assignment analysis following STRUCTURE based on microsatellite genotypes of *T. newnesi*, *T. hansoni* and *T. bernacchii*.

	Population[a]	Cluster 1	Cluster 2	Cluster 3
T. newnesi				
	TA1	0.249	**0.751**	
	TA2	0.207	**0.793**	
	CH	0.306	**0.694**	
	KGI	**0.830**	0.170	
	JI	**0.860**	0.140	
	SO	**0.868**	0.132	
T. hansoni				
	TA	0.139	**0.824**	0.037
	CH	0.223	**0.735**	0.041
	CR	0.349	**0.562**	0.089
	JI	**0.915**	0.053	0.032
	SO	**0.917**	0.058	0.025
	SG	0.225	0.029	**0.746**
T. bernacchii				
	CS	**0.939**	0.041	0.021
	TA	0.245	0.194	**0.561**
	CA	0.187	0.198	**0.614**
	CR	0.145	0.261	**0.595**
	RO	0.275	**0.644**	0.081
	DI	0.031	**0.857**	0.111
	JI	0.067	**0.702**	0.231
	EI	0.026	**0.901**	0.073
	SO	0.050	**0.719**	0.231

[a] Sample codes as in Table 1, bold values: statistical significant values after Bonferroni correction.

Please cite this article as: Van de Putte, A.P., et al., Comparative phylogeography of three trematomid fishes reveals contrasting genetic structure patterns in benthic and pelagic species, Mar. Genomics (2012), doi:10.1016/j.margen.2012.05.002

there was a distinct cluster for Casey (CS) as well as a further separation between a high Antarctic cluster consisting of the Ross Sea and Adelie Land samples (CA, CR, TA) and the cluster of the Peninsula region samples (RO, EI DI, JI,SO) (Table 3, Fig. 2). For all species the FCA clustering resulted in a similar grouping of populations with clear separation of Peninsular and the High Antarctic clusters (Fig. 3). Note that for *T. newnesi* both temporal samples from the same locality (TA1 and TA2) appeared close to each other.

The population structure according to the STRUCTURE and FCA analysis was tested in AMOVA. For the pelagic *T. newnesi* the analysis revealed that the among-group component (F_{ct}) explained only 0.24% of total genetic variation and this result was not significant. For both benthic species the AMOVA found significant amount of among-group partitioning of genetic variance, explaining 8.58% in *T. hansoni* and 3.51% for *T. bernacchii*.

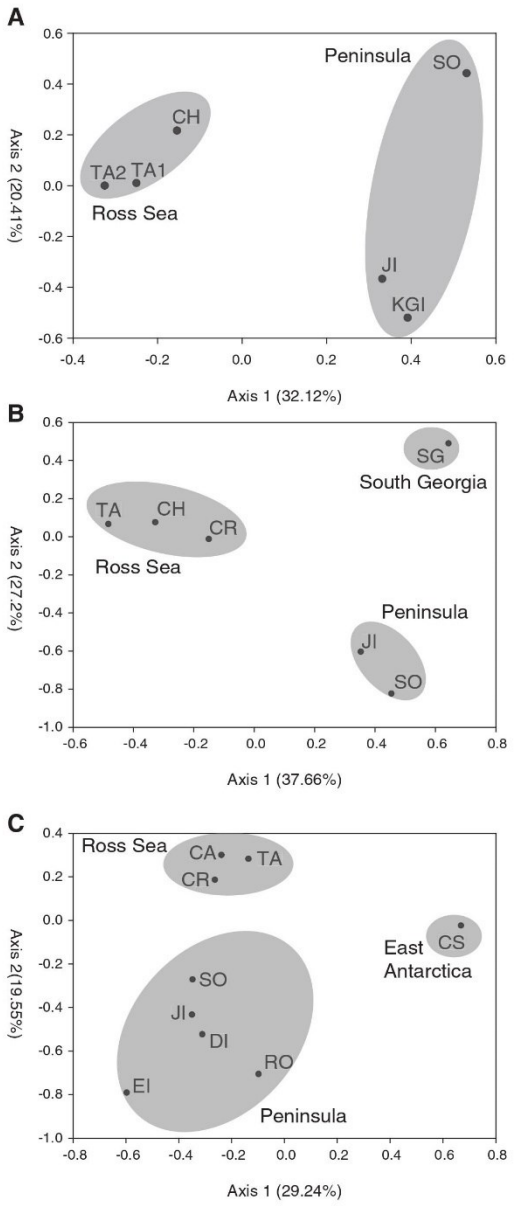


Fig. 2. Plot of the clustering analysis conducted in STRUCTURE (Pritchard et al., 2000) based on the microsatellite data of *Trematomus newnesi* (A) (K=2), *T. hansoni* (B) (K=3) and *T. bernacchii* (C) (K=3). Each individual is represented by a vertical bar indicating its estimated proportion of membership to each cluster. Sample codes as in Table 1.

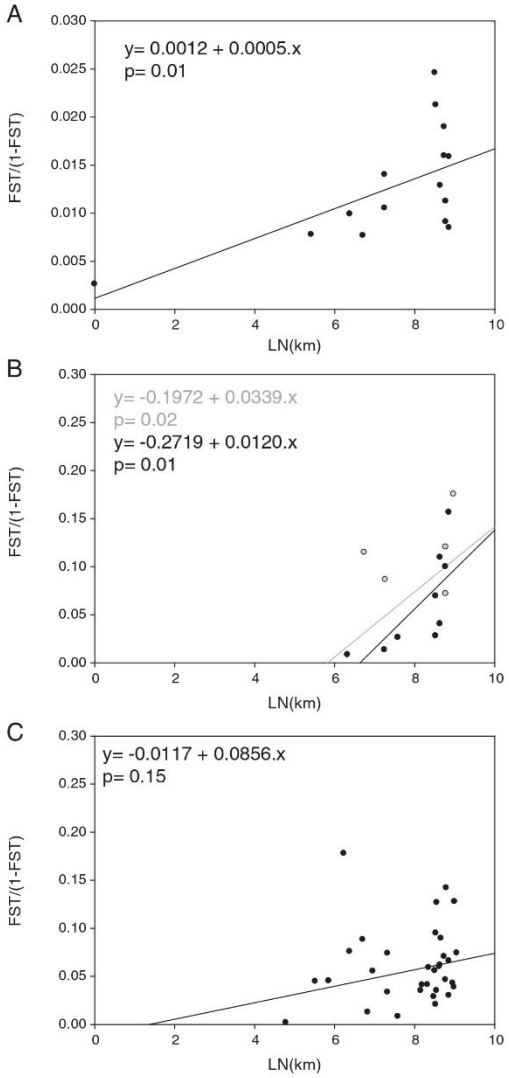


Fig. 3. Factorial correspondence analysis plot based on microsatellite data of *Trematomus newnesi* (A), *T. hansoni* (B), *T. bernacchii* (C). Sample codes as in Table 1.

Please cite this article as: Van de Putte, A.P., et al., Comparative phylogeography of three trematomid fishes reveals contrasting genetic structure patterns in benthic and pelagic species, Mar. Genomics (2012), doi:10.1016/j.margen.2012.05.002

Table 4
Population pairwise F_{ST} (Φ_{ST}) values of *T. newnesi* samples. The upper triangular matrix lists the values for microsatellite genotypes, and lower triangular lists the values for mtDNA genotypes.

T. newnesi[a]	CH	JI	KGI	SO	TA1	TA2
CH		**0.0208**	**0.024**	**0.0127**	**0.0104**	**0.0138**
JI	0.0854		0.0077	0.0098	**0.0090**	**0.0111**
KGI	0.0166	0.1926		0.0076	**0.0157**	**0.0186**
SO	−0.0453	0.1597	0.1535		**0.0084**	**0.0156**
TA1	−0.0164	−0.0032	−0.0339	−0.1425		0.0026

[a] Sample codes as in Table 1, bold values: statistical significant values after Bonferroni correction.

Table 5
Pairwise F_{ST} (Φ_{ST}) values of *T. hansoni* samples. The upper triangular matrix lists the values for microsatellite genotypes, and lower triangular lists the values for mtDNA genotypes.

T. hansoni[a]	CH	CR	JI	SG	SO	TA
CH		0.0081	**0.0647**	**0.1073**	**0.0986**	0.013
CR	**0.3032**		0.027	**0.0668**	0.0387	0.0254
JI	0.0485	**0.2881**		**0.0794**	−0.0077	**0.0907**
SG	0.0213	**0.4759**	**0.0805**		**0.1029**	**0.1492**
SO	−0.0064	**0.3913**	−0.0210	0.0070		**0.1352**
TA	−0.0094	**0.5478**	0.0396	−0.0632	−0.0367	

[a] Sample codes as in Table 1, bold values: statistical significant values after Bonferroni correction.

The pattern of the pairwise F_{ST} values of *T. newnesi* resembled the STRUCTURE analysis (Table 4): no significant differentiation was observed within the Peninsula region as well as between the two temporal samples from TA. Overall F_{ST} was 0.0104 (0.0071–0.0147). Global F_{ST} for *T. hansoni* was 0.0717 (0.0320–0.1240) or 0.0459 (0.0105–0.1046) when excluding the SG sample. All pairwise F_{ST} values that include SG were significant after Bonferonni correction. There were no significant pairwise differentiations within the High Antarctic or within the Peninsula clusters (Table 5). The Global F_{ST} of *T. bernacchii* was similar to that of *T. hansoni* excluding the SG samples: 0.0383 (0.01577–0.07169). No significant F_{ST} values were observed within both High Antarctic and Peninsula clusters (Table 6).

In all species the pairwise F_{ST} values correlated with the log transformed distance between sampling locations, suggesting a pattern indicative of isolation-by-distance (IBD; Fig. 4, Table 7). Although analysis of all *T. hansoni* samples revealed a significant IBD pattern we also excluded the most divergent SG population, in order to account for the possible effect of long-term isolation among populations (de Campos Telles et al., 2005). This lead to a strengthening of the signal ($r = 0.79$, $p < 0.01$). A Global Mantel test including all *T. bernacchii* samples revealed only a marginally significant pattern of IBD ($r = 0.27$, $p = 0.054$), which, however, became highly significant after excluding an outlying value related to the EI sample (Fig. 4, Table 7).

3.1.4. Demography

Bottleneck analysis found that both population clusters of *T. newnesi* had a normal L-shaped distribution of allele frequencies as expected under mutation-drift equilibrium and constant population size. For *T. hansoni* this analysis indicated recent growth in two population clusters including SG and those located around the Peninsula, while no deviations from equilibrium were detected in the High Antarctic cluster. We also found evidence of demographic fluctuations for *T. bernacchii* in the Peninsula region, while no deviations from mutation-drift equilibrium were detected in either High Antarctic clusters of the Ross Sea-Adelie Land and Casey (Fig. 5).

3.2. Mitochondrial DNA

jModeltest selected as best models for sequence evolution in *T. newnesi*, *T. hansoni* and *T. bernacchii*, respectively, the following mutational models: Tamura&Nei + Γ (Γ = 0.011), Kimura-2-parameter and Tajima&Nei + Γ (Γ = 0.1278). Treating each population separately, the mtDNA 2MOD analysis favoured the gene-flow-drift scenario over the pure-drift scenario; the probability of the model 1 equalled 0.98 in *T. newnesi*, 0.73 in *T. hansoni* and 0.79 in *T. bernacchii*. The probability of the model 1 equalled unity when populations were clustered according to the design suggested by the STRUCTURE. The haplotype diversity was highest in *T. newnesi* varying between 0.60 and 0.93, while in *T. hansoni* and *T. bernacchii* it ranged between 0.57–0.83 and 0–0.57, respectively. Similarly, the nucleotide diversities were highest among *T. newnesi* samples ranging between 0.0032 and 0.0930, while in *T. hansoni* and *T. bernacchii* we observed lower values between 0.0017–0.0046 and 0–0.0026, respectively (Table 2). Global (Φ_{ST}) values were lowest in *T. newnesi* (0.03), intermediate in *T. hansoni* (0.13) and highest in *T. bernacchii* (0.51).

The geographical partitioning of *T. newnesi* mtDNA variability was weak, since the F_{ct} component of AMOVA accounted for only 8% of the total variability and was not significant. Similarly, in *T. hansoni*, we found 0% of explained variance, indicating no detectable effect of population structure on mtDNA among the regions. In contrast, the F_{ct} component accounted for 44% of total variance in *T. bernacchii*, which was highly significant.

The global Mantel test for the *T. newnesi* mitochondrial dataset suggested a pattern corresponding to IBD which was not clearly significant ($p = 0.07$) (Table 7). Similarly, a global Mantel test including all *T. bernacchii* samples revealed only a marginally significant pattern of IBD ($r = 0.27$, $p = 0.054$). After excluding an outlying value related to the EI sample, we observed a highly significant pattern of IBD in this species (Table 7). On the contrary, the global Mantel test for *T. hansoni* did not reveal any IBD pattern, even when excluding the SG sample.

All datasets indicated the deviations from mutation-drift equilibrium suggestive of population oscillations, albeit with different intensity. We found negative but non-significant values of Tajima's D (D =

Table 6
Pairwise F_{ST} (Φ_{ST}) values of *T. bernacchii* samples. The upper triangular matrix lists the values for microsatellite genotypes, and lower triangular lists the values for mtDNA genotypes.

T. bernacchii[a]	CA	CR	CS	DI	EI	JI	SO	TA	RO
CA		0.0016	**0.0393**	0.0527	**0.1123**	0.0338	0.0819	−0.0016	**0.0556**
CR	**0.0523**		**0.0338**	0.0278	**0.0866**	0.0201	0.0579	0.0079	0.0394
CS	0.0064	−0.0360		**0.041**	**0.1131**	0.0372	0.0689	**0.0322**	**0.029**
DI	−0.3253	−0.2690	−0.2714		0.0431	−0.0085	0.0809	0.0657	−0.0003
EI	**0.8840**	**0.5259**	**0.5392**	1.0000		0.0426	0.1508	0.1242	0.0522
JI	**0.8840**	**0.5259**	**0.5392**	**1.0000**	0.0000		0.0702	0.0442	0.0123
SO	**0.8892**	**0.5456**	**0.5429**	**1.0000**	0.0000	0.0000		0.0618	0.0687
TA	**0.0002**	**0.0525**	0.0283	−0.3256	**0.8840**	**0.8840**	**0.8833**		**0.0564**
RO	**0.5946**	**0.3310**	**0.3846**	0.3773	−0.0833	−0.0833	−0.0414	**0.5946**	

[a] Sample codes as in Table 1, bold values: statistical significant values after Bonferroni correction.

Please cite this article as: Van de Putte, A.P., et al., Comparative phylogeography of three trematomid fishes reveals contrasting genetic structure patterns in benthic and pelagic species, Mar. Genomics (2012), doi:10.1016/j.margen.2012.05.002

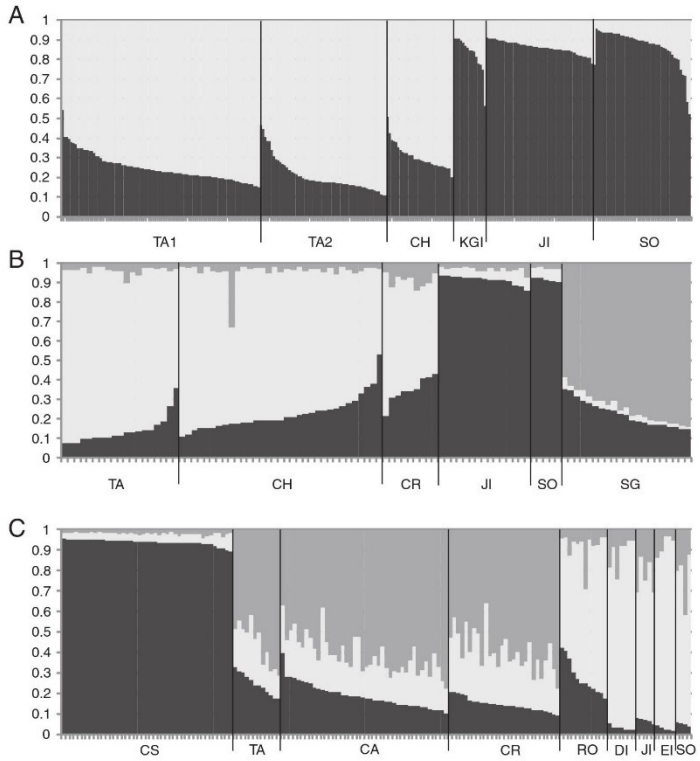


Fig. 4. Genetic isolation by distance inferred from F_{ST} genetic distances versus geographical distance for *Trematomus newnesi* (A), *T. hansoni* (B), *T. bernacchii* (C).

−1.06) in *T. newnesi* and the g parameter of the expansion model was 369 (3 SD = 89) and the lnL values of the MLE and zero growth were similar. However the Fu's F_s value was significantly negative (F_s = −8.96). On the other hand, *T. hansoni* and *T. bernacchii* mtDNA datasets significantly deviated from null expectations according to both Tajima's D (D = −1.76 and −1.49, respectively) and Fu's F_s (F_s = −7.36 and −4.06, respectively) tests and we found g values higher than three s.e. (1263; 3 SD = 189 respectively 3969; 3 SD = 378) indicating significant population growth in both species.

Table 7
Isolation by distance: Mantel test results genetic distance (F_{ST}) with geographic distance (ln(km)) for microsatellite and cytochrome *b* genotypes of *T. newnesi*, *T. hansoni* and *T. bernacchii*.

Trematomus species	Microsatellite		*cytb*	
	R	p	r	P
T. newnesi	**0.63**	**0.02**	0.6814	0.07
T. hansoni	**0.60**	**0.03**	−0.0078	0.49
T. hansoni (excl. SG[a])	**0.79**	**0.001**	−0.1046	0.73
T. bernacchii	*0.27*	*0.054*	**0.3109**	**0.03**
T. bernacchii (excl. EI[a])	**0.52**	**0.01**	**0.5165**	**0.01**

r: correlation coefficient, p: probability of correlation coefficient.
[a] For sample codes see Table 1, bold values: statistical significant values after Bonferroni correction.

4. Discussion

4.1. Quality of the data

Population structure inferred from nuclear as well as mtDNA markers was lower in *T. newnesi* than in either benthic species, which is consistent with the hypothesis that the genetic structure of marine fish is affected by ecological specialisation. However, before proceeding with a discussion on the biological relevance of our findings, we have to address several issues potentially interfering with the interpretation of the data. Significant deviations from HWE due to homozygote excess were observed at three out of six microsatellite loci in two species. These may be attributed to a number of factors, such as population substructuring or admixture, inbreeding, selection at or near a microsatellite locus, small sample size or null alleles, which are a common artefact of microsatellites (O'Reilly et al., 2004). However, none of the loci displayed homozygote excess in a large number of populations, which would be expected under the locus-specific problems and the overall results did not change when implementing corrected distances. Further, the power analysis suggested that our sampling scheme was adequate to detect deviations from panmixia under the observed range of F_{ST}'s.

Although differences in mitochondrial sequences between benthic and pelagic species observed from mitochondrial sequences are generally consistent with the microsatellites, global as well as pairwise Φ_{ST} values were usually higher than corresponding estimates of F_{ST} from nuclear markers. This difference is most notable in the case of *T. bernacchii*, where the overall Φ_{ST} and F_{ST} differed by an order of magnitude.

Please cite this article as: Van de Putte, A.P., et al., Comparative phylogeography of three trematomid fishes reveals contrasting genetic structure patterns in benthic and pelagic species, Mar. Genomics (2012), doi:10.1016/j.margen.2012.05.002

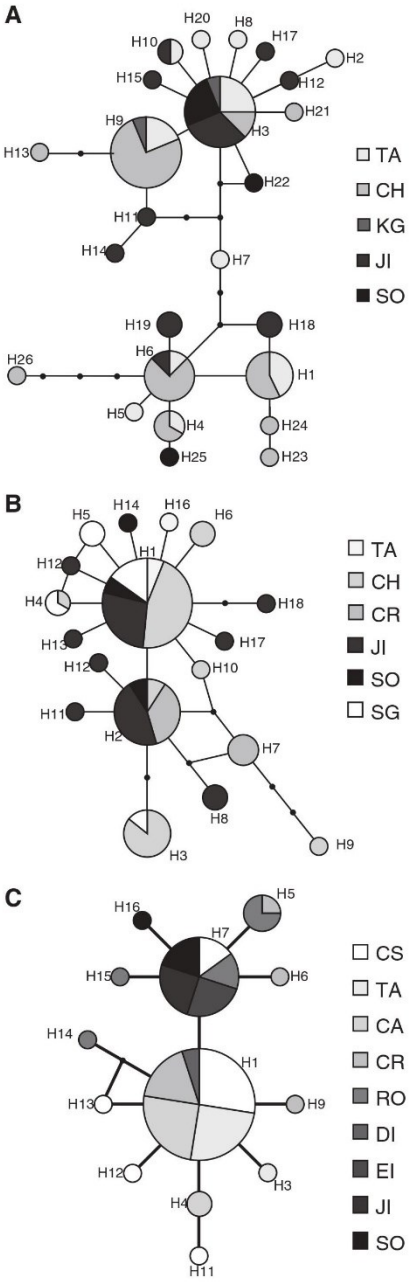


Fig. 5. Haplotype networks of data of *Trematomus newnesi* (A), *T. hansoni* (B) and *T. bernacchii* (C). Sample codes as in Table 1.

Contrasts in genetic differentiation between mitochondrial and nuclear markers are commonly observed in other organisms (Rogers, 2007; Larmuseau et al., 2010) and may be attributed to a number of non-neutral mechanisms, such as sex-specific dispersal or selection (Bazin et al., 2006). Buonaccorsi et al. (2001) demonstrated by simulation that substantial differences in F_{ST} estimates from nuclear and mtDNA markers might arise from different modes of inheritance and genomic sampling variances. Observed Φ_{ST} value of 0.43 in *T. bernacchii* is well within the 95% C.I. predicted by Buonaccorsi et al. (2001) simulations for a nuclear F_{ST} value of 0.038. Therefore, sex-specific dispersal or locus-specific selection do not have to be invoked to account for observed differences between mtDNA and nuclear markers. Indeed, we have no evidence for sex-specific dispersal in Trematominae. Janko et al. (2007) found no evidence of locus-specific selection when comparing *cytb* and nuclear DNA sequences in *T. bernacchii* and *T. newnesi*.

Finally, genetic measures of population differentiation are sensitive to demographic contraction/expansion events, and genetic drift and gene flow may take a long time to equilibrate (Hellberg, 2009). Traces of historical population fluctuations are evident in both benthic species and to a lesser extent also in the *T. newnesi* dataset given the negative mitochondrial Fu's F_s. Although such fluctuations might have obscured real isolation of contemporary populations, the 2MOD analysis unambiguously favours the model implying a balance between migration and drift in local demes, suggesting that analyses of genetic differentiation reflect on-going dispersal rather than past expansion/contraction/fragmentation events.

4.2. Spatio-temporal partitioning of genetic variability

There is increasing evidence that Southern Ocean fish display not only geographical variability, but also considerable temporal variability in the genetic composition of recruits (Zane et al., 2006; Papetti et al., 2007, 2009; Patarnello et al., 2003). This may result from sweepstakes effects. These effects are caused by stochastic larval mortality when only a small proportion of the available gene pool successfully contributes to the replenishment of the population (Hedgecock, 1994). However, we found no evidence of a sweepstakes effect in our study. This is shown by samples of *T. newnesi* from Adelie Land taken at different times at the same locality. These samples displayed the lowest pairwise F_{ST} among all samples. In short, to the best of our knowledge, the interspecific comparison of the species should not be largely influenced by a time-dependent effect on sampling.

According to our analyses of nuclear markers, patterns of geographical structure were similar among all three species. In *T. newnesi*, the STRUCTURE analysis identified two separated regions including the Peninsular sites and the sites in the High Antarctic (which is also generally consistent with the FCA). Both benthic species were separated into three clusters, two of which matched the above-mentioned population groupings, while the third cluster corresponded to a site that was marginal in our sampling scheme, i.e. the eastern-most site near the Casey station in *T. bernacchii* and the northernmost location in the South Georgia in the case of *T. hansoni*. In all species, significant pairwise F_{ST} values exclusively concerned comparisons between samples from distinct regions. Despite the large distance among sampling sites, 2MOD analyses revealed rather low modal F values in comparison with other organisms. The two populations with relatively high F values (EI in *T. bernacchii* and SG in *T. hansoni*) were also identified by pairwise F_{ST} estimates as the most aberrant ones. While the low sample size from EI may explain the former observation, the outlying character of the SG population may reflect effective isolation by the Polar Front (Shaw et al., 2004).

The geographical structure identified in this study matches traditional biogeographic separation of the Southern Ocean (Barnes and De Grave, 2000) implying the existence of barriers to dispersal among distinct regions. Alternatively, observed patterns may result from the process of IBD combined with the absence of samples from

Please cite this article as: Van de Putte, A.P., et al., Comparative phylogeography of three trematomid fishes reveals contrasting genetic structure patterns in benthic and pelagic species, Mar. Genomics (2012), doi:10.1016/j.margen.2012.05.002

the zones between Peninsular and High Antarctic sites. Significant results of the Mantel tests seem consistent with this possibility. Unfortunately, this issue may not be satisfactorily settled given the sampling limitations.

4.3. Ecology-driven differences in gene flow

Contemporary genetic studies of high-Antarctic notothenioids often report the lack of significant differentiation among sites separated by thousands of kilometres (see Matschiner et al., 2009; Patarnello et al., 2011; Papetti et al., 2012). Genetic homogeneity is supposed to be maintained across large distances passive by larval dispersal through currents. Indeed Matschiner et al. (2009) showed that such passive dispersal is sufficient to homogenise the populations of benthic fish over a transect spanning from the South Shetlands to South Georgia. We found significant interregional differences in all three species over a transect length rarely considered in this paper was rarely achieved in previous studies. For example, the analysis of Matschiner et al. (2009) and Papetti et al. (2012) were restricted to the region more or less corresponding to our "Peninsular population cluster". Here our samples generally did not significantly deviate from panmixia. The significant differentiation of the South Georgia population of *T. hansoni* contrasts with the non-significant results from *Gobionotothen gibberifrons* (Matschiner et al., 2009) but it is unclear whether such a difference reflects species-specific characteristics or simply insufficient sampling coverage (8 vs. 21 samples in this study). In any case, the present discovery of significant interregional differences suggests that processes determining geographical structure may operate on larger scales than usually considered in genetic studies in the Southern Ocean.

Our study revealed stronger population structuring in *T. hansoni* than in *T. newnesi*, despite the suspected much longer larval period in the former species. When excluding the South Georgia samples of *T. hansoni* from our analysis both benthic species displayed largely similar patterns. Furthermore recent barcoding studies indicate that *Trematomus vicarius*, which is endemic to South Georgia is genetically similar to *T. bernachii* and may constitute an isolated population of the former (Dettai et al., 2011; Lautredou et al., 2010). Thus our data do not entirely conform to the hypothesis that population connectivity of Southern Ocean fish is mainly driven by passive larval dispersal. The weaker level of spatial structuring found for the pelagic *T. newnesi* compared to both benthic species is apparent from lower global as well as pairwise F_{ST} and Φ_{ST} values. The interregional partitioning of genetic variability explained only a fraction (0.25%) of the total nuclear variability and was not significant for *T. newnesi*, while it explained more than 3% of the total variance in both benthic species. This finding suggests that factors other than length of larval period, such as ecology of adult stages or eggs, contribute to interspecific differences in population connectivity among notothenioid fish. However currently the knowledge of life history traits in Southern Ocean fish species is limited and would require further investigation in order draw more in depth conclusions. Differences between pelagic and benthic species found in this study are consistent with a microsatellite-based study of the mesopelagic lanternfish *Electrona antarctica* (Van de Putte et al., 2012), which covered a comparable portion of the Southern Ocean and showed no differentiation. Worldwide pelagic fishes show considerably lower levels of differentiation than benthic and coastal taxa (Ruzzante et al., 2006). Hence, our data are consistent with the hypothesis that the genetic structure of marine fish is affected by ecological specialisation of the species.

It remains unclear, however, whether the different dispersal potential of pelagic versus benthic notothenioids may translate into different adaptive potential or rates of speciation. In many adaptive radiations including notothenioids, allopatric or peripatric speciation might have been promoted by the interplay between the effects of adaptation to local conditions and barriers disrupting the genetic connectivity among populations (Rico and Turner, 2002). Population genetics tools allow the evaluation of population connectivity through estimates of the absolute number of migrants ($Nm \sim (1\text{-}F_{ST})/4F_{ST}$ (Lowe and Allendorf, 2010). Even though such estimates are based on simplifying assumptions and should be considered carefully (for example, the F_{ST}-based counts assume an infinite-island model with equal migration rates and population sizes among demes, while coalescent samplers are sensitive to the effects of ghost populations (Slatkin, 2005)), it is remarkable that our data indicate large levels of gene flow. Except for the mtDNA dataset in *T. bernacchii*, all estimates of geneflow (Nm) were greater than 1, which is usually assumed sufficient to ensure sharing of the same alleles among populations over long periods of evolutionary time. In fact, all Nm estimates were greater than 0.1, which is supposed to be enough for the spread of most adaptive alleles (Rieseberg and Burke, 2001). Hence, inferred levels of gene flow between contemporary populations seem sufficient to maintain genetic cohesiveness within all species.

In the past levels of gene flow may have changed dramatically during the periods, when available habitats have been reduced either by the expansion of the grounded ice sheets or mass wasting and turbidity currents, or through the shutdown of primary productivity under multiannual sea ice (Thatje et al., 2008). Footprints of population fluctuations putatively related to glacial periods are commonly observed in genetic studies of organisms from the Southern Ocean and there is evidence that some organisms not only experienced bottlenecks during the LGM but have been restricted into more or less isolated refugia (rev. in Rogers, 2007, 2012). Janko et al. (2007) showed that *T. bernacchii* mtDNA variability is consistent with the hypothesis of temporal fragmentation into inhabitable refugia whose isolation might have been further strengthened by a decrease of oceanic current activities during glacial periods (Kojima et al., 1997). Bottleneck analyses further indicate that population oscillations may occur asynchronously in distinct regions, since we found evidence of population expansion in northerly sites of both benthic species, while populations in the High Antarctic didn't deviate from mutation-drift equilibrium. Antarctic species therefore probably pass through periods of transient allopatry (Patarnello et al., 2011) similar to mid and high latitude taxa (Hellberg, 1998; Maggs et al., 2008).

Given that pelagic organisms probably were not as much affected by glaciations than benthic organisms (Janko et al., 2007), it is tempting to speculate that periods of transient allopatry might have promoted allopatric speciation especially in benthic feeders (Rogers, 2007, 2012). However, both mitochondrial phylogroups of *T. bernacchii* that putatively originated in isolated refugia underwent postglacial expansion with secondary admixture (Janko et al., 2007); note that both major *cytb* lineages are shared between the regions, albeit in different frequencies. A similar situation has been observed in Adelie penguins (Ritchie et al., 2004). In a multiocus analyses of the Trematominae, Janko et al. (2011) found no evidence for change of speciation or extinction rates in relation to Pleistocene glaciations. Altogether it seems that although the Quaternary climatic fluctuations had a dramatic effect on population size and structure especially in benthic fish (Zane et al., 2006; Janko et al., 2007; Rogers, 2007), they did not provoke mass extinctions or vicariant speciation and postglacial dispersal among temporarily fragmented populations was strong enough to prevent the achievement of reciprocal monophyly.

5. Conclusions

The present comparative analysis provides strong support for the hypothesis that the ecology of Antarctic marine species decisively influences their population structure, leaving benthic species more prone to local disturbances and sensitive to climatic fluctuations. This proves that the drivers of genetic differentiation in the Southern Ocean are similar to mid and high latitude environments (Maggs et al., 2008). Our study also conforms to a growing body of evidence that population connectivity in marine fish is not driven exclusively by the duration of passive larval dispersal but is influenced by other factors such as the active swimming of adults. It is unclear, however, whether inferred differences

Please cite this article as: Van de Putte, A.P., et al., Comparative phylogeography of three trematomid fishes reveals contrasting genetic structure patterns in benthic and pelagic species, Mar. Genomics (2012), doi:10.1016/j.margen.2012.05.002

between ecological groups translate into a different potential for local adaptation or extinction, since population connectivity was found to be quite high in all three species and the Pleistocene has not left notable traces on diversification rate (Janko et al., 2011). However, it is notable that most studies have focused on neutral markers in order to interpret the data in demographic rather than locus-specific context. Given that permeability of species barriers differs among the genes (Storchová et al., 2010), it becomes highly important to profit from recent technological and analytical progress in genome and transcriptome analysis (Bernatchez et al., 2011). Comparison of neutral and non-neutral markers should allow to elucidate the potential of local adaptation and speciation in the Southern Ocean.

Acknowledgements

We thank Mark Belchier from the British Antarctic Survey, and the Australian Antarctic Division for providing tissue samples; The Alfred Wegener Institute for logistic support; Bart Hellemans, Šárka Pelikánová and Jana Čechová for invaluable help in laboratory work. This study was supported by: the Belgian Science Policy (BELSPO projects no. EV/01/30B, "PELAGANT" and SD/BA/851, "PADI"); the Grant Agency of Academy of Sciences of the Czech Republic No. KJB600450903 to K.J. and L.Ch.; the Synthesys project conducted at the Muséum National d'Histoire Naturelle (Paris, France), part of the European sixth framework program FR-TAF-325 for K.J.; the Institute of Animal Physiology and Genetics AS CR that receives continuous support from IRP IAPG No. AV0Z50450515. G.E.M was funded by a post-doctoral fellowship provided by the Flemish Fund for Scientific Research (FWO Vlaanderen). J.S. was supported by the Polish Ministry of Science and Higher Education within the program 'Supporting International Mobility of Scientists' and a grant no. NN305376438. J.R. was supported by AFI 6/16 funded by the Natural Environment Resource Council UK. We thank the IPEV program 281 ICOTA coordinated by Philippe Koubbi and Catherine Ozouf-Costaz for samples from Adelie Land.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.margen.2012.05.002.

References

Barnes, D.K.A., De Grave, S., 2000. Biogeography of Southern polar bryozoans. Vie Milieu 50, 261–273.
Bay, L.K., Crozier, R.H., Caley, M.J., 2006. The relationship between population genetic structure and pelagic larval duration in coral reef fishes on the Great Barrier Reef. Mar. Biol. 149, 1247–1256.
Bazin, E., Glémin, S., Galtier, N., 2006. Population size does not influence mitochondrial genetic diversity in animals. Science 312, 570–572.
Belkhir, K., Borsa, P., Chikhi, L., Raufaste, N., Bonhomme, F., 2002. GENETIX 4.05, logiciel sous Windows TM pour la génétique des populations. Laboratoire Ge´nome, Populations, Interactions, CNRS UMR 5000. Universite´ de Montpellier II, Montpellier (France).
Bernatchez, L., St-Cyr, J., Normandeau, E., Maes, G.E., Als, T.D., Kalujnaia, S., Cramb, G., Castonguay, M., Hansen, M.M., 2011. Differential timing of gene expression regulation between leptocephali of the two Anguilla eel species in the Sargasso Sea. Ecol. Evol. 1, 459–467.
Bohonak, A.J., 1999. Dispersal, gene flow, and population structure. Q. Rev. Biol. 74, 21–45.
Bonhomme, F., Planes, S., 2000. Some evolutionary arguments about what maintains the pelagic interval in reef fishes. Environ. Biol. Fishes 59, 365–383.
Bradbury, I.R., Laurel, B., Snelgrove, P.V.R., Bentzen, P., Campana, S.E., 2008. Global patterns in marine dispersal estimates: the influence of geography, taxonomic category and life history. Proc. R. Soc. B. 275, 1803–1809.
Brookfield, J.F.Y., 1996. A simple new method for estimating null allele frequency from heterozygote deficiency. Mol. Ecol. 5, 453–455.
Buonaccorsi, V.P., McDowell, J.R., Graves, J.E., 2001. Reconciling patterns of inter-ocean molecular variance from four classes of molecular markers in blue marlin (*Makaira nigricans*). Mol. Ecol. 10, 1179–1196.
Carreras-Carbonell, J., Macpherson, E., Pascual, M., 2006. Population structure within and between subspecies of the Mediterranean triplefin fish *Tripterygion delaisi* revealed by highly polymorphic microsatellite loci. Mol. Ecol. 15, 3527–3539.
Ciofi, C., Beaumont, M.A., Swingland, I.R., Bruford, M.W., 1999. Genetic divergence and units for conservation in the Komodo dragon *Varanus komodoensis*. Proc. R. Soc. Lond. B Biol. Sci. 266, 2269–2274.
Cornuet, J.M., Luikart, G., 1997. Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. Genetics 144, 2001–2014.
Dettai, A., Lautredou, A.-C., Bonillo, C., Goimbault, E., Busson, F., Causse, R., Couloux, A., Cruaud, C., Duhamel, G., Denys, G., Hautecoeur, M., Iglesias, S., Koubbi, P., Lecointre, G., Moteki, M., Pruvost, P., Tercerie, S., Ozouf, C., 2011. The actinopterygian diversity of the CEAMARC cruises: Barcoding and molecular taxonomy as a multi-level tool for new findings. Deep-Sea Res. II Top. Stud. Oceanogr. 58, 250–263.
DeWitt, H.H., Heemstra, P.C., Gon, O., 1990. Nototheniidae. In: Gon, O., Heemstra, P.C. (Eds.), Fishes of the Southern Ocean. JLB Smith Institute of Ichtyology, Grahamstown, pp. 279–331.
de Campos Telles, M.P., Diniz-Filho, J.A.F., 2005. Multiple Mantel tests and isolation-by-distance, taking into account long-term historical divergence. Genet. Mol. Res. 4, 742–748.
Duhamel, G., Kock, K.-H., Balguerias, E., Hureau, J.-C., 1993. Reproduction in fish of the Weddell Sea. Polar Biol. 13, 193–200.
Evanno, G., Regnaut, S., Goudet, J., 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol. Ecol. 14, 2611–2620.
Excoffier, Laurent, Lischer, H.E.L., 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Mol. Ecol. Res. 10, 564–567.
Excoffier, L., Smouse, P.E., Quattro, J.M., 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131, 479–491.
Fu, Y.X., 1997. Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. Genetics 147, 915–925.
Goudet, J., 2001. FSTAT, a program to estimate and test gene diversities and fixation indices, version 2.9.3. , http://www.unil.ch/izea/softwares/fstat.html2001.
Hauser, L., Carvalho, G.R., 2008. Paradigm shifts in marine fisheries genetics: ugly hypotheses slain by beautiful facts. Fish Fish. 9, 333–362.
Hedgecock, D., 1994. Does variance in reproductive success limit effective population sizes of marine organisms? In: Beaumont, A.R. (Ed.), Genetics and evolution of aquatic organisms. Chapman and Hall, London, pp. 122–134.
Hedgecock, D., Barber, P.H., Edmands, S., 2007. Genetic approaches to measuring connectivity. Oceanography 20, 70–79.
Hellberg, M.E., 1998. Sympatric sea shells along the sea's shore: the geography of speciation in the marine gastropod *Tegula*. Evolution 52, 1311–1324.
Hellberg, M.E., 2009. Gene flow and isolation among populations of marine animals. Ann. Rev. Ecol. Evol. Syst. 40, 291–310.
Janko, K., Lecointre, G., DeVries, A., Couloux, A., Cruaud, C., Marshall, C., 2007. Did glacial advances during the Pleistocene influence differently the demographic histories of benthic and pelagic Antarctic shelf fishes? – Inferences from intraspecific mitochondrial and nuclear DNA sequence diversity. BMC Evol. Biol. 7, 220.
Janko, K., Marshall, C., Musilová, Z., Houdt, J.V., Couloux, A., Cruaud, C., Lecointre, G., 2011. Multilocus analyses of an Antarctic fish species flock (Teleostei, Notothenioidei, Trematominae): phylogenetic approach and test of the early-radiation event. Mol. Phylogenet. Evol. 60, 305–316.
Kock, K.-H., Pshenichnov, L.K., Devries, A.L., 2006. Evidence for egg brooding and parental care in icefish and other notothenioids in the Southern Ocean. Antarct. Sci. 18, 223–227.
Kojima, S., Segawa, R., Hayashi, I., 1997. Genetic differentiation among populations of the Japanese turban shell *Turbo* (*Batillus*) *cornutus* corresponding to warm currents. Mar. Ecol. Prog. Ser. 150, 149–155.
Kuhner, M.K., 2003. LAMARC: estimating population genetic parameter from molecular data. In: Salemi, M., Vandamme, A.M. (Eds.), The Phylogenetic Handbook; a practical approach to DNA and protein phylogeny. Cambridge University Press.
Kuhner, M.K., Yamato, J., Felsenstein, J., 1998. Maximum likelihood estimation of population growth rates based on the coalescent. Genetics 149, 429–434.
La Mesa, Mario, Ascoli, L., Caputo, V., 2006. Gametogenesis and reproductive strategies in some species of the Antarctic fish genus *Trematomus* (Nototheniidae) from Terra Nova Bay, Ross Sea. Polar Biol. 29, 963–970.
Larmuseau, M.H.D., Raeymaekers, J.A.M., Hellemans, B., Van Houdt, J.K., Volckaert, F.A.M., 2010. Mito-nuclear discordance in the degree of population differentiation in a marine goby. Heredity 105, 532–542.
Lautredou, A.-C., Bonillo, C., Denys, G., Cruaud, C., Ozouf-Costaz, C., Lecointre, G., Dettai, A., 2010. Molecular taxonomy and identification within the Antarctic genus Trematomus (Notothenioidei, Teleostei): How valuable is barcoding with COI? Polar Sci. 4, 333–352.
Leis, J.M., 2006. Are larvae of demersal fishes plankton or nekton? Adv. Mar. Biol. 51, 57–141.
Lessa, E.P., Cook, J.A., Patton, J.L., 2003. Genetic footprints of demographic expansion in North America, but not Amazonia, during the Late Quaternary. Proc. Nat. Acad. Sci. U. S. A. 100, 10031–10034.
Librado, P., Rozas, J., 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25, 1451–1452.
Lowe, W.H., Allendorf, F.W., 2010. What can genetics tell us about population connectivity? Mol. Ecol. 19, 3038–3051.
Maggs, C.A., Castilho, R., Foltz, D., Henzler, C., Jolly, M.T., Kelly, J., Olsen, J., Perez, K.E., Stam, W., Väinölä, R., Viard, F., Wares, J., 2008. Evaluating signatures of glacial refugia in North Atlantic benthic marine taxa. Ecol 89, 108–122.
Matschiner, M., Hanel, R., Salzburger, W., 2009. Gene flow by larval dispersal in the Antarctic notothenioid fish *Gobionotothen gibberifrons*. Mol. Ecol. 18, 2574–2587.
McGaughran, A., Torricelli, G., Carapelli, A., Frati, F., Stevens, M.I., Convey, P., Hogg, I.D., 2010. Contrasting phylogeographical patterns for springtails reflect different

Please cite this article as: Van de Putte, A.P., et al., Comparative phylogeography of three trematomid fishes reveals contrasting genetic structure patterns in benthic and pelagic species, Mar. Genomics (2012), doi:10.1016/j.margen.2012.05.002

evolutionary histories between the Antarctic Peninsula and continental Antarctica. J. Biogeo 37, 103–119.
O'Reilly, P.T., Canino, M.F., Bailey, K.M., Bentzen, P., 2004. Inverserelationship between F-ST and microsatellite polymorphism in the marine fish, walleye pollock (Theragra chalcogramma): implications for resolving weak population structure. Mol. Ecol. 13, 1799–1814.
Orsi, A.H., Whitworth, T., Nowlin, W.D., 1995. On the meridional extent and fronts of the Antarctic Circumpolar Current. Deep Sea Res. Part I 42, 641–673.
Papetti, C., Susana, Emanuele, La Mesa, M., Kock, K.-H., Patarnello, T., Zane, L., 2007. Microsatellite analysis reveals genetic differentiation between year-classes in the icefish *Chaenocephalus aceratus* at South Shetlands and Elephant Island. Polar Biol. 30, 1605–1613.
Papetti, C., Susana, E., Patarnello, T., Zane, L., 2009. Spatial and temporal boundaries to gene flow between *Chaenocephalus aceratus* populations at South Orkney and South Shetlands. Mar. Ecol. Prog. Ser. 376, 269–281.
Papetti, C., Pujolar, J.M., Mezzavilla, M., La Mesa, M., Rock, J., Zane, L., Patarnello, T., 2012. Population genetic structure and gene flow patterns between populations of the Antarctic icefish *Chionodraco rastrospinosus*. J. Biogeogr.
Patarnello, T., Marcato, S., Zane, L., Varotto, V., Bargelloni, L., 2003. Phylogeography of the *Chionodraco* genus (Perciformes, Channichthyidae) in the Southern Ocean. Mol. Phylogenet. Evol. 28, 420–429.
Patarnello, T., Verde, C., di Prisco, G., Bargelloni, L., Zane, L., 2011. How will fish that evolved at constant sub-zero temperatures cope with global warming? Notothenioids as a case study. Bioessays 33, 260–268.
Planes, S., Parroni, M., Chauvet, C., 1998. Evidence of limited gene flow in three species of coral reef fishes in the lagoon of New Caledonia. Mar. Biol. 130, 361–368.
Posada, D., 2008. jModelTest: phylogenetic model averaging. Mol. Biol. Evol. 25, 1253–1256.
Pritchard, J.K., Stephens, M., Donnelly, P., 2000. Inference of population structure using multilocus genotype data. Genetics 155, 945–959.
Purcell, J.F.H., Cowen, R.K., Hughes, R.C., Williams, D.A., 2006. Weak genetic structure indicates strong dispersal limits: a tale of two coral reef fish. Proc. R. Soc. B. 273, 1483–1490.
Ramos-Onsins, S.E., Rozas, J., 2002. Statistical properties of new neutrality tests against population growth. Mol. Biol. Evol. 19, 2092–2100.
Rico, C., Turner, G.F., 2002. Extreme microallopatric divergence in a cichlid species from Lake Malawi. Mol. Ecol. 11, 1585–1590.
Rieseberg, L.H., Burke, J.M., 2001. A genic view of species integration. J. Evol. Biol. 14, 883–886.
Ritchie, P.A., Millar, C.D., Gibb, G.C., Baroni, C., Lambert, D.M., 2004. Ancient DNA enables timing of the Pleistocene origin and Holocene expansion of two Adélie Penguin lineages in Antarctica. Mol. Biol. Evol. 21, 240–248.
Rogers, A.D., 2007. Evolution and biodiversity of Antarctic organisms: a molecular perspective. Philos. Trans. R. Soc. Lond. B Biol. Sci. 362, 2191–2214.
Rogers, A.D., 2012. Evolution and biodiversity of Antarctic organisms: a molecular perspective. In: Rogers, A.D., Johnston, N.M., Murphy, E.J., Clarke, A. (Eds.), Antarctic Ecosystems: An Extreme Environment in a Changing World. Blackwell Publishing Ltd.
Rousset, F., 2008. Genepop'007: a complete re-implementation of the genepop software for Windows and Linux. Mol. Ecol. Res. 8, 103–106.
Rutschmann, S., Matschiner, M., Damerau, M., Muschick, M., Lehmann, M.F., Hanel, R., Salzburger, W., 2011. Parallel ecological diversification in Antarctic notothenioid fishes as evidence for adaptive radiation. Mol. Ecol. 20, 4707–4721.
Ruzzante, D.E., Mariani, S., Bekkevold, D., André, C., Mosegaard, H., Clausen, L.A.W., Dahlgren, T.G., Hutchinson, W.F., Hatfield, E.M.C., Torstensen, E., Brigham, J., Simmonds, E.J., Laikre, L., Larsson, L.C., Stet, R.J.M., Ryman, N., Carvalho, G.R., et al., 2006. Biocomplexity in a highly migratory pelagic marine fish, Atlantic herring. Proc Biol Sci 273 (1593), 1459–1464.
Ryman, N., Palm, S., 2006. POWSIM: a computer program for assessing statistical power when testing for genetic differentiation. Mol. Ecol. Notes 6, 600–602.
Selkoe, K.A., Henzler, C.M., Gaines, S.D., 2008. Seascape genetics and the spatial ecology of marine populations. Fish Fish. 9, 363–377.
Shaw, P.W., Arkhipkin, A.I., Al-Khairulla, H., 2004. Genetic structuring of Patagonian toothfish populations in the Southwest Atlantic Ocean: the effect of the Antarctic Polar Front and deep-water troughs as barriers to genetic exchange. Mol. Ecol. 13, 3293–3303.
Skillings, D.J., Bird, C.E., Toonen, R.J., 2011. Gateways to Hawaii: Genetic Population Structure of the Tropical Sea Cucumber *Holothuria atra*. J. Mar. Biol. Article ID 783030 16 pages.
Slatkin, M., 2005. Seeing ghosts: the effect of unsampled populations on migration rates estimated for sampled populations. Mol. Ecol. 14, 67–73.
Storchová, R., Reif, J., Nachman, M.W., 2010. Female heterogamety and speciation: reduced introgression of the Z chromosome between two species of nightingales. Evolution 64, 456–471.
Tajima, F., 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics 123, 585–595.
Taylor, M.S., Hellberg, M.E., 2003. Genetic evidence for local retention of pelagic larvae in a Caribbean reef fish. Science 299, 107–109.
Thatje, S., Hillenbrand, C.-D., Mackensen, A., Larter, R., 2008. Life hung by a thread: endurance of antarctic fauna in glacial periods. Ecology 89, 682–692.
Thorrold, S.R., Zacherl, D.C., Levin, L.A., 2007. Population connectivity and larval dispersal : using geochemical signatures in calcified structures. Oceanography 20 (3), 80–89.
Thorson, G., 1950. Reproductive and larval ecology of marine bottom invertebrates. Biol. Rev. 25, 1–45.
Van de Putte, A.P., Van Houdt, J.K., Maes, G.E., Janko, K., Koubbi, P., Rock, J., Volckaert, F.A.M., 2009. Species identification in the trematomid family using nuclear genetic markers. Polar Biol. 32, 1731–1741.
Van de Putte, A.P., Van Houdt, J.K.J., Maes, G.E., Hellemans, B., Collins, M.A., Volckaert, F.A.M., 2012. High genetic diversity and connectivity in a common mesopelagic fish of the Southern Ocean: the myctophid *Electrona antarctica*. Deep-Sea Res. II Top. Stud. Oceanogr. 59–60, 199–207.
Van Houdt, J.K.J., Hellemans, B., Van De Putte, A., Koubbi, P., Volckaert, F.A.M., 2006. Isolation and multiplex analysis of six polymorphic microsatellites in the Antarctic notothenioid fish, *Trematomus newnesi*. Mol. Ecol. Notes 6, 157–159.
Van Oosterhout, C., Hutchinson, W.F., Wills, D.P.M., Shipley, P., 2004. Micro-checker: software for identifying and correcting genotyping errors in microsatellite data. Mol. Ecol. Notes 4, 535–538.
Volckaert, F.A.M., Rock, J., Van de Putte, A.P., 2012. Connectivity and Molecular Ecology in Antarctic Fish. In: di Prisco, G., Verde, C. (Eds.), Pole to Pole, Adaptation And Evolution In Marine Environments, vol. 1 (5). Springer Berlin, Heidelberg, pp. 75–96.
Waples, R.S., 1987. A multispecies approach to the analysis of gene flow in marine shore fishes. Evolution 41, 385–400.
Weir, B.S., Cockerham, C.C., 1984. Estimating F-statistics for the analysis of population structure. Evolution 38, 1358–1370.
Zane, L., Marcato, S., Bargelloni, L., Bortolotto, E., Papetti, C., Simonato, M., Varotto, V., Patarnello, T., 2006. Demographic history and population structure of the Antarctic silverfish *Pleuragramma antarcticum*. Mol. Ecol. 15, 4499–4511.

Please cite this article as: Van de Putte, A.P., et al., Comparative phylogeography of three trematomid fishes reveals contrasting genetic structure patterns in benthic and pelagic species, Mar. Genomics (2012), doi:10.1016/j.margen.2012.05.002

Preliminary report on fish diversity at the Prince Gustav Channel, the northern part of the James Ross Island, Antarctica

Šárka Mašová[1*], Eva Kašparová[2]

[1]*Department of Botany and Zoology, Faculty of Science, Masaryk University Brno, Kotlářská 2, 611 37 Brno, Czech Republic*
[2]*Institute of Animal Physiology and Genetics AS CR. v.v.i., Rumburská 89, 277 21 Liběchov, Czech Republic*

Abstract
Notothenioid fish were collected in the coastal part of the James Ross Island (Antarctica) mainly during Austral summer in 2012. In this paper, we report the list of species records and their basic biometric measurements as a reaction to missing ichthyofaunal investigation of this particular area. Collected fishes belong to the families Nototheniidae (*Trematomus bernacchii* Boulenger, 1902; *T. eulepidotus* Regan, 1914; *T. newnesi* Boulenger, 1902; *Gobionotothen gibberifrons* (Lönnberg, 1905); *Notothenia coriiceps* Richardson, 1844) and Bathydraconidae (*Gymnodraco acuticeps* Boulenger, 1902).

Key words: Notothenioid fish, Weddell Sea, Prince Gustav Channel

Introduction

Antarctica, the most remote continent surrounded by Southern Polar Ocean is bounded by Circum Antarctic Current (CAC). This mass of circulating water prevents the species migration northward and vice versa. The isolation of Antarctic continent is derived from the Gondwana breakage, which created CAC by opening of Drake passage in Miocene (Livermore et al. 2005). The Southern ocean waters, south of the Convergence are colder and thermally more stable than those in the north. Therefore, surface currents on both sides of the Convergence flow generally northward, away from Antarctica (DeWitt 1971). It represents an approximate northern boundary for the Antarctic Ocean as a natural biogeographic province (East-man et Grande 1989). As a reaction to the cooling from Oligocene, the Antarctic shelf became poor environment (Clarke et Crame 2010). Most of the fauna became extinct, or underwent bottleneck effect, due to iceberg scouring of the benthos (Thatje et al. 2005), freezing temperatures and seasonal food availability.

Received November 21, 2012, *accepted* December 19, 2012.
*Corresponding author: masova@sci.muni.cz
Acknowledgements: We thank all the people who helped us with the collecting of fishes and to the staff of the Czech Antarctic expedition to the James Ross Island for their help and support. The authors also acknowledge the ability to exploit the infrastructure of the Mendel station. This study was supported by the Czech Science Foundation (project No. P505/12/G112) and by the Department of Botany and Zoology, Faculty of Science, Masaryk University. We thank also to Karel Janko for the identification and verification of our identifications of species.

Notothenioids evolved a unique adaptations to the cold at morphological, physiological and biochemical levels that allowed them to survive subzero temperatures (Eastman et Grande 1989, Matschiner et al. 2011, Ingram et Mahler 2011, Patarnello et al. 2011). Therefore, they are the most abundant and diverse taxa with remarkable degree of endemism (Eastman et Sidell 2002, Verde et al. 2003, Sidell et O'Brien 2006). The prominent adaptation, causing remarkable adaptive radiation, was the gain of antifreeze glycolproteins in their body tissue, which decreased the freezing point (Cheng et Detrich 2007). Notothenioids derived from benthic ancestors and although lacking the swim bladder, they inhabit different bathymetry and evolved different life histories (benthic, pelagic and even cryopelagic) (Eastman et Eakin 2000) with polyphyletic origin (Rutschmann et al. 2011). The recent Southern Ocean fish fauna is taxonomically limited, highly endemic and dominated by the fishes of the suborder Notothenioidei not just by species number but also by abundance and biomass (Eastman et McCune 2000, Eastman 2005). No other oceanic ecosystem is so dominated by a single taxonomic group of fish (Clarke and Johnston, 1996). Eastman et Eakin (2000) mentioned that since the publication of Fishes of the Southern Ocean (by Gon et Heemstra in 1990), 11 new notothenioid species have been described and seven others placed in synonymy. The previous decades were an active time in the discoveries of new species of notothenioids.

Knowledge of species diversity is essential for understanding the structure, evolution and management of the Antarctic marine ecosystem (Eastman et Eakin 2000). The suborder Notothenioidei includes eight families with 43 genera and 122 species according to Eastman et Eakin (2000). Moreover, Eastman (2005) describes notothenioids as the most specialised taxa except of liparids and zoarcids, which account together for 88% of species diversity of the Antarctic fish fauna and states existence of eight notothenioid families (five of them are primarily Antarctic). The families include a total of 44 genera and 129 species, 101 Antarctic and 28 non-Antarctic. The 101 Antarctic species make up 45% of the benthic species diversity in the Antarctic region (Eastman 2005). The author enumerated in total 131 species (104 Antarctic and 27 non-Antarctic) and 44 genera in his not-yet-published Notothenioid classification list of species from 2010. He gained data from the electronic Catalog of Fishes by Eschmeyer et Fricke (2009). However, there are still some uncounted new species (*e. g.* Shandikov 2011) in the last version of the Catalog of Fishes by Eschmeyer (2012). Damerau et al. (2012) state similarly to Eastman (2005), that notothenioids consist of 131 species from 8 families, with 104 species. Out of 5 of these families is endemic in High-Antarctic region.

Our research was held in the northwestern Weddell Sea in the Prince Gustav Channel (PGC) in the region of the eastern Antarctic Peninsula margin. PGC is situated on the eastern coast of the Graham Land, between the James Ross Island and the Trinity Peninsula and is about 160 kilometers (100 miles) long and from 8 to 16 km wide (Reece 1950). Since 1995, the PGC has been free of ice (Correll 1998) during austral summer season.

Data of fish species composition in this area have not been revised yet. The aim of this paper is to fill the gap and create a pilot study of the James Ross Island and PGC nototheniods fish, their species list, respectively.

Material and Methods

Field investigations were carried out during the Czech expedition Antarctica 2011-2012 from January to March. Some fishes were caught in previous seasons with rods. Fifteen specimens of 5 notothenioid fishes (Perciformes, Notothenioidei) were examined for parasites during the expedition in 2011-2012. They were caught in several sites in the Prince Gustav Channel (63° 47' 58.37" S, 57° 55' 12.35" W; depth about 30 m), in front of Johann Gregor Mendel station on the James Ross Island. Fish were collected with the use of gillnet.

Fish nomenclature is in concordance with FishBase (Froese et Pauly 2012). Biometric measurements were done in millimetres (mm), weight in grams (g). Gonadosomatic index (GSI, %) was calculated as gonad weight divided by total weight multiplied by hundred.

Results and Discussion

A total of 6 fish species (Figs. 1 – 11) belonging to two families were collected during two Czech Antarctic expeditions and are listed in Table 1 with their biometric characteristics. All found species are mainly epibenthic, foraging mainly in benthos. *Trematomus eulepidotus* and *T. newnesi* have a pelagic lifestyle (Kock 1992, Eastman 1993, Near et al. 2009, Rutschmann et al. 2011). In Notothenioids, parental care of hatched eggs is not rare (Kock et al. 2006). Between populations, gene flow is mediated by CAC (Van de Putte et al. 2012; Matschiner et al. 2009). They were captured in the depth ranging from 20 to 30 m.

The measurements presented here came from 15 fish caught in 2012. Previous catching were tentative only and revealed one species not obtained in the next season (*see* below). *Gobionotothen gibberifrons* (Lönnberg, 1905) constituted 39% of weight of the specimens collected. *Notothenia coriiceps* Richardson, 1844; *Trematomus bernacchi* Boulenger, 1902; *T. eulepidotus* Regan, 1914 and *T. newnesi* Boulenger, 1902 accounted 35%, 13%, 11% and 2% of total biomass respectively. The family Nototheniidae was represented by the highest number of species. Only single species and specimen from Bathydraconidae (*Gymnodraco acuticeps* Boulenger, 1902) was recorded (the expedition in 2010-11, exclusively). *Gobionotothen gibberifrons* and *T. eulepidotus* were the most abundant species. *Notothenia coriiceps* and *T. newnesi* were represented only by one specimen in 2012. Their stomachs contained mainly krill, polychaeta, mussels and algae. The krill was the main component of nourishment, because the main spawning season of Antarctic krill is from January to March (Ross et Quetin 1986). Further detailed study of the trophic ecology and structure of coastal Antarctic fish communities would be conducted because it is needed to deepen an understanding of structural and functional aspects of these communities as mentioned by Targett (1981). The most of studies of Antarctic fishes come mainly from the Ross Sea and from the islands of the Scotia Arc. The studies done in the Weddel Sea area are scarce (*e. g.* Kock et al. 1984, Hubold 1985, Ekau 1990, Brenner et al. 2001, Artigues et al. 2003, Van der Molen et Matallanas 2003, Lombarte et al. 2003). Investigations of the James Ross Island or Prince Gustav Channel are completely missing.

Remarks to the records

Nototheniidae (Cod icefishes)
***Gobionotothen gibberifrons* (Lönnberg , 1905)**
syn.: *Notothenia gibberifrons* Lönnberg, 1905; *Notothenia vaillanti* Regan, 1913

Figs. 7, 8

Gobionotothen giberifrons is a sedentary, benthic species feeding on infauna and engibenthic animals (Gon et Heemstra 1990, Matschiner et al. 2009). It inhabits even seasonal packed ice. Mainly juveniles and part of the adult population occur in the inshore waters 15–30m deep. Adults occur in offshore, deeper waters, at the depths down to 450 m (Casaux et al. 1990, Kulesz 1994, Barrera-Oro 2002). We found mainly krill, bivalves and polychaetes in their stomachs. *Gobionotothen gibberifrons* is known from all the islands of the Scotia Arc, the northern part of the Antarctic Peninsula and the South Atlantic, near to the south Shetlands and the Heard Island (Bykov 1983, Gon et Heemstra 1990).

***Notothenia coriiceps* Richardson, 1844**
syn.: *Notothenia coriiceps neglecta* (non Nybelin, 1951); *Notothenia neglecta* (non Nybelin, 1951)

Figs. 9, 10

Notothenia coriiceps is a demersal, shallow-water benthic species (Gon et Heemstra 1990). One of the most abundant Antarctic coastal fishes distributed probably circum-Antarctic on the continental shelf, known from the western Ross Sea, Balleny Islands, Adélie Land, Antarctic Peninsula and associated islands (islands of the Scotia Arc, to South Georgia, the Weddell Sea, the Bouvet and the sub-Antarctic islands of the Indian Ocean sector- (Everson 1970 and 1977, Fischer et Hureau 1985, Gon et Heemstra 1990) in the depths down to 450–550 m according to (Kock 1989, Tiedke et Kock 1989, Gon et Heemstra 1990, Barrera-Oro et Casaux 2008, Jones et Kock 2006, Kock et al. 2007). Feeding strategies and behavior were studied by Fanta et Meyer (1998). *Notothenia coriiceps* is primarily a benthic predator. Its, main prey are amphipods, isopods and moluscs (Hureau 1970, Richardson 1975, Burchett et al 1983). Except from krill, we found also macroalgae in its stomach as was already mentioned by Gon et Heemstra (1990).

***Trematomus bernacchii* Boulenger, 1902**
syn.: *Notothenia bernacchii* (Boulenger, 1902); *Pagothenia bernacchii* (Boulenger, 1902); *Pseudotrematomus bernacchii* (Boulenger, 1902); *Trematomus bernachii* Boulenger, 1902

Figs. 1, 2

Benthic species, found in seafloors (Froese et Pauly 2012). It is a common representative of shallow-water fish communities in the High-Antarctic Zone (Hureau 1994), resides probably circum-Antarctic and offshore islands (Peter Island, South Shetland, Elephant and South Orkney islands) (Gon et Heemstra 1990). We found mainly krill in its stomachs. Vacchi et al. (2000) describes a hunt-and-peck strategy of this species,

employed to capture prey such as polychaetes and bivalves. Hureau (1970) mentioned that it may feed on polychaetes, gastropods, isopods, amphipods and few algae. The detailed dietary diversity is provided by La Mesa et al (2004).

***Trematomus eulepidotus* Regan, 1914**
syn. *Pseudotrematomus eulepidotus* (Regan, 1914)

Figs. 3, 4

This is a bentho-pelagic (Fischer et Hureau 1985, Gon et Heemstra 1990) or epibenthic (Eastman 1993) species with pelagic feeding (Klingenberg et Ekau 1996), it can rest in glass sponges (Gutt et Ekau 1996). Distributed circum-Antarctic in nearshore and continental shelf and nearby islands, including the South Orkneys (Froese et Pauly 2012). Schwarzbach (1988), Gon et Heemstra (1990) and Kailola et al. (1993) mention that they feed on salps, nudibranchs, amphipods, copepods, polychaetes, krill, crustaceans, chaetognaths, and fish.

***Trematomus newnesi* Boulenger, 1902**

Figs. 5, 6

Trematomus newnesi sometimes foraging on bottom, otherwise in water collumn, feeds preferently on plankton. It is even cryopelagic (Andriashev 1970). It occupies various habitats, such as benthos, the water column beneath ice or free from ice and the surface in conjunction with algae banks, they can perform vertical migration (DeWitt 1971, Fischer et Hureau 1985). Distribution probably circum-Antarctic, it is known from East Antarctica, Weddell Sea, west coast of the Antarctic Peninsula, the South Shetland Islands, the Elephant Island, the South Orkney Islands, the Mac Robertson, the George V, the Queen Mary, the Adelie Coasts, the Davis Sea and the Ross Sea (Tiedke et Kock 1989, Froese et Pauly 2012). Along the Antarctic Peninsula, especially juveniles often feed in the water column or at the undersurface of ice (Gon et Heemstra 1990). Its prey are mainly euphausiids and amphipods, less frequently polychaetes, gastropods and isopods (Daniels 1982). It feeds preferently on plankton (Vacchi et La Mesa 1995).

Bathydraconidae (Antarctic dragonfishes)
***Gymnodraco acuticeps* Boulenger, 1902**
syn.: *Gymnodraco victori* Hureau, 1963

Fig. 11

Gymnodraco acuticeps was caught in the season of 2010-11, therefore not measured. Demersal circum-Antarctic notothenioid, generally found in relatively shallow water, in contact with anchor ice (Bargelloni et al. 1998, Froese et Pauly 2012). Probably circum-Antarctic, found along the Antarctic Peninsula, the South Shetland Islands and the Antarctic continental shelf (Froese et Pauly 2012). They are active ambush predators feeding on a variety of organisms including fish, fish eggs, amphipods and polychaetes (Gon et Heemstra 1990, Bargelloni et al. 1998, Evans et al. 2005).



Plate 1. Photos of five species of Notothenioid fish caught in season 2011-12. Figs. 1, 2 - *Trematomus bernacchii* Boulenger, 1902; Figs. 3, 4 - *T. eulepidotus* Regan, 1914; Figs. 5, 6 - *T. newnesi* Boulenger, 1902; Figs. 7, 8 - *Gobionotothen gibberifrons* (Lönnberg, 1905); Figs. 9, 10 - *Notothenia coriiceps* Richardson, 1844 (Photos 2–6 and 8–10 by Jitka Míková).



Fig 11. Photo of Bathydraconidae fish caught in season 2010-11: *Gymnodraco acuticeps* Boulenger, 1902 (Photo by Kamil Láska).

Species	Common name	n	WT	GSI	L_S	L_T	Food compostion
Gobionotothen gibberifrons	Humped rockcod	5	59–294	1.5 (1F) 1.2 (1M)	170–284	250–331	krill bivalves polychaetes
Notothenia coriiceps	Black rockcod or Bullhead notothen	1	871	6.8 (1F)	344	395	krill macroalgae
Trematomus bernacchii	Emerald rockcod	3	28–170	2.8 (1F) 0.8 (1M)	120–204	140–230	krill
Trematomus eulepidotus	Blunt scalyhead	4	37–100	5.3–7.4 (3F) 0.6 (1M)	125–190	142–212	krill
Trematomus newnesi	Dusky rockcod	1	44	-	145	165	krill
Gymnodraco acuticeps	Ploughfish	1	-	-	-	-	-

Table 1. Information on fish species used in this study. *Table legend*: n – number of measured fish; WT – weight (g); GSI – gonadosomatic index in % (with sample number and indication of sex in parentheses, M – male, F – female); LS – standard length (mm); LT – total length (mm).

Conclusion

To monitor the species composition and also the diet of species, we started the investigation of fish in austral summer 2012. Six notothenioid fish species were caught (*Trematomus bernacchii*, *T. eulepidotus*, *T. newnesi*, *Gobionotothen gibberifrons*, *Notothenia coriiceps* and *Gymnodraco acuticeps*). Summer polar waters are rich in nutrition, which allows to develop trophic structures, where primary consumers, *e. g. Euphasia* sp. play a key role. Krill is the prominent nourishment for most of Antarctic marine organisms. Also notothenioids prefer krill, if available, as highly nutritious source. It can be the reason why we observed mostly krill in their stomachs. Besides krill, molluscs, polychaetes and algae were found in stomach. We emphasize the necessity of conducting further ichthyofaunal investigations as this will allow and help to monitor the status of the Prince Gustav Channel ecosystem. Eastman (2005) states that knowledge of species composition in an area or ecosystem is a fundamental prerequisite for subsequent work in evolutionary biology, ecology, biogeography and conservation.

Because the James Ross Island fishery has not yet been studied, we started with monitoring and arranging the equipment for next season work. Next austral summer, we want to proceed and watch the habitat and behavior of coastal fish up to 50-60 m using underwater camera. It is necessary to observe the shelf habitat to understand the species composition and explain the species life history. We want to pay closer attention to stomach content to prove the species food preferences.

References

ANDRIASHEV, A.P. (1970): Cryopelagic fishes of the Arctic and Antarctic and their significance in the polar ecosystems. *In*: M.V. Holdgate (ed.): Antarctic ecology. Academic Press, New York, pp. 287-304

ARTIGUES, B., MORALES-NIN, B. and BALGUERIAS, E. (2003): Fish length-weight relationships in the Weddell Sea and Bransfield Strait. *Polar Biology*, 26: 463-467.

BARGELLONI, L., MARCATO, S. and PATARNELLO, T. (1998): Antarctic fish hemoglobins: Evidence for adaptive evolution at subzero temperature. *Proceedings of The National Academy of Sciences of The United States of America*, 95: 8670-8675.

BARRERA-ORO, E.R. (2002): The role of fish in the Antarctic marine food web: Differences between inshore and offshore waters in the southern Scotia Arc and western Antarctic Peninsula. *Antarctic Science*, 14: 293-309.

BARRERA-ORO, E.R., CASAUX, R.J. (2008): General ecology of coastal fish from the South Shetland Island and west Antarctic Peninsula areas. *Berichte zur Polar- und Meeresforschung*, 571: 95-110.

BRENNER, M., BUCK, B.H., CORDES, S., DIETRICH, L., JACOB, U., MINTENBECK, K., SCHRÖDER, A., BREY, T., KNUST, R. and ARNTZ, W. (2001): The role of iceberg scours in niche separation within the Antarctic fish genus *Trematomus*. *Polar Biology*, 24: 502-507.

BURCHETT, M.S., SAYERS, P.J., NORTH, A.W. and WHITE, M.G. (1983): Some biological aspects of the nearshore fish populations at South Georgia. *British Antarctic Survey Bulletin*, 59: 63-74.

BYKOV, V.P. (1983): Marine Fishes: Chemical composition and processing properties. Amerind Publishing Co. Pvt. Ltd., New Delhi, 1983, 333 p.

CASAUX, R.J., MAZZOTTA, A.S. and BARRERA-ORO, E.R. (1990): Seasonal aspects of the biology and diet of nearshore notothenioid fish at Potter Cove South Shetland Islands, Antarctica. *Polar Biology*, 11: 63-72.

CHENG, C.-H.C., DETRICH, H.W. (2007): Molecular ecophysiology of Antarctic notothenioid fishes. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, 362: 2215-2232.

CLARKE, A., CRAME, J.A. (2010): Evolutionary dynamics at high latitudes: Speciation and extinction in polar marine faunas. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, 365: 3655-3666.

CLARKE, A., JOHNSTON, I.A. (1996): Evolution and adaptive radiation of Antarctic fishes. *Trends in Ecology & Evolution*, 11: 212-218.

CORRELL, R.C. (1998): Our Changing Planet, DIANE Publishing, Darby, PA, U.S.A., 1998, 162 p.

DAMERAU, M., MATSCHINER, M., SALZBURGER, W. and HANEL, R. (2012): Comparative population genetics of seven notothenioid fish species reveals high levels of gene flow along ocean currents in the southern Scotia Arc, Antarctica. *Polar Biology*, 35:1073-1086.

DANIELS, R.A. (1982): Feeding ecology of some fishes of the Antarctic Peninsula. *Fisheries Bulletin*, 80: 575-588.

DEWITT, H.H. (1971): Coastal and deepwater benthic fishes of the Antarctic. *In*: V.C. Bushnell (ed.): Antarctic Map, Folio Series. American Geographic Society Folio, New York, pp.1-10

EASTMAN, J., SIDELL, B. (2002): Measurements of buoyancy for some Antarctic notothenioid fishes from the South Shetland Islands. *Polar Biology*, 25: 753-760.

EASTMAN, J.T, GRANDE, L. (1989): Evolution of the Antarctic fish fauna with emphasis on the recent notothenioids. *In*: Crame, J.A. (ed.): Origins and evolution of the Antarctic biota. Geological Society of London, Special Publication, 47: 241-252.

EASTMAN, J.T., MCCUNE, A.R. (2000): Fishes on the Antarctic continental shelf: Evolution of a marine species flock? *Journal of Fish Biology*, 57: 84-102.

EASTMAN, J.T. (1993): Antarctic fish biology: Evolution in an unique environment. Academic Press, San Diego, 322 p.

EASTMAN, J.T. (2005): The nature of the diversity of Antarctic fishes. *Polar Biology*, 28: 94-107.

EASTMAN, J.T., EAKIN, R.R. (2000): An updated species list for notothenioid fish (Perciformes; Notothenioidei), with comments on Antarctic species. *Archive of Fishery and Marine Research*, 48: 11-20.

EKAU, W. (1990): Demersal fish fauna of the Weddell Sea, Antarctica. *Antarctic Science*, 2: 129-137.

ESCHMEYER, W.N., FRICKE, R. (eds.) (2009): Catalog of Fishes. (source: http://research.calacademy.org/ichthyology/catalog/fishcatmain.asp). Electronic version accessed September 2009.

ESCHMEYER, W. N. (ed.) (2012): Catalog of Fishes. California Academy of Sciences. (source: http://research.calacademy.org/research/ichthyology/catalog/fishcatmain.asp). Electronic version accessed November 2012.

EVANS, C.W., CZIKO, P., CHENG, C.-H.C. and DEVRIES, A.L. (2005): Spawning behaviour and early development in the naked dragonfish *Gymnodraco acuticeps*. *Antarctic Science*, 17: 319-327.

EVERSON, I. (1970): The population dynamics and energy budget of *Notothenia neglecta* Nybelin at Signy Island South Orkney Islands. *British Antarctic Survey Bulletin*, 23: 25-50.

EVERSON, I. (1977): The living resources of the Southern Ocean. Fisheries Survey Programme GLO/SO/77/1/FAO, Rome, 156 p.

FANTA, E., MEYER, A.A. (1998): Behavioural strategies for feeding of six species of the Antarctic fish family Nototheniidae (Pisces, Notothenioidei) in a tank. *Antarctic Record*, 42: 227-243.

FISCHER, W., HUREAU, J.C. (eds.) (1985): FAO species identification sheets for fishery purposes Southern Ocean: Fishing Areas 48, 58 and 88 (CCAMLR Convention Area) Prepared and published with the support of the Commission for the Conservation of Antarctic Marine Living Resources. Roma, FAO 1985. 2: 233-470.

FROESE, R., PAULY, D. (eds.) (2012): FishBase. World Wide Web electronic publication. (www.fishbase.org). Electronic version accessed October 2012.

GON, O., HEEMSTRA, P.C. (eds.) (1990): Fishes of the Southern Ocean. J.L.B. Smith Institute of Ichthyology, Grahamstown, South Africa, 462 p.

GUTT, J., EKAU, W. (1996): Habitat partitioning of dominant high Antarctic demersal fish in the Weddell Sea and Lazarev Sea. *Journal of Experimental Marine Biology and Ecology*, 206: 25-37.

HUBOLD, G. (1985): Stomach contents of the Antarctic Silverfish *Pleuragramma antarcticum* from the southern and eastern Weddell Sea (Antarctica). *Polar Biology*, 5: 43-48.

HUREAU, J.C. (1970): Biologie comparée de quelques poissons antarctiques (Notothenidae). *Bulletin de l'Institut océanographique de Monaco*, 68:1-244.

HUREAU, J.C. (1994): The significance of fish in the marine Antarctic ecosystems. *Polar Biology*, 14: 307-313.

INGRAM, T., MAHLER, D.L. (2011): Niche diversification follows key innovation in Antarctic fish radiation. *Molecular Ecology*, 20: 4590-4591.

JONES, C.D., KOCK, K.H. (2006): Standing stock, spatial distribution and biological features of demersal finfish from the 2006 US AMLR bottom trawl survey of the northern Antarctic Peninsula and Joinville/D'Urville Islands (Subarea 48.1). Commission for the Conservation of Antarctic Marine Living Resources. Hobart, Australia.

KAILOLA, P.J., WILLIAMS, M.J., STEWART, P.C., REICHELT, R.E., MCNEE, A. and GRIEVE, C. (1993): Australian fisheries resources. Bureau of Resource Sciences, Canberra, Australia, 422 p.

KLINGENBERG, C.P., EKAU, W. (1996): A combined morphometric and phylogenetic analysis of an ecomorphological trend: Pelagization in Antarctic fishes (Perciformes: Nototheniidae). *Biological Journal of the Linnean Society*, 59: 143-177.

KOCK, K., PSHENICHNOV, L. and DEVRIES, A. (2006): Evidence for egg brooding and parental care in icefish and other notothenioids in the Southern Ocean. *Antarctic Science*, 18: 223-227.

KOCK, K.H. (1989): Reproduction in fish around Elephant Island. *Archiv für Fischereiwissenschaft*, 39: 171-210.

KOCK, K.H. (1992): Antarctic Fish and Fisheries. Cambridge University Press, New York, 359 p.

KOCK, K.H., APPEL, J., BUSCH, M., KLIMPEL, S., HOLST, M., PIETSCHOK, D., PSHENICHNOV, L.V., RIEHL, R. and SCHÖLING, S. (2007): Composition and standing stock estimates of finfish from the Polarstern bottom trawl survey around Elephant Island and the South Shetland Islands (subarea 48.1, 19 December 2006 to 3 January 2007). *Commission for the Conservation of Antarctic Marine Living Resources Document*, WG-FSA-07/22. Hobart, Australia.

KOCK, K.H., SCHNEPPENHEIM, R. and SIEGEL, V. (1984): Contribution to the fish fauna of Weddell Sea. *Archiv für Fischereiwissenschaft*, 34: 103-120.

KULESZ, J. (1994): Seasonal biology of *Notothenia gibberifrons*, *N. rossii* and *Trematomus newnesi* as well as respiration of young fish from Admiralty Bay (King George, South Shetland Islands). *Polskie Archiwum Hydrobiologii*, 41: 79-102.

LA MESA, M., EASTMAN, J.T. and VACCHI, M. (2004): The role of notothenioid fish in the food web of the Ross Sea shelf waters: A review. *Polar Biology*, 27: 321-338.

LIVERMORE, R., NANKIVELL, A., EAGLES, G. and MORRIS, P. (2005): Paleogene opening of Drake Passage. *Earth and Planetary Science Letters*, 236: 459-470.

LOMBARTE, A., OLASO, I. and BOZZANO, A. (2003): Ecomorphological trends in the Artedidraconidae (Pisces : Perciformes : Notothenioidei) of the Weddell Sea. *Antarctic Science*, 15: 211-218.

MATSCHINER, M., HANEL, R. and SALZBURGER, W. (2009): Gene flow by larval dispersal in the Antarctic notothenioid fish *Gobionotothen gibberifrons*. *Molecular Ecology*, 18: 2574-2587.

MATSCHINER, M., HANEL, R. and SALZBURGER, W. (2011): On the Origin and Trigger of the Notothenioid Adaptive Radiation. *PLoS ONE* 6: e18911.

NEAR, T.J., JONES, C.D. and EASTMAN, J.T. (2009): Geographic intraspecific variation in buoyancy within Antarctic notothenioid fishes. *Antarctic Science*, 21: 123-129.

PATARNELLO, T., VERDE, C., DI PRISCO, G., BARGELLONI, L. and ZANE, L. (2011): How will fish that evolved at constant sub-zero temperatures cope with global warming? Notothenioids as a case study. *Bioessays*, 33: 260-8.

REECE, A. (1950): The ice of Crown Prince Gustav Channel, Graham Land, Antarctica. *Journal of Glaciology*, 1: 404-409.

RICHARDSON, M.G. (1975): The dietary composition of some antarctic fish. *British Antarctic Survey Bulletin*, 41/42: 113-120.

ROSS, R.M., QUETIN, L.B. (1986): How productive are Antarctic krill? *BioScience*, 36: 264-269.

RUTSCHMANN, S., MATSCHINER, M., DAMERAU, M., MUSCHICK, M., LEHMANN, M.F., HANEL, R. and SALZBURG, W. (2011): Parallel ecological diversification in Antarctic notothenioid fishes as evidence for adaptive radiation. *Molecular Ecology*, 20: 4707-4721.

SCHWARZBACH, W. (1988): Die Fischfauna des ostlichen und sudlichen Weddellmeeres: geographische Verbreitung, Nahrung und trophische Stellung der Fischarten. *Berichtezur Polarforschung*, 54: l-94.

SHANDIKOV, G.A. (2011): *Channichthys richardsoni* sp. n., a new Antarctic icefish (Perciformes: Notothenioidei: Channichthyidae) from the Kerguelen Islands area, Indian sector of the Southern Ocean. *Journal of V.N. Karazin Kharkiv National University. Series: Biology*. 14: 125-134.

SIDELL, B.D., O'BRIEN, K.M. (2006): When bad things happen to good fish: the loss of hemoglobin and myoglobin expression in Antarctic icefishes. *The Journal of Experimental Biology*, 209: 1791-1802.

TARGETT, T.E. (1981): Trophic Ecology and Structure of Coastal Antarctic Fish Communities. *Marine Ecology - Progress Series*, 4: 243-263.

THATJE, S., HILLENBRAND, C.-D. and LARTER, R. (2005): On the origin of Antarctic marine benthic community structure. *Trends in Ecology & Evolution*, 20: 534-540.

TIEDTKE, J.E., KOCK, K.H. (1989): Structure and composition of the fish fauna around Elephant Island. *Archiv für Fischereiwissenschaft*, 39: 143-169.

VACCHI, M., LA MESA, M. (1995): The diet of the Antarctic fish *Trematomus newnesi* Boulenger 1902 (Nototheniidae) in Terra Nova Bay, Ross Sea. *Antarctic Science*, 7: 37-38.

VACCHI, M., LA MESA, M. and GRECO, S. (2000): The coastal fish fauna of Terra Nova Bay, Ross Sea, Antarctica. *In*: F.M. Faranda, L. Guglielmo, A. Ianora (eds.): Ross Sea Ecology. Springer-Verlag, Berlin, pp. 457-468

VAN DE PUTTE, A.P., JANKO, K., KASPAROVA, E., MAES, G.E., ROCK, J., KOUBBI, P., VOLCKAERT, F.A.M., CHOLEVA, L., FRASER, K.P.P., SMYKLA, J., VAN HOUDT, J.K.J. and MARSHALL, C. (2012): Comparative phylogeography of three trematomid fishes reveals contrasting genetic structure patterns in benthic and pelagic species. *Marine Genomics*, 8: 23-34.

VAN DER MOLEN, S., MATALLANAS, J. (2003): Oocyte development and maturity classification of *Gerlachea australis* from the Weddell Sea, Antarctica. *Polar Biology*, 26: 653-658.

VERDE, C., PARISI, E. and DI PRISCO, G. (2003): The evolution of polar fish hemoglobin: A phylogenetic analysis of the ancestral amino acid residues linked to the root effect. *Journal of Molecular Evolution*, 57: pS258.

Systematics and Biodiversity (2013), 1–18

Taylor & Francis
Taylor & Francis Group

Research Article

Otostephanos (Rotifera, Bdelloidea, Habrotrochidae) with the description of two new species

NATALIIA S. IAKOVENKO[1,2], EVA KAŠPAROVÁ[3], MICHAEL PLEWKA[4] & KAREL JANKO[2,3]

[1]Department of Invertebrate Fauna and Systematics, Schmalhausen Institute of Zoology, NAS of Ukraine, Bogdana Khmelnyts'kogo 15, Kyiv 01601, Ukraine
[2]Department of Biology and Ecology, University of Ostrava, Chittusiho 10, 701 03 Ostrava, Czech Republic
[3]Laboratory of Fish Genetics, Institute of Animal Physiology and Genetics AS of Czech Republic, Rumburska 89, 277 21 Libechov, Czech Republic
[4]Department of Biology, State Gymnasium, Ochsenkamp 100, 58285 Gevelsberg, Germany

(Received 13 July 2013; revised 15 October 2013; accepted 16 October 2013)

We present a morphological feature-based key for the genus *Otostephanos* and describe two new species, *O. jolantae* sp. nov. and *O. ukrainicus* sp. nov. For *O. jolantae* sp. nov. we analysed the intraspecific morphological variability and provided the barcodes of mtCOX1 mitochondrial gene. Further, we developed a method to standardize measurements for the Philodina type of corona and trophi measurements of bdelloids. *Otostephanos jolantae* sp. nov. is a large rotifer with a smooth cuticle and bright red-orange gut; it can be distinguished from the known species by a high triangular upper lip with a tongue-like tip not divided into lobes, spade-shaped swollen rump, and 6/6 dental formula. It is found in *Sphagnum* collected in Poland, Ukraine and the Czech Republic. *Otostephanos ukrainicus* sp. nov. has a long body covered with coarse-grained cuticle on the last two neck segments, trunk and rump; it is distinguished by long narrow head and neck, saccular-like swollen trunk, spade-like rump, and a tiny foot. Unlike the other species of this genus, it has band-like upper lip with two narrow, sharp protrusions separated by an interspace, unusually small spurs and trophi with 5/5 major teeth. Thus far, it is only found in Ukraine, in pine and oak forest litter.

http://zoobank.org/urn:lsid:zoobank.org:pub:9FC484E3-67F5-43A9-8478-A7BE14FBE5E33

Key words: barcoding, bdelloid ecology, bdelloid taxonomy, biodiversity, DNA taxonomy, morphometry

Downloaded by [Univerzitni Knihovna Ostravske] at 08:07 05 December 2013

Introduction

Worldwide distributed genus *Otostephanos* Milne, 1916 of the family Habrotrochidae Bryce, 1910 encompasses 13 valid morphotaxa of the species rank inhabiting fresh waters, terrestrial mosses and soil, being one of the least species-rich genera of Subclass Bdelloidea, Phylum Rotifera (Segers, 2002, 2007). There are still no fossil records or phylogenies according to which the origin of the genus could be dated.

The type species of *Otostephanos*, *O. auriculatus* (Murray, 1911), was described from terrestrial moss collected in Eastern Africa (Kikuyu, Kenya) and Australia (Katoomba, N. S. Wales) as *Habrotrocha auriculata*; the peculiar feature that distinguished it from all known species of the genus *Habrotrocha* Bryce, 1910 (paired rings around the pedicels of the corona) was attributed to an optical artefact (Murray, 1911*a*). Two years later Bryce (1913) found rotifers with the same type of rotatory apparatus in the UK, confirmed the reality of the rings and described the specimens he found as *H. torquata*. Finally Milne (1916), in his paper on the Rotifera of South Africa, established a new genus, ascribing corona with two paired rings as its characteristic trait, and relocated *H. auriculata* into it as *Otostephanos auriculatus* and *H. torquata* as *O. torquatus*. In the same paper he described three new taxa, also found in Africa (*O. regalis* Milne, 1916; *O. torquatus amoenus* Milne, 1916; *O. monteti* Milne, 1916). Subsequently *Otostephanos* was found in Europe, Asia, North America, Antarctica and the Pacific region – New Zealand, Hawaii (Murray, 1911*b*; Harring, 1914; Bartoš, 1959; Haigh, 1963; Donner, 1965; Sohlenius

Correspondence to: Nataliia S. Iakovenko. E-mail: rotifer1@gmail.com

ISSN 1477-2000 print / 1478-0933 online
© The Trustees of the Natural History Museum, London 2013. All Rights Reserved.
http://dx.doi.org/10.1080/14772000.2013.857737

Downloaded by [Univerzitni Knihovna Ostravske] at 08:07 05 December 2013

et al., 1996, Song & Kim, 2000; Ustaoğlu *et al*., 2012). Five species (*O. annulatus* Koniar, 1955; *O. cuspidilabris* de Koning, 1947; *O. kostei* Donner, 1972; *O. macrantennus* de Koning, 1947; *O. mundiformis* de Koning, 1947) are still found only in Europe and one only in Africa (*O. jersabeki* Koste, 1996); the rest have been reported from two or more continents (Kutikova, 2005; Segers, 2007).

It should be noted that the known occurrence of *Otostephanos* seems to reflect the sampling efforts of researchers rather than the real distribution of the genus: the majority of finds were from Europe, the location of most bdelloid taxonomists. Within Europe *Otostephanos* have generally been found in the central part: four species-ranked taxa have been recorded in Austria, the same number is reported from Germany and the Netherlands, three have been reported in Czech Republic, Hungary and Romania, two have been reported in Switzerland and Italy and only one species of *Otostephanos* has been found in the UK, Sweden and Slovakia (Bartoš, 1959; Rudescu, 1960; Donner, 1965, 1972*a*, 1972*b*; Bancsi, 1986; Fontaneto & Melone, 2003*a*, 2003*b*; Fontaneto *et al*., 2007*a*). Seven species-ranked taxa of *Otostephanos* have been found in Ukraine during the past several years as a result of extensive faunistic search (Iakovenko & Ovander, 2011).

The lack of knowledge about the diversity and chorology of *Otostephanos* might also be attributed to the insufficient research efforts with respect to terrestrial rotifers, considering that eight of 13 species-rank taxa of *Otostephanos* inhabit drying mosses, litter and soil (Bryce, 1913; Milne, 1916; Donner, 1951; Schulte, 1954; Varga, 1957, 1966). Thus, the soil-dwelling species *O. jersabeki* is still known only from its type locality – Etosha National Park, Namibia, Africa (Koste, 1996). *Otostephanos regalis*, described from terrestrial moss (Southern Africa), have been subsequently found only once and outside of the African continent. Unfortunately, the single report of its occurrence in Germany was not confirmed by illustrations or slides (Hirschfelder *et al*., 1993). Another possible reason for the rare reports is the low local abundance of some species. This may explain, for example, why *O. kostei*, which has an unusual morphology, and thus should be difficult to overlook, has not been reported since its first record from Austria.

Assessing the distribution range of some *Otostephanos* species might be difficult, because of the taxonomical uncertainty, thus for the presumably cosmopolitan *O. auriculatus* (Murray, 1911), *O. donneri* (Bartos, 1959) and *O. torquatus torquatus* (Bryce, 1913), a number of morphological forms are known (Bryce, 1913; Bartoš, 1949, 1959; Donner, 1952, 1954, 1956, 1965; Varga, 1953; Schulte, 1954; Haigh, 1963; Yakovenko, 2000). These forms may potentially be good species (for asexual species delimitation criteria see Birky *et al*., 2005) with obscure biogeography.

All existing keys to the species of *Otostephanos* were never published as separate sources, but included into larger synopses (such as keys to Bdelloidea or the whole Rotifera). Two of them are in German (Voigt, 1956–57; Donner, 1965), two in Czech (Bartoš, 1951, 1959), one is in Romanian (Rudescu, 1960) and one is in Russian (Kutikova, 2005). No key in English has been available to date. No deposited types of any *Otostephanos* are known (Jersabek *et al.*, 2012), that is, until recently iconotypes have been used as substitutes and is the common taxonomical practice. Descriptions and redescriptions of taxa are made on the basis of external morphology observed under a light microscope, with the exception of *O. donneri*, for which the habitus, general view of the mastax, also fine trophi structure (SEM) is known and the mtCOX1 gene (556 bp, Ref. EF650595) have been published (Melone & Fontaneto, 2005).

Species-rank taxa of Bdelloidea, including morphologically distinguishable subspecies, have been suggested to be treated as clonal species (Segers, 2008), due to the obligatory parthenogenesis all individuals of separate lineages are reproductively isolated, but distinctive clusters can be considered species when using molecular and/or morphological analysis (Birky *et al*., 2005; Fontaneto *et al*., 2007*b*, 2007*c*). While the species concept for obligatory asexual organisms has been developed (Birky & Barraclough, 2009), using the taxonomic designation of subspecies for bdelloid rotifers makes no sense. However, no taxonomic revision has been made and triple names are still used for the subspecific taxa. In *Otostephanos* two subspecies names are accepted: *bilobatus* Hauer, 1939 of *O. auriculatus* and *amoenus* Milne, 1916 of *O. torquatus*. Obviously, these subspecies cannot be formally raised to the species rank. Changing taxonomic ranks requires detailed information about the external and internal morphology along with DNA analysis. This was recently done in the revision of *Abrochtha* Bryce, 1910 (Birky *et al*., 2011).

This study summarizes known information and presents new data on the taxonomy and morphology of genus *Otostephanos*. The research started with samples collected in Ukraine 12 years ago where uncommon bdelloids have been discovered. On the basis of the morphology of the corona we ascertained that these animals belonged to *Otostephanos*; however, they did not match any known species description. A few specimens found were designated as *Otostephanos* sp. and *O.* cf. *annulatus* (Iakovenko, 2005; Iakovenko & Ovander, 2011). Later, one of the two taxa was found in Poland by the first author and the research team of Professor Jolanta Ejsmont-Karabin. This discovery was noted as *Otostephanos* sp. in the faunistic survey of Polish Tatras (Bielańska-Grainer *et al*. 2011). Recently, additional material was acquired from two other European localities: (1) a peat bog in the Tábor District, Czech Republic; and (2) *Sphagnum* collected in the Austrian Alps.

Downloaded by [Univerzitni Knihovna Ostravske] at 08:07 05 December 2013

Materials and methods

Study area, methods of collection and preliminary laboratory procedures

Sampling was conducted in 2000 in Ukraine, during 2006–2009 in Poland, in 2011 in Czech Republic, and in 2012 in Austria (Fig. 1; Appendix I, see online supplemental material, which is available from the article's Taylor & Francis Online page at http://dx.doi.org/10.1080/14772000.2013.857737). With the appropriate permission for sampling in the protected areas, we collected aquatic vegetation, sphagnum, terrestrial mosses and forest litter in natural habitats including Lubche local reserve (Ukraine), Tatrzanski, Karkonosze, Bory Tucholskie, Wigry, Bialowezski National Parks and Mazurski Landscape Park (Poland). We used plastic or glass jars as samplers, and before examination the material was kept wet under room temperature without chemical preservation or freezing. Identification of rotifers was made within approximately 3 days after collection. The new species found in *Sphagnum* was mostly confined to acid waters (peat bogs, dystrophic lakes), but also occurred in ground *Sphagnum* growing in forests.

Animals were extracted (washed out) by rinsing the samples with distilled water several times, and then the extract was examined under dissecting microscope with the total magnification ×40. Rotifers were then put onto a glass slide by a micropipette and observed alive and active under the microscope with the total magnification ×300–600. For permanent slides we fixed rotifers with boiled water, pouring it into the embryo dish with the living rotifers (Pennak, 1978), then transferred the specimens sequentially to water–glycerin mix, to pure glycerin and to glycerin jelly, mounting on a slide as described in Kutikova (1970).

Clonal cultures of the first described species *O. jolantae* sp. nov. (PL0925OJ1–OJ5, CZ1137OJ1–OJ10, CZ1138OJ1–OJ10) started from a single animal were maintained in the laboratory under room temperature. Only three individuals of the second species described herein, *O. ukrainicus* sp. nov., were found and preserved shortly after observation, and no living cultures were established. Clonal progeny were numbered by lowercase letters added to the original individual code (for example, CZ1138OJ1a). We cultivated rotifers in 24-well tissue plates (Falcon™) filled with water from the correspondent samples that had been previously filtered through the mesh net #77 (mesh side length 64 μm) and additionally checked for rotifer eggs and other metazoans; this was done to provide rotifers with the specific feeding objects from the natural environment. Animals from the cultures were used for light microscope imaging, SEM preparation and DNA analysis: from each successful culture 2–3 specimens were used for extraction of trophi and 5–6 animals were prepared for DNA isolation. As a result, each original specimen was represented by at least one light microscope image, one SEM image and one successful sequence (clonal progeny are assumed to be morphologically and genetically identical to a mother); these data were used for the morphological and genetic comparison between individuals and populations.

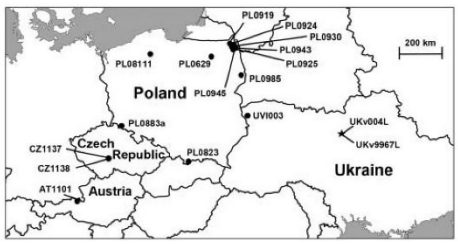


Fig. 1. Distribution of *Otostephanos jolantae* sp. nov. (circles) and O. ukrainicus sp. nov. (a star) in Europe. Labels near the points are codes of the collection points (see Appendix I, supplemental material online).

Terminology

The body of a bdelloid rotifer is divided into several main parts; head, trunk and foot are usually distinguished (Ricci & Melone, 2000; Fontaneto & Ricci, 2004), but some authors also mention neck and rump (Donner, 1965; Kutikova, 2005). The external metamerism of the body seems to be manifested in the cuticle and syncytial hypoderma, but not in the internal organs; these parts are referred to as joints or more precisely *pseudosegments*.

Head is the proximal part of the body consisting of 3 pseudosegments. First 2 pseudosegments form an elongated *rostrum* used for creeping and tactile sense; the apical dorsal rim of the rostrum has a transparent plate-like protrusion – *rostral lamella*. The third pseudosegment bears the complex of several ciliated and smooth parts used for locomotion and feeding – *corona*. The corona of Philodina type, which is found in families Habrotrochidae, Philodinidae, consists of 2 semispherical *trochi* placed on column-like protrusions (*pedicels*) that are often divided by a hollow (*sulcus*) and covered dorsally with a cuticular fold – *upper lip*. Each trochus might have a papilla in the middle of its top plane; papillae often carry long and stiff *sensitive bristles*. *Ligula* is a sharp tongue- or rod-like protrusion that may protrude from the sulcus or the top of the upper lip. Trochi form the first ciliary zone, and a bolster at the base of the pedicels (*cingulum*) comprises a second; on the ventral side, they merge into a continuous ciliary field. The mouth opening is located on the ventral side beneath the pedicels; its ventral rim is called the *lower lip*.

Downloaded by [Univerzitni Knihovna Ostravske] at 08:07 05 December 2013

Neck consists of 3 pseudosegments behind the head, of which the first one always bears on its dorsal side a sensitive organ – tubular *antenna* of 2–4 segments. *Trunk* follows the neck and consists of 6 pseudosegments. Fifth and sixth trunk pseudosegments together are called *rump*; rump is usually narrower than the rest of the trunk; it contains the rectum and excretory bladder, and the cloacal opening is located on its proximal rim. *Foot* is formed by 3–6 last pseudosegments; it is narrower than the rest of the body and bears two types of appendages: one or two that are called *spurs* are stiff and non-retractable, and are located on the penultimate pseudosegment; the soft and retractable *toes* (2–4) are located on the last pseudosegment of the foot. Two spurs might be present close together or separated with interspace. In some cases toes might be replaced by a sticky *adhesive plate*.

The digestive system of a bdelloid consists of three parts: (1) mouth opening, ectodermal ciliary buccal tube or *throat* with salivary glands, *oesophagus*, and masticatory apparatus (*mastax*) with digestive glands; (2) middle part — entodermal *gut*; (3) short ectodermal *intestine* opening into the cloaca ending with anus. Bdelloids of family Habrotrochidae have sack-like gut with thin walls and a wide internal cavity (*lumen*) filled with food formed into rounded lumps — *pellets*. *Trophi* are sclerotized parts of the mastax; in bdelloids they consist of three paired sections: the basis is formed by two massive *rami*, on which lay numerous rod-like sclerites — *unci*; laterally unci are surrounded with two sickle-like *manubria*. Thicker sclerites of unci are called *major teeth*; and the thinner ones are called *minor teeth*, respectively. Under a microscope, trophi may lie with the unci up and rami down (*cephalic view*) or vice versa (*caudal view*). The reproductive system has a pair of gonads — *germovitellaria*, each consisting of ovarium and vitellarium enveloped in follicular layer.

Abbreviations

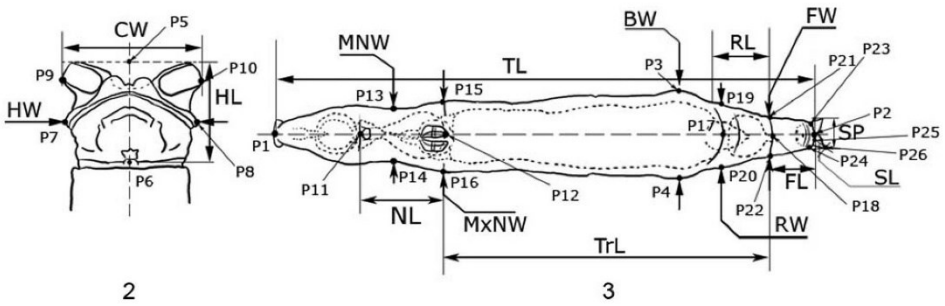
Explanation of the sample codes is given in Appendix I (see supplemental material online); for the explanation of the dimension abbreviations see Light microscopy section of Materials and methods. Other abbreviations used in the text: SIZ – Schmalhausen Institute of Zoology; LP – Landscape Park; LR – Local Reserve; NP – Nature Park; r. – region; vv. – voivodeship (province in Poland).

Light microscopy

Catching an image of an active bdelloid is difficult because of the animal's mobility. However, it is possible to properly observe animals when they are sluggish and the body is stretched. For *Otostephanos*, as the rest of Habrotrochidae, it is necessary to observe the rotifer feeding and the rotatory apparatus fully turned out of the mouth; this requires patience. Occasionally we used Marcain® to relax the animals at which point they would often turn out corona, but it had poor effect due to the deformation of the organ caused by the narcotizing agent. Up to 20 individuals (mature females with fully grown germovitellaria) were selected at random from each sample and pictured both creeping and feeding.

In our study we made an attempt to systematize and improve the aspects of the morphometric analysis of Bdelloidea relevant to external measurements, focusing on the families Habrotrochidae and Philodinidae that have rotatory apparatus that has been described as the Philodina type (Donner, 1965; Kutikova, 2005). Principal taxonomic characters of Bdelloidea have been described in many publications (Donner, 1965 and references therein; Ricci & Melone, 2000; Fontaneto & Ricci, 2004; Kutikova, 2005) and thorough geometric morphometric analysis was conducted on mastax of several bdelloid genera (Fontaneto *et al*., 2004, 2007*b*; Fontaneto & Melone, 2005; Melone & Fontaneto, 2005), but little has been done to standardize the external dimensions. However, progress has been made for Adinetidae by Segers & Shiel (2005). Measurement points were chosen so that they would be associated with the dimensions both in the classical keys on Bdelloidea (Bartoš, 1959; Donner, 1965) and recent publications (Segers & Shiel, 2005; Birky *et al*., 2011). The scheme of measuring of habrotrochid/philodinid rotifers is shown in Figs 2–3.

(1) *Total length* (TL) is the distance between two points on the longitudinal axis of symmetry of a fully stretched creeping bdelloid, one in the middle of anterior rim of the rostrum (P1) and the other on posterior rim of the spur segment (P2). The dimension includes rostrum, but does not include rostrum lamella and toes, since these organs are not always protruded and rarely fully visible on screenshots. *Greatest trunk width* (BW) is the distance between points P3 and P4 marking the widest trunk cross-section.
(2) *Head length* (HL) is the distance between two points on the longitudinal axis of symmetry of a head of a feeding rotifer, one at its intersection with the plane of the trochal discs (P5) and the other one on the anterior rim of the antennal pseudosegment (P6). *Collar width* (HW) is the distance between P7 and P8 marking lateral rims of the cingulum bolster. *Corona width* (CW) is its widest cross-section through the trochal discs (P9–P10).
(3) *Neck length* (NL) and its *smallest* (MNW) and *greatest* (MxNW) *width* may be useful measurements characterizing body shape of a bdelloid. NL is the distance between two points on the



Figs 2–3. Measurements of a feeding and a creeping bdelloid of Habrotrochidae or Philodinidae family. **2**, Head, dorsal view; **3**, Habitus, dorsal view. Abbreviations are explained in the section Materials and methods.

Downloaded by [Univerzitni Knihovna Ostravske] at 08:07 05 December 2013

longitudinal axis of symmetry of a fully stretched creeping rotifer, one marking anterior rim of the antennal pseudosegment (P11) and the other the anterior rim of the 1st trunk pseudosegment (P12). MNW is the distance between P13 and P14 designating the narrowest cross-section of the neck, and MxNW, respectively, stands for its widest cross-section (P15–P16). *Length of dorsal antenna* that is taking along the longitudinal axis of the organ has not been measured in this study.

(4) *Trunk length* as it is (see the definition of the trunk that is explained in the Terminology section) was not measured in this study. The measurement that is mentioned as trunk length in Segers & Shiel (2005) and Birky *et al*. (2011) is in fact the sum of trunk length and rump length. In this paper it is referred as *trunk and rump length* (TkL) and has been measured, to make our data comparable with the already published, as the distance between anterior rim of the 1st trunk pseudosegment (P12) and the cloacal opening (P18).

(5) *Rump length* (RL) is the distance between two points on the longitudinal axis of symmetry of a fully stretched, creeping bdelloid. This measurement is from the anterior rim of the preanal pseudosegment (P17) to the cloacal opening (P18).*Largest rump width* (RW) is the distance between P19 and P20 thus marking the widest cross-section of the rump.

(6) *Foot length* (FL) is the distance between two points on the longitudinal axis of symmetry of a fully stretched creeping rotifer, thus marking the cloacal opening (P18) and the posterior rim of the spur-bearing pseudosegment (P2). *Foot maximal width* (FW) is the distance between P21 and P22, thus marking the widest cross-section of the foot. *Spur-bearing pseudosegment width* (SSW) is the widest cross-section of it, or the distance between P23 and P24.

(7) *Spur length* (SL) will be difficult to measure when a spur is not symmetrical. Here we take SL as the distance between the middle of the spur in the area where it is attached to the foot (P25), and the spur tip (P26). *Distance between spur tips* (SP) is shown in the Fig. 3.

We also calculated the ratios of those parameters that are most commonly cited in the literature and ultimately proved to be useful in describing the body shape (see species diagnoses and Morphometric analysis section of the Results). Measurement ratios (body proportions) are more demonstrative than absolute dimensions; they are widely used in the keys for Bdelloidea as additional taxonomic features (Bartoš, 1959; Kutikova, 2005).

The trophi were first measured on digital images taken under the light microscope (Table 1) and as a consequence these numbers are approximate. Later the trophi were more precisely measured on SEM photos (Fig. 25, Table 2). The following dimensions were taken:

(1) *Ramus length* (RaL) is the distance between P27 and P28 on the cephalic view of a trophi, or between P29 and P30 on the caudal view.

(2) *Trophi width* (TrW) is the distance between P31and P32 on the cephalic and P33–P43 on the caudal view, marking a tip of the first major tooth and the point at which its longitudinal axis of symmetry crosses the external side of manubrium.

We used digital photos and video recordings for the preliminary observation of external morphology of the rotifers. The images were made using light microscopes Nikon E300, Nikon E600, Reichert/AO Diastar (PlanApo objective + differential interference contrast) under the total magnification ×300–600 with the digital cameras Pentax Optio M50 and Canon EOS 600d equipped with

Table 1. Body dimensions of *Otostephanos jolantae* sp. nov.

		Ukraine		Poland (Karkonosze/Wigry)			Czech Republic			Austria
Measurements	Holotype	min–max	mean ± SD	min–max	median	mean ± SD	min–max	median	mean ± SD	min–max
N[a]	1	5			10/15			20		3
TL[b]	330[c]	285–300	296 ± 6	305–366/ 301–381	330/333	331 ± 17/ 335 ± 23	296–395	326	330 ± 22	297–375
BW	61	–	–	50–66/47–69	60/57	59 ± 5/58 ± 7	46–72	63	62 ± 6	50–72
HL	39	37–39	38 ± 0.8	36–43/36–41	39/37	39 ± 2/38 ± 2	36–42	38	39 ± 2	35–37.5
HW	39	37–39	38 ± 0.9	37–44/39–42	40/41	40 ± 2/41 ± 1	37–43	39	40 ± 2	36–38
CW	37	36–37	36 ± 0.9	36–42/37–41	38/39	38 ± 2/39 ± 1	35–41	39	38 ± 2	35
NL	70	–	–	62–71/49–78	69/62	68 ± 3/64 ± 7	54–107	70	76 ± 17	54–67
MNW	34	–	–	30–37/30–35	34/33	33 ± 2/32 ± 2	30–36	34	34 ± 2	30–39
MXNW	40	–	–	40–45/34–46	41/41	42 ± 2/40 ± 4	36–54	45	45 ± 5	42–54
TkL	197	–	–	182–232/ 191–261	197/222	201 ± 18/ 231 ± 29	166–197	187	182 ± 12	199–231
RL	42	–	–	41–48/35–51	42/47	43 ± 2/46 ± 4	35–51	43	43 ± 4	43–57
RW	41	–	–	36–42/35–48	39/45	39 ± 2/43 ± 4	35–48	42	42 ± 3	40–58
FL	24	–	–	24–28/24–28	27/26	26 ± 1/26 ± 1	24–36	28	29 ± 5	25–39
FW	17	–	–	15–17/13–17	16/15	16 ± 0.7/ 15 ± 1	14–17	16	16 ± 0.5	16–28
SP	18	17	–	17–18/17–22	18/17	18 ± 0.4/ 18 ± 1	17–25	20	20 ± 1	14–20
SL	8	9	–	8–9/8–10	8/9	8 ± 0.4/ 9 ± 0.6	8–11	9	9 ± 1	10–11
SSW	13	10	–	10–13/10–13	12/12	12 ± 0.8/ 12 ± 0.9	8–14	12	12 ± 1	11–14
RaL[d]	21.6	22.0		– / 21.0–22.0	– / 21.2	– /21.4 ± 0.5	20.3–22.3	21.1	21.2 ± 0.7	20.1–22.8

[a]Number of measured individuals. [b]TL – total length, BW – greatest trunk width, HL – head length, HW – collar width, CW – corona width, NL – neck length, MNW – smallest neck width, MxNW – greatest neck width, TkL – trunk and rump width, RL – rump length, RW – largest rump width, FL – foot length, FW – foot width, SP – distance between spur tips, SL – spur length, SSW – spur–bearing pseudosegment width, RaL – ramus length. [c]All dimensions are given in μm. [d]Measured on photographs made under the light microscope.

the custom-built 'Stahlschmidt' photoflash. To detect minor morphological details, the rotifers were carefully examined and measured on screenshots produced from digital videos using Light Alloy 4.1 software. Adobe Photoshop CS3 Extended was used for making drawings and measurements, and for editing of the photographs.

Instrumental error was calculated in two ways. Firstly, three screenshots and six SEM images of rotifers representing three localities were measured on habitus dorsal view and, when possible, on cephalic and caudal view of trophi. Measurements were taken 10 times in a different way over 10 days, one measurement set per day separated

Table 2. Trophi dimensions of *Otostephanos jolantae* sp. nov. measured on SEM photos.

Measurements	Paratypes[a]	Poland, Karkonosze	Poland, Wigry	Czech Republic (typical specimens)	Czech Republic (CZ38OJ11)
N[b]	2	8	2	17	1
MJT (L)[c]	6/6[d]	6	6	6	6
MJT (R)	6/6	6	6	6	6/7
MNT (L)	29/29	26–29/28/28±1	28	23–31/26/26±3	26
MNT (R)	30/29	28–30/28/28±1	28–29	22–29/27/27±4	26
RaL (L, CE)	20.18/–	19.03–21.06/20.38/20.05±0.86	21.1–21.94	18.54–20.92/19.9/19.87±0.77	–
RaL (L, CA)	–/20.58[e]	20–58–20.9	–	19.64–21.59/20.71/20.58±0.58	21.4
RaL (R, CE)	20.19/–	19.1–21.16/20.13/20.13±0.82	21.2–21.84	18.32–21.01/19.91/19.87±0.87	–
RaL (R, CA)	–/20.26	20.26–20.89	–	19.6–21.78/20.45/20.54±0.61	21.22
TrW (L, CE)	9.94/–	9.94–11.89/11.05/11.0±0.6	11.07–11.58	10.5–11.64/11.16/11.13±0.42	11.28
TrW (L, CA)	–/10.1	10.09–10.95	–	10.65–11.85/11.23/11.24±0.33	–
TrW (R, CE)	10.33/–	10.33–11.9/10.96/11.07±0.68	11.08–11.5	10.47–11.25/11.11/11.0±0.29	11.0
TrW (R, CA)	–/10.26	10.25–11.07	–	10.6–11.64/11.2/11.16±0.36	–

[a]SIZ 49.4, SIZ 49.5. [b]Number of measured trophis. [c]MJT – number of major teeth in uncus, MNT – number of minor teeth, respectively; RaL – ramus length, TrW – trophi width, L – left uncus, R – right uncus, CE – cephalic view, CA – caudal view. [d]Number of teeth in uncus. [e]Here and further all dimensions are given in μm.

Downloaded by [Univerzitni Knihovna Ostravske] at 08:07 05 December 2013

Downloaded by [Univerzitni Knihovna Ostravske] at 08:07 05 December 2013

by one day between successful takes, as described in Fontaneto *et al*. (2004), with modifications corresponding to the aim of the current research. Screenshots from the light microscope gave instrumental error that consisted 0.6–4.74% of the value depending on the dimension. For the SEM photographs instrumental error was 0.01–2.86% of the value. Secondly, for 10 individuals of the same population (Czech Republic) five best light microscope video screenshots showing habitus were chosen and measured to evaluate the error produced by variable level of stretching of a soft-bodied animal on sequentially taken screenshots. For several measurements (BW, NL, RL and RW) the error was high – up to 10% of the value; however, for TL, CW, HL, HW, FL and FW it did not exceed 3%.

Scanning electronic microscopy

Fine structure of the masticatory apparatus was examined by SEM. Trophi were extracted from animals of the clonal cultures (at least one trophi per culture, representing one original individual) and mounted on glass slides according to the protocol of De Smet (1998). Soft tissues were dissolved with Savo® Perex commercial bleach under the dissecting microscope OLYMPUS SZX7, after that the trophi were washed with deionized water, mounted on circular cover slips attached to 10 mm aluminium tables, dried on air and coated with gold. The photos were made using SEM JEOL 6380 LV electron microscope of the Charles University in Prague, Czech Republic.

We analysed both cephalic and caudal view of the trophi and calculated the number of major (MJT) and minor teeth (MNT) in each uncus. MJT may be species-specific in bdelloids (Donner, 1965) while the total number of teeth is positively related to the trophi size (Melone & Fontaneto, 2005); the trophi size, in turn, significantly differs both between traditional species and (at least in some cases) between cryptospecies (Fontaneto *et al.*, 2007*b*, 2007*c*). Trophi were measured as described above in the Light microscopy section. Statistical analysis of all measurements was performed in R Studio 0.97.316 software according to Crawley (2005).

DNA sequencing and alignment

Target locus was successfully amplified only from rotifers collected in Czech Republic; the DNA extraction/amplification failed in those collected in Poland. Selected animals from clonal cultures were vouchered, i.e. digitally photographed and videotaped alive and active (see the section Light microscopy of the Materials and methods) before starting the extraction that causes total destruction of a rotifer; then the animals were thoroughly washed in deionized water and transferred into 25 μl plastic tubes, single rotifer per tube. DNA extraction was conducted using Biorad Chelex® 100 Molecular Biology Grade resin according to the protocol of Fontaneto *et al.* (2007*b*) with minor changes −56 °C for 20 min and 100 °C for 10 min (D. Fontaneto, pers. comm.).

Partial mitochondrial cytochrome c oxidase subunit I gene (mtCOX1) was amplified following the procedures described in Kaya *et al.* (2009), with the minor modification of the cycler protocol. The 657-bp fragment of mtCOX1 was amplified using universal primers LCO1490 and HCO2198 (Folmer *et al.*, 1994). PCR was conducted with the initial denaturation at 94 °C for 5 min, followed by 40 cycles of denaturation at 94 °C for 40 s, annealing at 50 °C for 1 min, and elongation under 72 °C for 40 s, and a final extension at 72 °C for 7 min (D. Fontaneto, pers. comm.).

Fragments were sequenced and five successful mtCOX1 sequences that we obtained were submitted to GenBank under accession numbers KC520782 (3 identical ones) and KC520783 (2 identical ones, see Appendix II, see supplemental material online). Sequences were visually checked using SeqMan II 5.05 and aligned in BioEdit 7.1.9 using implemented ClustalW.

To ascertain the species status of the described species we applied the method 4× rule by Birky *et al.* (2005) and Birky & Barraclough (2009) following the procedure described in Birky *et al.* (2011). The method implies identification of the putative species as mtDNA sequence clusters followed by estimation of the ratio of nucleotide diversity between such clusters (represented as mean pairwise corrected sequence distances, K) to the parameter θ (calculated as $\theta = \pi/(1-4\pi/3)$, where π is sequence difference within the clade). We used the unique published sequence from other *Otostephanos* species, *O. donneri* along with sequences of 14 other bdelloid species from different genera (Appendix II, see supplemental material online) to estimate the sequence difference within and between species. K and π were calculated using MEGA 5.10 (Tamura *et al*., 2011).

Results

Alpha taxonomy

Type Rotifera Cuvier, 1817
Class Eurotatoria De Ridder, 1957
Subclass Bdelloidea Hudson, 1884
Order Philodinida Melone & Ricci, 1995
Family Habrotrochidae Bryce, 1910
***Otostephanos* Milne, 1916**

Milne, 1916: 173; Voigt, 1956: 36; Donner, 1965: 96; Kutikova, 2005: 108.

The distinctive feature of the genus is paired transversal rings in the middle of each pedicel of the corona. The rings are continuous on the dorsal side and interrupted on the ventral side.

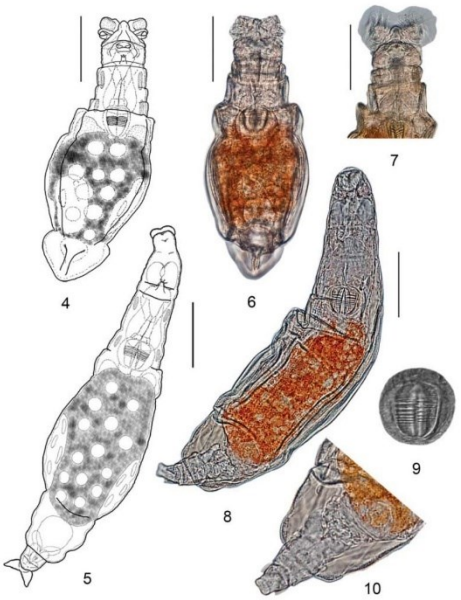
Downloaded by [Univerzitni Knihovna Ostravske] at 08:07 05 December 2013

No eyespots. Integument is thin, smooth or sculptured, occasionally with protrusions, but never with transversal ventral ribs. Corona is usually equal to the collar in its width, may also be somewhat narrower or wider. Upper lip never forms an envelope-like structure enclosing pedicels and trochal discs, it is whole or divided into lobes. Foot short, of 3 or 4 pseudosegments. Three toes. Oviparous. Trophi usually has numerous thin major teeth in unci, but may also have 2 or more thick ones. Gut with thin walls and wide lumen filled with rounded food pellets – a typical feature of the Habrotrochidae. In fresh waters or terrestrial habitats; marine species, commensals or parasites are not known.

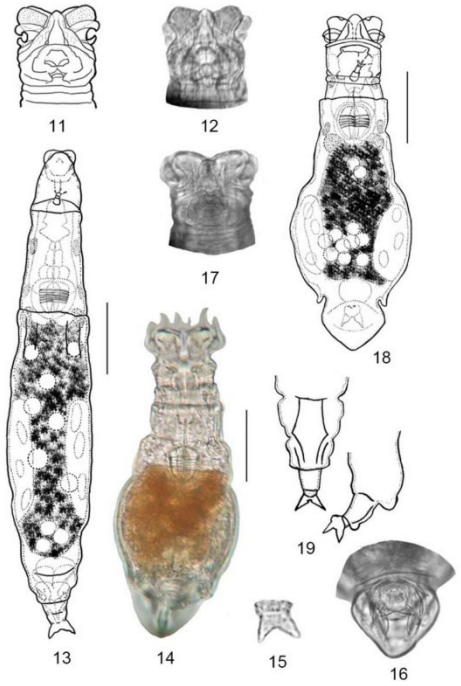
Type species: *Otostephanos torquatus* (Bryce, 1913)

Otostephanos jolantae sp. nov. (Figs 4–25)

HOLOTYPE: SIZ 49.1 (CZ1138OJ9b, 31.10.2011)
PARATYPES: SIZ 49.2 (CZ1138OJ9c, 31.10.2011), SIZ 49.3 (CZ113809d, 31.10.2011); SIZ 49.4 (CZ1138OJ9e, 31.10.2011), SIZ 49.5 (CZ1138OJ6c, 31.10.2011).



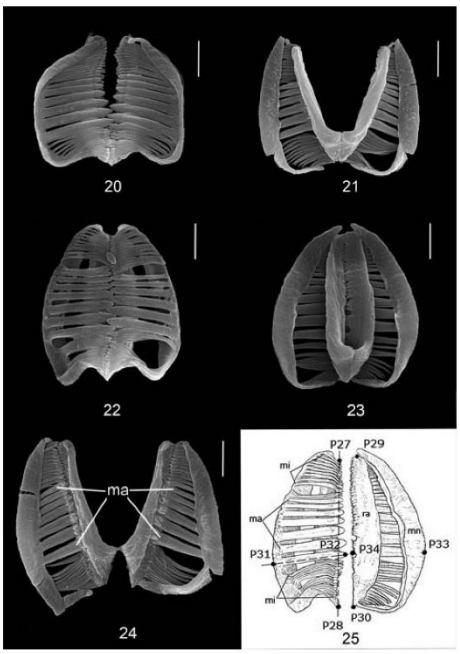
Figs 4–10. *Otostephanos jolantae* sp. nov. Holotype: **4**, habitus, dorsal view, feeding; Paratype SIZ 49.2: **5**, habitus, dorsal view, creeping. Specimen from Austria (AT11OJ1): **6**, habitus, ventral view, feeding; **7**, head, dorsal, feeding; **8**, habitus, creeping; **9**, trophi, enlarged; **10**, rump and foot, enlarged. Scale bars represent 50 μm.



Figs 11–19. *Otostephanos jolantae* sp. nov. Specimen from Poland, Karkonosze (PL0883aOJ1): **11–12**, head, dorsal view, feeding; **13**, habitus, dorsal view, creeping; **14**, habitus, ventral view, feeding; **15**, spurs; **16**, rump and foot. Specimen from Poland, Wigry (PL0919OJ1): **17**, head, dorsal view, feeding. Specimen from Ukraine (Vl003): **18**, habitus, dorsal view, feeding; **19**, rump and foot. Scale bars represent 50 μm.

TYPE LOCALITY: peat bog near v. Borkovice, 418 m asl, South-Bohemian Region, Czech Republic, N49°12.477′ E14°38.606′. ADDITIONAL MATERIAL: SIZ 46.1 (PL0883aOJ1, 22.07.2008), SIZ 46.2 (PL0883aOJ2, 22.07.2008), SIZ 46.3 (PL0883aOJ3, 22.07.2008), SIZ 46.4 (PL0883aOJ4, 22.07.2008), SIZ –OJ12, 10 trophi; SIZ 47.1 (PL0919OJ1, 23.07.2009), SIZ 47.2 (PL0919OJ2, 23.07.2009), SIZ 47.3 (PL0919OJ3, 23.07.2009), SIZ 47.4 (PL0919OJ4, 23.07.2009); SIZ 48.1 (PL0925OJ1, 23.07.2009); SIZ 48.2 (PL0925OJ3, 23.07.2009), SIZ 50.1–50.10 (CZ1137OJ1b–CZ1137OJ10b, 31.10.2011), SIZ 49.6–49.9 (CZ1138OJ1c-OJ4c, 31.10.2011), SIZ 49.10–49.15 (OJ1138OJ11, 31.10.2011); voucher images Vl003, CZ1137OJ1a, CZ1138OJ3a, CZ1138OJ5a, CZ1138OJ7a, CZ1138OJ9a; partial mtCOX1 sequences deposited under GenBank accession numbers KC520782, KC520783 (Appendix II, see supplemental material online).

Downloaded by [Univerzitni Knihovna Ostravske] at 08:07 05 December 2013



Figs 20–25. *Otostephanos jolantae* sp. nov. Paratypes SIZ 49.4 and SIZ 49.5, trophi: **20**, cephalic view; **21**, caudal view. Specimens from Poland, Karkonosze, trophi: **22**, PL0883aOJ3, cephalic view; **23**, PL0883a, caudal view. Specimen from Czech Republic with 6/6 + 1 major teeth (CZ1138OJ11): **24**, caudal view, ma – major teeth. Schematic image showing the points of measurement (explained in the text): **25**, ma – major teeth, mi – minor teeth, ra – ramus, mn – manubrium. Scale bars represent 5 μm.

HABITAT AND DISTRIBUTION: *Sphagnum* spp., peat bogs, dystrophic lakes, wet moss in streams and forests; temperature 15–21 °C, humidity 73–100% and pH 4.5–7.5 (mean ± SD 5.8 ± 0.9); Ukraine: Volyn r.; Poland: Kuyavian-Pomeranian, Lesser Poland, Lower Silesian, Podlaskie, and Warmian-Mazurian vv.; Czech Republic: type locality; Austria: Tyrol.

ETYMOLOGY: named after the Polish ecologist and rotifer researcher Prof., Dr. Hab. Jolanta Ejsmont-Karabin, manager of the project 'Taxonomy and ecology of bdelloid rotifers (Rotifera, Bdelloidea) in the territory of Poland' in the course of which the species was found and described.

DIAGNOSIS: *O. jolantae* sp. nov. has a large body with the smooth or gently dotted cuticle and bright-red or red-orange gut colouration, spade-like protrusion on rump and 6/6 major teeth in trophi. The gut colour is permanent, not disappearing even in offspring raised in culture and always being present in rotifers sampled in natural environment. The other large and brightly coloured species, *O. regalis*, has lemon-coloured stippled integument on trunk, rump and foot, while the integument of *O. jolantae* n. sp. is smooth or gently dotted, and colourless. The corona of *O. jolantae* is large, of the same width than the head base, but its narrow sulcus is not covered by membrane even in the lower part (differently from *O. torquatus* that may also have 6/6 teeth). The upper lip of *O. jolantae* is wide triangular, high and going over the trochal disc plane, its rounded tip is often elongated as a tongue-like lobe (Figs 4, 11, 12, 17, 18), but is whole and not divided into three or two parts like in *O. auriculatus* s. str. or *O. auriculatus bilobatus*. The peculiar feature of *O. jolantae* n. sp. is rump shape: the first rump segment is heavily swollen and in its lower part has a large angle-shaped dorsal protrusion, so that the rear part of the feeding animal attached to substrate looks like a spade (Figs 4, 6, 14, 16, 18). The first foot segment of *O. jolantae* n. sp. has a longitudinal dorsal knob (Fig. 19) while in *O. auriculatus* it is absent.

Description

BODY: large, terete.

INTEGUMENT: smooth or very gently dotted, thin, colourless. No hard or soft case covering the body, neither organic no mineral.

APPENDAGES: no knobs, humps or spines except a hump on the foot.

EYESPOTS: absent.

ROSTRUM: thick, short, lamella divided into two semicircular lobes.

CORONA: slightly narrower than the cingulum (CW 92–99% of HW). Feeding head length is variable (HW 87–116% of HL). Head base rectangular, its width/height is 151–185%. Pedicels rather short and thick, with two massive lateral pairs of rings. Trochi slightly bent inside, depressed in the middle, without papillae or sensitive bristles, their retractors are not prominent. Sulcus narrow, not covered with a membrane, without a knob or ligula. Upper lip wide triangular with the tongue-like rounded tip going over the plane of trochi, the tip is single and not divided into lobes. Cingulum bolster (collar) massive. Lower lip not prominent.

NECK: moderately long (NL about 18–28% of TL), stout. Dorsal antenna thick, its length is about 1/3 of the antennal segment width. Trunk cylindrical, massive, BW 16–22% of TL.

TRUNK: massive, swollen, separated from neck and rump.

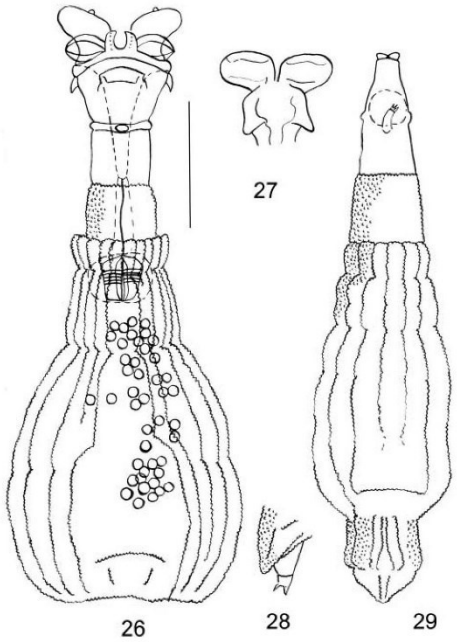
RUMP: of moderate length (RL 12–16% of TL), heavily swollen, its first segment has a spade-like or angular dorsal protrusion directed backwards.

Downloaded by [Univerzitni Knihovna Ostravske] at 08:07 05 December 2013

FOOT: short, slim, of 4 segments, FL 7–12% of TL, with longitudinal knob on the first pseudosegment.
SPURS: of moderate length (SL/SSW 61.5–94%, SP/SSW 141–217%), flat, triangular with inner ridges cut out bracket-like, divergent, pointed, with the short interspace.
TOES: Three, short.
INNER ORGANS: throat voluminous, oesophagus of moderate length and without loop, gut bright-red or red-orange, food pellets large, round, colourless; 2 pairs of protonephridia in the neck; 6 nuclei in each germovitellarium.
TROPHI: ramate, large, heart-shaped, 19–22 μm long and 10–12 μm wide; rami massive, with the indentation (scleropilia) on the interior margin of each ramus, articulated in the ventral part; the region of articulation is a short protrusion of the interior side of the rami, it is straight, continuous and has no incisures; manubria crescent-shaped, wide, flat; major teeth thick, with the rounded tip, dental formula (in populations from Poland and Czech Republic) is 6/6 major teeth; 23–31 minor teeth in the left uncus and 22–30 in the right one.
MEASUREMENTS: Tables 1 and 2.
REMARKS: In the sample from Czech Republic (CZ1138), along with the typical form having 6/6 major teeth, we found a specimen morphologically identical to the described *O. jolantae*, with the size parameters within the general range (TL 318 μm, BW60 μm, CW 37 μm, HW 38 μm, NL 67 μm, RL 37 μm, FL 36 μm, SL 7.5 μm, SSW 11 μm, RaL 21.4/21.2 μm, RaW 11.0/11.3 μm), but with 6/6+1 major teeth (L/R) in unci (Table 2). The additional tooth in the right uncus was thinner than the rest. Individual was photographed and processed for SEM of trophi (Fig. 24), but no culture and DNA extraction was made. Four individuals found in the sphagnum sample collected in Austria (AT11OJ1, Figs 6–10, Table 1) were morphologically identical to the new species with the exception that they had evidently dotted cuticle and at least one of the specimens had a foot ending with an adhesive plate instead of three toes.

Otostephanos ukrainicus sp. nov. (Figs 26–29)

HOLOTYPE: SIZ 51.1 (UKv9967L, 21.09.1999).
PARATYPES: SIZ 51.2, 51.3 (UKv004L, 15.04.2000).
TYPE LOCALITY: Pushcha-Voditsa forest near Kyïv, 171 m a.s.l., Kyïv r., Ukraine; N 50°31.078′, E 30°25.006′.
HABITAT AND DISTRIBUTION: pine and oak litter in the type locality, temperature 8–15 °C, humidity of the litter 51–53%, pH 5.6–5.7.
ETYMOLOGY: species is named for the country in which it is found.
DIAGNOSIS: *O. ukrainicus* sp. nov. has large yellowish body with the coarse-grained cuticle on 2 last neck segments, trunk and rump, and by this it differs from the most of the congeners. The new species does not have integument of a bright lemon colour, as is reported for *O. regalis*, the congeneric species with granulated cuticle; instead, it has band-like upper lip with two sharp lobes while *O. regalis* has semicircular upper lip with a single lobe and a ligula on the tip; the foot, spurs, trophi and 'food pellets' of the new species seem to be smaller than in *O. regalis*. *O. ukrainicus* sp. nov. has 5/5 major teeth in trophi that was not observed so far in any known *Otostephanos*.



Figs 26–29. *Otostephanos ukrainicus* sp. nov. Holotype: **26**, habitus, feeding, dorsal view; **27**, head, feeding, ventral view; **28**, habitus, creeping, dorsal view; **29**, rump and foot, creeping, lateral view. Scale bar represents 50 μm.

Description

BODY: yellowish, long, with small head, narrow neck, large saccular trunk, pentagonal rump and tiny foot.
INTEGUMENT: covered with coarse granules on the last 2 neck segments, whole trunk and rump; the rest of the body is smooth.
APPENDAGES: without any knobs, humps or spines.
EYESPOTS: absent.

Downloaded by [Univerzitni Knihovna Ostravske] at 08:07 05 December 2013

ROSTRUM: very short and narrow, lamella with 2 small rounded lobes.
HEAD: rather large while feeding (HL is 11–12% of TL, HW is 90–92% of HL), head base trapezoid. Corona distinctively wider than the collar (CW is 113–116% of HW). Pedicels of moderate length, with massive paired rings in the middle, separated from each other by deep sulcus not covered with membrane; retractors unnoticeable; trochi bent toward each other, each with large rounded papilla in the middle, but sensitive bristles are not visible. Upper lip narrow, band-like, with two horn-like pointed protrusions curved towards each other and separated by an interspace as wide as the width of a protrusion. Cingulum bolster not prominent. Lower lip with two large elongated horn-like corners, well-visible even from the dorsal side.
NECK: long (NL is 20–24% of TL), narrow. Antenna small, as long as 1/2 of the bearing pseudosegment width.
TRUNK: massive, wide, pear-like while feeding.
RUMP: of moderate size (RL is 15% of TL, RW is 13–14% of RL), of unusual shape: its first segment is cylindrical and the second one has blunt triangular protrusion pointing backwards; the protrusion covers the foot completely.
FOOT: very short and narrow (FL 9% of TL, FW 56–57% of FL), of three pseudosegments, visible only from lateral view of a creeping animal.
SPURS: tiny sharp cones separated by interspace, SL is 32–33% of SSW.
TOES: three, tiny and very difficult to observe.
INNER ORGANS: throat large, long; oesophagus long, but without a loop; food pellets very small, round.
TROPHI: of moderate length (18.0–18.5 μm), uncus width 7.9 μm, with 5/5 thick and well-visible major teeth.
MEASUREMENTS: (holotype/paratypes): TL 410/425–442 μm, BW 106/101–127 μm, HL 48/47–49 μm, CW 51/50 μm, HW 44/44 μm, NL 86/85–100 μm, MNW 34/34–35 μm, MXNW 63/64–70 μm, TkL 237/238–245 μm, RL 62/62–67 μm, RW 56/58–60 μm, FL 37/37–38 μm, FW 21/21 μm, SL 6/6 μm, SSW 19/18 μm, TrL (light microscopy) 18.0/18.1–18.4 μm.

Morphometric analysis

In this section, we consider only *O. jolantae*, since the data on *O. ukrainicus* were limited to only 3 individuals.

We evaluated the variation produced by instrumental error (1) measured on the same screenshot and (2) measured between screenshots of the same animal, using linear mixed effects model. The results showed that (1) was negligible in comparison with variation in size between individuals for both video screenshots and SEM images (less than 0.01% of the total variation), and that (2) was higher (up to 16.8% of total variation for the most variable dimensions, see Materials and methods), but still much smaller than the variation of the size between individuals.

We found no striking difference neither in size of individuals (at least the mature ones), nor in average values of the body measurements for each location (Table 1). The exception was Ukrainian population, where rotifers were in average smaller (total length 285–300 μm), though all animals but one were still within general size range (295–395 μm); however, this could not be verified statistically for the sample of 5 specimens. No statistically significant differences in size were found for the most external measurements, neither between populations (F_{sample}) nor between individuals ($F_{individual}$), namely (see Appendix III, see supplemental material online; two-way ANOVA F-test): from $F_{sample} = 0.440$, $P = 0.667$ and $F_{individual} = 0.827$, $P = 0.653$ for head length to $F_{sample} = 7.785$, $P = 0.029$ and $F_{individual} = 2.760$, $P = 0.131$ for foot width. However, neck length and distance between spurs were significantly different within and between populations (NL: $F_{sample} = 34.160$, $P = 0.0002$ and $F_{individual} = 12.10$, $P = 0.001$; SP: $F_{sample} = 24.467$, $P = 0.0002$ and $F_{individual} = 5.301$, $P = 0.006$). Rump and spur length differed significantly between populations (RL: $F_{sample} = 11.488$, $P = 0.003$; SL: $F_{sample} = 10.685$, $P = 0.006$).

Some minor variability between individuals (but not between populations) in the shape of body parts was observed (Figs 4, 12, 17, 18): the upper lip may be elongated or rounded; the rump protrusion may be more or less prominent.

The measurement data showed slight asymmetry of the trophi of *O. jolantae*: right half appeared to be longer (ramus) and wider (uncus) regardless of the position of the first major tooth (closer or farther to the rami articulation); it also had higher number of minor teeth (Table 2). However, these differences were not statistically significant (paired *t*-test, length: $t = 0.236$, $P = 0.816$; width: $t = 0.57$, $P = 0.574$). Ramus length did not significantly increase with increasing of the number of minor teeth (GLM with Poisson errors: $z = 0.004$, $P > 0.997$). Neither length nor width of the trophi had significant difference within and between populations (see Appendix III, see supplemental material online; two-way ANOVA F-test): for the left ramus length $F_{sample} = 0.064$, $P = 0.939$ and $F_{individual} = 0.665$, $P = 0.767$; for the right ramus length $F_{sample} = 0.020$, $P = 0.980$ and $F_{individual} = 0.467$, $P = 0.907$; for the left ramus width $F_{sample} = 0.952$, $P = 0.442$ and $F_{individual} = 0.497$, $P = 0.889$; for the right ramus width $F_{sample} = 0.394$, $P = 0.685$ and $F_{individual} = 0.647$, $P = 0.781$.

Measurement ratios (body proportions) were estimated as linear functions of body (or particular organs) width or length (Fig. 30) and compared between populations using ANCOVA (Appendix IV, see supplemental material online). Czech population had statistically significant differences from two Polish ones in HL/TL, MNW/BW, RL/

Downloaded by [Univerzitni Knihovna Ostravske] at 08:07 05 December 2013

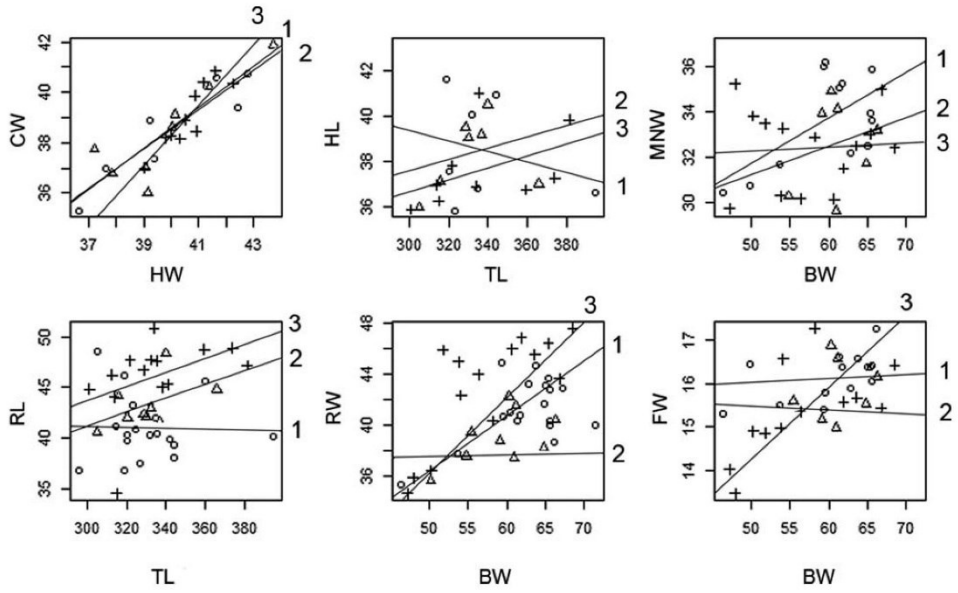


Fig. 30. Dimensions ratios plotted as linear functions of the correspondent body measurement (in μm) to the total body length or width. Abbreviations are explained in the section Materials and methods. '1' and circles indicate measurements for rotifers collected in Czech Republic, '2' and triangles indicate individuals from Polish Karkonosze, and '3' and plus signs indicate individuals from Polish Wigry, respectively.

TL, RW/BW, FW/BW and SP/SSW (ANCOVA t-test for the difference between intercepts: $t_{HL/TL} = 4.588$, $P = 0.0003$; $t_{MNW/BW} = 4.166$, $P = 0.0003$; $t_{RL/TL} = 3.602$, $P = 0.001$; $t_{RW/BW} = 4.132$, $P = 0.0002$; $t_{FW/BW} = 4.132$, $P = 0.0002$; $t_{SP/SSW} = 10.191$, $P < 0.0001$). Population from Polish Wigry was significantly different from the rest by MXNW/BW and SP/SSW (ANCOVA t-test for the difference between intercepts: $t_{MXNW/BW} = 2.918$, $P = 0.006$; $t_{SP/SSW} = 2.918$, $P = 0.006$). However, as is seen from the table in Appendix IV (see supplemental material online) and Fig. 30, the analysis showed that the degree of scatter was high and correlation between covariates, accordingly, was weak for all parameters except CW/HW (coefficient of determination $r^2 = 0.751$–0.851 for CW/HW, $r^2 < 0.550$ for the rest of the ratios).

DNA taxonomy

The 4× rule proposed by Birky and coauthors (Birky *et al.*, 2005; Birky & Barraclough, 2009) implies calculation of K/θ ratio (see Materials and methods section) to separate independently evolving groups of individuals (i.e. evolutionary species) based on the theoretical stipulation that K/$\theta < 4$ within a group and K/$\theta \geq 4$ between such groups. This method can be used to distinguish between cryptic species (Birky *et al.*, 2011), and to verify integrity of a morphospecies (Marrone *et al.*, 2010).

We obtained and sequenced PCR products from the clonal cultures representing five original specimens – CZ1137OJ1, CZ1138OJ3, CZ1138OJ9, CZ1138OJ5 and CZ1138OJ7. The original specimens were not produced by the same female, but were descendants of different females from one large population that might emerge either from a single founder or several founders brought to the sampled peatbog from different localities. The mean pairwise corrected sequence differences within the species (π) for *O. jolantae* were 0.000 (identical) to 0.0054, and calculated θ were more than one order of magnitude lower than K estimated between *O. jolantae* and the other species (0.1309–0.6225) including the congeneric *O. donneri* (0.1779). We consider these results a confirmation of genetic integrity of the population we studied, as well as genetic integrity of the new species as a whole and its separation from the other bdelloid species.

Discussion

To our knowledge, no records of any bdelloid rotifer that is identical to *O. jolantae* and *O. ukrainicus* are cited in

Downloaded by [Univerzitni Knihovna Ostravske] at 08:07 05 December 2013

the literature, neither among described species nor among their varieties or unidentified individuals.

Otostephanos jolantae is one of the three species of *Otostephanos* with the dental formula 6/6 (Table 3). In most specimens 6 strong major teeth are present in each uncus, although we found a single specimen with 6/6+1 teeth. Unlike *O. torquatus* and the rest of congeneric species, *O. jolantae* seems to have this number stable, never reaching 7/7 or more. The major teeth of *O. torquatus* are thinner and the trophi is smaller, being 15–18 μm long (Bryce, 1913; Schulte, 1954) versus 19–22 μm in *O. jolantae*. *O. regalis* Milne, 1916, another species of the genus with a similar dental formula, has the major teeth differentiated by thickness: the first three are considerably stronger than the rest (3 + 3/3 + 3). Moreover, *O. regalis* seems to have larger trophi (22–22.5 μm long), and its upper lip is semicircular and does not reach the trochal disc plane (Milne, 1916). Hitherto *O. jolantae* has been found only on *Sphagnum* or other aquatic moss, while *O. torquatus* and *O. regalis* are reported only from terrestrial mosses and soil (Milne, 1916; Hauer, 1939; Schulte, 1954; Kutikova, 2005; Iakovenko & Ovander, 2011). *Otostephanos auriculatus*, which is similar to *O. jolantae* by the corona shape and also occurs in ponds and swamps (Harring, 1914) has the tip of upper lip divided into 2–3 lobes, its gut is never of red or bright-orange colour and its dental formula is 10/10 thinner teeth.

Thus far only two *Otostephanos* species, apart from *O. ukrainicus*, were known to have granulated cuticle, *O. donneri* and *O. regalis*. However, they can easily be delimitated from each other by the character of sculpturation, dental formula, foot and its appendages, and spur length and shape (Table 3). *Otostephanos donneri* has 7/7 up to 12/12 thin major teeth, short foot with a transversal knob on the first segment, and long needle-shaped spurs with bulbous bases; the granulation on the trunk and rump seems to be very fine, if present at all (Bartoš, 1959; Yakovenko, 2000). *Otostephanos regalis* has a dental formula 3 + 3/3 + 3 (or possibly 3 + 4/3 + 4, according to Milne, 1916), with thicker and thinner major teeth; its foot and spurs are small, but not particularly tiny and seem to be well-visible on the dorsal view; the last two neck segments are smooth, but the first foot segment is granulated, without any knobs. Only *O. ukrainicus* has 5/5 thick teeth in unci along with the granulation on neck and triangular protrusion on the rump that completely covers peculiarly small foot from above; there is no knob on the first foot segment.

The paired rings on the pedicels are a characteristic feature of the genus, and the animals were carefully observed both directly under the microscope and by obtaining digital videos to confirm that they are not an optical artefact. Similar species in the genus *Habrotrocha* belonging to the same family (like *Otostephanos*, they have food in the gut formed into rounded lumps, or pellets, and corona of the similar shape, but do not possess paired ring structure) were also assessed. The only species with the dental formula 6/6 resembling *O. jolantae*, *H. megalocephala* de Koning, 1947, is apparently smaller (TL 250 μm, trophi length 16 μm); the corona of *H. megalocephala* is narrower, and the upper lip has no high tongue-like tip; the rump is without a spade-shaped protrusion. There is no known *Habrotrocha* species that have granulated integument in combination with 5/5 major teeth and the peculiar protrusions of the upper lip as found in *O. ukrainicus*.

The body size of rotifers is reported to vary widely (up to several times) among populations of the same morphospecies (Ricci, 1991, 2001; Kutikova, 2005), as well as between individuals of the same population (Theilacker & McMaster, 1971). Our results showed that, in spite of certain minor variation in shape, size and proportions of some body parts of *O. jolantae*, there was no significant difference between most of the size parameters, including total length, corona size, and trophi length and width.

We found a specimen of *O. jolantae* with the dental formula 6/6 + 1 instead of 6/6. Although the variation by 1–3 or even more major teeth in unci is well-known for habrotrochids (Kutikova, 2005 and references therein; Table 3) and animals that have variable dental formula are usually considered to be of the same species when the rest of the features are uniform, this idea has to be questioned for the reasons stated above. Unfortunately, we did not obtain mtCOX1 sequences of the deviant individual with 6/6 + 1, and further research involving combined morphological and molecular approach is needed to clarify the taxonomical status of this morphotype.

The method based on representing the size of body parts as a linear function of the total length or width with subsequent comparison of the groups by analysis of covariance (ANCOVA) proved to be sensitive enough to distinguish even minor interpopulational variations in rotifer shape. We expect that this method would be useful for more precisely describing and distinguishing bdelloid morphospecies.

The observation of *Otostephanos* with an adhesive plate instead of toes is the first case reported for this genus. Toe loss in xerophile Habrotrochidae was observed by Donner (1953) and interpreted as an adaptation to edaphic habitat. Nevertheless, such phenomenon was not reported for hydrophile bdelloids and seems unlikely to be an adaptive morphological change since the species have remained in the same habitat (sphagnum). Further, whether this change affects only phenotype or is genetically inherited is not known: in our experiment the single animal found with the adhesive plate died without reproducing. The trophi size of the individual with the adhesive plate was slightly larger than in nominal *O. jolantae*, but it was measured under the light microscope. Such trophi dimensions are

Downloaded by [Univerzitni Knihovna Ostravske] at 08:07 05 December 2013

Table 3. Morphological characters and ecology of *Otostephanos*.

Species	TL[a]	RaL	BW/ TL,%	CW/ HW,%	FL/ TL,%	SL/ SSW,%	Dental formula	Upper lip	Spurs	Skin and appendages	Habitat
O. annulatus	260[b]	17	14	100–115	14	98	7/7, 8/8, 10/10, 2+4+5 /2+4+5	3 small lobes	triangular, no interspace	smooth, no appendages	aquatic moss
O. auriculatus auriculatus	400	15, 28	29	121, 135	11	86, 122	10/10 or higher	1 lobe, tip divided in 3	triangular, no interspace	smooth, no appendages	terrestrial moss, litter, sphagnum
O. auriculatus bilobatus	380–400	22–28	~28	134	~8	87–119	10/10	1 lobe, tip divided in 2	triangular, interspace	smooth, no appendages	sphagnum, wet moss, litter
O. cuspidilabris	230	20	18	97–114	10	108	8/8	1 arcuate lobe	divergent, no interspace	smooth, a hump on foot	aquatic sphagnum, terrestrial habitats
O. donneri	290–344	17.5–21	19	100–105	8	123	7/7, 9/9, 10/9, 12/12	1 triangular lobe	needle–like, base swollen	granulated or smooth, a hump on foot	waterplants
O. jersabeki	300	15	17	122	8	91–100	7/7	1 lobe, tip with a bulge	triangular, interspace	smooth, a hump on foot	soil
O. jolantae sp. nov.	285–381	19–22	15–22	92–99	7–9	62–94	6/6	1 tongue-like lobe	triangular, interspace	smooth, a hump on foot	aquatic & terrestrial sphagnum
O. kostei	290	19	7–8	138	3	135	3/3	strip-like	sickle-like	smooth	sediment, waterplants
O. macrantennus	~ 275	20–21	~23	96	~8	60	9/9	1 triangular lobe	triangular, interspace	smooth, no appendages	waterplants
O. monteti	260–306	18	21	100–107	10	60–90	7/7, 8/8	1 lobe and ligula	triangular, interspace	smooth except spinules on neck	waterplants, aquatic sphagnum
O. mundiformis	240	21	?	114–115	?	72–100	8/8	1 triangular lobe	triangular, interspace	smooth, a hump on foot	sphagnum
O. regalis	390	22–22.5	21	129	8	72	3+3/3+3 (or 3+4/3+4?)	1 semicircular lobe with ligula	triangular, no interspace	granulated trunk, rump and 1st foot segment	terrestrial moss
O. torquatus torquatus	260–410	15	~24	67–123	11	78–87	6/6, 7/7, 8/8	1–3 small lobes	conical or triangular	smooth, knob on foot	terrestrial moss, litter
O. torquatus amoenus	225–290	13.5	18–19	91–115	7–9	74–92	7/7	3 small lobes	triangular, no interspace	smooth, no appendages	terrestrial moss, litter
O. ukrainicus sp. nov.	410–442	18.0–18.4	24–26	113–116	9	32–33	5/5	strip with 2 protrusions	conical, with interspace	granulated neck, trunk, rump	forest litter
O. cf. torquatus[c]	218	16	15	107	7	92	more than 10/10	1 lobe	sword-like	knobs on rump and foot	bottom sediment

[a]TL – total length, RaL – ramus length, BW – greatest trunk width, CW – corona width, HW – collar width, FL – foot length, SL – spur length, SSW – spur–bearing pseudosegment width. [b]Dimensions are given in μm. [c]Described and depicted in Donner (1972a) as a form morphologically similar to *O. torquatus*.

Downloaded by [Univerzitni Knihovna Ostravske] at 08:07 05 December 2013

rather biased and cannot be precisely compared; we also do not have any mtCOX1 sequences of this morphotype to analyse. The taxonomic affiliation of the Austrian specimens remains unclear until such data are obtained.

Otostephanos jolantae is the ninth species of *Otostephanos* known to inhabit water bodies and the fourth one (along with *O. auriculatus*, *O. cuspidilabris* de Koning, 1947 and *O. mundiformis* de Koning, 1947) found in *Sphagnum*. It is usually very abundant in the spots of its occurrence, reaching 120–500 ind/cm^2 of moss. It was only once found in a lotic environment (in submerged aquatic moss growing in a shallow stream), and never detected in litter, soil, or terrestrial moss other than wet *Sphagnum*. *Otostephanos ukrainicus* has only been recorded from a single locality (pine and oak forest) and is one of the three *Otostephanos* found in soil or litter, along with *O. jersabeki* and *O. torquatus* (Table 3). Using the gradation proposed by Bartoš (1946) and developed by Koniar (1955) for water content preference of rotifers, *O. jolantae* might be classified as a hydrophile and *O. ukrainicus* as a hygrophile or xerophile. In relation to pH *O. jolantae* might be classified as acidophil or acidic (Wulfert, 1950; Bērziņš & Pejler, 1987; Pejler & Bērziņš, 1993); it usually occurs along with other acidophil bdelloids such as *Adineta gracilis* Janson, 1893, *Habrotrocha lata* (Bryce, 1892) and *H. angusticollis* (Murray, 1905). *Otostephanos ukrainicus* might have low pH preference as well, since the needle litter in which it was found usually has low pH (5–6, pers. obs.). However, the species was not abundant in needle litter and this might indicate that the conditions were not specifically suitable for it.

Both *O. jolantae*, with the exception of the two deviant forms (taxonomic status of those is to be verified), and *O. ukrainicus* are morphologically, ecologically and genetically uniform entities that can be easily distinguished from the rest of the *Otostephanos* species – by morphology and, in the case of *O. jolantae*, by 4× rule applied to the mtCOX1 sequences. The data on interpopulation variability are only limited to morphological features and these species do not show high level of interpopulation variability, except the two cases related to *O. jolantae*, for which more data on heritability of the deviant trait and molecular difference from the nominal species are needed.

Considering evolution of the genus *Otostephanos*, we hypothesize that it involved a change or repeated changes of habitat from strictly aquatic (hydrophile species) to limnoterrestrial (xerophile species) ecological mode, or vice versa. Indeed, within *Otostephanos*, all transition steps between these modes can be found (Table 3): *O. donneri*, *O. kostei*, *O. macrantennus* and *O. monteti* inhabit lentic waters; *O. annulatus*, *O. cuspidilabris*, *O. mundiformis* and *O. jolantae* occur in semi-aquatic conditions (submerged semi-aquatic moss); and the rest of species can survive under significant drought and populate terrestrial edaphic or epiphytic environment. However, these scenarios cannot be traced in morphology: there is the whole spectrum of variation in size and structure of organs used for feeding (corona, mastax), locomotion (rotatory apparatus, foot) and attachment to a substrate (spurs) within each ecological group, hence, attributing any of the morphotypes as an adaptation to aquatic or terrestrial environment is not possible.

We conclude that a natural key of *Otostephanos*, correctly reflecting all phylogenetic relationships within the genus, cannot be constructed solely on the basis of morphological features. However, the proposed phenotypic key that summarizes available morphological data is the first step for understanding the phylogeny of *Otostephanos*. We intend to continue identifying robust genetic markers allowing determination of the interspecific relationships within the genus, the integrity of *Otostephanos* as a taxon and its position within Bdelloidea.

Phenotypic key to the species of the genus *Otostephanos* Milne, 1916

1a. Spurs needle-shaped with bulb-like swollen base, about 1/4 longer than the width of spur bearing pseudosegment; body width is about 18–21% of the length; rectangular transverse dorsal knob on the first foot pseudosegment; corona is equal to the collar in its width; rostrum short, lamella with 2 lobes; dental formula 7/7–12/12 very fine teeth ..... *O. donneri*

1b. Spurs of different shape 2

2a. Spurs sickle-like, curved towards each other, only slightly longer than bearing pseudosegment width; body width is 7–8% of the length; corona much wider than the collar; very long thin rostrum with a single-lobed lamella; dental formula 3/3 thick major teeth ..... *O. kostei*

2b. Spurs triangular, conical or blade-shaped, shorter or equal to spur bearing pseudosegment width; more than 3/3 major teeth in unci 3

3a. Dorsal antenna 1.25–1.5 times longer than the first neck pseudosegment width; the ventral side of the 1st neck pseudosegment has sculpturation in the form of small spinules 4

3b. Dorsal antenna shorter than the first neck pseudosegment width; no spinule-like sculpturation on the neck 5

4a. Integument on the ventral side of the first neck pseudosegment with the numerous rows of small short spinules; upper lip tongue-like with a rod-like ligula behind it in the sulcus; corona width 110–115% of head length; spurs triangular with interspace; dental formula 7/7–8/8 ..... *O. monteti*

4b. Length of the dorsal antenna is the same as in *O. monteti*, but only single row of spinules on the ventral side of

Downloaded by [Univerzitni Knihovna Ostravske] at 08:07 05 December 2013

the first neck pseudosegment; upper lip wide triangular, ligula in sulcus very short, button-like and on the membrane connecting pedicels; corona width 140–160% of head length; spurs triangular without interspace; dental formula 9/9 ..... *O. macrantennus*
5a. Rotifers large (body length of a creeping animal more than 300 μm) and brightly coloured; dental formula 6/6 or less thick teeth 6
5b. Rotifer colourless, yellowish, bluish or reddish, size usually smaller than 300 μm; dental formula higher than 6/6, teeth thin 8
6a. Integument colourless; rump with a triangular or spade-like dorsal protrusion 7
6b. Integument of lemon or pinkish yellow colour, stippled on trunk, rump and foot; upper lip not reaching trochal discs plane, semicircular with a small ligula; rump without triangular or spade-like protrusion; longitudinal knob on 1st foot pseudosegment; dental formula 3+3/3+3 or 3+4/3+4 (thick + thin major teeth) ..... *O. regalis*
7a. Integument granulated on the two last neck pseudosegments, trunk and rump; body yellowish; upper lip band-like with two sharp thin protrusions separated by interspace; rump with triangular dorsal protrusion; no knob on foot; dental formula 5/5. ..... *O. ukrainicus* sp. nov.
7b. Integument all smooth or gently dotted; upper lip wide triangular with the tongue-like tip going over trochal discs plane; gut of bright red or red-orange colour with large 'food pellets'; rump with a spade-like protrusion; longitudinal knob on 1st foot pseudosegment ..... *O. jolantae* sp. nov.
8a. Length of a creeping animal less than 250 μm; knob on the first foot pseudosegment; usually in waterbodies 9
8b. Creeping animal is longer than 250 μm; knob on the first foot pseudosegment may be absent; usually terrestrial 11
9a. Integument on trunk, rump and foot dotted; rump with lateral humps on each pseudosegment; knob on the 1st foot pseudosegment semispherical; pedicels merged, sulcus covered with membrane; upper lip pointed, triangular with convex sides; dental formula at least 10/10 very thin teeth ..... *O.* cf. *torquatus* (in Donner, 1972*a*)
9b. Integument smooth; no humps on rump; knob on the first foot pseudosegment longitudinal; dental formula 8/8 10
10a. Corona about 1.15 times wider than the collar; pedicels very short, merged and sloped dorsally, so that transversal paired rings in corona are visible mostly from the ventral side; upper lip triangular; head base more than 1.5 wider than its height; a papilla in sulcus; rump conical ..... *O. mundiformis*
10b. Corona as wide as the collar or slightly narrower; pedicels short, but not merged and bent, paired rings distinctively visible from the dorsal side; upper lip semicircular; no papilla in sulcus; rump swollen ..... *O. cuspidilabris*
11a. Upper lip high, triangular 12
11b. Upper lip low, arcuate 14
12a. Upper lip not reaching trochal discs plane, framed at the top by a thick bulge and a bolster; head base width is smaller than its length; longitudinal knob on the 1st foot pseudosegment; rump conical; dental formula 7/7 ..... *O. jersabeki*
12b. Upper lip reaching trochal disc plane, its tip is divided into rounded lobes; head base wider than its length; no knob on the first foot pseudosegment; dental formula 10/10 or more fine teeth 13
13a, Tip of the upper lip divided into 3 lobes; rump swollen; two lateral bosses on the 1st foot pseudosegment; spurs shorter than the bearing pseudosegment width ..... *O. auriculatus auriculatus*
12b. Tip of the upper lip divided into 2 lobes; rump conical; no lateral bosses on foot; spurs equal or slightly longer than the bearing pseudosegment width ..... *O. auriculatus bilobatus*
14a. Body plump, colourless; upper lip almost triangular and cut out forming 3 low angular lobes. Sulcus not covered with membrane even in its lower part, no ligula; rump conical; no knob on the first foot pseudosegment; dental formula 8/8, but also reported to be 7/7, 10/10, 11/11 ..... *O. annulatus*
14b. Body cylindrical or spindle-shaped, often yellowish; upper lip ribbon-like or arcuate with protrusions or just concaved in the middle; sulcus partly or completely covered with a membrane, often with a small ligula in the middle; knob on first foot pseudosegment may be absent; dental formula 6/6-8/8 15
15a. Upper lip ribbon-like, not divided into lobes, may be concaved in the middle (according to the first description) or have rounded protrusions; no papillae or setae on the trochal discs or in the rostrum; first foot pseudosegment usually bears a dorsal knob ..... *O. torquatus torquatus*
15b. Upper lip arcuate with 3 blunt or rounded protrusions in the middle; a papilla bearing a long seta in the middle of each trochal disc; in a creeping rotifer 2 long setae are visible protruding both sides of the rostrum; no knob on first foot pseudosegment ..... *O. torquatus amoenus*

Acknowledgements

We thank Dr V.M. Virchenko (Kholodnyy Institute of Botany NASU) for providing sphagnum samples collected in Ukraine. We acknowledge Prof. Jolanta Ejsmont-Karabin, the head of Hydrobiological Station PAS and the manager of the project 'Taxonomy and ecology of bdelloid rotifers (Rotifera, Bdelloidea) in the territory of Poland' for the resources granted to collect and process the material and the opportunity of publishing a new

species description; we also thank Prof. I. Bielańska-Grajner for her help in carrying out the project. Drs M. Holyńska, Ch.D. Jersabek, D. Fontaneto, M. Devetter and Prof. L. Kutikova are gratefully acknowledged for suggestions and providing some literature sources, especially for providing copies of the older literature on Bdelloidea. We thank Dr R. Vargovich for their comments and suggestions on the manuscript.

Funding

The research in Poland was performed under the grants No. N304 0341 33 'Taksonomia i ekologia wrotków (Rotifera) z rzędu pijawczaków (Bdelloidea) na terenie Polski' and INTAS Young Scientist Fellowship Grant Ref. Nr 06-1000014-5639. The funding for SEM and DNA sequencing came from the grant KJB600450903 of Grant Agency AS ČR.

Supplemental data

Supplemental data for this article can be accessed here.

References

Bancsi, I. 1986. *A kerekesférgek – Rotatoria – kishatarozoja I.* Vizgazdalkodasi Intezet, Budapest, Hungary.

Bartoš, E. 1946. Rozbor drobnohledné zvířeny českých mechů. *Věstnik Československe zoologické společnosti* **10**, 55–80.

Bartoš, E. 1949. Mikroskopická zvířena šumavských mechů. – I. Mechy okolí Plešného jezera. *Věstnik Československe zoologické společnosti* **13**, 10–29.

Bartoš, E. 1951. The Czechoslovak Rotatoria of the order Bdelloidea. *Věstnik Československe zoologické společnosti* **15**, 241–500.

Bartoš, E. 1959. *Vířnici – Rotatoria. Fauna ČSR.* Sv.15. ČCSAV, Praha, Czech Republic.

Běrziņš, B. & Pejler, B. 1987. Rotifer occurence in relation to pH. *Hydrobiologia* **147**, 107–116.

Bielańska-Grainer, I., Ejsmont-Karabin, J. & Yakovenko, N. 2011. Bdelloidea (Rotifera) from the Tatra National Park, with some species newly found in Poland. *Fragmenta Faunistica* **54**, 103–111.

Birky, C.W. & Barraclough, T.G. 2009. Asexual speciation. In: Shön, I., Martens, K. & Van Dijk, P., Eds., *Lost Sex: The Evolutionary Biology of Parthenogenesis*, Springer, Dordrecht, Germany, pp. 201–216.

Birky, C.W., Wolf, C., Maughan, H., Herbertson, L. & Henry, E. 2005. Speciation and selection without sex. *Hydrobiologia* **181**, 29–45.

Birky, C.W., Ricci, C., Melone, G. & Fontaneto, D. 2011. Integrating DNA and morphological taxonomy to describe diversity in poorly studied microscopic animals: new species of the genus *Abrochtha* Bryce, 1910 (Rotifera: Bdelloidea: Philodinavidae). *Zoological Journal of the Linnean Society* **161**, 723–734.

Bryce, D. 1913. On five new species of Bdelloid Rotifera. *Journal of the Quekett Microscopical Club* **2**, 83–94.

Crawley, M. J. 2005. *Statistics: An Introduction using R*. John Wiley & Sons, Chichester, UK.

De Smet, W.H. 1998. Preparation of rotifer trophi for light and scanning electronic microscopy. *Hydrobiologia* **387/883**, 117–121.

Donner, J. 1951. Erste Übersicht über die Rotatorienfauna einiger Humusböden. *Österreiche Zoologische Zeitschrift Wien* **3**, 175–240.

Donner, J. 1952. Die Rädertiergemeinschaft auf einem Burgturm. *Mikrokosmos* **41**, 126–128.

Donner, J. 1953. Einige Fälle von Reduktion der Zehen zu einer Haftscheihe bei bdelloiden Rotatorien des Bodens. *Österreighische Zoologische Zeitschrift* **4**, 346–353.

Donner, J. 1954. Zur Rotatorienfauna Südmährens. *Österreighische Zoologische Zeitschrift* **5**, 30–117.

Donner, J. 1956. Über die Mikrofauna, besonders die Rotatorienfauna des Bodens. *Acta Zoologica Academiae Scientiarum Hungaricae Budapest* **8**, 303–333.

Donner, J. 1965. *Ordnung Bdelloidea. (Rotatoria, Rädertiere). Bestimmungsbücher zur Bodenfauna Europas 6*. Akademie Verlag, Berlin, Germany.

Donner, J. 1972*a*. Die Rädertierbestande submerserer Moose und weiterer Merotope im Bereich der Stauraume der Donau an der deutch-österreichlischen Landsgrenze. *Archivum Hydrobiologie Suppl.* **44**, 49–114.

Donner, J. 1972*b*. Rotatorien aus einigen Böden des östlichen Osterreich. *Revue Écologie et Biologie Sol* **9**, 671–682.

Folmer, O., Black, M., Hoeh, W., Lutz, R. & Vrijenhoek, R.C. 1994. DNA primers for amplifcation of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* **3**, 294–299.

Fontaneto, D. & Melone, G. 2003*a*. On some rotifers new for the Italian fauna. *Italian Journal of Zoology* **70**, 253–259.

Fontaneto, D. & Melone, G. 2003*b*. Bdelloid rotifers from lakes above 1700 m in western Italian Alps, with taxonomic notes on *Dissotrocha macrostyla*. *International Review of Hydrobiology* **88**, 594–601.

Fontaneto, D. & Melone, G. 2005. Do rotifer jaws grow after hatching? *Hydrobiologia* **546**, 213–221.

Fontaneto, D. & Ricci, C. 2004. Rotifera: Bdelloidea. In Yule, C.M. & Yong, H.S., Eds., *Freshwater Invertebrates of the Malaysian Region*. Academy of Sciences Malaysia, Kuala Lumpur, Malaysia.

Fontaneto, D., Melone, G. & Cardini, A. 2004. Shape diversity in the trophi of different species of *Rotaria* (Rotifera, Bdelloidea): a geometric morphometric study. *Italian Journal of Zoology* **71**, 63–72.

Fontaneto, D., Herniou, E.A., Barraclough, T.G. & Ricci, C. 2007*a*. On the global distribution of microscopic animals: new worldwide data on bdelloid rotifers. *Zoological Studies* **46**, 336–346.

Fontaneto, D., Herniou, E., Barraclough, T.G., Ricci, C. & Melone, G. 2007*b*. On the reality and recognisability of asexual organisms: morphological analysis of the masticatory apparatus of bdelloid rotifers. *Zoologica Scripta* **36**, 361–370.

Fontaneto, D., Herniou, E.A., Boschetti, C., Caprioli, M., Melone, G., Ricci, C. & Barraclough, T.G. 2007*c*. Independently evolving species in asexual bdelloid rotifers. *Public Library of Science Biology* **5**, 914–921.

Haigh, S.B. 1963. The bdelloid rotifers of New Zealand. *Journal of the Quekett Microscopical Club* **29**, 161–170.

Harring, H.K. 1914. A list of the Rotatoria of Washington and vicinity, with descriptions of a new genus and ten new

Downloaded by [Univerzitni Knihovna Ostravske] at 08:07 05 December 2013

species. *Proceedings of the United States National Museum* **46**, 387–405.

Hauer, J. 1939. Zur Kenntnis subterraner Rotatorien. *Zoologisher Anzeiger* **126**, 41–44.

Hirschfelder, A., Koste, W. & Zucci, H. 1993. Bdelloid rotifers in aerophytic mosses: influence of habitat structure and habitat age on species composition. *Hydrobiologia* **255/652**, 443–343.

Iakovenko, N. S. 2005. *Bdeloidni kolovertky (Rotifera, Bdelloidea) Ukrajiny (systematyka, fauna, ekologiia)*. Candidate Biological Science Dissertation, Schmalhausen Institute of Zoology, Kyiv, Ukraine.

Iakovenko, N.S. & Ovander, E.N. 2011. Anotovanyj spysok bdeloidnych kolovertok (Rotifera: Eurotatoria, Bdelloidea) fauny Ukrajiny. *Bulletin of the Kyiv Taras Shevchenko National University, Biological series* **58**, 25–29.

Jersabek, C.D., De Smet, W.H., Fischer, C., Fontaneto, D., Michaloudi, E., Wallace, R.L. & Segers, H. 2012. List of Available Names in Zoology, Candidate Part Phylum Rotifera, species group names established before 1 January 2000. http://www.mapress.com/zootaxa/2012/f/zt03179p068.pdf, accessed 31 March 2012.

Kaya, M., Hernıou, E.A., Barraclough, T.G. & Fontaneto, D. 2009. Inconsistent estimates of diversity between traditional and DNA taxonomy in bdelloid rotifers. *Organisms, Diversity & Evolution* **9**, 3–12.

Koniar, P. 1955. Prispevok k poznania virnikov (Rotatoria) Vysokých Tatier. *Biologia* **10**, 449–463.

Koste, W. 1996. On soil Rotatoria from a lithotelma near Halali Lodge in Etosha National Park in N-Namibia, South Africa. *Internationale Revue der gesamten Hydrobiologie und Hydrographie* **81**, 353–365.

Kutikova, L.A. 1970. *Kolovratki fauny SSSR (Rotatoria)*. Podklass Eurotatoria. Nauka, Leningrad, Russia.

Kutikova, L. 2005. *Bdelloidnyje kolovratki fauny Rossii*. KMK Scientific Press Ltd., Moscow, Russia.

Melone, G. & Fontaneto, D. 2005. Trophi structure in bdelloid rotifers. *Hydrobiologia* **546**, 197–202.

Milne, W. 1916. On the bdelloid Rotifera of South Africa. *Journal of Quekett Microscopical Club* **2**, 47–83.

Marrone, F., Lo Brutto, S. & Arculeo, M. 2010. Molecular evidence for the presence of cryptic evolutionary lineages in the freshwater copepod genus *Hemidiaptomus* G.O. Sars, 1903 (Calanoida, Diaptomidae). *Hydrobiologia* **644**, 115–125.

Murray, J. 1911*a*. Some African rotifers: Bdelloidea of tropical Africa. *Journal of Royal Microscopical Society London*, 1–18.

Murray, J. 1911*b*. Bdelloid Rotifera of South Africa. *Annales of the Transvaal Museum Pretoria* **3**, 1–19.

Pejler, B. & Bērziņš, B. 1993. On the ecology of mire rotifers. *Limnologica* **23**, 295–300.

Pennak, R.W. 1978. *Freshwater invertebrates of the United States*, 2nd edition. John Wiley & Sons, New York, USA.

Ricci, C. 1991. Comparison of five strains of a parthenogenetic species, *Macrotrachela quadricornifera* (Rotifera, Bdelloidea). I. Life history traits. *Hydrobiologia* **211**, 147–155.

Ricci, C. 2001. A reconsideration of the taxonomic status of *Macrotrachela quadricornifera* (Rotifera, Bdelloidea). *Journal of Zoology London* **255**: 273–277.

Ricci, C. & Melone, G. 2000. Key to the identification of the genera of bdelloid rotifers. *Hydrobiologia* **418**, 73–80.

Rudescu, L. 1960. *Rotatoria. Fauna republicii populare Romine. Trochelmintes*. Academiei Republicii Populare Romine, Bucharest, Romania.

Schulte, H. 1954. Beitrage zur Ökologie und Systematik der Bodenrotatorien. *Zoologischer Jahrbucher* **82**, 497–654.

Segers, H. 2002. Contribution to the nomenclature of Rotifera: Annotated checklist of valid species and genus-group names. *Journal of Natural History* **36**, 631–640.

Segers, H. 2007. Annotated checklist of the rotifers (Phylum Rotifera), with the notes on nomenclature, taxonomy and distribution. *Zootaxa* **1564**, 1–104.

Segers, H. 2008. Global diversity of rotifers (Rotifera) in freshwater. *Hydrobiologia* **595**, 49–59.

Segers, H. & Shiel, R. J. 2005. Tale of a sleeping beauty: a new and easily cultured model organism for experimental studies on bdelloid rotifers. *Hydrobiologia* **546**, 141–145.

Sohlenius, B., Boström, S. & Hirschfelder, A. 1996. Distribution patterns of microfauna (nematodes, rotifers and tardigrades) on nunataks in Dronning Maud Land, East Antarctica. *Polar Biology* **16**, 191–200.

Song, M.O. & Kim, W. 2000. Bdelloid Rotifers from Korea. *Hydrobiologia* **439**, 91–101.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. 2011. MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony method. *Molecular Biology and Evolution* **28**, 2731–2739.

Theilacker, G.H. & McMaster, M.F. 1971. Mass culture of the rotifer *Brachionus plicatilis* and its evaluation as a food for larval anchovies. *International Journal on Life in Oceans and Coastal Waters* **10**, 183–188.

Ustaoğlu, M.R., Altındağ, A., Kaya, M. Akbulut, N., Bozkurt, A., Özdemir Miş, D., Atasagun, S., Erdoğan, S., Bekleyen, A., Saler, S. & Okgerman, H. C. 2012. A checklist of Turkish Rotifers. *Turkish Journal of Zoology* **36**, 607–622.

Varga, L. 1953. Die Rotatorien der Ozberek-Quelle bei Diosjeno. *Annales Instituti Biologici (Tihany) Hungaricae Academiae Scientiarum* **22**, 155–169.

Varga, L. 1957. Untersuchungen über die Mikrofauna der Waldstreu einiger Waldtypen im Bükkgebirge (Ungarn). *Acta Zoologica Academie Scientiarum Hungaricae* **3**, 443–478.

Varga, L. 1966. *Kerekesférgek I. Rotatoria I. Magyarorszag Allatvilaga – Fauna Hungaricae*. Akademiai Kiado, Budapest, Hungary.

Voigt, M. 1956–1957. *Rotatoria: Die Rädertiere Mitteleuropas*. Berlin-Nikolassee, Germany.

Wulfert, K. 1950. Das Naturschutzgebiet auf dem Glatzer Schneeberg. Die Rädertiere des Naturschutzgebietes. *Archiv für Hydrobiologie* **44**, 441–471.

Yakovenko, N.S. 2000. New for the fauna of Ukraine rotifers (Rotifera, Bdelloidea) of Adinetidae and Habrotrochidae families. *Vestnik zoologii* **34**, 11–19.

Associate Editor: Elliot Shubert

Downloaded by [Univerzitni Knihovna Ostravske] at 08:07 05 December 2013

View publication stats

PLOS | ONE

RESEARCH ARTICLE

Lifestyle and Ice: The Relationship between Ecological Specialization and Response to Pleistocene Climate Change

Eva Kašparová[1,2]*, Anton P. Van de Putte[3], Craig Marshall[4], Karel Janko[1,2,5,6]*

1 Laboratory of Fish Genetics, Institute of Animal Physiology and Genetics, Academy of Sciences of the Czech Republic, Rumburska 89, 27721 Libechov, Czech Republic, **2** Institute of Vertebrate Biology, Academy of Sciences of the Czech Republic, Kvetna 8, 603 65, Bmo, Czech Republic, **3** Laboratory of Biodiversity and Evolutionary Genomics, Katholieke Universiteit Leuven, Ch. Deberiotstraat 32, B-3000 Leuven, Belgium, **4** Department of Biochemistry, and Genetics Otago, University of Otago, P.O. Box 56, Dunedin, New Zealand, **5** Centre for Polar Ecology, University of South Bohemia in Ceské Budejovice, Na Zlate stoce 3, 370 05, Ceske Budejovice, Czech Republic, **6** Life Science Research Centre, Department of Biology and Ecology, Faculty of Natural Sciences, University of Ostrava, Chittussiho 10, 710 00 Silesian Ostrava, Czech Republic

* kasparova@iapg.cas.cz (EK); janko@iapg.cas.cz (KJ)

CrossMark
click for updates

OPEN ACCESS

Citation: Kašparová E, Van de Putte AP, Marshall C, Janko K (2015) Lifestyle and Ice: The Relationship between Ecological Specialization and Response to Pleistocene Climate Change. PLoS ONE 10(11): e0138766. doi:10.1371/journal.pone.0138766

Editor: Lorenzo Zane, University of Padova, ITALY

Received: November 26, 2014

Accepted: September 3, 2015

Published: November 4, 2015

Copyright: © 2015 Kašparová et al. This is an open access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Data Availability Statement: All sequence files are available from the GenBank database (accession number(s) for cyt b, KC153538 - KC153654; and for S7 intron, KC520782- KC520827, KC833756 - KC833898.

Funding: This work has been supported by the Royal Belgian Institute for Natural Sciences, OD Nature, Vautierstraat 29, B-1000 Brussels, Belgium. Grant Agency of the Czech Academy of Sciences (GA AS CR) (No. KJB600450903), European Centre of Ichtyoparasitology. (No. P505/12/G112), Ministry of Education, Youth and Sport of Czech Republic (Centre for Polar Ecology University of South Bohemia in Ceske Budejovice) (LM2010009,

Abstract

Major climatic changes in the Pleistocene had significant effects on marine organisms and the environments in which they lived. The presence of divergent patterns of demographic history even among phylogenetically closely-related species sharing climatic changes raises questions as to the respective influence of species-specific traits on population structure. In this work we tested whether the lifestyle of Antarctic notothenioid benthic and pelagic fish species from the Southern Ocean influenced the concerted population response to Pleistocene climatic fluctuations. This was done by a comparative analysis of sequence variation at the cyt *b* and S7 loci in nine newly sequenced and four re-analysed species. We found that all species underwent more or less intensive changes in population size but we also found consistent differences between demographic histories of pelagic and benthic species. Contemporary pelagic populations are significantly more genetically diverse and bear traces of older demographic expansions than less diverse benthic species that show evidence of more recent population expansions. Our findings suggest that the lifestyles of different species have strong influences on their responses to the same environmental events. Our data, in conjunction with previous studies showing a constant diversification tempo of these species during the Pleistocene, support the hypothesis that Pleistocene glaciations had a smaller effect on pelagic species than on benthic species whose survival may have relied upon ephemeral refugia in shallow shelf waters. These findings suggest that the interaction between lifestyle and environmental changes should be considered in genetic analyses.

CZ.1.07/2.2.00/28.0190) and the Belgian Science Policy (BELSPO contract n°BR/132/A1/vERSO). The authors' team receives continuous support from the Institute of Animal Physiology and Genetics (RVO: 67985904). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

Abbreviations: APF, Antarctic Polar Front; LGM, Last Glacial Maximum; AFGP, antifreeze glycoproteins; cyt b, cytochrome *b*; HKA test, Hudson–Kreitman–Aguade´ Test; EBSP, Extended Bayesian skyline plot; BEAST, Bayesian Evolutionary Analysis Sampling Trees; HKY, Hasegawa, Kishino and Yano DNA substitusion model; AIC, Akaike information criterion; MCMC, Markov chain Monte Carlo method; GLM, Generalized linear models; ANOVA, Analysis of variance; WMW, Wilcoxon-Mann-Whitney test.

Introduction

Climate change has always been a feature of the natural world. Although we can trace some of the physical effects of climate change, identifying the biological effects of past climatic change is complicated. Studies identifying 'genetic wakes'[1] carrying signals of dispersal, extinction, and speciation correlating with Pleistocene glacial cycles have provided evidence of dramatic worldwide effects on species. However, no simple pattern of population response to these climatic changes has been found. Although explanations for different responses among species to Pleistocene climate change have been proposed in comparative studies [2,3] general patterns of response are not always present. Even closely-related organisms exposed to common events may have quite distinct population histories with no immediately obvious reasons for the differences [4]. Polar regions provide attractive natural laboratories to address such issues since polar ecosystems are relatively simpler than low-latitude ones and the direct effects of climatic changes are more easy to detect compared to lower latitudes (e.g. physical eradication of habitats by expanding glaciers). Despite ongoing research, our knowledge of evolution at the poles remains limited [5]. Moreover, studies tend to focus on the more easily accessible Northern Hemisphere than on the Southern. This is especially important since diversity patterns are not symmetrical between the two hemispheres [6] with much higher levels of endemism within the Southern polar circle, suggesting that different mechanisms apply. In the Arctic, despite evidence of high-latitude refuges in some organisms (e.g. cloudberry (*Rubus chamaemorus*) [7] both marine and terrestrial organisms were apparently able to move along a latitudinal cline in response to changes in the Pleistocene climate (2,5 Mya to 12 Kya) (e.g. relict moss *Rhytidium rugosum* [8] or molluscs [9]). In the Antarctic and the Southern Ocean, the situation is quite different. Populations are relatively isolated from other parts of the globe by the Antarctic Polar Front (APF) and by physiological and competitive constraints on potential invaders [5,9,10]. To understand the effects of future climate change it is useful to study how Antarctic organisms survived in isolation and coped with climate fluctuations and oscillating ice sheet cover during the Pleistocene.

Contemporary population genetic studies reveal no simple pattern of population history in the species on the Antarctic continental shelf and Southern Ocean. Some species demonstrate population structure implying historical fragmentation into isolated glacial refuges in the high-Antarctic that led either to partial reproductive isolation (e.g. crinoids, icefish, octopods [5,10–14] and secondary hybridization during interglacial periods (e.g. in notothenioids [15]), or to allopatric speciation (e.g. sea slug [16]). Other species show a 'classical' pattern suggesting survival of inhospitable periods in lower-latitude refugia and subsequent recolonisation of Antarctica from the north (e.g. bull kelp [17]). Some species show recent expansions associated with glacial retreat after the Last Glacial Maximum (LGM) (15–12 Kya) [18] (e. g. the limpet *Nacella* and trematomid fish [19,20] whereas shrimps, sea spiders and deep-sea echinoids demonstrate much older expansion timings [21–23]. Strikingly different population dynamics have been detected even among closely-related species e.g. within the Trematominae [20] and among springtails (Collembola) [24].

Similar discrepancies were found in studies that address interspecific processes. It has been suggested that ice ages might have caused the extinction of some species [25], or conversely, increased the speciation rate during the Pleistocene (glaciation as a diversity pump; [16]). On the other hand, a recent macroevolutionary study of the diversification rate among the fish tribe Trematominae demonstrated it was largely unchanged during the Pleistocene [26].

There may be several reasons for this diversity of patterns. Firstly, the genetic markers and analytical tools used in different studies may not be equivalent, and difficulties in calibrating

the molecular clock of Antarctic organisms may hamper clear-cut comparisons among unrelated taxa [27] regardless of any underlying patterns. Alternatively, stochastic variability induced by asynchronous patterns of ice expansion and retreat in glacial periods may account for the variety of patterns of population structure when geographically isolated refugia on the continental shelf repeatedly appeared and disappeared. Differences in the timing of these may have allowed species to migrate among refugia [28]. In addition, the response of a species to glaciations may depend upon its distribution range and the local conditions within each of the Antarctic biogeographic provinces [24,29,30]. In contrast, several studies show that the ability of a species to cope with climatic changes mainly depends on ecological factors such as niche width, habitat preference, and depth range [20,23].

In order to understand the processes that lead to diverse population genetic patterns among marine species, Hellberg [4] asked: "Do closely related species differ in inferred effective population size? What are life histories or ecological attributes of this variation?" In this study we have addressed both of Hellberg's questions. Specifically focusing on thirteen closely related but ecologically diverse fish species (Perciformes, Notothenioidei), we looked for different demographic responses to Pleistocene ice sheet expansions and tested whether the long-term effective population size and timing of population expansions has been governed by species-specific lifestyles such as the use of benthic or pelagic habitats.

The notothenioid fish are an excellent model for assessing the role of lifestyle in influencing demographic history. The origins of modern notothenioid fish date from Southern Ocean cooling during the Oligocene [31,32] when the Eocene fish fauna went extinct. This allowed the subsequent occupation of "vacant" niches by ancestral notothenioid fish that avoided freezing because of antifreeze glycoproteins (AFGP) (reviewed in Eastman & McCune [33]) [32]. These notothenioid fish radiated during the Miocene from a putative benthic ancestor without a swim bladder into an array of species strikingly diverse in morphology and lifestyle. A pelagic lifestyle seems to have arisen independently several times in the suborder [26,34] indicative of significant plasticity in body size and conformation. Notothenioid fish comprise closely related, monophyletic, and ecologically diverse species with a polyphyletic distribution of traits (including cryopelagic, pelagic and benthic lifestyles). These characteristics make them suited for comparative studies and minimize the effect of "phylogenetic constraints" sensu Gould and Lewontin [35] i.e. the risk that the differences among species result from intrinsic characteristics of particular phylogenetic lineages rather than the presence of some potentially adaptive trait.

This study extends previous results by re-analyzing the data from four species from Janko et al. [20] and by adding datasets from nine newly-sequenced species. Two genetic loci (mitochondrial cyt *b* and nuclear S7) were sequenced from all species and subjected to a number of analytical approaches to look for evidence of historical population changes. We sorted the thirteen fish species into distinct eco-groups according to their habitat specialization and predicted that if habitat use specialization affects species responses to climatic events, there should be evidence of consistent patterns in inferred demographic histories among similar eco-groups. Pelagic species are likely to be less affected by scouring of the shelf by glaciers and ice sheet advance, and may have even experienced population expansions during ice ages as the polar pelagic habitat expanded north with movement of the Antarctic Polar Front during the LGM [18,36]. By contrast, populations of benthic species are potentially more vulnerable to local extinctions caused by increasing glaciation and might have suffered more recent and severe bottlenecks associated with the last glaciation that led, for example, to reductions in the extent of shallow bottom.

Results

Genetic variability of species

Two markers, cytochrome *b* (cyt *b*) and nuclear S7 gene were PCR amplified and tested for selection effects. The S7 dataset was analysed as unrecombined regions including the gametic phases of heterozygous individuals.

The cyt *b* sequences showed no evidence of indels or stop codons. Recombination events were detected in several S7 datasets; these were subsequently pruned to obtain the longest non-recombined portion of that locus for each species (Table 1). In *Pagetopsis macropterus*, the high number of recombination events prevented phasing and obtaining sufficiently long sequences. Therefore, the S7 dataset of *Pagetopsis macropterus* was excluded from all analyses. No departures from neutral expectations for any species were found in the McDonald-Kreitman and HKA tests within cyt *b* and across both loci (Table 1).

Since geographical subdivisions can bias estimates of historical demography, AMOVA analyses were performed to check population connectivity between regions. Significant evidence of population structuration were found in *T. hansoni* cyt *b* and S7 datasets and in the *L. nudifrons* S7 dataset (see Table A in S1 File). For those species with evidence of geographical structure, we performed tests of genetic variability and neutrality tests according to the geographic subdivision for both markers (see Table B in S1 File). The detection of structure generally reflected a distinction between biogeographic provinces in *T. hansoni* (high-Antarctic vs Ant. Peninsula), whereas in *L. nudifrons* we noticed significant differences among individual sampling sites.

Deviations from the constant population size model

A number of different methods were used to assess the deviation from a constant population size model. Significant deviations were observed in all cyt *b* datasets (Table 1) as suggested by significantly negative values of Fu's F_s in all datasets and of Tajima's D in all but one case (Table 1). This is consistent with the star-shaped haplotype networks (Fig 1) expected for species undergoing recent demographic expansion. Generally, we found that the haplotype networks of pelagic species are more complex than in benthic species. We did note however, that species with geographic structure (*T. hansoni*, *L. nudifrons* and also *G. gibberifrons*) possessed more complex patterns than other benthic species. Arlequin's goodness-of-fit statistics did not reject a sudden expansion model in any dataset suggesting that all species may have undergone an increase in population size. The estimated expansion time (in mutational units) differed for pelagic and benthic species. Estimates from DnaSP for pelagic species ranged from 1.22 to 2.23, and for benthic from 0.08 to 1.53 (see Table 2). Estimates from SITES matched those of DnaSP very closely except for *G. acuticeps* and *T. nicolai* where SITES estimates exceeded those of DnaSP almost seven-fold. On the other hand, although Arlequin estimates were similar to those of SITES and especially those of DnaSP in most species, they differed by as much as twenty-fold for *T. pennellii*, *T. nicolai* and *G. acuticeps* (see S1 Fig).

Datasets for S7 also displayed negative values of Tajima's D in all cases and in all but one case for Fu's F_s. These values were significantly different from zero in five cases of Fu's F_s, and in one case of Tajima's D (Table 1). Arlequin goodness-of-fit statistics did not reject a sudden expansion model in any dataset. Expansion time estimates were older for the S7 intron than for mitochondrial cyt *b* locus—a pattern observed also in Janko et al. [20] (see Table 2).

Expansion time estimates from geographical regions detected as isolated by AMOVA in *T. hansoni* show similar or even younger dating as expansion time estimate obtained from combined datasets. In contrast, the estimate from SITES indicated an old expansion, but the model

Table 1. Results of neutrality tests and expansion rate estimation.

		Cyt *b*					Tests using both loci		S7 intron			
Species	**E**	**N**	**L**	**Tajima´s D**	**Fu´s F_s**	**McD&K**	**HKA**	**LAMARC**	**N**	**L**	**Tajima´s D**	**Fu´s F_s**
Aethotaxis mitopteryx	P	33	553	• -2.42273	• -14.642	0.175	5.51	795.41 +/- 294	46	571	•-2.36578	•-17. 377
Notothenia rossii	I	30	723	• -2.02488	• -9.332	0.763	0.0936	4325.29 +/-1324	26	188	-1.77006	•-7.83
Trematomus eulepidotus	I	35	399	• -2.29808	• -15. 249	0.763	5.63	610.8747 +/- 122	42	622	-1.7435	•-33.793
Gobionotothen gibberifrons	B	29	640	• -2.08520	• -12.746	0.002	0.268	35.09154 +/- 58	29	340	-1.82666	•-9.4
Gymnodraco acuticeps	B	23	732	• -1.97778	• -4.152	0.175	4.32	270.7582 +/- 236	42	273	-0.66043	-0.05
Lepidonotothen nudifrons	B	40	463	• -1.97088	• -8.233	1.586	0.1959	5682.365 +/- 1253	66	625	-0.07802	0.739
Pagetopsis macropterus	B	25	687	• -1.82397	• -4.992	0.763	NA	NA	NA	NA	NA	NA
Trematomus hansoni	B	76	399	-1.8338	• -8.681	3.643	4.43	403.455 +/- 240	98	479	-1.6701	•-16.978
Trematomus nicolai	B	34	353	• -1.72673	• -3.595	1.263	0.3768	49.74308 +/- 261	58	443	-1.04706	-0.465

Results here are for nine newly sequenced species. E—eco-group (P—pelagic, I—intermediate, B—benthic), N—number of sequenced species, L—length in base pairs of the non-recombined locus, McD&K—G value with Williams' correction of the McDonald & Kreitman test, HKA—sum of deviations of the HKA test, LAMARC—g with 95% support intervals with averaged errors, Symbol • indicates values significant after sequential Bonferroni correction (α = 0,05), the *P. macropterus* S7 dataset was not available for this analysis.

doi:10.1371/journal.pone.0138766.t001

fitted the data poorly and these estimates are unreliable. For the *Lepidonotothen nudifrons* S7 dataset, estimates of expansion times were also more recent than for the combined dataset.

Further evidence for past population changes comes from generalized skyline plots. Most cyt *b* datasets showed ε values that grouped adjacent intervals into two or more composite population growth intervals indicating more or less strong population growth (Fig 2A). However, the datasets for *T. nicolai*, *T. pennellii*, *T. bernacchii* and *G. acuticeps* contain too little variability for analysis by generalized skyline plots, and produced optimal ε values that grouped all coalescent events into single composite intervals. This prevented any demographic resolution in those species. The generalized skyline plot for the S7 intron showed more coalescence events than for the cyt *b* locus and the resolution extended over longer periods but provided less clear information about the population history (S2 Fig).

Finally, two methods were used to reconstruct demographic history using both loci simultaneously. Lamarc 2.1.10 was used to estimate the parameter g of the growth rate under the model of exponential growth. In all species except *T. nicolai*, *G. gibberifrons* and *G. acuticeps*, 95% support intervals of g excluded zero indicating that populations have been growing (Table 1). In three species (*G. gibberifrons*, *G. acuticeps and T. nicolai*) Lamarc failed to converge even after an extended run (more than 400 000 interactions) and the 95% support intervals for g ranged from negative to positive values. These data should therefore be considered with care, but even in these species, the MLE (maximum likelihood estimate) was always positive (Table 1).

Extended Bayesian skyline plots (EBSP) combining nuclear and mtDNA loci using the branch-specific mutation rates estimated by BEAST indicated that the recent population size of most species increased by at least an order of magnitude from the ancestral population size. (Fig 2B, S3 Fig). However, recent population size estimates of four species (*T. newnesi*, *T. nicolai*, *T. pennellii* and *G. acuticeps*) showed only minimal signals of population increase.

Ecological correlates of demographic differences among species

Estimates of the genetic diversity (hd, π, θ_W) and the relative timings of changes of population size varied considerably among species (Table 2, Fig 3). However, unequal sampling does not

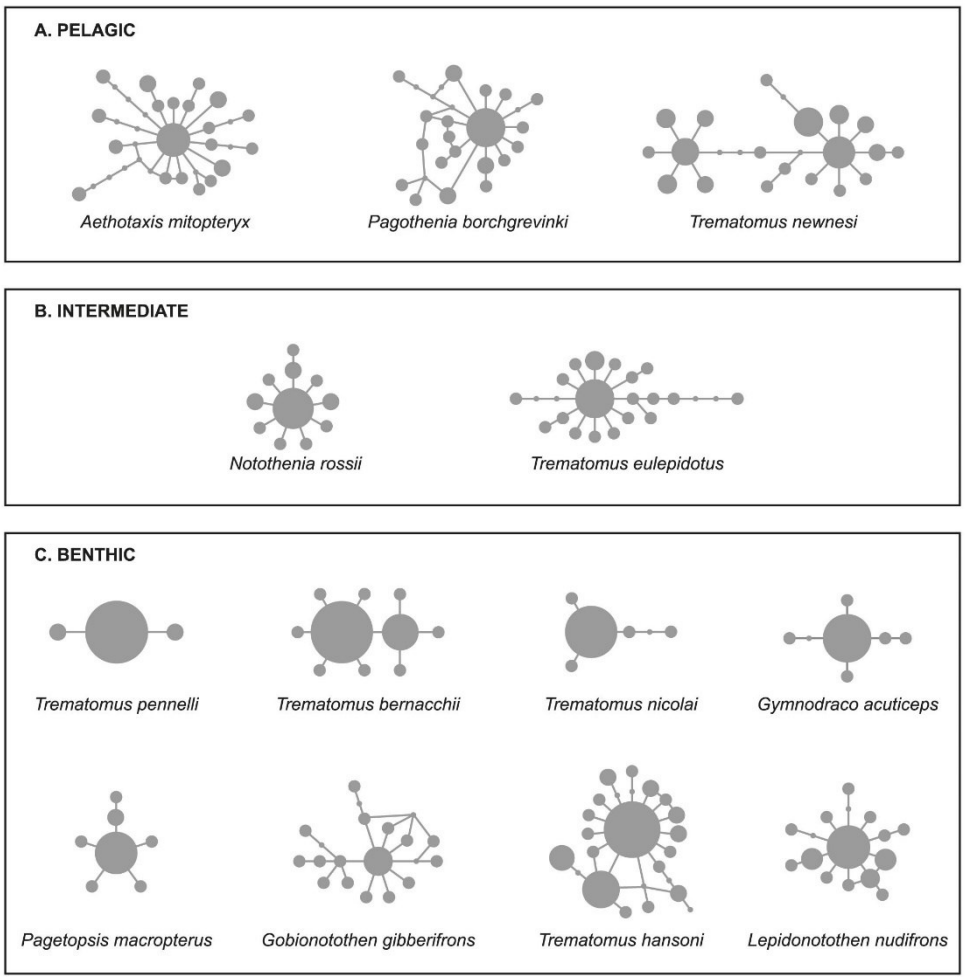


Fig 1. Unrooted networks of cyt *b* constructed by statistical parsimony. Unique haplotypes are represented by circles and their absolute frequency is indicated by circle size. Internodes represent unsampled inferred intermediate haplotypes.

doi:10.1371/journal.pone.0138766.g001

account for the among-species variability observed since the GLM (generalised linear model) incorporating three factors—sample size, geographical sampling coverage and transect length —did not explain the distribution of the data well and generated higher AIC scores than corresponding null models.

On the other hand, the ecological grouping of the species accounted for a significant portion of the variability in genetic diversity indices and demographic parameters for the cyt *b* dataset.

Table 2. Summary of genetic diversity estimates and of population expansion times for cyt b and S7 of a combined dataset of 13 species.

		Cyt b					
Species	**E**	**hd**	**π *10^3**	**ΘW*10^3**	**T(ex) (DSP)/T(ex) (S) /T(ex) (A)*10^3**	**T(ex) (DSP)/T(ex) (S) /T(ex) (A) (Kya)**	**DS**
Aethototaxis mitopteryx	P	0.87	4.8	14.95	1.22/ 2.32/ 1.15 (0.18–4.39)	148.53/ 282.38/ 140.24 (21.95–535.37)	N
Pagothenia borchgrevinki	P	0.88	3.6	8.5	1.68/ 1.79/ 1.46 (0.98–2.06)	166.16/ 168.73/ 144.55 (97.03–203.96)	J
Trematomus newnesi	P	0.91	6.9	9.77	2.23/ 4.69/ 5.52 (0.86–9.67)	300.77/ 633.71/ 745.94 (116.22–1306.756)	J
Notothenia rossii	I	0.68	1.3	3.49	0.63/ 0.65/ 0.73(0.32–1.33)	62.93/ 65.17/ 73 (32–133)	N
Trematomus eulepidotus	I	0.86	7.6	21.35	1.47/ 4.4/ 2.51(0.00–7.7)	179.42/ 492.14/ 306.1 (0–939.022)	N
Gobionotothen gibberifrons	B	0.86	3.1	7.22	1.53/ 2.21/ 1.63 (0.00–3.71)	160.61/ 232.2/ 171.58 (0–390.52)	N
Gymnodraco acuticeps	B	0.32	0.7	2.12	0.08/ 0.55/ 2.05 (0.25–2.82)	9.99/ 67.26/ 250 (30.49–343.90)	N
Lepidonotothen nudifrons	B	0.63	2	5.75	0.98/ 2.23/ 1.05 (0.52–2.26)	160.39/ 365.75/ 172.13 (85.26–370.492)	N
Pagetopsis macropterus	B	0.49	0.9	2.32	0.45/ 0.93/ 0.49 (0.08–0.94)	48.52/ 99.94/ 52.69 (8.60–101.075)	N
Trematomus bernacchii	B	0.48	1.3	4.28	0.67/ 0.49/ 0.78 (0.43–1.29)	83.23/ 60.67/ 96.3 (53.09–159.26)	J
Trematomus hansoni	B	0.89	4.2	10.82	1.18/ 1.51/ 1.84 (0.00–57.33)	135.32/ 173.94/ 211.49 (0–6589.66	N
Trematomus nicolai	B	0.17	0.5	2.11	0.25/ 0.87/ 4.23 (0.60–4.25)	27.7/ 96.18/ 470 (66.67–472.23)	N
Trematomus pennellii	B	0.13	0.3	1.8	0.12/ 0.12/ 3 (0.46–3.50)	10.61/ 10.79/ 263.16 (40.35–307.02)	J
		S7					
Species	**E**	**hd**	**π *10^3**	**ΘW*10^3**	**T(ex) (DSP)/ T(ex) (S)/ T(ex) (A)*10^3**	**T(ex) (DSP)/ T(ex) (S)/ T(ex) (A) (Kya)**	**DS**
Aethototaxis mitopteryx	P	0.77	4.22	14.19	0.67/ 1.92/ 0.88	1117.92/ 3200.67/ 1459.43	N
Pagothenia borchgrevinki	P	0.84	3.995	8.5	0.6/ 2.96/ 2.21	1005.12/ 4934.21/ 3691.52	J
Trematomus newnesi	P	0.79	7.41	9.62	1.47/ NA/ 10.45	2102.25/ NA/ 14930.72	J
Notothenia rossii	I	0.59	6.88	16.66	1.46/ 5.6/ 11.4	2429.08/ 8428.19/ 19002.66	N
Trematomus eulepidotus	I	0.99	11.28	23.85	4.27/ 3.94/ 5.57	8530.21/ 7870.09/ 11137.46	N
Gobionotothen gibberifrons	B	0.82	17.88	37.4	0.09/ 10.82/ 3.66	121.85/ 15453.78/ 5231.09	N
Gymnodraco acuticeps	B	0.85	24.37	32.59	1.17/ 16/ 45.27	1956.65/ 26669.72/ 75454.82	N
Lepidonotothen nudifrons	B	0.54	16.79	23.24	0.53/ 8.56/ 36.28	1060.3/ 17115.58/ 72557.79	N
Pagetopsis macropterus	B	NA	NA	NA	NA/ NA/ NA	NA/ NA/ NA	N
Trematomus bernacchii	B	0.51	2.1	4.28	0.31/ 1.14/ 1.17	515.74/ 1900.11/ 1954.4	J
Trematomus hansoni	B	0.91	6.95	15.1	1.74/ 2.77/ 1.22	3486.43/ 5532.36/ 2434.24	N
Trematomus nicolai	B	0.67	5.23	8.56	0.42/ 4.33/ NA	692.25/ 7223.48/ NA	N
Trematomus pennellii	B	0.52	1.4	1.8	0.47/ 2.8/ 1.28	790.96/ 4661.02/ 2139.83	J

E-eco-group. hd—haplotype diversity. π—nucleotide diversity. W—Watterson's estimate of theta per site. T(ex) (DSP)/ T(ex) (S)/ T(ex) (A)*103—time of the expansion estimated in DnaSP/ SITES/ Arlequin in mutational time units. T(ex) (DSP)/ T(ex) (S)/ T(ex) (A) (Kya)–absolute time of the expansion estimated in DnaSP/ SITES/ Arlequin in Kya. For time of expansion estimated in Arlequin are confidence intervals in brackets. DS—Data source (N—new in this study. J—Janko et.al. 2007)

doi:10.1371/journal.pone.0138766.t002

GLMs incorporating eco-group, sample size, sampling region, and geographical transect as a parameter always produced a lower AIC value than the null model (Table 3). Moreover, the ΔAIC was significant in F-tests in ANOVA for the parameter π and the expansion time estimates from DnaSP. The WMW (Wilcoxon-Mann-Whitney test) test suggested significantly lower values in the benthic group for all indices except the estimate from Arlequin.

Population expansion was consistently shown in the EBSP. In particular, for those species where considerable population growth is indicated, we observed a more recent onset of population expansion in benthic species when compared with the pelagic ones. The intermediate group appeared most heterogeneous where *T. eulepidotus* showed the oldest signal of population growth (Fig 2B, see S3 Fig for demographic histories with credibility intervals). A similar picture is shown in the generalized skyline plot for cyt *b* (Fig 2A).

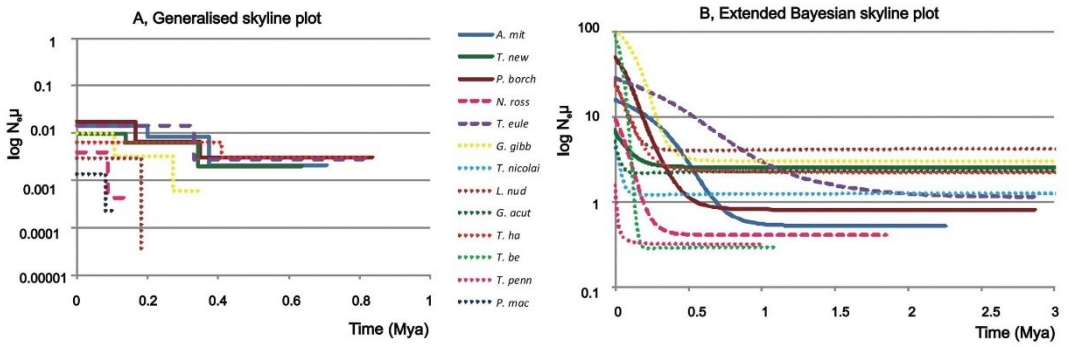


Fig 2. A- Generalised skyline plot of cyt *b* genealogies, B- mean of extended Bayesian skyline plot of combined datasets of cyt *b* and S7 gene. The vertical axis shows the estimated log of $N_e\mu$ and the horizontal axis represents time in Mya; the time is zero now. The pelagic eco-group is indicated by solid lines, intermediate by dashed lines, and benthic by dots (solid lines: blue – *Aethotaxis mitopteryx*, brown – *Pagothenia borchgrevinki*, green – *Trematomus newnesi*, dashed lines: pink – *Notothenia rossii*, violet – *Trematomus eulepidotus*, dots: yellow *Gobionotothen gibberifrons*, dark green – *Gymnodraco acuticeps*, brown – *Lepidonotothen nudifrons*, grey – *Pagetopsis macropterus*, light green – *Trematomus bernacchii*, red – *Trematomus hansoni*, light blue – *Trematomus nicolai*, pink – *Trematomus pennellii*).

doi:10.1371/journal.pone.0138766.g002

Discussion

The population size of species rarely remains unchanged over long periods. Population genetic studies in Southern Ocean species demonstrate significant deviations from neutral expectations in most of the species studied here. In previous studies this has commonly been attributed to past population fluctuations (reviewed in [5]). Consistent with this expectation, we found significantly negative values of neutrality indices in all cyt *b* datasets and in five of eight S7 datasets (Table 1) coupled with star-shaped haplotype networks (Fig 1) implying population expansion. Lack of significance in values of the HKA and McDonald-Kreitman tests (Table 1) suggest that such patterns were caused by demographic events rather than by locus-specific selection pressure or selective sweeps. Significantly positive g values from multilocus analysis by LAMARC support such a conclusion in all species except *T. nicolai* and *G. acuticeps* where the confidence intervals included 0. We should also carefully consider the signal in *G. gibberifrons* where the chains did not converge after a large number of iterations. More or less recent population growth is also suggested by EBSP (of combined unlinked loci) in all but two species from this study and two from Janko et al. [20] (*T. newnesi*, *T. pennellii*, *T. nicolai*, and *G. acuticeps*) (Fig 2B).

Coalescent genealogy samplers like LAMARC or EBSP allow very detailed insights into population histories when datasets contain sufficient variability to parameterize the underlying model or to group adjacent coalescent intervals and to estimate historical population sizes. However, in several cases LAMARC or EBSP did not detect large changes in population size although other demographic analyses (Tajima´s D, Fu´s Fs) indicated likely population expansion in these species. This highlights the value of using a number of different statistical approaches (see also below). It is likely that a number of identical sequences and limited genetic variability resulting in few coalescent events led to the loss of demographic signal [37]. In *T. pennellii T. nicolai*, and *G. acuticeps*, the generalized skyline analysis found only one composite interval, supporting this interpretation. On the other hand, the genetic variability of *T. newnesi* was large compared to other species and therefore ESBP plots show that populations of cryopelagic *T. newnesi* probably did not dramatically fluctuate in size during the Pleistocene. This is

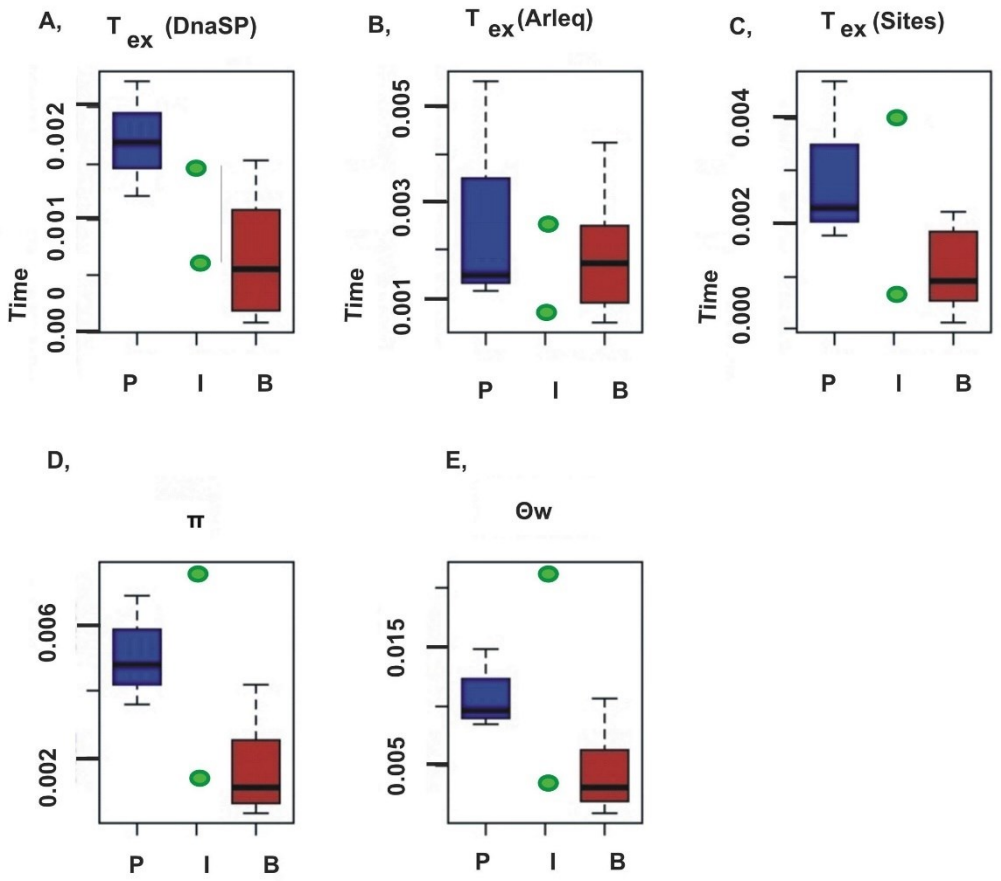


Fig 3. A- C: Comparisons of eco-groups based on cyt *b*: A—$T_{(ex)}$ DnaSP: time of the expansion onset estimated in DnaSP in mutational units, B—$T_{(ex)}$ Arlequin: time of expansion onset estimated in Arlequin in mutational units, C—$T_{(ex)}$ SITES: time of the expansion onset estimated in SITES in mutational units, D—π: nucleotide diversity of cyt *b*, E—θ_W: Watterson's estimate of theta per site for cyt *b*. The pelagic group is indicated in blue, intermediate in green and benthic in brown.

doi:10.1371/journal.pone.0138766.g003

consistent with the finding of Janko et al. [20], who reported a lack of population expansion signals in *T. newnesi*.

Sources of interspecific variability in genetic diversity and demographic patterns

We observed considerable among-species variation in parameters of genetic diversity (hd, π, θ_W) and demographic history (Table 2, Fig 3). This is typical for studies of Southern Polar fish taxa and for marine species in general [4]. However, in this study we found a clear correlation

Table 3. The effect of ecological groupings, of sample sizes (n), of sampling region (sample) and of geographical transect (dist) on the distribution of parameter values estimated from cyt *b*.

GLM Formula	AICglm1	AICglm0	ΔAIC	P	WMW
GLM eco-group					
Hd	4.736	7.136	-2.4	0.085	0.04848
π	59.134	63	-3.866	0.048	0.02424
θW	83.593	85.78	-2.187	0.092	0.04848
T(ex) (DnaSP)	25.021	29.088	-4.067	0.045	0.02424
T(ex) (Sites)	46.652	48.31	-1.658	0.11	0.04848
T(ex) (Arleq)	52.8	49.7	3.099	0.7	0.7758
GLM n					
Hd	8.3	6.33	1.97	0.9	NA
π	-114.7	-116.7	2	0.8	NA
θW	-91.7	-93.56	1.86	0.74	NA
T(ex) (DnaSP)	169.01	167.13	1.88	0.86	NA
T(ex) (Sites)	189	187.2	1.8	0.7	NA
T(ex) (Arleq)	205.75	203.77	1.98	0.9	NA
GLM sample					
Hd	8.2	6.33	1.87	0.73	NA
π	-114.8	-116.7	1.9	0.72	NA
θW	-91.6	-93.56	1.96	0.86	NA
T(ex) (DnaSP)	168.6	167.13	1.47	0.5	NA
T(ex) (Sites)	189.16	187.2	1.96	0.65	NA
T(ex) (Arleq)	205.61	203.77	1.84	0.72	NA
GLM dist					
Hd	8.1	6.33	1.77	0.69	NA
π	-114.8	-116.7	1.9	0.76	NA
θW	-91.6	-93.56	1.96	0.84	NA
T(ex) (DnaSP)	168.5	167.13	1.37	0.47	NA
T(ex) (Sites)	189.25	187.2	2.05	0.97	NA
T(ex) (Arleq)	205.67	203.77	1.9	0.78	NA

GLM Formula describes the design of each Generalised Linear Model (genetic data were fitted against eco-group, sample size (n), biogeographic provinces (sample) and distance of sampling transect (dist), AICglm1—AIC score for GLM incorporating the eco-group parameter, AICglm0—AIC score of the null model, ΔAIC—difference in AIC values between the two models, P—p value of the ANOVA F test, WMW—provides p-values of Wilcoxon-Mann-Whitney test for differences between pelagic and benthic groups. For the codes of the parameters see Table 2 and for sample sizes see S1 Table.

doi:10.1371/journal.pone.0138766.t003

of lifestyle with among-species variation. This variation is unlikely to result from either unequal geographical sampling or from geographical structuring of these species, either of which can theoretically affect demographic inference. Although AMOVA indicated that some species are structured to a significant level, GLMs incorporating sampling sizes and transect lengths as well as biogeographic coverage, did not account for the distribution of the data. Looking at expansion time estimates of significantly structured fish populations, we did not observe many differences between demographic estimates from sequences originating from particular regions and demographic estimates from total sample sizes. This further suggests that geographical structure does not significantly affect our inferences. On the other hand, partitioning the species according to their life style into the three eco-groups did account for much of the observed variability in population genetic and demographic estimators and greatly improved the model fit to the cyt *b* datasets (Table 3). Pelagic species have significantly higher

mtDNA genetic diversity (hd, π) and effective population size (θ_W) than do benthic ones (parameter values in Table 2, p values in Table 3, Fig 3). The two species with significant AMOVA test values (*T. hansoni and L. nudifrons*) belong to a benthic group. Population structure within a species tends to increase genetic diversity and inflate the expansion time, but the benthic group still proved significantly less genetically variable and had a more recent expansion time compared to the pelagic group. This supports our view that among-species differences are not caused by geographical structure but instead correlate with ecological clustering.

The higher diversities of pelagic species may be trivially explained by the higher carrying capacity of the pelagic realm than the shallow benthos—therefore supporting higher population sizes of pelagic species. However, given that the estimates of genetic diversity are sensitive to historical population fluctuations, we think that the differences reflect different demographic histories for each eco-group rather than their actual population sizes. Support for this interpretation comes from our finding that a significant portion of the variance in expansion time estimates from cyt *b* (except those from Arlequin) can be attributed to the division of species into the three eco-groups (Table 3). WMW tests also indicated significant differences among population expansion times of pelagic and benthic species. Generalized skyline plots of cyt *b* as well as EBSP analysing both loci simultaneously also show that the onset of demographic expansion of pelagic species generally started earlier than in benthic species (with the exception of *T. hansoni* in the cyt *b* dataset and *G. gibberifrons*; Fig 2, Table 2). This suggests that benthic and pelagic species tend to consistently differ in their demographic histories.

Three potential problems could complicate the role of ecology on demographic response. First, we observed disagreement between Schneider and Excoffier's estimator of population expansion time (implemented in Arlequin software [38] and other methods). The moment method of Rogers [39] implemented in DnaSP is sensitive to deviations from the infinite sites model [38] and may underestimate τ in the case of saturated DNA or multiple substitutions occurring at the same site. However, we observed the greatest discordance between τ estimates in species with very low variability, i.e. *T. pennellii* (number of segregating sites S = 2), *T. nicolai* (S = 3) and *G. acuticeps* (S = 6) (see S1 Fig) where such a problem should not occur. Low-variability datasets were also characterized by large confidence intervals around Arlequin τ estimates suggesting that this discrepancy is probably caused by insufficient variability in these datasets.

Second, stable isotope analysis by Rutschmann et al. [34] raised questions about the ecological classification of *T. nicolai*. Although there is a possibility that stable isotope analysis does not necessarily provide accurate information about the ecology of the species [40] we repeated the GLM and WMW analysis treating *T. nicolai* as a species with an unclear ecological position. We still found significant differences between pelagic and benthic species, as the corresponding GLMs always resulted in lower AIC scores than the null models for all demographic parameters (Hd, π, θ_W and estimates of population expansion times).

Finally, when analysing the S7 nuclear locus separately, we found no significant differences between pelagic and benthic species and estimated genetic variability and population expansion times were higher than those from *mt*DNA [39,41], a pattern also seen in other studies [20,42]. The most likely explanation of this finding is that the differences among loci result from the variable properties of each marker. Relatively fast-evolving mitochondrial loci such as cyt *b* [20] are likely to incorporate traces of more recent demographic events than more slowly evolving markers like S7. Moreover, substantial differences in genetic differentiation estimated from nuclear and *mt*DNA markers may arise from different modes of inheritance and ploidy variances [43]. Nuclear markers have longer coalescent times relative to mtDNA due to higher effective population sizes and are therefore expected to preserve demographic history over longer periods. It is therefore possible that deviations from the simple stepwise growth model caused by cyclical population expansions and contractions have a greater effect on the

reconstruction of demographic history from the nuclear marker than for the mitochondrial locus. Indeed, Rogers [39] demonstrated that when a population oscillated in size rather than monotonically expanded, the τ estimate of the population growth would mostly reflect the initial growth period during the coalescent history of a given marker. In any case, the combined analysis of both markers (EBSP) indicated a generally more recent onset of expansions in pelagic species.

In a summary, our comprehensive comparative analysis of thirteen fish species provides evidence that lifestyle has a major effect on population genetic structure as well as on the responses to important environmental events such as climate change.

Role of species ecology in the evolution of the Southern Polar biota

Populations of shallow benthic organisms are likely to have been reduced primarily by physical damage of benthic habitats during glacial maxima. This is likely to have included a combination of lower sea levels, grounded ice, mass wasting, and general ecological factors such as lower food availability, less advection and increased inter-specific competition [28,44]. The response of pelagic species to these processes is less clear. Thatje et al. [25] postulated that Southern Ocean pelagic organisms were probably also restricted to a few areas of local marine productivity (such as polynyas) and that the pelagic realm was somewhat reduced by complete, multi-annual sea-ice coverage resulting in decreased ecosystem productivity. On the other hand, pelagic communities might have profited from the expansion of the pelagic habitat of the Southern Ocean during glacial maxima due to the northward shift of the APF [18]. Smetacek and Nicol [44] also suggested that the apparent reduction of productivity in the seasonal sea-ice zone during cold periods may have been the result of an expansion in the populations of grazers and predators. Our data support these views and suggest that populations of closely related species that differ in their benthic and pelagic specialization had significantly different histories and population expansions most likely driven by different external stimuli.

Applying the fossil-based molecular clock calibration of Near [27] to their data led Janko et al. [20] to propose that major population expansions of the two benthic species coincided with the retreat of the grounded ice, whereas expansion of pelagic populations largely predated the LGM; consistent with our estimated expansion dates (Table 2). However, given the critique by Patarnello et al. [45] we refrain from linking absolute dating of the expansions to presumed climatic events. This is mainly because the application of molecular clocks calibrated with ancient nodes (i.e. older than several Mya as in Near [27] may lead to overestimates in the dating of recent (intraspecific) events [46] making such absolute time estimates biased, especially to recent events. Our absolute time estimates thus probably represent probable upper limits of the true values.

Nonetheless, given that the markers assessed here do not deviate from rate constancy [20], our analysis of closely related species is informative even in relative terms since the degree of overestimation due to the time-dependency of molecular clocks is negatively correlated with the true time of the event [46]. The estimates of relatively young expansion times in benthic species should thus be biased towards even more recent values than those of older events in the pelagic taxa. This suggests that the true differences between the eco-groups may be even greater than detected in our study.

Therefore, our conclusion that much of the among-species variance can be accounted for by differences in lifestyle is valid even without absolute dating. This suggests that reactions to important extrinsic events such as climate change, depend on species-specific traits. This conforms to the hypothesis that benthic populations were reduced by negative effects of glaciations

and recovered recently after these negative effects vanished. On the other hand, pelagic species probably reacted differently as shown by more ancient estimates of population expansions.

Combining current population demographic data with previously published macro-evolutionary patterns allows deeper insights into how Southern Oceanic species might have survived the Pleistocene. Although such climatic events probably increased the risk of local population extinctions—as suggested by signals of population expansion from low effective population sizes [29,42,47]–the overall net diversification rate was probably not altered. A multilocus phylogenetic analysis by Janko *et al.* [26] found no evidence for either an increase or decrease in the diversification rate of the Trematominae during the Pleistocene. This indicates that although local populations probably went extinct or were drastically reduced in size during unfavourable periods, the extinction rates of entire species overall may not have changed.

Thatje et al. [28] provided an explanation as to how species might have survived Antarctic glaciations. They suggested that asynchronous patterns of ice expansion and retreat in different Antarctic regions during glacial periods allowed the survival of benthic species by migration between temporarily available refugia along the continental shelf. Although Van de Putte et al. [48] found greater geographical structure in benthic Trematominae species compared to pelagic *T. newnesi*, and Damerau et al. [12] reported restricted gene flow among populations of *Lepidonotothen larseni*, the population structure of notothenioid species is generally weak [12,14,36,48–51]. This supports the view that these species had a sufficiently high dispersal rate to allow effective migration among temporarily available refugia along the continental shelf. Combined microevolutionary (this study) and macroevolutionary [26] analysis of Trematominae is therefore in line with Thatje et al.'s [28] hypothesis and suggests that species that survived inhospitable periods and glacier-induced extinctions may have experienced significant local effects.

Overall, our data support the hypothesis that benthic species contain both lower genetic diversity and were subject to more recent major population expansions than pelagic species. This may reflect population reductions during glacial periods followed by population expansions during climate warming. These findings indicate that the ecological specialization of a species may have an important effect on its response to climatic shifts.

Materials and Methods

Sampling

Approval to catch fish to take fin clips and to return the fish alive was granted by the Animal Ethics Committee of the University of Otago AEC 56/2002 and the Ministry of Foreign Affairs and Trade who issued permits under the Antarctic Marine Living Resources Act. Collecting samples was also permitted by the Ministry of Environment of the Czech Republic, as the competent governmental authority pursuant to Sections 8 and 24 of Act No. 276/2003 Coll.

Samples of shelf notothenioid fish were collected by bottom trawl or fishing rod from eight Antarctic locations including the Antarctic Peninsula, maritime Antarctic and Ross Sea area (see Fig 4 and S1 Table) during the cruise ANT-XXIII/8 with RV Polarstern in 2006, and during expeditions supported by Antarctica New Zealand (event K066), and the Ukrainian Antarctic programs. The data for four species from Janko et al. [20] were added to the analyses described here (two cryopelagic species: *Pagothenia borchgrevinki*, *Trematomus newnesi*, and two benthic species: *Trematomus pennellii* and *Trematomus bernacchii*).

Species were allocated into one of three ecological groups (eco-groups) according to their lifestyle (see S2 Table) determined by such characteristics as adult foraging behaviour, spawning strategy, egg type, and larval and juvenile dependence on the marine bottom (substrate). Guarding and incubation time of benthic eggs was judged as a key trait for the benthic eco-

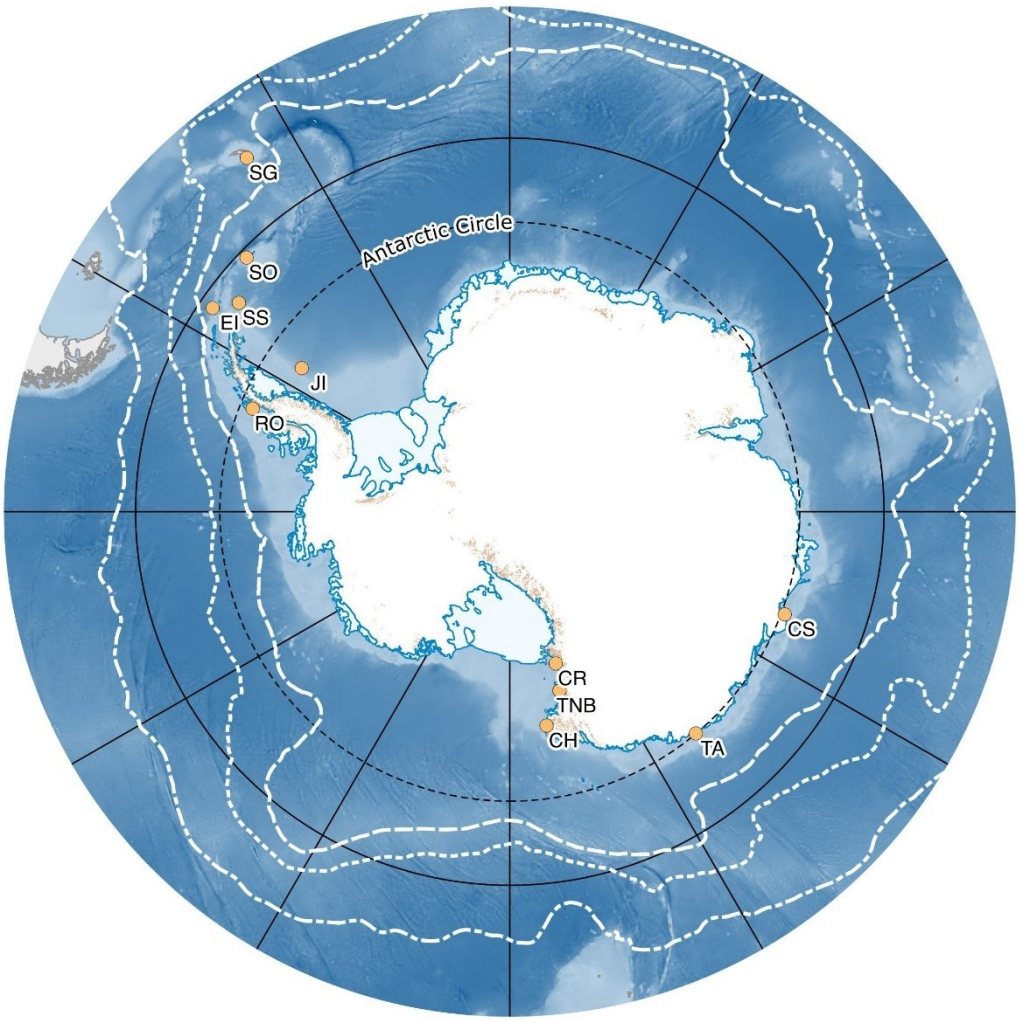


Fig 4. Map of Antarctica showing sampling locations. SO—South Orkney, SG—South Georgia, EI—Elephant Island, SS—South Shetlands, JI—Joinville Island, CR—Cape Roberts, CH—Cape Hallett, TA—Terre Adelie, TNB—Terra Nova Bay, Rothera—Rothera research station, CS—Casey, CA—Cape Armitage.

doi:10.1371/journal.pone.0138766.g004

group classification [52] because it predetermines the vital dependence of the species on available benthic habitat.

The pelagic/cryopelagic (P) eco-group comprises species foraging in the water column and with pelagic eggs (*Aethotaxis mitopteryx*, *Pagothenia borchgrevinki*, *Trematomus newnesi*).

The benthic eco-group (B) comprises species foraging in the benthos and with benthic eggs (*Trematomus hansoni*, *Trematomus nicolai*, *Gobionotothen gibberifrons)* and species known to guard nests (*Lepidonotothen nudifrons*, *Gymnodraco acuticeps*, *Pagetopsis macropterus*, *Trematomus pennellii*, *Trematomus bernacchii*).

Finally, the intermediate (I) eco-group was developed for species where the classification is either ambiguous or unknown and so were classified separately. This group includes *Trematomus eulepidotus*, which is known to forage in the water column but is reproductively bound to the bottom as it lays eggs in sponges, and *Notothenia rossii*, where there is a switch from pelagic eggs to benthic juveniles and semipelagic adults (see S2 Table).

The classification of *Trematomus nicolai* is not without ambiguity, since stable isotope analysis by Rutschmann [34] suggested that it is pelagic. However, given the limitations of the isotope-based inference [40] we have used the traditional benthic classification of *Trematomus nicolai* [53] (see S3 Table), further supported by its low buoyancy comparable to other clearly benthic fish [54].

Molecular markers and techniques

Two loci were PCR amplified; the mtDNA gene for the cyt *b*, and the first intron in the nuclear S7 gene. Cyt *b* was amplified according to two species-specific amplification protocols and primer combinations (see S2 Table) that gives an approximately 600 bp fragment. Approximately 700 bp of the S7 intron were amplified according to the protocol of Janko et al. [20]. Homologous sequences were aligned using CLUSTALX software and any ambiguities resolved by inspection of the sequencing chromatograms. Sequences were deposited in the GenBank database, accession numbers: cyt *b*, KC153538—KC153654; S7 intron, KC520782- KC520827, KC833756—KC833898. The S7 intron sequences of *Notothenia rossii* and *Lepidonotothen nudifrons* were not long enough to be deposited in the Genbank, as the length of the longest fragment without recombination was below the minimum length required by NCBI (>200 bp) (enclosed as S3 Table).

Phase resolution

Recombination may complicate both the resolution of gametic phases and the inference of demography. To avoid this problem, we initially aligned only S7 sequences from homozygous individuals within each species-specific dataset and applied the "four-gamete test" [55] implemented in DnaSP v. 5.0 [56] to detect potential recombination events. When intralocus recombination was detected, we discarded the sites left or right of the putative recombination events as appropriate in order to retain the longest possible contiguous unrecombined sequence. We subsequently used a Bayesian statistical method [57] provided by fastPHASE implemented in DnaSP to reconstruct the gametic phases of heterozygous individuals in the unrecombined alignment. For a detailed explanation see Janko et al. [26].

Detection of selection and potential geographic structure

To test whether the variability of each locus was significantly affected by selection rather than by demographic processes, we performed two tests. The McDonald-Kreitman test [58] assesses whether the ratio of silent to replacement polymorphisms in the cyt *b* is the same as the ratio of silent to replacement fixed differences. The significance of departures from neutral expectations was evaluated by the 'G test of independence' implemented in DnaSP. We also used the HKA test [59] using both cyt *b* and S7 data for each species. This test compares the observed number of segregating polymorphisms within species to the number of differences between species. In a neutral case, the number of intraspecific polymorphisms and interspecific

divergence are correlated in all loci. The significance of the sums of the deviations was evaluated against a neutral distribution generated from 1000 coalescent simulations with the software HKA. In all cases, *Eleginops maclovinus*, a basal species to all AFGP-containing notothenioids [27] was used as an outgroup. The significance of the outcome (0.05) was evaluated after sequential Bonferroni correction for multiple testing [60].

We used Analysis of Molecular Variance (AMOVA) [61] implemented in Arlequin to test for potential geographic subdivision of examined species using the best-fitting model of sequence evolution for each species. We estimated the level of genetic structuration within populations, between populations within a region (in all species sampled at at least two sites) and between regions (wherever sampling was from separate regions). We defined the regions according to biogeographic provinces defined by Barnes et al. [62].

Genetic diversity and inferences of past population size change

Each alignment was characterized by haplotype diversity (hd), mean nucleotide divergence (π) and Watterson's estimate of effective population size θ_W estimated with the program DnaSP. In order to robustly evaluate possible differences among eco-groups, we adopted several approaches to test whether the populations of studied species remained constant in size or fluctuated over their demographic history. For all species, minimum spanning networks were reconstructed using the algorithm of statistical parsimony implemented in the TCS program, version 1.06 [63]. Recent population expansions were expected to show star-like phylogenetic patterns in this analysis.

Neutrality tests are often used to test population stability due to their sensitivity to past population size changes that may cause deviations from the mutation-drift equilibrium. Tajima's D [64] and Fu's F_S test [65] are considered to be two of the strongest tools in detecting traces of past population expansion [56] and were performed in DnaSP with 1000 coalescent simulations to evaluate whether the observed values might result from a population in mutation-drift equilibrium. The significance of p-values was evaluated by sequential Bonferroni corrections for multiple testing [60].

Estimates of times of the major demographic events were obtained by fitting models of sudden population expansion using two methods. First, we used mismatch distribution for the timing of expansion using the parameter τ [39] with a stepwise population growth model. The relationship between absolute time (t) and τ is: $t = \tau/2\mu$ (where μ equals the substitution rate per locus, rather than per site). Values of τ were estimated by two programs; DnaSP and Arlequin version 3.11 [66]. The latter program also allows an estimate of τ [38] with confidence intervals obtained by a parametric bootstrap approach based on 1000 replicates.

Second, the maximum likelihood method implemented in SITES [67] takes into account the distribution of polymorphisms in a population and estimates three parameters of a model of sudden population expansion, these are $\theta_{ancestral}$, θ_{final} and τ.

Due to known problems in dating of recent events in general [46] and in the application of molecular clocks to notothenioids in particular [27], we have used mutational time units to compare expansion times estimates among species (see Discussion). Since expansion times provided by mismatch analyses and SITES are scaled by per-locus mutation rates, rather than per site, we divided our τ values by the locus sequence length in each species to obtain comparable values. We also used the estimates of branch-specific mutation rates inferred with BEAST (see below how BEAST was used) to translate the mutation time estimates into absolute dating.

Likelihood Analysis with Metropolis Algorithm using Random Coalescence, LAMARC *2.1.10* [68] was used to estimate effective population exponential growth rate. To reduce a bias of growth rate (g) both genes were analysed simultaneously. The relative effective population size was set to 1 for mitochondrial gene and to 4 for nuclear. These settings allowed us to

estimate Θ on the *mt*DNA scale. The relative mutational rate used was four times higher for cyt *b* than for S7 gene.

LAMARC analyses were performed more than once with a different random number seed each time to assess whether 95% of the ML point estimates fell within each other's confidence intervals. LAMARC was run for at least 400 000 iterations, and longer in datasets where convergence was difficult to reach.

As the final approach, the demographic history of the sampled DNA sequences was investigated through two versions of skyline plots; the generalized skyline plot and the extended Bayesian skyline plot (EBSP). The generalised skyline plot [37] analyses each locus separately and is helpful in testing whether the variability of datasets is sufficient for reconstruction of more than one coalescence interval. We generated ultrametric genealogies for each species in PAUP* 4.0 [66] under the strict clock model and used Genie v3.0 [69] for skyline plot reconstruction. The optimal value of the parameter ε, which controls the grouping of adjacent coalescence intervals, was chosen by maximizing the log L_{AICc} function of the skyline plot. Since each locus was analysed separately for each species, the population size estimates of the generalized skyline plots are scaled in mutation time units.

The EBSP [70] implemented in BEAST v. 1.8.0. [71] enables the combination of multiple loci for population dynamics assessment, which can reduce the variability in estimating the population size function. The combination of both loci requires an estimate of relative locus-specific substitution rates in each species. To assess the substitution rate estimates of each marker, a reduced dataset of cyt *b* and S7 was produced. Datasets of both markers, cyt *b* and S7, comprised one sequence chosen at random from each of the thirteen studied species and one sequence of the basal Trematominae species *Trematomus scotti* [26]. The divergence time of *T. scotti* from the remaining Trematominae was used according to the timing of Near [27]. The sequence evolution model was set to HKY (estimated by jModelTest v 2.1.2 [72] based on the Akaike information criterion (AIC)). Given that Janko et al. [26] found no evidence for significant background extinction rate in Trematominae, a Yule process of speciation was used with an uncorrelated lognormal relaxed clock. The normally-distributed priors for uncorrelated lognormal relaxed clock mean substitution rate were applied: 0.98% for cyt *b* and 0.28% for S7 gene per site per Mya with standard deviation of 0.5 for cyt *b* and 1 for S7 marker. The standard deviation of the mean was left at the default. Four independent MCMC runs for each locus and species were performed with the length set to 10^7 and MCMC chain convergence was assessed by Tracer 1.5 [73]. Runs were subsequently combined with LogCombiner using 10% burnin, and substitution rate of the terminal branch leading to a species was recorded for each of thirteen species for both markers.

Branch-specific median substitution rates and corresponding 95% HPD intervals obtained with BEAST were used as clock rate priors with normal distribution in the subsequent EBSP analyses combining both loci in each species. Substitution rates for the nuclear locus were scaled relative to the mtDNA rate. A strict clock [74] and piecewise linear model was applied. The best-fitting substitution model was estimated using AIC in jMmodelTest v2.1.2. [72]. The length of the MCMC chain was set to 5×10^8 and convergence was assessed using Tracer 1.5. Due to the combination of two loci with different mutation rates, the EBSP were scaled in years as provided by BEAST rather than in mutation time units.

The effects of ecological specialization and geographical sampling on estimates of genetic diversity

To evaluate whether differences in genetic diversity and demographic patterns among species are caused by unequal sampling bias or by differences among fish eco-groups, we characterized

each species by four traits: a) number of samples per species, b) length of sample transect measured as a coastline distance with Google Earth [75] c) the biogeographic provinces covered by the sampling (provinces were defined according to more detailed biogeographic segmentation [62]: Eastern Antarctica, Antarctic Peninsula and the Scotia Arc) and d) the eco-group.

Generalized linear models (GLM) with Gaussian error distribution were used to detect traits explaining among-species variability in genetic indices and demographic patterns. We performed GLM forward selection starting from the null model expecting no dependence of measured values on species-specific traits. To do so, the best model was considered to be that with the lowest AIC score and we evaluated its significance by ANOVA using the F test. We also used a non-parametric Wilcoxon-Mann-Whitney test (WMW) to test the differences between the groups of clearly benthic and pelagic species (i.e. not considering the intermediate group).

Supporting Information

S1 Fig. Analyses showing the discrepancies among time of expansion in mutational units estimated from different demographic methods from cyt *b*. The Y-axis shows the logarithm of the ratio of expansion time estimates (in mutational time units) obtained with different methods (DSP—DnaSP, S—SITES, A—Arlequin); X-axis indicates the number of segregating sites within the dataset. The species with highest discrepancies between Arlequin and other estimates are denoted by coloured points: red–*Trematomus pennellii*, green–*Trematomus nicolai*, blue–*Gymnodraco acuticeps*.
(TIF)

S2 Fig. Skyline plot of S7 intron genealogies of the pelagic, intermediate, benthic eco-group. The vertical axis shows estimated $N_e\mu$ and the horizontal axis represents time in mutational time units; time is zero in the present. Low variability at this locus resulted in resolution of just one composite interval for several species (*T. bernacchii*, *T. pennellii*, *G. acuticeps*, *L. nudifrons* and *P. macropterus*), thus preventing their demographic reconstruction.
(PDF)

S3 Fig. Demographic changes of studied species estimated by EBSP showing changes in effective population size (Neμ) and credibility intervals over time (measured in Mya). The Y- axis shows estimated effective population size (Neμ), the X-axis represents time in Mya, time is zero at the present. *Pagetopsis macropterus* is not included in the analysis because of the high number of recombination sites in the S7 dataset.
(PDF)

S1 File. Table A: AMOVA analysis of all newly sequenced species with specimens from more than one locality (*Aethotaxis mitopteryx* was sampled just at EI) (supplement of Janko et al. (2007)); *Trematomus hansoni* geographic subdivision was used according to the structure found by Van de Putte et al. (2012) and locality JI with two samples for *Lepidonotothen nudifrons* was omitted. **Table B:** neutrality tests for species with identified geographical structure separately for both markers. E—eco-group (P—pelagic, I—intermediate, B—benthic). P value—P value of certain fixation indices. Locality: SO—South Orkney, SG—South Georgia, EI—Elephant Island, SS—South Shetlands, JI—Joinville Island, CR—Cape Roberts, CH—Cape Hallett, TA—Terre Adelie, Rothera—Rothera research station, CS—Casey. N—number of haplotypes. L—length in base pairs of the non-recombined locus. hd—haplotype diversity. π – nucleotide diversity. ΘW—Watterson's estimate of Theta per site. T(ex) (DSP)/ T(ex) (S)/ T (ex) (A)$^{*}10^{3}$ –time of the expansion estimated in DnaSP/ SITES/Arlequin in mutational time units. T(ex) (DSP)/T(ex) (S)/T(ex) (A) (Kya)–absolute time of the expansion estimated in DnaSP/SITES/Arlequin in Kya. NS—not significantly different from zero. #–values significant

at $\alpha = 0.05$ after sequential Bonferroni correction. * model fits poorly.
(XLSX)

S1 Table. Specimen numbers for cyt *b*/ S7 sampled at different locations. (see Fig 4 captions for location details).
(XLSX)

S2 Table. References to eco-groups characteristics and primers used in this work. The amplification protocols and primers used for cyt *b* are indicated by references: [1], [2], Reference to E—references to eco-groups integration. The S7 intron was amplified using primers S7RPEX1F and S7RPEX2R [3].
(DOCX)

S3 Table. *Notothenia rossii* and *Lepidonotothen nudifrons* haplotypes of S7 intron without recombination events.
(TXT)

Acknowledgments

We are grateful to Michael E. Hellberg, Huw J. Griffiths, Joseph Eastman and Karl-Hermann Kock for useful comments on this manuscript. We thank Academic Editor Lorenzo Zane, referee M. Matschinner and one anonymous referee for constructive and helpful comments.

Thanks to Catherine Ozouf-Costaz and Jenny Rock for providing us with material from the Terre Adélie station, Dumont d'Urville. We also thank the French polar institute "Institut Paul Emile Victor" for technical support and the IPEV program 281 (ICOTA). We thank Antarctica New Zealand and the Latitudinal Gradients Project for supporting the work of K066 and the Alfred Wegener Institute, Bremerhaven, Germany, for supporting the Polarstern Cruise ANT XXIII-8 and the Ukrainian National Antarctic Scientific Centre.

Author Contributions

Conceived and designed the experiments: KJ CM AVP EK. Performed the experiments: EK. Analyzed the data: EK KJ. Contributed reagents/materials/analysis tools: KJ. Wrote the paper: KJ CM AVP EK.

References

1. Krosby M, Rohwer S. A 2000 km genetic wake yields evidence for northern glacial refugia and hybrid zone movement in a pair of songbirds. Proc R Soc B. 2009; 276: 615–621. doi: 10.1098/rspb.2008.1310 PMID: 18986973
2. Fauvelot C, Bernardi G, Planes S. Reductions in the mitochondrial DNA diversity of coral reef fish provide evidence of population bottlenecks resulting from Holocene sea-level change. Evolution. 2003; 57: 1571–1583. PMID: 12940362
3. Lessa EP, Cook JA, Patton JL. Genetic footprints of demographic expansion in North America, but not Amazonia, during the Late Quaternary. PNAS. 2003; 100: 10331–10334. PMID: 12913123
4. Hellberg ME. Gene Flow and Isolation among Populations of Marine Animals. Annual Review of Ecology, Evolution, and Systematics. 2009; 40: 291–310.
5. Rogers AD. Evolution and Biodiversity of Antarctic Organisms. In: Rogers AD, Johnston NM, Murphy EJ, Clarke A, editors. Antarctic Ecosystems. John Wiley & Sons, Ltd; 2012. pp. 417–467
6. Chown SL, Sinclair BJ, Leinaas HP, Gaston KJ. Hemispheric asymmetries in biodiversity—a serious matter for ecology. PLoS Biol. 2004; 2: e406. PMID: 15547645
7. Ehrich D, Alsos IG, Brochmann C. Where did the northern peatland species survive the dry glacials: cloudberry (*Rubus chamaemorus*) as an example. Journal of Biogeography. 2008; 35: 801–814.
8. Sabovljević M, Frahm J-P. Genetic diversity of the relict moss Rhytidium rugosum (Hypnales) in Europe inferred from the ITS region (nrDNA). Biologia. 2011; 66: 42–49.

9. Raffi S. The significance of marine boreal molluscs in the Early Pleistocene faunas of the Mediterranean area. Palaeogeography, Palaeoclimatology, Palaeoecology. 1986; 52: 267–289.
10. Aronson RB, Thatje S, Clarke A, Peck LS, Blake DB, Wilga CD, et al. Climate Change and Invasibility of the Antarctic Benthos. Annual Review of Ecology, Evolution, and Systematics. 2007; 38: 129–154.
11. Strugnell JM, Watts PC, Smith PJ, Allcock AL. Persistent genetic signatures of historic climatic events in an Antarctic octopus. Mol Ecol. 2012; 21: 2775–2787. doi: 10.1111/j.1365-294X.2012.05572.x PMID: 22494503
12. Damerau M, Matschiner M, Salzburger W, Hanel R. Comparative population genetics of seven notothenioid fish species reveals high levels of gene flow along ocean currents in the southern Scotia Arc, Antarctica. Polar Biol. 2012; 35: 1073–1086.
13. Hemery LG, Eléaume M, Roussel V, Améziane N, Gallut C, Steinke D, et al. Comprehensive sampling reveals circumpolarity and sympatry in seven mitochondrial lineages of the Southern Ocean crinoid species *Promachocrinus kerguelensis* (Echinodermata). Mol Ecol. 2012; 21: 2502–2518. doi: 10.1111/j.1365-294X.2012.05512.x PMID: 22469156
14. Papetti C, Pujolar JM, Mezzavilla M, La Mesa M, Rock J, Zane L, et al. Population genetic structure and gene flow patterns between populations of the Antarctic icefish *Chionodraco rastrospinosus*. Journal of Biogeography. 2012; 39: 1361–1372.
15. Marino IAM, Benazzo A, Agostini C, Mezzavilla M, Hoban SM, Patarnello T, et al. Evidence for past and present hybridization in three Antarctic icefish species provides new perspectives on an evolutionary radiation. Mol Ecol. 2013; 22: 5148–5161. doi: 10.1111/mec.12458 PMID: 23962255
16. Wilson NG, Schrödl M, Halanych KM. Ocean barriers and glaciation: evidence for explosive radiation of mitochondrial lineages in the Antarctic sea slug *Doris kerguelenensis* (Mollusca, Nudibranchia). Mol Ecol. 2009; 18: 965–984. doi: 10.1111/j.1365-294X.2008.04071.x PMID: 19207248
17. Fraser CI, Nikula R, Spencer HG, Waters JM. Kelp genes reveal effects of subantarctic sea ice during the Last Glacial Maximum. PNAS. 2009; 106: 3249–3253. doi: 10.1073/pnas.0810635106 PMID: 19204277
18. Anderson RF, Chase Z, Fleisher MQ, Sachs J. The Southern Ocean's biological pump during the Last Glacial Maximum. Deep-Sea Res Part II-Top Stud Oceanogr. 2002; 49: 1909–1938.
19. González-Wevar CA, David B, Poulin E. Phylogeography and demographic inference in *Nacella* (Patinigera) *concinna* (Strebel, 1908) in the western Antarctic Peninsula. Deep Sea Research Part II: Topical Studies in Oceanography. 2011; 58: 220–229.
20. Janko K, Lecointre G, DeVries A, Couloux A, Cruaud C, Marshall C. Did glacial advances during the Pleistocene influence differently the demographic histories of benthic and pelagic Antarctic shelf fishes?—Inferences from intraspecific mitochondrial and nuclear DNA sequence diversity. BMC Evol Biol. 2007; 7: 220. PMID: 17997847
21. Arango CP, Soler-Membrives A, Miller KJ. Genetic differentiation in the circum—Antarctic sea spider *Nymphon australe* (Pycnogonida; Nymphonidae). Deep Sea Research Part II: Topical Studies in Oceanography. 2011; 58: 212–219.
22. Diaz A, Feral J-P, David B, Saucede T, Poulin E. Evolutionary pathways among shallow and deep-sea echinoids of the genus *Sterechinus* in the Southern Ocean. Deep-Sea Res Part II-Top Stud Oceanogr. 2011; 58: 205–211.
23. Raupach MJ, Thatje S, Dambach J, Rehm P, Misof B, Leese F. Genetic homogeneity and circum-Antarctic distribution of two benthic shrimp species of the Southern Ocean, *Chorismus antarcticus* and *Nematocarcinus lanceopes*. Mar Biol. 2010; 157: 1783–1797.
24. McGaughran A, Torricelli G, Carapelli A, Frati F, Stevens MI, Convey P, et al. Contrasting phylogeographical patterns for springtails reflect different evolutionary histories between the Antarctic Peninsula and continental Antarctica. Journal of Biogeography. 2010; 37: 103–119.
25. Thatje S, Hillenbrand C-D, Mackensen A, Larter R. Life hung by a thread: endurance of Antarctic fauna in glacial periods. Ecology. 2008; 89: 682–692. PMID: 18459332
26. Janko K, Marshall C, Musilova Z, Van Houdt J, Couloux A, Cruaud C, et al. Multilocus analyses of an Antarctic fish species flock (Teleostei, Notothenioidei, Trematominae): Phylogenetic approach and test of the early-radiation event. Mol Phylogenet Evol. 2011; 60: 305–316. doi: 10.1016/j.ympev.2011.03.008 PMID: 21402163
27. Near TJ. Estimating divergence times of notothenioid fishes using a fossil-calibrated molecular clock. Antarctic Science. 2004; 16: 37–44.
28. Thatje S, Hillenbrand C-D, Larter R. On the origin of Antarctic marine benthic community structure. Trends in Ecology & Evolution. 2005; 20: 534–540. PMID: 16701431
29. Allcock AL, Strugnell JM. Southern Ocean diversity: new paradigms from molecular ecology. Trends in Ecology & Evolution. 2012; 27: 520–528. doi: 10.1016/j.tree.2012.05.009 PMID: 22727016

30. Kaiser S, Brandao SN, Brix S, Barnes DKA, Bowden DA, Ingels J, et al. Patterns, processes and vulnerability of Southern Ocean benthos: a decadal leap in knowledge and understanding. Mar Biol. 2013; 160: 2295–2317.
31. Eastman JT. The nature of the diversity of Antarctic fishes. Polar Biol. 2005; 28: 93–107.
32. Near TJ, Dornburg A, Kuhn KL, Eastman JT, Pennington JN, Patarnello T, et al. Ancient climate change, antifreeze, and the evolutionary diversification of Antarctic fishes. PNAS. 2012; 201115169. doi: 10.1073/pnas.1115169109 PMID: 22331888
33. Eastman JT, McCune AR. Fishes on the Antarctic continental shelf: evolution of a marine species flock? J Fish Biol. 2000; 57: 84–102.
34. Rutschmann S, Matschiner M, Damerau M, Muschick M, Lehmann MF, Hanel R, et al. Parallel ecological diversification in Antarctic notothenioid fishes as evidence for adaptive radiation. Mol Ecol. 2011; 20: 4707–4721. doi: 10.1111/j.1365-294X.2011.05279.x PMID: 21951675
35. Gould SJ, Lewontin RC. The Spandrels of San Marco and the Panglossian Paradigm: A Critique of the Adaptationist Programme. Proc R Soc Lond B. 1979; 205: 581–598. PMID: 42062
36. Zane L, Marcato S, Bargelloni L, Bortolotto E, Papetti C, Simonato M, et al. Demographic history and population structure of the Antarctic silverfish *Pleuragramma antarcticum*. Mol Ecol. 2006; 15: 4499–4511. PMID: 17107479
37. Strimmer K, Pybus OG. Exploring the Demographic History of DNA Sequences Using the Generalized Skyline Plot. Mol Biol Evol. 2001; 18: 2298–2305. PMID: 11719579
38. Schneider S, Excoffier L. Estimation of Past Demographic Parameters From the Distribution of Pairwise Differences When the Mutation Rates Vary Among Sites: Application to Human Mitochondrial DNA. Genetics. 1999; 152: 1079–1089. PMID: 10388826
39. Rogers AR, Harpending H. Population growth makes waves in the distribution of pairwise genetic differences. Mol Biol Evol. 1992; 9: 552–569. PMID: 1316531
40. Post DM. Using stable isotopes to estimate trophic position: Models, methods, and assumptions. Ecology. 2002; 83: 703–718.
41. Rogers AR. Genetic Evidence for a Pleistocene Population Explosion. Evolution. 1995; 49: 608–615.
42. Eytan RI, Hellberg ME. Nuclear and mitochondrial sequence data reveal and conceal different demographic histories and population genetic processes in Caribbean reef fishes. Evolution. 2010; 64: 3380–3397. doi: 10.1111/j.1558-5646.2010.01071.x PMID: 20584072
43. Buonaccorsi VP, McDowell JR, Graves JE. Reconciling patterns of inter-ocean molecular variance from four classes of molecular markers in blue marlin (*Makaira nigricans*). Mol Ecol. 2001; 10: 1179–1196. PMID: 11380876
44. Smetacek V, Nicol S. Polar ocean ecosystems in a changing world. Nature. 2005; 437: 362–368. PMID: 16163347
45. Patarnello T, Verde C, di Prisco G, Bargelloni L, Zane L. How will fish that evolved at constant sub-zero temperatures cope with global warming? Notothenioids as a case study. Bioessays. 2011; 33: 260–268. doi: 10.1002/bies.201000124 PMID: 21290397
46. Ho SYW, Phillips MJ, Cooper A, Drummond AJ. Time Dependency of Molecular Rate Estimates and Systematic Overestimation of Recent Divergence Times. Mol Biol Evol. 2005; 22: 1561–1568. PMID: 15814826
47. Dambach J, Thatje S, Rödder D, Basher Z, Raupach MJ. Effects of Late-Cenozoic Glaciation on Habitat Availability in Antarctic Benthic Shrimps (Crustacea: Decapoda: Caridea). PLoS ONE. 2012; 7: e46283. doi: 10.1371/journal.pone.0046283 PMID: 23029463
48. Van de Putte AP, Janko K, Kasparova E, Maes GE, Rock J, Koubbi P, et al. Comparative phylogeography of three trematomid fishes reveals contrasting genetic structure patterns in benthic and pelagic species. Mar Genom. 2012; 8: 23–34.
49. Bortolotto E, Bucklin A, Mezzavilla M, Zane L, Patarnello T. Gone with the currents: lack of genetic differentiation at the circum-continental scale in the Antarctic krill *Euphausia superba*. BMC Genetics. 2011; 12: 32. doi: 10.1186/1471-2156-12-32 PMID: 21486439
50. Matschiner M, Hanel R, Salzburger W. Gene flow by larval dispersal in the Antarctic notothenioid fish *Gobionotothen gibberifrons*. Mol Ecol. 2009; 18: 2574–2587. doi: 10.1111/j.1365-294X.2009.04220.x PMID: 19457182
51. Papetti C, Susana E, Mesa ML, Kock K-H, Patarnello T, Zane L. Microsatellite analysis reveals genetic differentiation between year-classes in the icefish *Chaenocephalus aceratus* at South Shetlands and Elephant Island. Polar Biol. 2007; 30: 1605–1613.
52. Kock KH, Pshenichnov LK, Devries AL. Evidence for egg brooding and parental care in icefish and other notothenioids in the Southern Ocean. Antarct Sci. 2006; 18: 223–227.

Hydrobiologia
DOI 10.1007/s10750-015-2463-2

BIOLOGY OF THE ROSS SEA

Antarctic bdelloid rotifers: diversity, endemism and evolution

N. S. Iakovenko · J. Smykla · P. Convey · E. Kašparová · I. A. Kozeretska · V. Trokhymets · I. Dykyy · M. Plewka · M. Devetter · Z. Duriš · K. Janko

Received: 9 March 2015 / Revised: 17 July 2015 / Accepted: 25 July 2015
© European Union 2015

Abstract Antarctica is an isolated continent whose conditions challenge the survival of living organisms. High levels of endemism are now known in many Antarctic organisms, including algae, tardigrades, nematodes and microarthropods. Bdelloid rotifers are a key, widespread and abundant group of Antarctic microscopic invertebrates. However, their diversity, regional distribution and endemism have received little attention until recently. We provide the first authoritative review on Antarctic Bdelloidea, based on published data and new collections. Our analysis reveals the extreme levels of bdelloid endemism in Antarctica. Sixty-six bdelloid morphospecies are now confirmed from the continent, and 83–91 putative species are identified using molecular approaches (depending on the delimitation method used). Twelve previously unknown species are described based on both morphology and molecular analyses. Molecular analyses indicate that only two putative species found in Antarctica proved to be truly cosmopolitan. The level of endemism based on the available data set (95%) is higher than that in any other continent, with many bdelloid species occurring only in maritime or continental Antarctica. These findings are consistent with the long-term presence of Bdelloidea in Antarctica, with their considerable isolation facilitating intraregional radiation, providing further evidence that does not support the microbial global ubiquity hypothesis that "everything is everywhere."

Guest editors: Diego Fontaneto & Stefano Schiaparelli / Biology of the Ross Sea and Surrounding Areas in Antarctica

Electronic supplementary material The online version of this article (doi:10.1007/s10750-015-2463-2) contains supplementary material, which is available to authorized users.

N. S. Iakovenko · Z. Duriš · K. Janko
Department of Biology and Ecology, Faculty of Science, University of Ostrava, Chittussiho 10, 71000 Ostrava, Czech Republic

N. S. Iakovenko (✉)
Department of Invertebrate Fauna and Systematics, Schmalhausen Institute of Zoology NAS of Ukraine, Bogdana Khmelnits'kogo Str. 15, Kiev 01601, Ukraine
e-mail: rotifer1@gmail.com

J. Smykla
Department of Biodiversity, Institute of Nature Conservation, Polish Academy of Sciences, Mickiewicza 33, 31-120 Kraków, Poland

Present Address:
J. Smykla
Department of Biology and Marine Biology, University of North Carolina Wilmington, 601 S. College Rd., Wilmington, NC, USA

P. Convey
British Antarctic Survey, NERC, High Cross, Madingley Road, Cambridge CB3 0ET, UK

E. Kašparová · K. Janko
Laboratory of Fish Genetics, Institute of Animal Physiology and Genetics AS ČR, Rumburská 89, 27721 Liběchov, Czech Republic

Published online: 05 October 2015

Keywords Bdelloidea · DNA taxonomy · Molecular biogeography · 4× rule · Generalized mixed Yule coalescent · Poisson tree processes

Introduction

Antarctica's ecosystems are characterized by the challenges of extreme environmental stresses, including low temperatures, desiccation and high levels of solar radiation, all of which have led to the evolution and expression of well-developed stress tolerance features in the native terrestrial biota (Convey, 1996; Peck et al., 2006). The availability of liquid water, as well as its predictability, is considered to be the most important driver of biological and biodiversity processes in the terrestrial environments of Antarctica (Block et al., 2009; Convey et al., 2014). Antarctica's extreme conditions and isolation combined with the over-running of many, but importantly not all, terrestrial and freshwater habitats by ice during glacial cycles underlie the low overall levels of diversity that characterize the contemporary faunal, floral and microbial communities of the continent (Convey, 2013). Nevertheless, in recent years it has become increasingly clear that these communities contain many, if not a majority of species that have survived multiple glacial cycles over many millions of years and undergone evolutionary radiation on the continent itself rather than recolonizing from extra-continental refugia (Convey & Stevens, 2007; Convey et al., 2008; Fraser et al., 2014). With this background, high levels of endemism characterize the majority of groups that dominate the Antarctic terrestrial fauna, including in particular Acari, Collembola, Nematoda and Tardigrada (Pugh & Convey, 2008; Convey et al., 2012).

The continent of Antarctica is ice-bound, and surrounded and isolated from the other Southern Hemisphere landmasses by the vastness of the Southern Ocean. The 1000-km Drake Passage separates it from South America and distances of 4–5000 km from Australia/New Zealand and South Africa. Terrestrial ecosystems reach their greatest development in the coastal regions, where most of the continent's biodiversity is found, most evidently along the Antarctic Peninsula and parts of the coastline of East Antarctica. Terrestrial communities are also present on isolated nunataks and the major mountain ranges inland, as well as in the 'dry valleys' of southern Victoria Land, which are the single largest ice-free areas of the continent (Convey, 2013). However, most ice-free areas are small and isolated by tens to hundreds of kilometers from neighboring areas.

Bdelloids, microscopic water-dwelling invertebrates belonging to the Subclass Bdelloidea of the Phylum Rotifera, account for 11–100% of all rotifer species recorded in Antarctic waterbodies and for 40–100% of species from terrestrial habitats (e.g., Dougherty & Harris, 1963; Sudzuki, 1964; Everitt, 1981; Sohlenius et al., 1996; Smykla et al., 2010). The evolutionary success of Bdelloidea in the extreme Antarctic environment is underlain by their parthenogenetic mode of reproduction and their ability to survive drying and/or freezing in an anabiotic state (cryptobiosis). Populations of bdelloids usually consist of a mix of reproductively isolated clonal lineages, often apparently morphologically uniform, but which are genetically distinguishable evolutionary entities (Birky et al., 2005). At least some clonal lineages can be identified by detailed examination of external morphological characteristics (Birky et al., 2011) and/or by the size and shape of hard parts of the masticatory apparatus (Fontaneto et al., 2007). To date only seven bdelloid morphospecies have been recognized as being endemic to the Antarctic and sub-Antarctic (Segers, 2007), although a recent preliminary molecular analysis has suggested that this number should be considerably greater (Velasco-Castrillón et al., 2014a). Unfortunately, many studies (including recent) use only superficial identification of rotifers, often incomplete or misleading when based on identification keys (Donner, 1965; Kutikova, 2005) for mostly European fauna. Much of the early

I. A. Kozeretska · V. Trokhymets
Educational and Scientific Centre "Institute of Biology", Taras Shevchenko National University of Kiev, Volodymyrs'ka Str. 64, Kiev 01033, Ukraine

I. Dykyy
Department of Zoology, Ivan Franko National University of Lviv, Grushevs'kogo Str. 4, Lviv 79005, Ukraine

M. Plewka
Department of Biology, State Gymnasium, Ochsenkamp 100, 58285 Gevelsberg, Germany

M. Devetter
Biology Centre, Institute of Soil Biology AS ČR, Na Sádkách 7, 37005 Ceske Budejovice, Czech Republic

literature on Antarctic Bdelloidea is inevitably in journals with limited access, and hence much relevant information is not easily accessible to contemporary researchers.

With this background, the aims of this study are: (1) to review contemporary knowledge of aspects of diversity, ecology and reproductive biology of Antarctic bdelloid rotifers, (2) to advance knowledge of morphological and molecular diversity of Bdelloidea in Antarctica and (3) to evaluate the level of endemicity of Antarctic bdelloids.

Current state of knowledge of bdelloid diversity and biology in Antarctica

Early studies

The history of bdelloid research in Antarctica and the sub-Antarctic dates back more than a 100 years. Early records of Bdelloidea date to the start of the twentieth century, obtained from material collected by the First German Antarctic (1901–1903), Swedish (1901–1904), British (1907–1909) and Second French (1908–1910) Antarctic Expeditions. Richters (1907, 1908) was the first to record bdelloids from terrestrial mosses. However, the only two species unequivocally recognizable from his records, *Callidina angusticollis* (=*Habrotrocha angusticollis* Murray, 1905) and *C. longirostris* [=*Rotaria sordida* (Western, 1893)], were found further north, between 35° and 40° S (St. Paul and Amsterdam islands). The remaining 13 bdelloids, also attributed to the genus *Callidina*, are now unidentifiable to species. The illustrations available, depicting contracted bodies, jaws and foot appendages, suggest that these rotifers are most likely correctly referred to the genera *Habrotrocha* and/or *Macrotrachela*.

Scottish biologist, microscopist and polar explorer J. Murray was the first to describe new species of Antarctic Bdelloidea. In the excellently illustrated report on the British Expedition, Murray (1910) listed 12 bdelloid species from mosses and pools of Ross Island and one species [*Callidina tridens* = *H. tridens* (Milne, 1886)] from terrestrial moss from the Stranded Moraines of McMurdo Sound. Five species of the 12 found were previously unknown: *Philodina gregaria* Murray, 1910, *Ph. antarctica* Murray, 1910, *Ph. alata* Murray, 1910, *Habrotrocha* (as *Callidina*) *angularis* (Murray, 1910) and *Adineta grandis* Murray, 1910. Four species that Murray identified as cosmopolitan, *A. barbata* Janson, 1893, *A. longicornis* Murray, 1906, *Callidina constricta* [=*Habrotrocha constricta* (Dujardin, 1841)] and *C. habita* [=*Macrotrachela habita* (Bryce, 1894)], were noted to have morphological differences from the original descriptions of these species as found in Europe. One further species, *Philodina* sp., while apparently new to science, was not further described.

Murray (1910) also discussed the tolerance of bdelloids to desiccation, salinity and extreme temperatures, their habitat and possible feeding preferences, presumed cosmopolitanism and possible dispersal mechanisms, and the origin of the Antarctic rotifer fauna. He noted the predominance of Bdelloidea over other rotifers in the habitats examined and the remarkably high proportion of species that appeared to be known only from Antarctica, which were fully adapted to the conditions of the Antarctic environment. He noted that the two most abundant species, *A. grandis* and *Ph. gregaria*, were both viviparous (possibly a means of increasing progeny survival under extreme conditions), although the only exclusively viviparous bdelloid genus, *Rotaria* Scopoli, 1777, would not be found in Antarctica for some time yet. Murray (1910) considered wind to be the main vector of bdelloid dispersal, also noting that the characteristics of air currents around the Antarctic continent made transportation of rotifers from sources to the north impossible. Waterbirds, along with wind, were also considered as dispersal vectors on the local scale, between different water bodies on Ross Island. Murray (1910) also included the first report of "watermelon snow," a phenomenon caused by aggregations of *Ph. gregaria*, a large bdelloid rotifer with a bright-red colored stomach.

Early taxonomic studies of the Bdelloidea of the maritime Antarctic and sub-Antarctic islands were carried out by de Beauchamp (1913, 1940), who investigated terrestrial habitats of Jenny Island and Îles Kerguelen. However, the description of a new viviparous bdelloid *Philodina* (?) *jeanelli* Beauchamp, 1940, from Kerguelen was based only on contracted individuals and has possibly hampered identification of this species by subsequent researchers.

Further taxonomic studies

More recent taxonomic studies were made by Donner (1972a, 1980) using collections made by Dougherty and Harris (1963) on Ross Island and by Jennings (1976a) on Signy Island (South Orkney Islands; material initially erroneously attributed to the Falkland Islands). The Ross Island material allowed redescription of three species previously found by Murray—*A. grandis*, *Ph. gregaria* and *M. insolita* var., the latter apparently being identical with *M. habita* as described by Murray (1910). The Signy Island material included the previously undescribed species *Mniobia ostensa* Donner, 1980, and 11 other bdelloid species thought to be cosmopolitan. Sudzuki (1964), examining material from Langhovde on the continental Antarctic coastline, depicted 11 unidentified bdelloids from the genera *Adineta*, *Habrotrocha*, *Macrotrachela* and *Mniobia*, which cannot now be reliably attributed to any known species since many important characters (corona, trophi shape, oviparity/viviparity) were missing in the images presented. Dartnall (1983, 1995a, b) and Dartnall & Hollowday (1985) reported a total of 32 bdelloid species, depicting and redescribing 15 species from the maritime Antarctic and the continent (Princess Elizabeth Land), among which were nine previously unknown representatives of *Adineta*, *Habrotrocha*, *Macrotrachela* and *Philodina*. Notwithstanding some uncertainty over details of the corona, most of the specimens described were clearly different from known species, while specimens identified as *A. gracilis* and the viviparous *Rotaria rotatoria* (Pallas, 1766) showed morphological inconsistencies with the original descriptions of non-Antarctic material. *Macrotrachela* (=*Callidina*) *papillosa* (Thompson, 1892) was erroneously listed as *Habrotrocha papillosa* and *M. insolita* de Koning, 1947, as *M. insolata*.

Life cycle

Several studies have described the life cycles of endemic Antarctic bdelloids. Dougherty (1964) investigated reproductive features of *Ph. gregaria* cultivated in the laboratory and found its maturation time to be 28–110 days (in a laboratory refrigerator). This suggests a life span considerably longer than in any other cultivated bdelloid, including another Antarctic endemic, *A. grandis* (Dartnall, 1992; Ricci, 2001). The fecundity of the viviparous *Ph. gregaria* (up to 24 offspring; Dougherty, 1964), was also much lower than those of various oviparous bdelloid species in cultures maintained at room temperature (Ricci & Caprioli, 2005). Dartnall & Hollowday (1985) recorded that *Ph. gregaria* could produce up to 32 young per female, a number close to that of many oviparous bdelloids but still lower than others. Dougherty (1964) stated that most *Ph. gregaria* offspring started to reproduce 27–90 days after birth. Dartnall (1992) confirmed the unusually long life span of *Ph. gregaria*—up to 89 days at 4°C, and twice that of *A. grandis* at the same temperature (40–50 days). Ruttner-Kolisko & Kronsteiner (1979, cited in Dartnall, 1992) reported that at 6°C *Ph. gregaria* lived longer than at 10°C (60 days vs 26) and produced more offspring (15 vs. 7). Also, Dartnall (1992) found the age at the first reproduction to be 36–37 days for *Ph. gregaria*, about ten times more than typical oviparous non-Antarctic bdelloids cultivated at room temperature (Ricci & Caprioli, 2005).

Ecology

A number of studies have described the interactions of Antarctic bdelloids with their substrata (moss, algal mats and soil), comparing these with other terrestrial microinvertebrates, while others have addressed seasonal changes in their populations. Davis (1981) evaluated the role of bdelloids in bryophyte communities of Signy Island by estimating their dry biomass from data presented by Jennings (1976b, 1979) on density and species composition. Both the average and maximum biomasses of Bdelloidea were comparable with or higher than those of Nematoda, though considerably lower than those of Tardigrada. The biomass of bdelloids in mosses could reach up to 29.5 mg dry mass m^{-2}—fourfold greater than that of monogonont rotifers, with about a half of the bdelloid biomass being attributed to *Adineta* species. Davis' (1981) data on feeding preferences suggested that the diet of bdelloids consisted entirely of dead organic matter, contrasting with Dougherty (1964), who stressed the importance of unicellular algae in the diet of *Ph. gregaria*. However, members of the genus *Adineta* are also known to feed predominantly on dead organic matter elsewhere (Örstan, 1992). Everitt (1981) observed cyclical changes in abundance throughout the year in the bdelloid population of a

saline continental lake in the Vestfold Hills. Rotifers overwintered in a cryptobiotic state and during the summer reproduced with abundance peaks occurring at 3-week intervals. Dougherty (1964) and Dartnall (1992) reported that the time between recovery from cryptobiosis, or birth, and the first reproduction in *Ph. gregaria* was at least 1 month in the laboratory, but that it could be shorter in the natural environment. In the relatively stable lake environment, the abundance peaks observed could be successive new generations, especially as Bdelloidea, unlike another rotifer group, Monogononta, do not possess specific larval or programmed dormant stages. However, the largest abundance peak described by Everitt (1981) corresponded to a massive inflow of N and P compounds into the lake, indicating that environmental influences are also important. In the more unstable (in terms of water availability) terrestrial habitats environmental factors seem to be the major driver of bdelloid abundance dynamics (Iakovenko, 2004). Priddle & Dartnall (1978), investigating the microflora and microfauna of aquatic moss and algal communities in lakes of Signy Island, observed three- to seven-fold decreases in the abundance of *Philodina* sp. during winter compared to summer. They also reported that two non-sessile bdelloid species showed distinctive space distribution patterns inside moss cushions, dominating in different zones of stems and leaves. Cathey et al. (1981) found *Ph. gregaria* and *Ph. alata* to be able to colonize artificial substrata (polyurethane foam) in eight lakes of southern Victoria Land, the former being present in all the lakes and the latter in only three.

Based on recent studies, most or all rotifer species that have been recorded in Antarctic soil communities are bdelloids (Smykla et al., 2010). Even in soils of the McMurdo Dry Valleys, one of the driest places in Antarctica, rotifers were present in all sampled locations (Courtright et al., 2001). Confirming Murray's (1910) speculations of almost a century ago, Nkem et al. (2006) concluded that wind plays an important role in the dispersal of soil rotifers, and this has been proposed as the primary mechanism behind their colonization of remote ice-free areas such as isolated valleys and nunataks, where they can reach abundances of up to 135 ind g^{-1} dry substrate (Sohlenius et al., 1996). In some soil types at Edmondson Point, Victoria Land, Smykla et al. (2010, 2012) found bdelloid rotifers to be the dominant group of microinvertebrates, reaching over 8000 ind 100 g^{-1} fresh soil. Smykla et al. (2010, 2012) also reported that bdelloids reached high abundances in wet soils under moss and algal and cyanobacterial mats, while being absent in both barren fellfields and heavily nutrient-enriched penguin colonies. In contrast, Porazinska et al. (2002) reported rotifers to be present and even dominant in terms of abundance (>4000 ind kg^{-1} dry soil) in ornithogenic soils collected on Ross Island. Sohlenius & Boström (2008) similarly noted that rotifers were the most frequently encountered and abundant group of invertebrates in both ornithogenic soils and fellfields of Dronning Maud Land. Velasco-Castrillón et al. (2014b) reported bdelloid rotifers to be the most widespread and abundant taxon in soils from multiple locations in East Antarctica, being present in 87% of sampled sites and reaching 44 ind g^{-1} dry soil. In this latter study, bdelloids were present in soils with widely varying particle size composition (from fine to coarse), both with and without vegetation, and with a broad variety of abiotic and geochemical parameters, all observations that are consistent with the high tolerance of this group toward extreme conditions.

As also noted in the Arctic (De Smet & Van Rompu, 1994), Bdelloidea play an important role in Antarctic cryoconite communities. In cryoconites on glaciers of the McMurdo Dry Valleys, rotifers were dominant, reaching over 3500 ind 100 g^{-1} dry sediment, although abundance decreased with elevation and was also influenced by pH, nutrient concentrations and cryoconite area (Porazinska et al., 2004). Rotifer and tardigrade abundances were also positively correlated in these cryoconites.

"Watermelon snow" and similar phenomena on the surface of water, ice or algal mats resulting from the massive accumulation of red-coloured *Ph. gregaria* was originally described by Murray (1910) and later addressed briefly by Dougherty & Harris (1963), Dougherty (1964) and in more detail by Dartnall (1992). These accumulations can create very noticeable red patches on the surface of such substrata, ranging from a few centimeters to many meters in diameter (Dartnall, 1983). For a patch to grow to a size of about 10 m may take only a week, with the abundance of *Ph. gregaria* reaching up to over 20 million ind. m^{-2}.

Diversity and endemism

Studies that have included or provided compilations of the rotifer fauna of Antarctica and the sub-Antarctic, in particular terrestrial Bdelloidea, have been published by Dartnall (1983), Dartnall & Hollowday (1985), Sudzuki (1988), Adams et al. (2006), Segers (2007), Velasco-Castrillón et al. (Velasco-Castrillón et al. 2014a, b, c) and Fontaneto et al. (2015). These sources should be referred to for details on species diversity of particular regions. A few studies have been carried out at the same location over time. For example, Dougherty & Harris (1963), investigating Ross Island and the McMurdo Dry Valleys, found virtually the same species as recorded by Murray (1910). A number of previously unrecorded bdelloid species have been reported from Antarctica and sub-Antarctic by Jennings (1976a), Sudzuki (1979), Everitt (1981), Sohlenius et al. (1996) and Sohlenius & Boström (2005). These studies reported, along with the indigenous Antarctic bdelloids, some 20 morphospecies similar to species first described from Europe, thus considering Antarctic bdelloid fauna to include many cosmopolitan species. No Antarctic endemic bdelloid families or genera have been reported.

Velasco-Castrillón et al. (2014a) consider that the known Antarctic Bdelloidea diversity comprises 36 morphospecies. However, this figure does not include three species-level taxa identified by Murray (1910), Jennings (1976a) and Cathey et al. (1981) or ten further undescribed species reported by Dartnall & Hollowday (1985), Dartnall (1995a,b) and Sohlenius et al. (1996): *Adineta vaga minor* Bryce, 1893, *Ceratotrocha cornigera* (Bryce, 1893), *Philodinavus* sp., *A.* sp., *Habrotrocha* sp., *Macrotrachela* sp. "A", *Macr.* sp. 1, *Macr.* sp. 2., *Mniobia* sp. N, *Philodina* sp. "A", *Ph.* sp. "B", *Ph.* sp. 1. and *Ph.* sp. 2. Including these taxa, in total 49 bdelloid morphospecies have been recorded in Antarctica and the sub-Antarctic over the last century.

Based on classical taxonomy, only seven endemic bdelloids [five described by Murray (1910), *plus Ph. jeanelli* and *Mn. ostensa*] have been reported for Antarctica, with the remainder being cosmopolitan and previously known from other continents including Europe (Donner, 1965; Segers, 2007). In contrast, the application of contemporary molecular approaches (Velasco-Castrillón et al., 2014a) suggests that the bdelloid fauna of Antarctica comprises mostly endemic species, or at least species not yet recorded from any other continent.

Molecular approaches

A number of genomic and molecular phylogeographic studies have been performed during the last decade on various groups of Antarctic microfauna, mainly microarthropods and nematodes (Stevens et al., 2006; Stevens & Hogg, 2006; McGaughran et al., 2008, 2010; Velasco-Castrillón & Stevens, 2014) as well as various microbial groups (see Vyverman et al., 2010) and mosses (Pisa et al., 2014). However, the application of such studies to bdelloid rotifers in Antarctica remains at an early stage. Fragmentary sequence data on Antarctic bdelloids have been published in studies of the evolution and global biogeography of Bdelloidea (Barraclough et al., 2007; Fontaneto et al., 2008, 2012). Velasco-Castrillón et al. (2014a) recently evaluated molecular diversity of Antarctic and sub-Antarctic bdelloids across a wide area. Their study identified 47 putative species, counting both sequence clusters, and singletons (entities with only one sequence obtained). All of the putative species were designated as Antarctic or Tierra del Fuego endemics based on percentage sequence similarities in comparison with representatives of eight bdelloid genera from other continents. The study also indicated that the true number of taxa in the genera *Adineta* and *Philodina* determined from the sequence data analyzed using the Poisson tree processes (PTP) model (Zhang et al., 2013) must be considerably higher than can apparently be determined by morphological approaches alone.

The current study shows that when appropriately analyzed, the morphological diversity of Antarctic bdelloids is sufficient to reveal most diversity detected by contemporary molecular markers. We also reevaluate previously published data on Antarctic bdelloid rotifer endemism and determine the ratio of endemic to cosmopolitan bdelloid species in comparison with such from other continents.

Materials and methods

Sampling and extraction of rotifers

New samples included in this study were obtained from both maritime and continental regions at sites between 63°60′S and 77°55′S (Fig. 1; Table 1). In continental Antarctica 11 sampling locations were

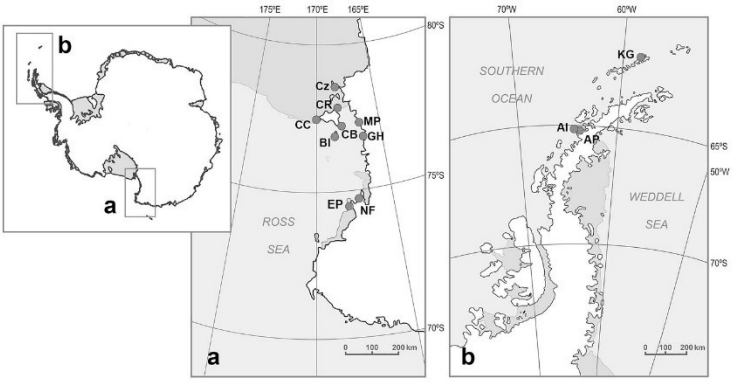


Fig. 1 Map indicating sampling locations in Antarctica. **a** Ross Sea area; **b** Antarctic Peninsula and adjacent islands. *BI* Beaufort Island. Ross Island: *CB* Cape Bird, *CC* Cape Crozier, *CR* Cape Royds. Coastal zone of Victoria Land: *Cz* Cape Chocolate, *GH* Granite Harbor, *MP* Marble Point, *NF* Northern Foothills. Maritime Antarctica: *AI* Argentine Islands archipelago, *KG* King George Island, *AP* Mount Demaria, Kiev Peninsula, Graham Land

Table 1 Locations sampled in the Antarctic, including altitudes and the number of collected samples

Area	Locality[a]	Latitudes	Longitudes	Altitudes, m	Habitats
Antarctic Peninsula	AP	65°12–65°17′S	64°06–64°08′W	15–146	Soil, moss
Argentine Islands	AI	65°09–65°56'S	64°03'– 66°08'W	3–59	Soil, moss, lichens
King George Island	KG	63°60'41–44''S	60°42'05–29"W	5–11	Soil, waterbodies
Victoria Land	BI	76°55'–76°59'S	166°54'–166°56'E	6–141	Soil, moss, algal mats
-"-	CB	77°12'– 77°15'S	166°22'–166°27'E	5–100	Soil, moss, algal mats
-"-	CC	77°27'21–39"S	169°13'–169°15'E	61–201	Soil, moss
-"-	CR	77°32'– 77°34'S	166°08'–166°10'E	6–28	Soil, moss, algal mats
-"-	Cz	77°56'21–26"S	164°30'–164°32'E	20	Soil, moss, algal mats
-"-	EP	74°19'45–60"S	165°07'– 165°09'E	15–24	Soil
-"-	GH	77°00'25–60"S	162°28'–162°32'E	10	Soil
-"-	MP	77°25'–77°27'S	163°40'–163°51'E	14–38	Soil, moss, algal mats
-"-	NF	74°42'25–46"S	164°06'11–54E	34–113	Soil

[a] See Fig. 1

AP Antarctic Peninsula, *AI* Argentine Island Archipelago, *KG* King George Island, *BI* Beaufort Island, *CB* Cape Bird, *CC* Cape Croizier, *CR* Cape Royds, *Cz* Chocolate Point, *EP* Edmondson Point, *GH* Granite Harbour, *MP* Marble Point, *NF* Northern Foothils

visited in the Ross Sea area, including the Victoria Land coast, Beaufort and Ross Islands (Fig. 1a, Supplementary file I). The fieldwork and sampling in the Ross Sea area were conducted during five austral summer seasons between 2003/04 and 2011/12 within the project of J. Smykla (Smykla et al., 2010, 2011, 2012). Soil and moss were collected at all localities. The soil samples were obtained from barren fellfields, bryophyte communities, wetlands with algal and cyanobacterial mats, and the vicinity of active and relict penguin colonies. Algal mats were collected in coastal areas and on Ross Island. Most of the collected material was stored frozen (−20°C), but some terrestrial mosses were dried and stored at room temperature; details of collection methods and primary sample processing are given in Smykla et al. (2010, 2012, 2015).

In the maritime Antarctic 237 samples were obtained during the summers of 2004/2005, 2006/2007 and 2009/2010 from the Argentine Islands

archipelago, King George Island and coastal areas of the Antarctic Peninsula under the projects of K. Janko, I. Kozeretska and V. Trokhymets. These included 50 soil and 183 moss samples, one sediment sample from a pool on King George Island and three lichen samples from the Argentine Islands archipelago (Fig. 1b; Table 1; Supplementary file I). Methods of collection, storage and rotifer extraction were as used for the continental samples, except for mosses, which were washed directly along with wet sieving and sugar gradient centrifugation as described by Freckman & Virginia (1993).

Alpha taxonomy procedures

Detailed procedures of rotifer sorting, identification, digital imaging and the preparation of type material (glycerin jelly slides and SEM mounts) are described in Iakovenko et al. (2013). We used the keys of Donner (1965) and Kutikova (2005) as a primary guide for identification, but detailed taxonomic analysis was based on the first descriptions (cited in Donner (1965) and further specific studies (Haigh, 1965, 1966; Donner, 1972a, b, 1980; Örstan, 1995; Koste, 1996a; Ricci et al., 2001, 2003; Birky et al., 2011).

Rotifer trophi (hard parts of the mastax) were extracted using Savo®Perex bleach and prepared for SEM according to De Smet (1998). Trophi measurements (ramus length and trophi width) were made as described by Iakovenko et al. (2013). Type material for newly described species (holotypes, paratypes and additional specimens) are deposited in the collections of the Schmalhausen Institute of Zoology, National Academy of Sciences of Ukraine, Kiev, Ukraine.

Additionally, we investigated and described rotifers depicted in photographs in Velasco-Castrillón et al. (2014a). To reliably distinguish, both morphologically and genetically, between several similar European and Antarctic species, we used material from our collections in Bulgaria, Czech Republic, Germany and Poland (Supplementary file I). Previously unpublished data on the morphology and morphometry of *A. barbata* Janson, 1893, *A. gracilis* Janson, 1893, *A. vaga* (Davis, 1873) and *Habrotrocha thienemanni* Hauer, 1924, from these collections, as well as COX1 sequences of the voucher specimens from which the morphometric data were taken, were used for these analyses.

Morphometric analyses

External rotifer body dimensions were taken on screenshots from digital videos, and trophi were measured on SEM photos, as described in Iakovenko et al. (2013). Total length (TL) in the case of adinetid rotifers was taken as the distance between the middle of the anterior rim of the head and the posterior rim of the spur pseudosegment, i.e., not including the rostrum, as it was usually bent under the head (Fig. 2).

To distinguish some Antarctic species from morphologically similar European ones, we measured specimens from clonal cultures, from which we subsequently obtained some COX1 sequences: 113 specimens of Antarctic *Adineta*, 69 specimens of European *Adineta* and 16 specimens of Antarctic *Habrotrocha*. We used the Linear Mixed Effects Model (LME) and Principal Components Analysis (PCA) to compare body and trophi measurements. The results of PCA were visualized as the two first principle components of variation plotted against each other. All statistical analyses were performed in R 2.15.1 following Crawley (2007).

DNA taxonomy procedures

The DNA extraction protocol followed Fontaneto et al. (2007) and Iakovenko et al. (2013). The target locus of the mitochondrial COX1 gene (355 bp in length) was amplified and sequenced from 194 bdelloid specimens (Supplementary file III) using universal primers LCO1490 and HCO2198 (Folmer et al., 1994) with the subsequent reamplification to increase the outcome of the product, using bdelloid-specific primers Bdell_CO1_FW (5′-CGTACWGAGTTAGGAATRGTA-3′) and Bdell_CO1_Rev (5′-CCAAAATTWCGATC TAAYA-3′) (Robeson et al., 2011).

To construct phylogenies, we downloaded available sequences of the taxonomically assigned bdelloid species from GenBank, available from all continents except South America (977 COX1 sequences, their detailed descriptions are given in the Supplementary file II). We used EMBL online version of MAFFT software (Katoh et al., 2002) to construct one total alignment of both newly obtained sequences and those downloaded from GenBank and four separate alignments for four genus-specific data sets (*Adineta* Hudson and Gosse, 1886, *Habrotrocha* Bryce, 1910, *Macrotrachela* Milne, 1886, *Philodina* Ehrenberg,

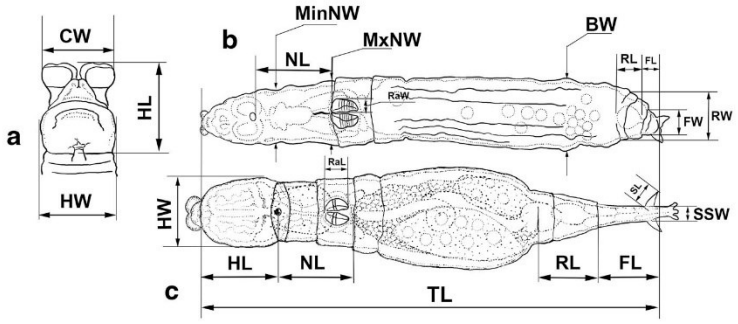


Fig. 2 Measurements of bdelloids of the families Habrotrochidae and Philodinidae (**a**, **b**) and Adinetidae (**c**). *BW* body width, *CW* corona width, *FL* foot length, *FW* foot width, *HL* head length, *HW* head width, *MinNW* minimal neck width, *MxNW* maximal neck width, *NL* neck length, *RaL* ramus length, *RaW* ramus width, *RL* rump length, *RW* rump width, *SL* spur length, *SSW* spur pseudosegment width, *TL* total length

1830). The monogonont rotifer *Brachionus calyciflorus* Pallas, 1755, was used as outgroup in each of these alignments, and each genus-specific data set also contained a member of another bdelloid genus as an additional outgroup: *Bradyscela clauda* (Bryce, 1893) for *Adineta*, *M. ehrenbergii* (Janson, 1893) for *Habrotrocha* and *H. constricta* (Dujardin, 1841) for *Macrotrachela* and *Philodina*.

We constructed phylogenetic trees in MrBayes 3.2.3 (Ronquist et al., 2012), running 8–20 million generations and sampling every 1000 generations. The optimal nucleotide substitution model (GTR+I+G) was chosen for each data set in jModelTest 2.1.6 (Darriba et al., 2012). The analysis was stopped when the standard deviation of split frequencies was below 0.01, with the PSRF being 1.00 for all the parameters. Effective sample size (ESS) sufficiency for the model parameters, process stationarity and the number of burn-in trees were checked using both MrBayes and Tracer 1.6 software (Rambaut et al., 2013). The resulting consensus trees constructed in MrBayes were visualized using FigTree 1.4.2 (Rambaut, 2012), and the full-size Bayesian trees are included in Supplementary file IV.

Three independent approaches were used for species delimitation based on DNA sequence data: 4× rule (Birky et al., 2005; Birky & Barraclough, 2009), Generalized Mixed Yule Coalescent Approach (GMYC; Fujisawa & Barraclough, 2013) and PTP with Bayesian support (bPTP; Zhang et al., 2013). The 4× rule identifies as putative species those monophyletic clades whose genetic distances (K) to other sequences on the phylogenetic tree are larger than four times the intra-clade divergence (θ). To assess this, we constructed matrices of mean pairwise correlated sequence distances for each clade in the Bayesian trees in MEGA6 software (Tamura et al., 2013), calculating θ and estimating the K/θ ratio within and between the clades (Supplementary file V).

The GMYC method likewise identifies species as independently evolving entities represented by a number of clades on a phylogenetic tree. However, each clade is delimited by optimizing the tree nodes indicating transitions between inter- and intraspecific evolutionary processes. The maximum likelihood optimum is found between models of species diversification (based on the Yule model) and branching events within species (based on the neutral coalescent model). The initial tree should be time-calibrated (ultrametric), unrooted and not contain polytomies or zero-length branches. We used a single-threshold version of the method implemented in GMYC species delimitation software available online (http://species.h-its.org/gmyc/). The uploaded coalescent trees were produced from Bayesian unrooted trees in R 3.1.2 (http://www.r-project.org/) using the *chronopl* function of the "ape" package. This function utilizes a semiparametric method based on penalized likelihood (Sanderson, 2002) to estimate the tree node ages through a trade-off between contiguous and non-contiguous branch rates.

Unlike GMYC, the bPTP method does not require a time-calibrated and unrooted tree as input. In this method, the number of substitutions κ between intra- and interspecific events is used instead of time as a tree-calibrating parameter. Assuming that each substitution (which is independent of other substitutions) has a probability ρ of generating a speciation event, κ substitutions generate η speciations in a continuous process, and in a population of the size η the number of substitutions is sufficient; the process proceeds at the rate $\rho \times \eta$ and follows a Poisson distribution. The number of substitutions is calculated from the branch lengths of the input tree. We used online implementation of bPTP (http://species.h-its.org/ptp/) and the trees produced in MrBayes as the input.

Results

In total, we identified 60 morphospecies, including 20 taxa currently identified to the generic level only and still under investigation, and ten listed as "conformis" that show minor morphological differences from known species. Only 13 of the morphospecies found occurred in both maritime and continental Antarctica. The material examined included six of the seven known Antarctic endemics: *A. grandis*, *H. angularis*, *Mn. ostensa*, *Ph. alata*, *Ph. jeanelli* and *Ph. gregaria*. We have identified ten morphospecies reported by other researchers from Antarctica as *A. barbata* Janson, 1893, *A. vaga* (Davis, 1873), *H. gulosa* Milne, 1916, *H. vicina* Donner, 1980, *Macr. ambigua* Donner, 1965, *Macr. concinna* (Bryce, 1912), *Macr. habita* (Bryce, 1894), *Macr. musculosa* (Milne, 1886), *Macr. nixa* Donner, 1962, and *Rotaria rotatoria* (Pallas, 1766). These species are considered cosmopolitan, or at least are known from locations other than Antarctica. However, of these ten species, those resembling *A. barbata* and *A. vaga* s. str. are shown to be distinct new taxa and therefore currently endemic to Antarctica, based on both minor but consistent morphological differences and molecular analyses.

In *Alpha taxonomy*, below, we describe 12 new for science Antarctic bdelloid species. For some of them we also provide statistical analysis of morphometric data confirming their delimitation from morphologically similar described species occurring in Europe (*Morphometric analyses*). New records for the Antarctic, yet to be verified by molecular analyses belonging to already described cosmopolitan species, included *H. angusticollis* (Murray, 1905), *Macr. nana* (Bryce, 1912), *Mniobia incrassata* (Murray, 1905), *Mn. scabrosa* Murray, 1911 and *Pleuretra lineata* Donner, 1962. The genus *Scepanotrocha* (*S.* cf *semitecta* Donner, 1951) is reported from Antarctica for the first time. The list of known Antarctic bdelloids is therefore extended to 66 *morphospecies* (49 already known and reported in the existing literature, 12 new for science and 5 new for Antarctica).

Phylogenetic trees constructed using 194 original COX1 sequences and 977 sequences downloaded from GenBank gave similar results on the delimitation of independently evolving entities (IEE) according to the 4× rule, GMYC and bPTP models. These results are discussed in detail below (*DNA taxonomy* subsection). The 4× rule gave 140 IEEs: 44 of *Adineta*, 22 of *Habrotrocha*, 26 of *Macrotrachela* and 48 of *Philodina*. In total, 132 IEEs were identified by GMYC: 44 of *Adineta*, 20 of *Habrotrocha*, 18 of *Macrotrachela* and 50 of *Philodina*. Finally, bPTP generated a somewhat higher number of IEEs (160): 47 of *Adineta*, 26 of *Habrotrocha*, 29 of *Macrotrachela* and 58 of *Philodina*. Most of the IEEs identified by GMYC and bPTP were confirmed by the 4× rule. Delimitation according to the GMYC approach gave the best correspondence with rotifer morphology, considering both major and minor external features and morphometric data. Geographical distribution of the identified IEEs is discussed in *Biogeography*.

The integrity of most species identified by morphology, with the exception of *A. grandis*, *Ph. gregaria* and two new species of *Habrotrocha*, was confirmed by molecular analyses (*DNA taxonomy*). According to the molecular data, *A. grandis* consists of at least two cryptic species, one of which is described below as new for science. Ten putative species (IEEs) were identified from molecular data only, obtained from both the new material examined in this study and COI sequences downloaded from GenBank.

Alpha taxonomy

The list of locations is given after both the literature sources (cited in the Introduction) and our data (marked with *). Full descriptions of the examined samples, mentioned in Type material and Additional material below (as sample codes), are given in the Supplementary file I.

Abbreviations BW, body width; HL, head length; HW, head width; NL, neck length; MinNW, minimal neck width; MxNW, maximal neck width; RL, rump length; RW, rump width; FL, foot length; FW, foot width; SL, spur length; SSW, spur pseudosegment width; TL, total length. The abbreviations of the localities are explained in the Fig. 1 and Table 1, with the exception of the data from literature: DM, Dronning Maud Land; EB, Enderby; FI, Francis Island; HI, Haswell Island; LH, Langhovde; MM, McMurdo Sound; QM, Queen Mary's Land; SI, Signy Island; TF, Tierra del Fuego; WK, Wilkes Land.

Phylum Rotifera Cuvier, 1817

Class Eurotatoria De Ridder, 1957

Subclass Bdelloidea Hudson, 1884

Order Philodinida Melone & Ricci, 2005

Family Adinetidae Hudson & Gosse, 1889

Genus *Adineta* Hudson and Gosse, 1886

***Adineta coatsae* sp. nov. (Figs. 3a, 4)**

Murray, 1910 (*A. barbata*?), pp. 53–54, Pl. XII Fig. 9a–c. Dartnall & Hollowday, 1985 (*A. barbata*), p. 30, Fig. 24a, b. Velasco-Castrillón et al., 2014a (*A.* sp. Bd24), p. 8 (main text), 2, Fig. 6 (Annex S1).

Type locality Chocolate Point (Victoria Land), 20 m asl., S77°56.400′, E164°30.693′.

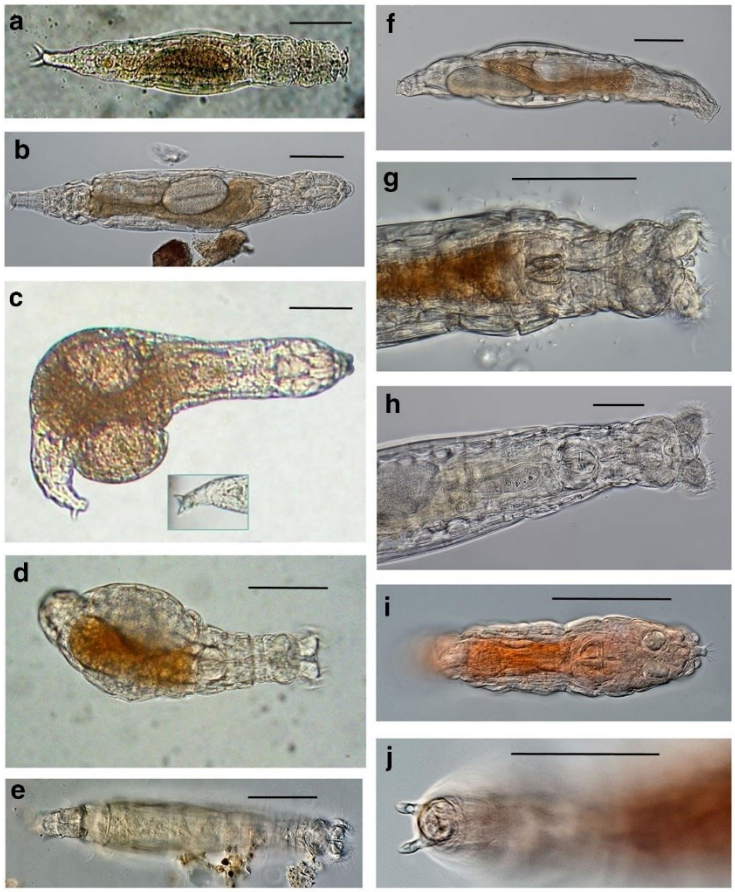


Fig. 3 New species of Antarctic bdelloids (photographs M. Plewka and N. Iakovenko): **a** *Adineta coatsae* sp. nov., holotype, habitus, dorsal view; **b** *A. editae* sp. nov., habitus, dorsal view; **c** *A. grandis*, habitus, dorsal view; **d** *H. antarctica* sp. nov., holotype, habitus, feeding, dorsal view; **e** *H. vernadskii* sp. nov., habitus, feeding, ventral view; **f** *M. jankoi* sp. nov., habitus, creeping, ventral view; **g** same, head, feeding, ventral view; **f** *M. ioannae* sp. nov., habitus, feeding, dorsal view; **i** *Ph. dartnallis* sp. nov., habitus, creeping, dorsl view; **j** same, spurs. *Scale bar* 50 µm

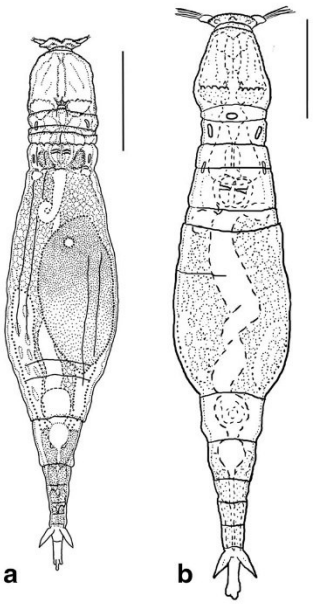


Fig. 4 *Adineta coatsae* sp. nov. (Antarctica): **a** holotype, habitus, dorsal view. *A. barbata* (Europe, BG0715): **b** habitus, dorsal view. *Scale bar* 50 µm

Type habitat Algal and cyanobacterial mats.

Type material Holotype: SIZ 55.1 (CzM3NCmatAC1, 23.1.2010, Leg. J. Smykla), mounted in glycerin jelly. Paratypes: SIZ 55.2-55.3 (CzM3NCmatAC2-3), trophi mounted for SEM. *Additional material.* SIZ 55.4-10 (CzM3AS1-2, MPM4mossAC1, V10AC1-2, KG2AC1, V10AC1), digital photos and videos.

Etymology Named in honor of a mountaineer and Antarctic researcher, Dr. Larry Coats, who assisted in the fieldwork done in the Ross Sea area.

Barcodes GenBank ID KJ543629-30.

ZooBank LSID. urn:lsid:zoobank.org:act:22DAD23A-DD71-4FB7-828C-59DCD677EAB7.

Diagnosis Similar to *A. barbata* (Fig. 4b) by flat laterally widened rostrum with two protrusions ending with a bundle of long thin sensory bristles and long sword-like spurs. However, the protrusions are leaf-like, while in *A. barbata* they are tubular. Frontal rim of the rostrum is concaved and has a notch in the middle, while in *A. barbata* the notch is absent and the frontal rostral rim is prominently convex (Fig. 4b). Spurs gradually tapering from their base to the points, shorter than in *A. barbata*. The new species seems to have substantially smaller body (TL 220–292 µm) than *A. barbata* (TL 280–400 µm according to Donner, 1965 and 223–374 µm according to our data).

Description Body of moderate size, not very wide, flattened dorsoventrally, transparent, stomach usually of brown-yellow color. Dartnall & Hollowday (1985) report the color of this rotifer as grayish-brown. Integument smooth, thin, without sculpturation, spines, knobs or bolsters. Head trapezoid, wider in the posterior part, HL is 15–19% of TL, HW is 76–94% of HL. Distal rostral pseudosegment flat, lobe-like widened, with a V-shaped shallow and wide notch in the middle. Rostral lamella shaped as two lateral leaf-like narrow protrusions with a bundle of long sensory bristles under each protrusion. Eight rectangular teeth in each rake. Neck of moderate length and width, NL is 12–17% of TL, antenna about 1/4–1/5 of bearing pseudosegment. Trunk oval, BW 17–28% of TL. Rump conical, first pseudosegment slightly swollen, RL is 12–17% of TL, RW is 75–92% of RL. Slim foot of five pseudosegments, of moderate length, FL is 28–35% of TL, FW is 40–62% of FL. Spurs sword-like, long, gradually tapering from the base to the points; SL is 115–181% of SSW. Three short unsegmented toes. No eyespots. Throat and straight esophagus of moderate size. Trophi small, round, 11–12 µm long and 13–15 µm wide; 2/2 major teeth and 26/26 minor teeth in unci. Oviparous; egg oval, 101 × 46 µm, smooth, 1–6 round knobs on both poles and the sides.

Measurements See Table 2. Body length 120 µm (possibly in contracted state) according to Velasco-Castrillón et al. (2014a), and 325 µm according to Dartnall & Hollowday (1985).

Distribution Maritime Antarctica: AI*, KG*, SI. Continental Antarctica: EB, VL (CR, CH, GH*, MP*), possibly also DM and MM (Dougherty & Harris, 1963; Sohlenius et al., 1996). *Habitat* Algal and cyanobacterial mats in wetlands; terrestrial moss, soil.

Adineta editae sp. nov. Iakovenko (Figs. 3b, 5)

Dartnall & Hollowday, 1985 (*A. gracilis*), p. 31, Fig. 24c. Fontaneto et al., 2008 (*A. gracilis*), p. 3139. Velasco-Castrillón et al., 2014a (*A.* cf *gracilis* Bd8), p. 8 (main text); 1, Figs. 2–5 (Annex S1).

Table 2 Body dimensions (measured from light microscope photographs) and trophi dimensions (measured from SEM photographs) of the described bdelloid species

Species	Measurements (μm)					
	TL[a]	BW	HL	HW	CW	NL
Adineta coatsae sp. nov.	220–292, 257 ± 27[b]	39–62, 52 ± 9	35–55, 42 ± 7	28–46, 36 ± 6	–	30–49, 40 ± 7
Holotype	248	56	44	38	–	40
A. editae sp. nov.	206–382, 285 ± 41	40–90, 58 ± 12	39–56, 45 ± 4	30–47	–	34–63, 45 ± 9
Holotype	340	90	56	35 ± 5	–	55
A. emsliei sp. nov.	206–352, 294 ± 44	40–87, 69 ± 15	32–61, 49 ± 8	27–47, 39 ± 5	–	34–72, 50 ± 10
Holotype	332	84	50	38	–	47
A. grandis	304–505, 414 ± 61	60–152, 94 ± 22	57–92, 70 ± 9	48–71, 56 ± 7	–	43–118, 82 ± 19
Type locality	500–505	81–90	73–92	64–71	–	84–103
A. fontanetoi sp. nov.	471–509	81–98	67	51–61	–	79–87
Holotype	471	98	67	51	–	79
Habrotrocha antarctica sp. nov.	225–299, 270 ± 25	35–58, 48 ± 8	29–36, 33 ± 2	28–37, 33 ± 3	25–32, 29 ± 2	37–54, 46 ± 6
Holotype	249	40	31	31	27	88
H. devetteri sp. nov.	209–282, 252 ± 26	38–60, 48 ± 7	22–31, 27 ± 6	29–34, 32 ± 4	30–41, 35 ± 7	37–56, 46 ± 7
Holotype	216	38	22	29	30	42
H. vernadskii sp. nov.	216–298, 256 ± 17	29–62, 43 ± 8	32–35, 33 ± 1	28–38, 33 ± 2	22–28, 25 ± 1	38–61, 50 ± 6
Holotype	258	41	32	28	22	47
Macrotrachela donneri sp. nov.	252–415, 325 ± 44	39–87, 59 ± 13	33–48, 41 ± 6	41–52, 46 ± 4	46–58, 52 ± 5	38–72, 53 ± 11
Holotype	306	61	43	43	48	47
M. ioannae sp. nov.	284–488, 409 ± 80	38–85, 61 ± 18	55–59	59–60, 59 ± 1	60–64, 61 ± 2	50–83, 64 ± 11
Holotype	283	38	59	60	60	50
M. jankoi sp. nov.	245–570, 327 ± 88	43–107, 61 ± 18	34–54, 43 ± 7	33–64, 45 ± 9	40–73, 52 ± 10	52–91, 61 ± 13
holotype	265	56	37	41	55	55
Philodina dartnallis sp. nov.	226–349, 275 ± 44	45–87, 57 ± 12	27–38, 33 ± 4	21–31, 26 ± 25	37–55, 45 ± 8	39–62, 48 ± 7
Holotype	226	49	36	42	50	45
Ph. shackletoni sp. nov.	360–507, 451 ± 64	66–148, 94 ± 37	63–87, 75 ± 17	71–120, 96 ± 35	83–131, 107 ± 33	60–118, 87 ± 30
Holotype	474	71	63	71	83	84

Table 2 continued

Species	Measurements (μm)					
	RL	RW	FL	SL	SSW	RaL
Adineta coatsae sp. nov.	29–47, 37 ± 7	25–38, 31 ± 5	28–35, 31 ± 2	9–16, 12 ± 2	7–11, 8 ± 1	11.1–12.3
Holotype	31	28	30	13	7	–
A. editae sp. nov.	30–58, 40 ± 8	22–43, 33 ± 6	28–48, 36 ± 6	6–10, 7 ± 1	7–13, 10 ± 1	15.0–18.6, 16.7 ± 1
Holotype	52	44	35	10	13	–
A. emsliei sp. nov.	27–55, 41 ± 8	23–48, 35 ± 7	27–46, 36 ± 5	6–9, 7 ± 1	8–12, 9 ± 1	14.7–19.3, 15.7 ± 1
Holotype	49	48	39	8	9	–
A. grandis	36–72, 52 ± 10	27–64, 46 ± 10	28–69, 51 ± 12	7–14, 11 ± 2	9–17, 13 ± 2	23.2–31.0, 25.4 ± 1
Type locality	53–68	54–64	61–65	10–12	16–17	24.8–31.0
A. fontanetoi sp. nov.	56–59	51–58	49–63	8–10	13–14	24.1–28.3, 26.8 ± 1
Holotype	59	58	63	8	13	–
Habrotrocha antarctica sp. nov.	29–39, 34 ± 4	27–37, 32 ± 4	22–37, 30 ± 5	6–9, 7 ± 1	8–15, 11 ± 2	14.5–19.3
Holotype	29	31	29	7	11	–
H. devetteri sp. nov.	27–42, 35 ± 5	27–37, 32 ± 4	23–33, 26 ± 3	4–9, 6 ± 1	6–13, 10 ± 2	16.0–17.0
Holotype	37	34	30	5	12	–
H. vernadskii sp. nov.	26–46, 37 ± 4	24–43, 32 ± 5	19–34, 24 ± 4	6–11, 9 ± 1	10–17, 13 ± 2	15.0–17.4
Holotype	39	30	23	10	15	–
Macrotrachela donneri sp. nov.	34–51, 42 ± 6	27–46, 37 ± 7	24–38, 30 ± 4	9–14, 11 ± 1	9–13, 11 ± 1	21.2–24.8, 23.3 ± 1
Holotype	40	39	30	11	10	–
M. ioannae sp. nov.	41–72, 55 ± 11	33–64, 55 ± 11	31–37, 35 ± 2	10–14, 13 ± 2	13–21, 19 ± 3	25.9
Holotype	41	33	33	14	21	–
M. jankoi sp. nov.	32–74, 46 ± 11	28–54, 39 ± 8	19–46, 28 ± 8	5–8, 7 ± 1	9–15, 12 ± 2	18.1–21.9, 19.6 ± 1
holotype	55	45	32	5	9	–
Philodina dartnallis sp. nov.	32–63, 40 ± 10	26–47, 34 ± 6	21–47, 30 ± 10	5–10, 7 ± 1	9–16, 12 ± 2	24.7
Holotype	32	26	21	6	9	–
Ph. shackletoni sp. nov.	60–78, 69 ± 9	46–66, 55 ± 10	39–76, 58 ± 15	17–24, 15 ± 3	11–21, 15 ± 4	24.2
Holotype	69	53	59	19	14	–

[a] See explanations in "Materials and methods"

TL total length, *BW* body width, *HL* head length, *HW* head width, *CW* corona width, *NL* neck length, *RL* rump length, *RW* rump width, *FL* foot length, *SL* spur length, *SSW* spur pseudosegment width, *RaL* ramus length

[b] Min–max, mean ± SD

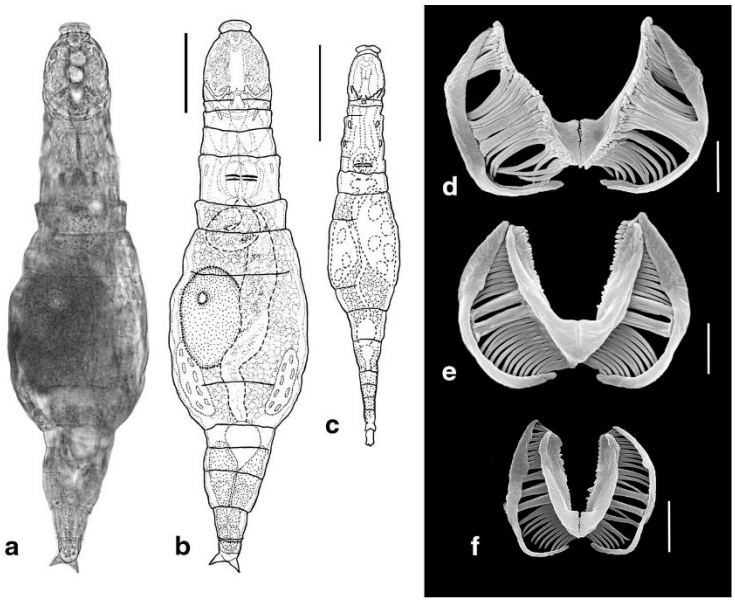


Fig. 5 *Adineta editae* sp. nov. (Antarctica): **a**, **b** holotype, habitus, dorsal view; **d** paratype, trophi, cephalic view; **e** paratype, trophi, caudal view. *A. gracilis* (Europe, PL0924): **c** habitus, dorsal view; **f** trophi, caudal view. *Scale bar* 50 µm (**a**–**c**) or 5 µm (**d**–**f**)

Type locality Rocka Islands (Argentine archipelago), 15 m asl, S65°10.738′, W64°29.522′

Type habitat Soil.

Type material Holotype: SIZ 53.1 (MRockaAED1a, 15.02.2010, Leg. K. Janko), mounted in glycerin jelly. Paratypes: SIZ 53.2-7 (MRockaAED2-7), in glycerin jelly on a separate slide; SIZ 53.8-21 (MRockaAED1b-e, MRockaAED8-18), trophi mounted for SEM. *Additional material* SIZ 53.22-25 (870_1AED1, V12AED1-3), trophi mounted for SEM; SIZ 53.26-33 (870_1AED2, VRA01AED2-3, VS03AED1-3), digital photos and videos.

Etymology Named after the Czech biologist Dr. Edita Drdová-Janková, wife of the collector and project leader Dr. Karel Janko.

Barcodes Gen Bank ID EF173189-91, EF173193, KJ543598-600, see also Supplementary file III.

ZooBank LSID urn:lsid:zoobank.org:act:21EBEA58-A1F3-4D91-9249-CA4E6607986B.

Diagnosis Resembles *A. gracilis* s. str. Janson, 1893 (Fig. 5c, f) and *A. bartosi* Wulfert, 1960 known from Europe, by the short narrow rostrum, the arcuate rostral lamella not divided into lobes and without long sensory bristles underneath and the structure of rakes. Unlike other *Adineta*, all three mentioned species have rod-like, V-shaped rakes with only two claw-like teeth in each rake directed toward each other. The new species differs from *A. gracilis* and *A. bartosi* by the shape of the head and spurs. The first head pseudosegment not bubble-like swollen as in *A. bartosi*. Head not elongated in the proximal part and not widened (hexagonal) in the distal part as in *A. gracilis* s. str. The head of the new species is larger and wider than in *A. gracilis* s. str. Differently from *A. gracilis* s. str., the new species has somewhat larger and stouter body. Spurs of the new species narrow conical, pointed, with short interspace, while *A. gracilis* s. str. has isoceles triangular spurs without an interspace, and the spurs of *A. bartosi* are narrow, peg-like and without an interspace. Trophi much larger than in *A. gracilis* s. str. with larger number of minor teeth in unci.

Description Body of moderate size, dorsoventrally flattened, transparent, colorless except the yellow–brown stomach. Integument smooth, thin, without knobs, spines or bolsters. Rostrum very short, of moderate width; its lamella wide, semicircular, not divided into lobes. Two claw-like sharp teeth pointing toward each other in each thin rod-like rake. Head wide oval, of regular shape or slightly narrowed toward rostrum, HL is 13–19% of TL. Neck rather short and

wide, NL 34–62% of TL, antenna about 1/3 of the bearing pseudosegment width. Trunk wide, oval. Rump somewhat swollen in the middle part, RL is 11–16% of TL. Foot of moderate length, five pseudosegments, FL is 10–16% of TL. Spurs short, conical, pointed, divergent, with tiny interspace, SL is 60–83% of SSW. Three short unsegmented toes. No eyespots. Trophi round, 15–19 μm long and 16–20 μm wide; 2/2 major and 28–34 minor teeth in unci. Oviparous. Eggs oval, smooth without knobs or spines. Egg size 71–89 × 45–61 μm by our data and 70 × 50 μm as reported by Dartnall & Hollowday (1985).

Measurements See Table 2. TL 300 μm by Dartnall & Hollowday (1985) and 220–300 μm according to Velasco-Castrillón et al. (2014a).

Distribution Maritime Antarctica: AI*, SI, AP*. Continental Antarctica: DM, EB, LH, MM, VL (Cz*, CR*), WK.

Habitat Soil, terrestrial moss and lichens, pools.

Comments Most likely all the researchers, except Murray (1910), have been reporting this species under *A. gracilis*—which, in spite of presumed cosmopolitanism, is very unlikely to inhabit dry and cold Antarctic, being a strict acidophile most common in sphagnum bogs (Bērziņš, 1987).

The head of the new species is 45 ± 4 μm long and 34 ± 4 μm wide, HW/HL is 69–90%. According to our data, *A. gracilis* s. str. has the head 40 ± 7 μm long and 29 ± 4 μm wide ($N = 42$), HW/HL is 53–70%. By our data, *A. gracilis* s. str. has TL 247 ± 45 μm, BW/TL 13–23%, RW/RL 54–82%, FW/FL 27–40% ($N = 42$). The new species TL is 286 ± 41 μm, BW/TL is 13–23%, RW/RL is 67–99%, and FW/FL is 38–58%. By our data, the trophi of *A. gracilis* 11.1 ± 0.4 μm long, 13.7 ± 0.9 μm wide ($N = 14$), 20–24 minor teeth in each uncus. The new species has trophi of 16.6 ± 1 μm long and 18.4 ± 0.7 μm wide, with 28–34 minor teeth in each uncus.

Adineta emsliei sp. nov. Fig. 6a, b, d, e

Dartnall, 1995a (*A.* sp.), p. 13, Fig. 7a. Velasco-Castrillón et al., 2014a (*A.* sp. Bd1), p. 8.

Type locality Cape Royds (Ross Island), 27 m asl, S77°32.500′, E166°8.933′.

Type habitat Cyanobacterial mats in wetlands.

Type material Holotype SIZ 52.1 (CR23matAE1a, 14.1.2010, Leg. J. Smykla) mounted in glycerin jelly. Paratypes: SIZ 52.2-52.16 (CR23matAE2-16) in glycerin jelly on a separate slide; SIZ 52.16-17 (CR23-matAE1b-c), trophi mounted for SEM. *Additional material* SIZ 52.18-52.22 (CR23matAE1e-j, CBM 2AE1), trophi mounted for SEM; SIZ 52.23-31 (CR23-matAE17-21, CBM2matAE1-4), digital photos and videos.

Barcodes Gen Bank ID KJ543570-80, see also Supplementary file III.

ZooBank LSID urn:lsid:zoobank.org:act:45FA650B-0086-4E3C-BCCD-A3F228E987F3.

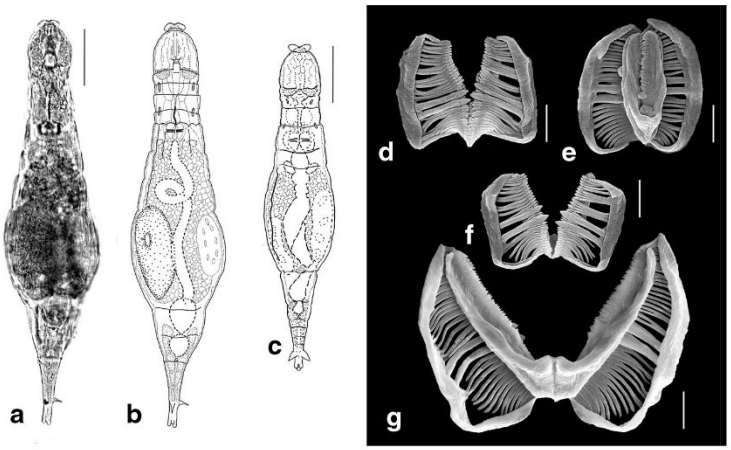


Fig. 6 *Adineta emsliei* sp. nov. (Antarctica): **a**, **b** holotype, habitus, dorsal view; **d** paratype, trophi, cephalic view; **e** paratype, trophi, caudal view. *A. vaga* (Europe, PL0838): **c** habitus, dorsal view; **f** trophi, cephalic view. *A. grandis* Murray, 1910 (Antarctica): **g** trophi, caudal view. *Scale bar* 50 μm (**a**–**c**) or 5 μm (**d**–**g**)

Etymology Named in honor of the leading Antarctic researcher Dr. Steven D. Emslie for his invaluable support in the Ross Sea project.

Diagnosis Resembles *A. grandis* by the bright orange body color, but it is smaller and not viviparous. By our data, the new species is larger than the similar oviparous species *A. vaga* s. str. (Davis, 1873) (Fig. 6c). Trophi size is intermediate between *A. vaga* s. str (Fig. 6f) and *A. grandis* (Fig. 6g). Spurs are needle-like with bulb-like swollen bases, while *A. vaga* s. str. has straight triangular spurs. From *A. vaga major* Bryce, 1893, and *A. vaga minor* Bryce, 1893, the new species differs by the shape of the spurs, and the intermediate head size (it is larger than *A. vaga minor*, but smaller than *A. vaga major*). From *A. vaga* s. lat. the new species differs by the orange body (*A. vaga* s. lat. is colorless inclusive stomach).

Description Body of moderate size, wide, flattened, of bright orange color. Integument smooth, thin, transparent, without knobs, spines, bolsters or other appendages. Rostrum short, sickle-like, distal rostral pseudosegment not plate-like flattened. Two short semicircular rostrum lobes, no stiff sensory bristles, only short cilia under the lobes. Wide-oval head of a moderate size, HL is 13–18% of TL, HW is 71–94% of HL. Six thin peg-like teeth in each massive scoop-like rake. Neck of moderate length and width, slightly contracted behind the head, NL is 14–21% of TL, antenna about 1/3 of the bearing pseudosegment width. Trunk oval, wide, BW is 19–27% of TL. Rump conical, somewhat swollen in the middle, RL is 11–16% of TL, RW is 74–98% of RL. Relatively short slim foot of five pseudosegments, FL is 10–16% of TL, FW is 29–45% of FL. Spurs short (SL 60–94% of SSW), pointed, needle-shaped with bulb-like swollen bases, divided by straight interspace of ~2 spur widths. Three short unsegmented toes. No eyespots. Trophi ramate, round, 15–18 μm long and 14–18 μm wide. Rami massive, the region of articulation is straight, protruding backwards, without incisure. Interior margins of rami with long numerous peg-like scleropili. Manubria thin, sickle-like. Two major teeth and 29–33 minor teeth in each uncus. Throat small, esophagus short, straight. Stomach glands of moderate size. Eight nuclei (3–7 according to Murray) in each germovitellarium. Oviparous. Eggs oval, 60–70 × 39–44 μm, shell smooth, without knobs or spines.

Measurements See Table 2. TL 350 μm according to Dartnall (1995a, b).

Distribution Maritime Antarctica: AI*. Continental Antarctica: EB, HI, VL (CR*, CB*, MP*), WK.

Habitat Cyanobacterial mats wetlands, terrestrial moss, soil.

Comments According to our data, the new species has TL 294 ± 44 μm, while TL is 414 ± 61 μm in *A. grandis* ($N = 20$) and 274 ± 14 μm in *A. vaga* s. str. ($N = 15$). The new species has trophi 15.7 ± 1.1 μm long with 29–32 minor teeth in each uncus, while *A. grandis* has trophi 25.4 ± 1.4 μm long with 36–44 minor teeth ($N = 53$), and *A. vaga* s. str. has it 13 ± 0.7 μm long with 25–27 minor teeth ($N = 14$).

Adineta grandis Murray, 1910 (Figs. 3c, 6g, 7a)

Murray (1910, pp. 51–53, Pl. XII Fig. 10). Voigt (1956–1957, p. 71, Taf. 5 Abb. 24, Taf. 8 Abb. 19, Taf. 14 Abb. 16). Donner (1965, p. 273, Fig. 200a). Donner (1972a, p. 252, Abb. 1). Koste (1996b) (as *A. grandis*, but most likely sibling species), p. 243, Abb. 5. Dartnall & Hollowday (1985, p. 31, Fig. 24d–f). Kutikova (2005, p. 275, Ris. 299). Velasco-Castrillón et al. (2014a) (*A.* sp. Bd2): 8 (main text); 2, Fig. 8 (Annex S1).

Type locality Cape Royds

Type habitat "Brown vegetation" (algae?) in lake.

Barcodes GenBank ID KJ543581-88, see also Supplementary file III.

Material examined BI11, 1 ind.; BI23, 10 ind.; CBM1CYmat, 6 ind.; CBM2mat, 4 ind.; CBC1mat, 5 ind.; CBPc2mat, 1 ind.; CRL21, 2 ind.; CR24, 2 ind.; CRL24, 1 ind.; CzM2Cymat, 8 ind.; CzM3CYmat, 9 ind.; EPL23, 11 ind.; MPM3, 1 ind.; MPM5, 34 ind.; MPM5CYmat, 10 ind.

Description The largest species of the genus, and the only known viviparous one. Reported TL is 306–750 μm (Murray, 1910; Donner, 1965; Dartnall & Hollowday 1985) and 304–505 μm according to our data. Its foot is shorter than in other species of *Adineta*. Trophi length 23–29 μm (our data). Body pale orange or brownish yellow, sometimes reddish ("light brown or yellowish, darker in the alimentary tract" according to Murray). Integument smooth, thin, transparent, without knobs, spines or other appendages. Rostrum short, of moderate width, distal rostral pseudosegment not strongly widened or flattened. Rostral lamella divided into two small semicircular lobes. No stiff sensory bristles under rostrum lobes, only short soft

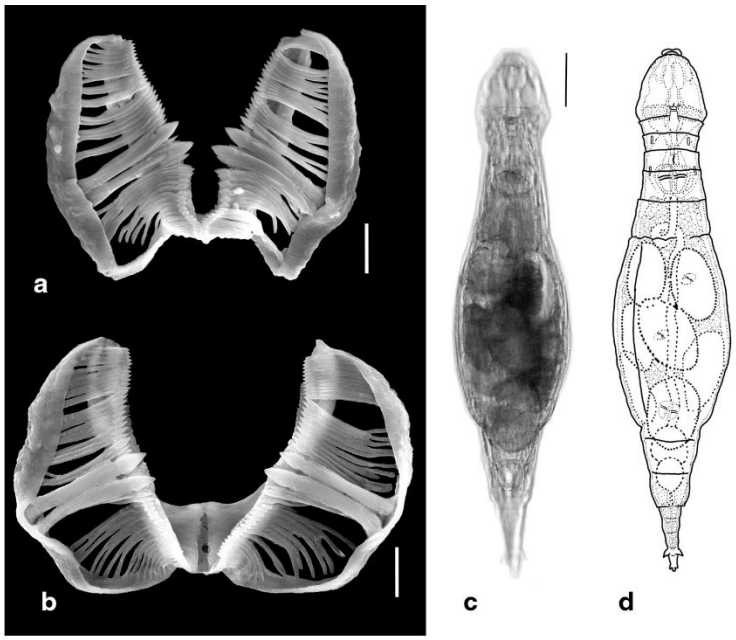


Fig. 7 *Adineta grandis*: **a** trophi, cephalic view. *A. fontanetoi* sp. nov.: **b** paratype, trophi, cephalic view; **c**, **d** holotype, habitus, dorsal view. *Scale bar* 50 μm (**c**, **d**) or 5 μm (**a**, **b**)

cilia. Head not large (HL is 13–19% of TL), wide oval ("ovate" by Murray), tapering toward rostrum, HW is 66–97% of HL. 6–10 teeth in each massive scoop-like rake. Neck massive, long (NL is 11–27% of TL). Dorsal antenna thick, about 1/4 of width of the antennal pseudosegment. Trunk wide (its width depends on the number of embryos inside), BW is 16–31% of TL. Rump conical, with both pseudosegments somewhat swollen laterally (in some specimens the lateral swellings look like knobs), gradually tapering into a very short narrow foot. RL is 7–16% of TL, RW is 74–103% of RL. Foot short, of 5 pseudosegments, FL is 6–15% of TL, FW is 40–56% of FL. Spurs conical, widened at the base (according to Murray, "short broad cones," "stout and subacute"), pointed, narrow, divergent, divided by the straight interspace equal to 1–2 spur widths, SL is 60–98% of SSW. Three short unsegmented toes. No eyespots. Trophi ramate, large, round or elongate. Rami massive, interior margin with numerous peg-like scleropili. Articulation protruding to the ventral part, straight and without incisure. Manubria wide, flat, crescent-shaped. Major uncinal teeth thick, dental formula 2/2; 38–41 minor teeth. Trophi unusually large for *Adineta*: 30 μm long according to Donner (1965), 24–31 μm long and width is equal to the length, according to our data. Throat voluminous, esophagus short, straight. Stomach glands large. Eight nuclei in each of germovitellaria. Viviparous, up to 4 embryos with developed trophi can be seen inside trunk.

Measurements See Table 2. TL up to 750 μm according to Murray (1910).

Distribution Maritime Antarctica: SI. Continental Antarctica: EB, HI, MM, VL (BI*, CB*, CC*, CR, Cz*, EP*, MP*). Africa (questionable): Madagascar (Koste, 1996a).

Habitat Algal mats and sediment in pools and seepages, soil, terrestrial moss.

Comments Velasco-Castrillón et al. (2014a) erroneously attributed this rotifer to "wheel-bearers" (although *A. grandis* has no trochi), and described it as "ovoviviparous" although the species is viviparous.

Adineta fontanetoi sp. nov. Fig. 7b–d

Type locality Beaufort Island, 9 m asl, S76°58.147′, E166°54.217′.

Type habitat Soil.

Type material Holotype: SIZ 54.1 (BI27AG1a, 29.1.2010, Leg. J. Smykla), mounted in glycerin jelly. Paratypes: SIZ 54.2-4 (BI27AG1b-d), SIZ 54.5-22 (BI27AG2-19), trophi mounted for SEM.

Etymology The species is named after colleague rotiferologist Dr. Diego Fontaneto who first sequenced this species (as *A. grandis*).

Barcodes GenBank ID EF173184-85, KP869896.

ZooBank LSID urn:lsid:zoobank.org:act:15A138A9-A20D-41BE-A7E6-0EC4BF2F59B0.

Diagnosis By external morphology the new species does not differ from *A. grandis* (see the description above) and may be easily confused with the latter under the light microscope. However, it has somewhat larger trophi (ramus length mean ± SD 26.8 ± 1.2 μm in *A. fontanetoi* sp. nov. and 25.4 ± 1.4 μm in *A. grandis*).

Description Viviparous. 8 teeth in each rake. Trophi 24–28 μm long and 27–28 μm wide; 2/2 major uncinal teeth, 38–43 minor teeth in the left uncus and 39–42 in the right one.

Measurements See Table 2.

Distribution Maritime Antarctica: SI. Continental Antarctica: BI*.

Habitat Soil.

Order Philodinida Melone & Ricci, 2005

Family Habrotrochidae Bryce, 1910

Genus *Habrotrocha* Bryce, 1910

Habrotrocha antarctica sp. nov. (Figs. 3d, 8)

Murray, 1910 (*Callidina constricta*), pp. 48–49, Pl. XII Fig. 13a, b; Dartnall & Hollowday, 1985 (*H. constricta*), p. 32; Fig. 25a–c. Velasco-Castrillón et al., 2014c (Bd12), p. 8.

Type locality Cape Royds, 18 m asl, S77°32.532′, E166°8.855′.

Type habitat Soil.

Type material Holotype: SIZ 56.1 (CRL23HE1a, 14.01.2010, Leg. J. Smykla), mounted in glycerine jelly, encircled in green ink. Paratypes: SIZ 56.2-4 (CRL23HE1b-d), on the same slide as holotype, encircled in black ink; SIZ 56. 5-11 (CRL23HE1e-k), trophi mounted for SEM. *Additional material* SIZ 56.12-23 (EPL24M51-5, CzL4CYmatHE1-7), digital videos and photos; SIZ 56.24 (CzL4CYmatHE8), trophi mounted for SEM.

Etymology Named after the Antarctic continent where it was first found.

Barcodes GenBank ID EF650588-90, KJ543609-11, see also Supplementary file III. *ZooBank LSID.* urn:lsid:zoobank.org:pub:C3618A2A-F710-4318-B8EA-58C285EC6CDA.

Diagnosis Very similar to *H. elusa* s. lat. Milne, 1916, except of the integument sculpturation, rump shape and trophi structure. The foot is much wider and the spurs thicker and longer than in *H. elusa vegeta* Milne, 1916. Differently from *H. elusa* s. str. Milne, 1916, it has no lateral knobs on the first rump pseudosegment. The integument on the trunk and rump is not dotted or granulated, unlike it is reported for *H. elusa* s. str. (Milne, 1916; Donner, 1965). The same as in *H. elusa* s. str., corona width of the new species is almost equal to the cingulum, while in *H. elusa vegeta* it is substantially narrower. Similar to *H. constricta* by the size and body shape, however distinguished by the upper lip with a notch in the middle (so that the tip is divided into two small lobes), while in *H. constricta* the tip is whole. Dental formula 7/7 major uncinal teeth (the last 2–3 thinner than the rest), while in *H. elusa* s. str. it is reported to be 6/6. In *H. elusa vegeta* it is 3 + 4/3 + 4 and in *H. constricta* usually has 6/6 major teeth in unci and rarely 7/7 or 8/8 (Donner, 1965). Murray (1910) reports this species to have 4/4 thicker teeth, succeeded by several finer ones, what can be sometimes observed in our specimens.

Description Body of moderate size, transparent, spindle-shaped, colorless but usually with yellow-brown or bright orange stomach. Integument smooth, thin, without knobs, ribs or spines. No knob on the first foot pseudosegment. Rostrum short, lamella divided into two small semicircular lobes. Corona narrower than the oval head base, CW/HW 79–91%, HW is 96–100% of HL. Pedicels short, straight, divided by a narrow sulcus without membrane or ligula. Trochal discs kidney shaped in apical view. No papillae or sensory bristles on trochi. Upper lip triangular, reaching plane of trochal discs, upper rim thickened by cuticular bolster, tip divided by a notch into two small rounded lobes. Lower lip not wide, not projecting laterally. Cingulum bolster very narrow. Neck of moderate length and width, NL is 15–35% of TL. Trunk slim, BW is 15–20% of TL. Rump conical, first pseudosegment swollen, RL is 11–15% of TL, RW is 80–110% of RL. Foot very short, 4 pseudosegments,

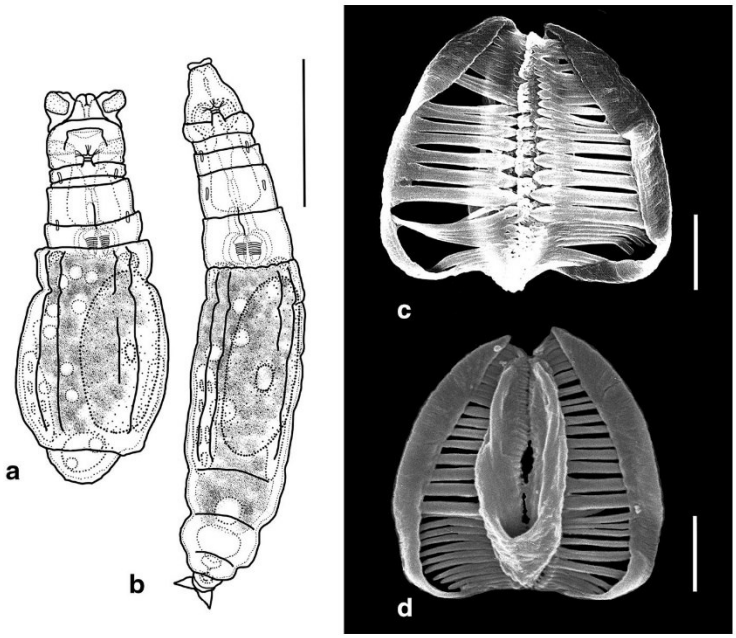


Fig. 8 *Habrotrocha antarctica* sp. nov.: **a** holotype, habitus, feeding, dorsal view; **b** same, creeping, dorsal view; **c** paratype, trophi, cephalic view; **d** paratype, trophi, caudal view. *Scale bar* 50 µm (**a**, **b**) or 5 µm (**c**, **d**)

FL is 10–12% of TL, FW is 49–74% of FL. Spurs short, triangular with elongated narrow tips and slightly swollen middle part, divergent, without interspace, SL is 55–74% of SSW. Three short unsegmented toes. No eyespots. Throat narrow, esophagus short, straight. Stomach glands small, round. Food pellets rounded, small. Trophi ramate, heart-shaped, 15–19 µm long and 17–20 µm wide. Rami thick, with numerous short scleropili along the inner rims. Articulation straight, wide, without incisure. Manubria narrow, sickle-like. Dental formula 7/7 or 4 + 3/4 + 3, with 24–25 minor teeth in each uncus. Oviparous. Eggs oval, 65–70 × 33–41 µm, shell smooth, without knobs or spines.

Measurements See Table 2. TL 250 µm by Murray (1910) or 375 µm (Dartnall & Hollowday, 1985).

Distribution Maritime Antarctica: SI. Continental Antarctica: EB, MM, VL (CR, EP*, Cz*).

Habitat Soil, algal mats, sediment in pools.

Comments Murray (1910) first depicted this species, but erroneously identified it as *C.* (=*H.*) *contstricta*. In Murray's image the notch in the middle of the upper lip (absent in *H. constricta*) is clearly visible, and the dental formula seems to be 7/7 or 8/8 (though in the description Murray mentions only 4/4 major teeth). *H. antarctica* sp. nov. was identified as *H. constricta* by Dartnall & Hollowday (1985), but the specimen depicted by these authors has a two-lobed upper lip, while *H. constricta* has only one lobe.

Habrotrocha devetteri sp. nov. Iakovenko (Fig. 9a–d)

Velasco-Castrillón et al., 2014a (Bd42), p. 8 (main text); 5, Figs. 21–22 (Annex S1).

Type locality Cape Bird (Ross Island), 77 m asl, S77°13.207′, E166°26.568′.

Type habitat: Soil.

Type material Holotype: SIZ 57.1 (CBM2HD2a, 19.01.2010, Leg. J. Smykla), mounted in glycerin jelly, incircled with green ink. Paratypes: SIZ 57.2-5 (CBM2HD1, CBM2HD3-5), mounted on the same slide as the holotype, incircled with black ink; SIZ 57.6 (CBM2HT2b), trophi mounted for SEM. *Additional material* SIZ 57.7-14 (CBC4HD1, CBM2HD6-9, CBM2matHD1-3), digital photos and videos; SIZ 57.15 (CzL4CymatHD2), trophi mounted for SEM.

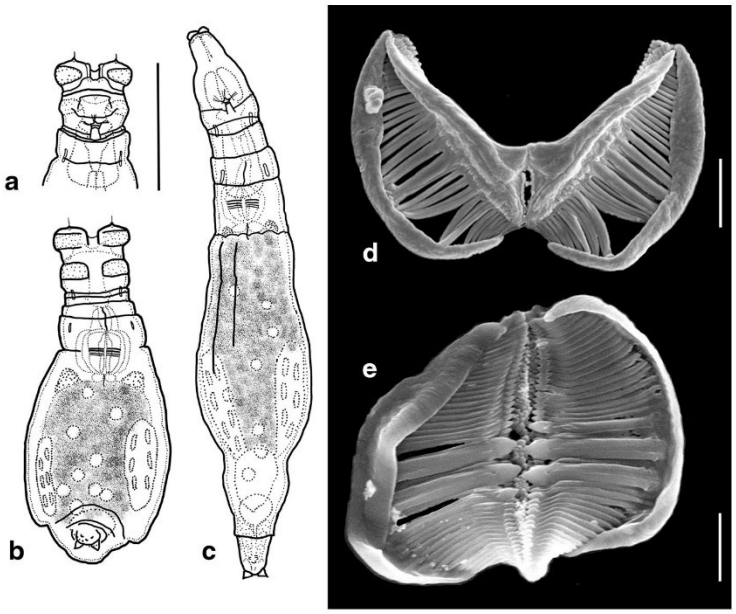


Fig. 9 *Habrotrocha devetteri* sp. nov. (Antarctica): **a** holotype, head, feeding, dorsal view; **b** same, habitus, feeding, ventral view; **c** habitus, creeping, dorsal view; **d** paratype, trophi, caudal view. *H. thienemanni* (Europe): **e** trophi, cephalic view. *Scale bar* 50 μm (**a**–**c**) or 5 μm (**d**, **e**)

Etymology Named after colleague rotiferologist Dr. Miloslav Devetter participating in this study.

Barcodes GenBank ID KJ543668-74, see also Supplementary file III.

ZooBank LSID urn:lsid:zoobank.org:act:C39F702E-3A94-4529-879F-7093793196D2.

Diagnosis Similar to *H. thienemanni* s. lat. by the shape and size of the corona, trunk and spurs. Like *H. thienemanni* s. lat., its upper lip has two small lobes; however, the lobes are rounded and divided by a broad interspace, while in *H. thienemanni* s. lat. the lobes are often pointed and divided by a notch. The new species has 2 + 2/2 + 2 major teeth in the unci, while *H. thienemanni* s. lat. has 2 + 1/2 + 1 major teeth (Fig. 9d, e). It differs from *H. crassa* Donner, 1949, another species with a two-lobed upper lip, by the corona being wider than the head base, the smooth integument, body outline and dental formula (4/4 in *H. crassa*). It differs from *H. tranquilla* Milne, 1916, by its smaller size. The TL of the new species is 209–282 μm, while for *H. tranquilla* it is 340–402 μm. The lower lobes of the upper lip are divided by an interspace, while in *H. tranquilla* they are higher and divided by a notch. Dental formula is not 7/7−9/9 as in *H. tranquilla*.

Description Body of moderate size, spindle-shaped, colorless, transparent. Integument thin, smooth, without knobs, spines or bolsters. Rostrum short, lamella with two small semicircular lobes. Corona wider than the oval head base, CW is 103–118% of HW, HL is 22–31% of TL. Pedicels short, straight. Sulcus very narrow, half-covered with membrane. Trochal discs with papillae and sensory bristles. Upper lip goes up to a half of the pedicels, it is arcuate with two small semicircular lobes divided by an interspace. Lower lip slightly protruding laterally. Cingulum narrow. Neck of moderate length, NL is 15–26% of TL. The length of the antenna is about 1/3 of the bearing pseudosegment width. Trunk plump, BW is 17–30% of TL. Rump conical, RL is 12–17% of TL, RW is 81-98% of RL. Foot short, slim, 4 pseudosegments, FL is 8–14% of TL, FW is 51–88% of TL. Spurs short, triangular, divided by interspace as broad as one spur width, SL is 44–76% of SSW. Three short unsegmented toes. No eyespots. Throat and esophagus short, food pellets round, of moderate size. Stomach glands of medium size. Trophi ramate, heart-shaped, 16–19 μm long and 16–19 μm wide. Rami thin, with numerous short scleropili along the inner rim, articulation straight and without incisure. Manubria thin, sickle-like. 2 + 2/

2 + 2 major teeth, 30–33 minor teeth in each uncus. Oviparous, egg oval, 67 × 39 µm, shell smooth, without knobs or spines.

Measurements See Table 2. TL 250–360 µm (Velasco-Castrillón et al., 2014a).

Distribution Continental Antarctica: EB, VL (CC*, CB*), WK.

Habitat Soil, algal mats.

Habrotrocha vernadskii sp. nov. (Figs. 3e, 10)

Type locality Galindez Island (Argentine Islands archipelago), 4 m asl, S65°15.060′, W64°14.558′.

Type habitat Soil.

Type material Holotype: SIZ 58.1 (V12HE2a, 1.03.2010, Leg. K. Janko), mounted in glycerin jelly, encircled in green ink. Paratypes: SIZ 58.2-4 (V12HE2a-c), on the same slide as holotype, encircled in black ink; SIZ 58. 5-8 (V12HE4a-b, V12HE13a-b), trophi mounted for SEM. *Additional material* SIZ 58. 9-15 (CCA2HE1-2, CCA4HE1-5), digital photos and videos.

Etymology Named after the Ukrainian polar research base "Academician Vernadsky" in the vicinity of which it was found.

Barcodes GenBank ID—see Supplementary file III.

ZooBank LSID urn:lsid:zoobank.org:act:6FF7FEAF-BC8C-46FB-AA46-F09ADF2D2147.

Diagnosis Resembles *H. elusa* s. str. Milne, 1916 by the yellowish body with thicker integument on trunk and rump, the triangular upper lip with the tip divided by a notch into two rounded lobes, by lateral knobs on the rump and by the short triangular spurs. However, the knobs seem to be smaller and sharper than in *H. elusa* s. str., and their number is 4, not 6. Milne (1916) states that *H. elusa* s. str. has "thick, leathery but smooth skin" that is stippled, but not granulated, on the trunk, rump and foot excluding spurs. Contradictory to this, the new species has a trunk, rump and first foot pseudosegment covered with minute granulae, as in "*H. elusa* s. str." found by Donner (1965). Similarly to *H. elusa vegeta*, which lacks granulated culticle, corona of the new species is distinctively narrower than the head base, CW/HW is 71–84%. Milne reports *H. elusa*'s s. str. corona to be

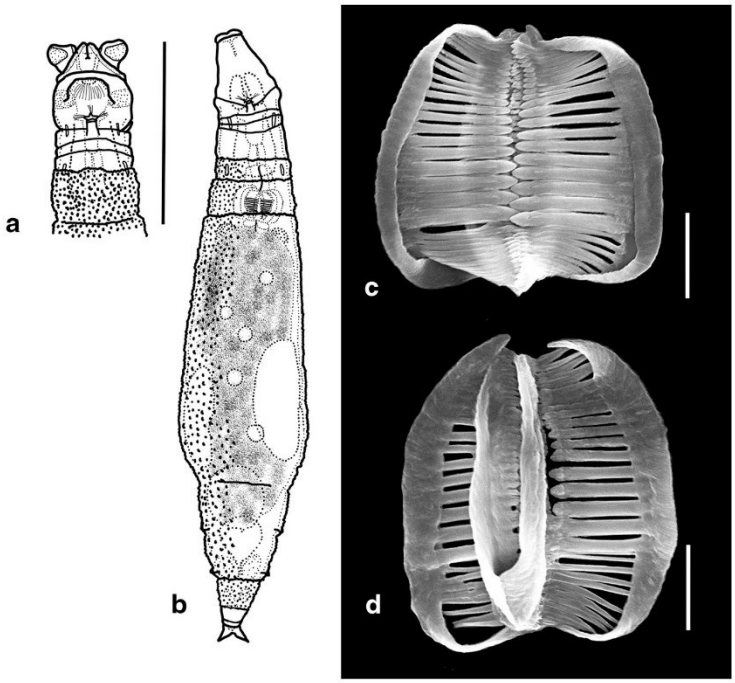


Fig. 10 *Habrotrocha vernadskii* sp. nov.: **a** holotype, head, feeding, dorsal view; **b** same, habitus, creeping, dorsal view; **c** paratype, trophi, cephalic view; **d** paratype, trophi, caudal view. *Scale bar* 50 µm (**a**, **b**) or 5 µm (**c**, **d**)

equal to or slightly wider than the head base (CW/HW is 100–111%). Dental formula 9/9 major uncinal teeth (the last two almost as thin as minor teeth), while in *H. elusa* s. str. it is 6/6, and in *H. elusa vegeta* is 3+4/3+4 (Donner, 1965). Differs from *H. crenata* s. lat. by the shape of the upper lip (in *H. crenata* s. lat. the tip of the upper lip is not divided into lobes) and by the pattern of sculpturation. In *H. crenata* s. str. the whole foot and spurs are granulated, and the first foot pseudosegment has a rounded knob absent in the new species. Dental formula of *H. crenata* is 7/7 or 8/8 (Donner, 1965). Differs from *H. antarctica* sp. nov. by the granulated integument, lateral knobs on rump, narrower corona, and slightly longer and narrower spurs. Also, it has a larger number of major and minor teeth in the unci (9/9) than *H. antarctica* sp. nov. (7/7 or 4+3/4+3).

Description Body slim, spindle-shaped, yellowish. Integument granulated on the last neck pseudosegment, trunk, rump and the first foot pseudosegment, and smooth on the rest of the body. Four small pointed lateral knobs on rump (2 on the distal rim of the first and 2 on the second pseudosegment). No knobs on foot. Rostrum very short, lamella with two small semicircular lobes. Corona narrower than rectangular head base, HL is 11–15% of TL. Upper lip triangular, reaches plane of trochal discs, tip divided by a notch into two small semicircular lobes. Trochi without papillae and sensory bristles. Pedicels short, straight. Sulcus narrow, partly covered by prominent retractors of trochi. Lower lip not protruding laterally. Neck rather long, of moderate width, NL is 38-61% of TL. Antenna is 1/3–1/4 of the bearing pseudosegment's width. Trunk narrow, BW is 29–62% of TL depending on the amount of eggs in a female. The first rump pseudosegment swollen, RL is 10–15% of TL, RW is 71–99% of RL. Foot short, 4 pseudosegments, FL is 8–11% of TL, FW is 54–85% of FL. Spurs of moderate length, narrow triangular, bases merged but seem to form short interspace, SL is 56–84% of SSW. Three short unsegmented toes. No eyespots. Throat small, esophagus short, straight. Food pellets small, of irregular shape. Oviparous, eggs oval, shell smooth, without knobs or spines. Trophi ramate, heart shaped, 15–17 μm long and 14–16 μm wide. Articulation straight, without incisure. Numerous short scleropili on inner rims of rami. Manubria narrow, sickle-like. Unci with 9/9 major teeth, gradually diminishing in thickness, the last ones hardly distinguishable from minor teeth (26–27 in each uncus).

Measurements See Table 2.

Distribution Maritime Antarctica: AI*. Continental Antarctica: CC*.

Habitat Soil, terrestrial moss.

Family Philodinidae Ehrenberg, 1838

Genus *Macrotrachela* Milne, 1886

Macrotrachela donneri sp. nov. Fig. 11

Murray, 1910 (*Callidina habita*): Pl. IX Fig. 3, Pl. XI Fig. 8a. Donner, 1965 (*Macr. insolita* var. 3), p. 132, Fig. 96l, m. Donner, 1972a (*Macr. insolita* var.), p. 252, Abb. 2. Iakovenko & Tyshenko, 2006 (*Macr. hewitti*), p. 2, Ris. 2.

Type locality Marble Point (Victoria Land), 4 m asl, S77°25.597′, E163°45.148′.

Type habitat Soil.

Type material Holotype: SIZ 59.1 (MPM5MD1a, 25.01.2010, Leg. J. Smykla), on slide in glycerine jelly, encircled with green ink. Paratypes: SIZ 59.2-6 (MPM5MD2-6), in glycerine jelly on the same slide as the holotype, encircled with black ink; SIZ 59.7-9 (MPM5MD1b-d), trophi mounted for SEM. *Additional material* SIZ 59.10-15 (KG1MD1, EPL25MD1, MPL1MD1, MPL3MD1-3), digital photos and videos; SIZ 59.15-18 (MPL3MD1-4), trophi mounted for SEM.

Barcodes GenBank ID KP869998.

ZooBank LSID urn:lsid:zoobank.org:act:86AC6997-0625-4B56-BEC9-FECCF2379EC5.

Etymology Named after the Austrian rotiferologist Dr. J. Donner who first depicted this species as *M. insolita* var.

Diagnosis Similar to *Macr. concinna* (Bryce, 1912), *Macr. habita* (Bryce, 1894), *Macr. hewitti* (Murray, 1911), *Macr. insolita* de Koning, 1947 and *M. plicata* s. str. (Bryce, 1892) by the upper lip with two rounded lobes. Alike *Macr. habita, Macr. hewitti* and *Macr. insolita*, the new species has a knob on the first foot pseudosegment, which is lacking in *Macr. concinna* and *Macr. plicata*. Ligula in the sulcus and knobs on rump typical for *Macr. plicata* are absent in the new species. The integument is smooth, while in *M. habita* it is very finely stippled (Bryce, 1894). The foot is rather stout (what distinguishes all three species

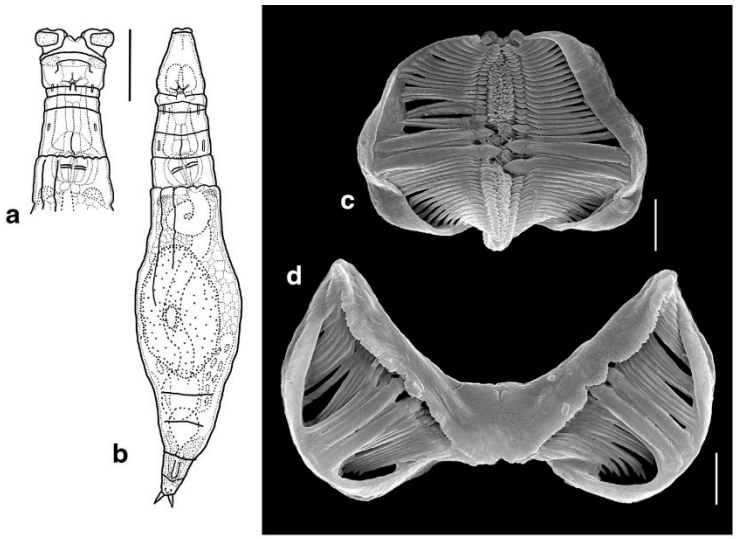


Fig. 11 *Macrotrachela donneri* sp. nov.: **a** holotype, head, feeding, dorsal view; **b** same, habitus, creeping, dorsal view; **c** paratype, trophi, cephalic view; **d** paratype, trophi, caudal view. *Scale bar* 50 μm (**a**, **b**) or 5 μm (**c**, **d**)

from *Macr. plicata* s. lat.). Similarly to *Macr. insolita*, the dental formula of the new species is 2/2, while in *Macr. habita* it is 2+1/1+2 (with an additional thinner teeth), and in *Macr. hewitti* it is 4/4 or 5+1/5+1 (Donner, 1965). It is also distinguished by the peg-like or narrow conical spurs with broad interspace—in *Macr. habita* and *Macr. insolita* the spurs are triangular, wide, and flat, the interspace is shorter; in *Macr. hewitti* the interspace is lacking (Donner, 1965; Murray, 1911). Larger than *Macr. insolita* (TL 325 ± 44 μm, TL of *Macr. insolita* is about 250 μm). CW/HW ratio (107–119%) is intermediate between *Macr. habita* (120–125%) and *Macr. insolita* (100–103%), and the head seems to be shorter and wider than in *Macr. insolita*. The upper lip is shorter, and the lobes are more separated from each other than in *Macr. concinna* and *Macr. insolita*. The head base is rectangular, while it seems to be trapezoid in *Macr. habita*, and wide-oval in *Macr. insolita*.

Description Body large, transparent, colorless, but often with bright-orange stomach. Integument smooth, without spines or bolsters, no knobs except a large longitudinal knob on the first foot pseudosegment. Rostrum short, thick, lamella with two semicircular lobes. Head wide, corona wider than head base, CW is 107–119% of HW, HL is 10–15% of TL. Pedicels short, straight. Sulcus as wide as 1/2 of a trochus, covered with protruding trochi retractors. No papillae or sensory bristles on trochi. Head base rectangular, shorter than its width. Upper lip arcuate with two large semicircular lobes not divided by interspace, reaching 1/2 of the pedicels' height. Lower lip protrudes laterally. Neck of moderate length and width, NL is 15–21% of TL. Length of antenna is about ¼ of bearing pseudosegment width. Trunk thick, BW is 15–25% of TL. Rump large, swollen, RL is 12–15% of TL, RW is 65–97% of RL. Foot short, of 4 pseudosegments, first pseudosegment with dorsal elongated knob. Spurs rather long, rod-like, pointed, gradually tapering from the base to tips, interspace equal to 2 spur widths, SL is 86–111% of SSW. Three thick unsegmented toes. No eyespots. Throat voluminous, esophagus short, straight. Lumen long, thick, often with a loop. Stomach glands large, round. Trophi ramate, large, heart-shaped, 21–25 μm long and 24–28 μm wide. Rami massive, inner rim with numerous scleropili. Articulation long, flat, straight, without incisure. Manubria long, wide, crescent-like. Dental formula 2/2, with 42–43 minor teeth in each uncus. Oviparous. Egg lemon-shaped, shell smooth with two round knobs on each pole.

Measurements See Table 2. TL up to 570 μm, CW 95 μm in Murray (1910). According to Donner (1965), TL 410 μm, CW 66 μm, SL 29 μm, trophi 30 μm long.

Distribution Maritime Antarctica: AI*, KG*, SI. Continental Antarctica: VL (CB*, CC*, CR, Cz*, BI*, EP*, MP*).

Habitat Soil, algal mats in seepages, terrestrial moss.

Comments Murray (1910) first depicted this rotifer from Cape Royds, though he apparently described two different species under the name *C. habita*.

Macrotrachela ioannae sp. nov. Iakovenko (Figs. 3h, 12)

Type locality Rocka Islands (Argentine Islands Archipelago), 15 m asl, S65°10.738′, W64°29.522′.

Type habitat Soil.

Type material Holotype: SIZ 60.1 (MRockaMI1a, 15.02.2010, Leg. K. Janko), mounted in glycerin jelly, encircled with green ink. Paratypes: SIZ 60.2-3 (V12MI1-2), on the same slide with holotype, encircled with black ink. *Additional material* SIZ 60.4-6 (MRockaMI2-3, CrulsBMI1), digital photos.

Etymology Named after Mgr. Ioanna Vaňkova, a friend and a specialist in linguistics, who gave much advice on creating Latin names for the new rotifer species.

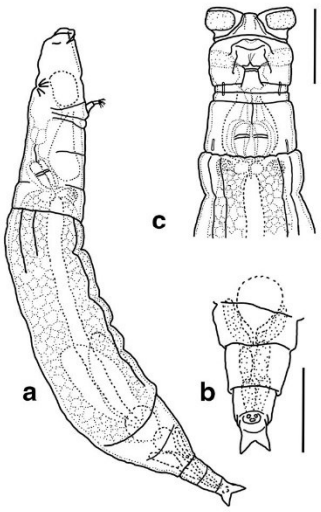


Fig. 12 *Macrotrachela ioannae* sp. nov.: **a** holotype, habitus, creeping, lateral view; **b** same, head, feeding, dorsal view; **c** same, foot, ventral view. *Scale bar* 50 μm

Barcodes GenBank ID KP869995-97.

ZooBank LSID urn:lsid:zoobank.org:act:05D141A6-F494-45F3-B144-755BBCAAB31F.

Diagnosis Similar to *Macr. ehrenbergii* (Janson, 1893), *Macr. timida* s. lat., *Macr. induta* Donner, 1951, and *Macr. allani* (Murray, 1911). Differs from all these species by the shape of spurs with papillae-like tips, and dental formula (additional thinner tooth in each uncus). The head base is shorter and wider than in *Macr. ehrenbergii*. The corona is almost equal to the head base, while in *Macr. timida* and *Macr. allani* it is substantionally wider. Spurs shorter than the bearing pseudosegment width, while in these species they are, on the contrary, longer. Unlike in *Macr. timida* s. lat., no knobs on foot or rump. Differs from *Macr. induta* also by the shape of the upper lip, which in *Macr. induta* is wide arcuate, with a low rounded lobe in the middle. The new species has a narrow arcuate upper lip with a high trapezoid lobe with a rounded tip in the middle, bearing a thin bolster along the upper rim.

Description Body large, colorless, transparent. Integument smooth, without knobs, spines or bolsters. Rostrum thick, of moderate length, lamella with two semicircular lobes. Corona not wide, CW is 100–108% of HW, HL is 11–21% of TL. Pedicels short, straight. Sulcus of moderate width, without ligula, not covered with membrane or trochi retractors. Head base rectangular, its width larger than height. Upper lip arcuate with single trapezoid lobe, its rounded tip has a bolster along the upper rim. Lower lip not protruding laterally. Neck of moderate length and width, NL is 12–18% of TL. Length of antenna is 1/3 of the bearing pseudosegment's width. Trunk cylindrical, BW is 13–17% of TL. First rump pseudosegment swollen, RL is 12–15% of TL, RW is 78–96% of RL. Foot short, 4 pseudosegments, FL is 8–11% of TL, FW is 52–77 of FL. Spurs short, flat, triangular, divergent, with bases merged and tips separated as small papillae. Three unsegmented toes. No eyespots. Throat voluminous, esophagus short, straight. Lumen wide, with a loop. Stomach glands round, not large. Trophi ramate, heart-shaped, 26 μm long and 20–21 μm wide. Dental formula 2 + 1/1 + 2 major teeth (with an additional thinner teeth) and about 30 minor teeth in each uncus. Oviparous. Eggs oval, 124 × 66 μm. Egg shell smooth, without knobs or spines.

Measurements See Table 2.

Distribution Maritime Antarctica: AI*.

Habitat Soil, terrestrial moss.

***Macrotrachela jankoi* sp. nov. Iakovenko (Figs. 3f, g, 13)**

Velasco-Castrillón et al., 2014a (Bd7), p. 8.

Type locality Squa Island (Argentine Islands archipelago), 20 m asl, S65°25.117′, W64°26.583′.

Type habitat Soil.

Type material Holotype: SIZ 61.1 (VS02MJ1a, 15.03.2010, Leg. K. Janko), mounted in glycerine jelly. Paratypes: SIZ 61.2-3 (VS02MJ2, VS02MJ3), mounted in glycerine jelly; SIZ 61.4 (VS02MJ4), trophi mounted for SEM. *Additional material* SIZ 61.5-17 (V08MJ1, V11MJ1, V12MJ1-2, VRA01MJ1-7, VRA02MJ1), digital photos and videos; SIZ 61.18-26 (V12MJ3, MRockaMJ1-8), trophi mounted for SEM.

Barcodes GenBank ID KJ543594-97, KJ543597, KP869999, KP870000.

ZooBank LSID urn:lsid:zoobank.org:act:6E2BA135-65A8-4256-9B82-54E86D84865B.

Etymology Named after Dr. Karel Janko, the leader of the project conducted on Vernadsky Base and collector of the material.

Diagnosis Resembles *Macr. insulana* Donner, 1962, by the shape of the corona and upper lip, the characteristic sulcus with two denticles on the dorsal side, and the absence of a knob on the first foot pseudosegment. Differs by the flat and short triangular spurs, which are longer and peg-like in *Macr. insulana*. Dental formula of the new species is 2/2, while in *Macr. insulana* it is 1 + 2/2 + 1.

Description Body large, transparent, stomach of bright red or orange color. Integument smooth, thin, without knobs, spines or bolsters. Rostrum stout, of moderate length, lamella with two large rounded lobes. Corona wider than oval head base, CW is 100–133% of HW, HL is 35–54% of TL. Pedicels short, straight. Sulcus wide, not covered with membrane. On dorsal side of head two short denticles divided by interspace visible in sulcus. Trochi large, with papillae and sensory bristles. Upper lip with two triangular lobes reaching about a half of the sulcus and divided by interspace. Lower lip not protruding laterally. Neck stout, of moderate length, NL is 16–21% of TL. Antenna about 1/3 of the bearing pseudosegment. Trunk plump, BW is 15–21% of TL. Both pseudosegments of the rump swollen, RL is 12–21% of TL, RW is 73–93% of RL. Foot short, stout, 4 pseudosegments, without a dorsal knob, FL is 10–12% of TL, FW is 50–82% of FL. Spurs small,

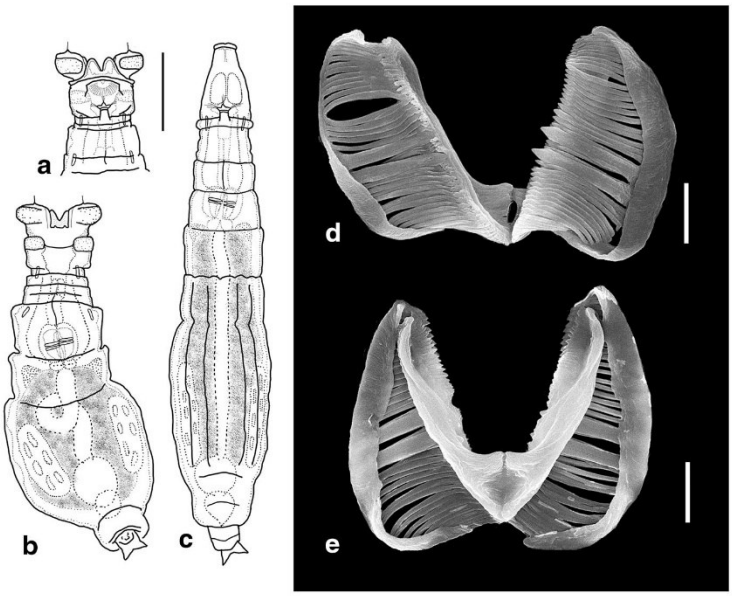


Fig. 13 *Macrotrachela jankoi* sp. nov.: **a** holotype, head, feeding, dorsal view; **b** same, habitus, feeding, ventral view; **c** same, habitus, creeping, dorsal view; **d** paratype, trophi, cephalic view; **e** paratype, trophi, caudal view. *Scale bar* 50 μm (**a**–**c**) or 5 μm (**d**, **e**)

short, isosceles triangular, divided by an interspace almost equal to spur width, SL is 44–79% of SSW. Three short unsegmented toes. No eyespots. Throat voluminous, esophagus short, straight. Stomach glands round. Trophi ramate, 18–22 µm long and wide. Rami massive, with numerous short scleropili along the inner rim. Articulation somewhat concaved in the middle, but without incisure. Manubria thin, sickle-like. Dental formula 2/2, 39–41 minor teeth in each uncus. Oviparous. Eggs oval, shell smooth, without knobs or spines.

Measurements See Table 2.

Distribution Maritime Antarctica: AI*, AP*, KG*. Continental Antarctica: EB, VL (Cz*).

Habitat Soil, terrestrial moss.

Genus *Philodina* Ehrenberg, 1830

Philodina dartnallis sp. nov. (Figs. 3i, j, 14)

Priddle & Dartnall, 1978 (? *Philodina*), p. 475. Dartnall & Hollowday, 1985 (*Philodina* sp. 'A'), p. 24, Fig. 27a–e. Velasco-Castrillón et al., 2014a (*Ph.* sp. Bd46), p. 8.

Type locality Cape Bird, 77 m asl, S77°13.207′, E166°26.568′.

Type habitat Soil.

Type material Holotype: SIZ 63.1 (CBM2PHD1a, 19.01.2010, Leg. J. Smykla), mounted in glycerin jelly, encircled with green ink. Paratypes: SIZ 63.2-4 (CBM2PHD1b-d), on the same slide with holotype, encircled with black ink. *Additional material* SIZ 63.5-12 (CzM4PHD1-5, CzM4matPHD1, VDM2 PHD1-2), digital photos and videos.

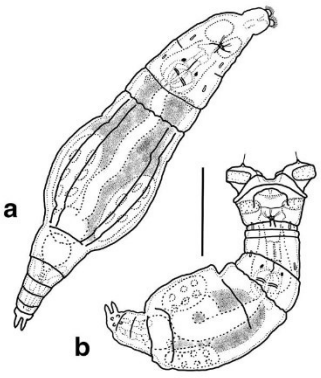


Fig. 14 *Philodina dartnallis* sp. nov.: **a** holotype, habitus, creeping, dorsal view; **b** same, feeding, dorsal view. *Scale bar* 50 µm

Barcodes GenBank ID KJ543683-86, see also Supplementary file III.

ZooBank LSID urn:lsid:zoobank.org:act:6706ED3E-BBFF-4390-A602-4595C122986E.

Etymology Named after Antarctic researcher Dr. H. Dartnall who first depicted this species as *Ph.* sp. 'A'.

Diagnosis Similar to *Ph. flaviceps* Bryce, 1906, *Ph. australis* Murray, 1911, and some specimens of *Ph. brevipes* Murray, 1902. Resembles *Ph. flaviceps* by the shape of corona and spurs; however, the spurs of the new species are longer and with broader interspace. Differs from *Ph. australis* by the spur shape, which in the new species is peg-like with a broad interspace, but narrow triangular without an interspace in *Ph. australis*. Differs from *Ph. brevipes* by the shape of the upper lip (rounded lobes in the new species, pointed in *Ph. brevipes*) and by the absence of long sensory cilia in the rostrum. The foot seems to be shorter than in *Ph. brevipes*.

Description Body of moderate size, colorless, transparent. Integument smooth, thin, without knobs, spines or bolsters. Rostrum of moderate length, thick, lamella with two very small semicircular lobes. Corona wider than head base, CW is 102–119% of HW, HL is 27–38% of TL. Upper lip wide, arcuate, with two large low rounded lobes not reaching the plane of the trochal discs and divided by a broad interspace. Pedicels short, trochi large, with papillae and sensory bristles, retractors visible. Sulcus wide, not covered with membrane, without ligula. Lower lip not protruding laterally. Neck wide, of moderate length. Antenna 1/3 of bearing pseudosegment width. Trunk plump, BW is 14–25% of TL. Rump conical, first pseudosegment slightly swollen, without protrusions, RL/TL is 10–18%, RW/RL is 74–96%. Foot of moderate length, stout, 5 pseudosegments, without knobs or protrusions, FL is 8–15% of TL, FW is 13–22% of FL. Spurs peg-like, parallel to each other, divided by very narrow interspace, SL is 51–72% of SSW. Four thick unsegmented toes. Two cerebral orange or bright-red eyespots. Throat and esophagus of moderate length. Trophi ramate, round, 3/2. Stomach bright red, lumen wide. Egg oval, with

rounded knob on one pole, shell without spines or sculpturation, egg size 50–59 × 34–42 µm.

Distribution Maritime Antarctica: AI*, AP*. Continental Antarctica: EB, VL (CB*, Cz*), WK.

Habitat Soil, terrestrial moss, lakes.

Philodina shackletoni sp. nov. (Fig. 15)

Velasco-Castrillón et al., 2014a (*Ph.* sp. Bd45), p. 8 (main text); 5, Figs. 23–27 (Annex S1).

Type locality Cape Royds, 18 m asl, S77°32.532′, E166°08.855′.

Type habitat Soil.

Type material Holotype: SIZ 62.1 (CRL25PHC1a, 14.01.2010, Leg. J. Smykla), mounted in glycerine jelly. Paratypes: SIZ 62.2-3 (CRL25PHC2-3), the same. *Additional material* SIZ 62.4 (CRL21PHC1), digital photos.

Barcodes GenBank ID KJ543677-86, see also Supplementary file III.

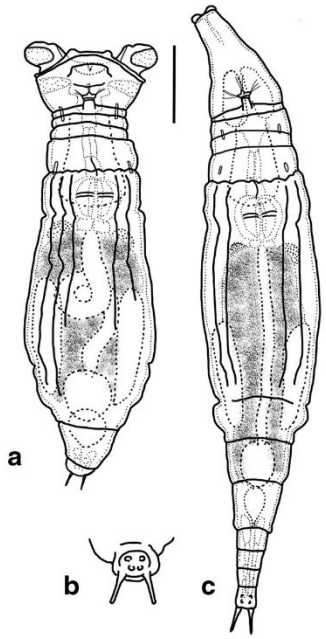


Fig. 15 *Philodina shackletoni* sp. nov.: **a** holotype, habitus, feeding, dorsal view; **b** foot, ventral view; **c** holotype, habitus, creeping, dorsal view. *Scale bar* 50 µm

ZooBank LSID urn:lsid:zoobank.org:act:B2616FE6-B7B9-45D0-9F3A-DF065BE96A4F.

Etymology Named in honor of the leading Antarctic explorer, Sir Ernest Henry Shackleton, who in 1909 established his base on Cape Royds where the species was discovered.

Diagnosis Most closely resembles *Ph. flaviceps* Murray, 1906, by the shape of spurs and corona, however lacking eyespots. Spurs are longer than in *Ph. flaviceps* and divided by broader interspace.

Description Body large, spindle-shaped, colorless with yellow–brown stomach. Integument smooth, thin, without knobs, spines or bolsters. Rostrum of moderate size, with crescent-like lamella. Corona wider than trapezoid head base, CW is 109–117% of HW, HL is 13–17% of TL. Pedicels short, slightly bent inwards. Sulcus wider than diameter of a trochus, not covered with membrane. Trochi with papillae and sensory bristles. Upper lip very low, arcuate, with two small rounded lobes divided by interspace. Lower lip not protruding laterally. Neck of moderate length and width, NL is 18–23% of TL. Antenna long, almost equal to the bearing pseudosegment width. Trunk slim, BW is 15–20% of TL. Rump large, swollen, RL is 15–17% of TL, RW is 11–13 of RL. Foot long, slim, 5 pseudosegments, FL is 10–15% of TL, FW is 27–32% of FL. Spurs long, needle-like, SL is 115–164% of SSW. Four unsegmented toes. No eyespots. Throat small, esophagus short. Lumen wide. Stomach glands small, round. Trophi ramate, 24 µm long and wide, dental formula 2/2. Oviparous. Eggs oval, shell smooth, without knobs or spines.

Measurements See Table 2. TL 400 µm (Velasco-Castrillón et al., 2014a).

Distribution Maritime Antarctica: SI. Continental Antarctica: EB, VL (CR*), WK.

Habitat Soil, terrestrial moss, pools.

Morphometric analyses

Adineta editae sp. nov. differed from the similar European species *A. gracilis* by larger size of body and mastax, and longer spurs. LME demonstrates that the variation of body measurements between species represented over 60% of total variation for the parameters describing the width along the rotifer body (HW, MinNW, MxNW, FW, SSW) and for the spur length. The variation between localities and individuals was not significant for FW, SSW and SL

(ANOVA on LME output: LR = 11.1 to 13.1, $P > 0.1$). This was in correspondence with our visual observation that *A. editae* sp. nov. had a distinctively stouter body than *A. gracilis* s. str. In the PCA plot (Fig. 16a) external measurements of the two species did not overlap along PC1 (correlating with all measurements) and PC2 (correlating with HW and SL).

The difference in trophi measurements between *A. editae* sp. nov. and *A. gracilis* represented over 90% of total variation in the number of minor teeth and trophi length, and over 80% in the case of trophi width. The variation between localities and individuals was insignificant for all measurements (LR = 0.2 to 2.1, $P > 0.5$). The trophi measurements of these species were completely separated on the PCA plot (Fig. 16b) and did not overlap along PC1 (correlates with all measurements) and PC2 (correlates with the number of minor teeth and the unci width).

The body and trophi of the Antarctic species *A. grandis* and *A. fontanetoi* sp. nov. are indistinguishable by external morphology and did not differ significantly in any of the measured parameters. The Antarctic *A. emsliei* sp. nov. and the European *A. vaga* s. str. noticeably differed by at least one trophi measurement (the number of minor teeth in unci). The difference by this parameter consisted of over 80% total variation, with the variation between localities and individuals being insignificant (LR = 0.7 to 1.5, $P > 0.5$). The antarctic species *A. grandis* and *A. emsliei* sp. nov. were distinguished by all trophi measurements, the difference between species being over 90% of total variation. The variation between localities and individuals was not

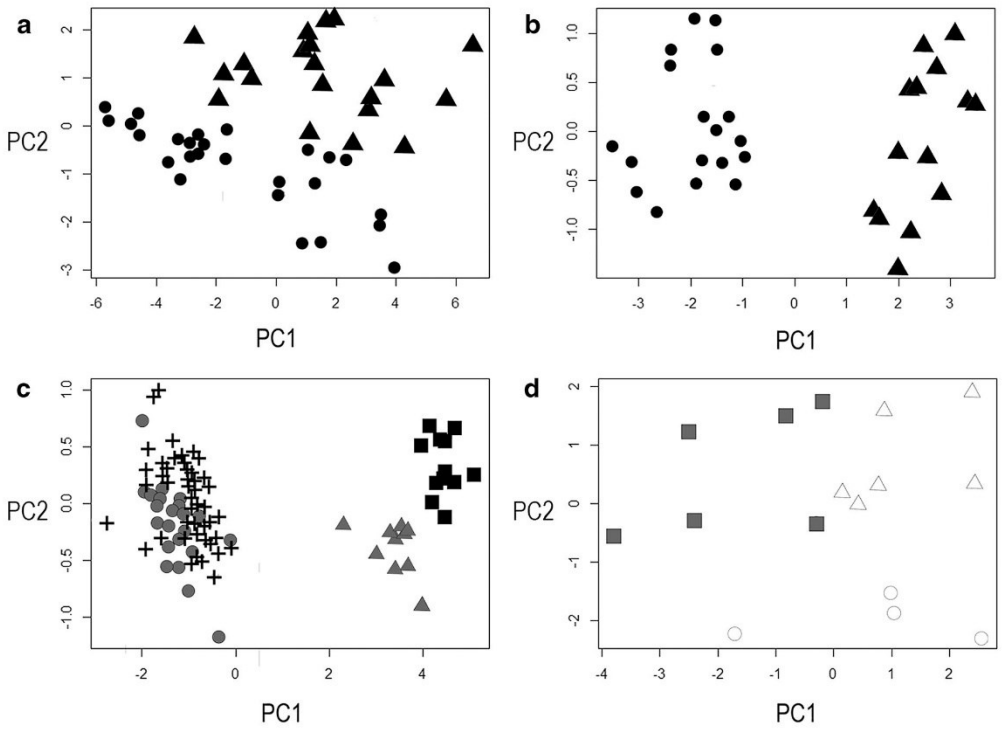


Fig. 16 Principal components analysis of rotifer body and trophi measurements: **a** *A. gracilis* (*circles*) and *A. editae* sp. nov. (*triangles*), body dimensions; **b** same, trophi dimensions; **c** *A. grandis* (*crosses*), *A. fontanetoi* sp. nov. (*circles*), *A. emsliei* sp. nov. (*squares*) and *A. vaga* (*triangles*), trophi dimensions; **d** *H. antarctica* sp. nov. (*squares*), *H. vernadskii* sp. nov. (*circles*) and *H.* sp. 4 (*triangles*), trophi dimensions

significant except for rami length (LR = 0.3 to 4.5, $P > 0.1$). In the PCA plot (Fig. 16c) the samples of trophi of *A. grandis* and *A. fontanetoi* sp. nov. overlapped completely on both PC1 (correlating with all trophi measurements) and PC2 (correlating with the number of minor teeth and trophi width), but the samples of *A. emsliei* sp. nov. did not overlap with any of the other species.

The Antarctic *H. antarctica* sp. nov. and *H. vernadskii* sp. nov. did not show any significant difference in trophi length and width, but could be distinguished by the number of minor teeth of the unci. Variation between the species on the latter measurement represented over 70%, the variation between localities and individuals being insignificant (LR = 3.6, $P > 0.1$). *Habrotrocha* sp. 4 is indistinguishable from *H. antarctica* sp. nov. by the external morphology, but has an intermediate trophi size between *H. antarctica* sp. nov. and *H. vernadskii* sp. nov., with the variation between species by all trophi parameters representing 50% or less of total variation. On the PCA plot of the samples of trophi meausrements (Fig. 16d) *H. antarctica* sp. nov. and *H. vernadskii* sp. nov. did not overlap with each other on any axis. *Habrotrocha* sp. 4 did not overlap with either of the two other species.

DNA taxonomy

On the phylogenetic tree of *Adineta* (Fig. 17a) consisting of 46 IEEs and 28 singletons according to GMYC, the sequences of Antarctic rotifers grouped into 1 singleton and 8 independently evolving entities (IEE) identified by the GMYC and 4× rule approaches (32 singletons and 6 IEEs according to bPTP). Twenty-five IEEs of *Adineta* identified by GMYC contained sequences only from Europe, 6 IEEs—sequences from North America, 3 IEEs—sequences from each of Africa, Australia and New Zealand, and 2 IEEs—sequences from Asia. None of the Antarctic IEEs contained rotifers originating from any other continents, while three cosmopolitan IEEs (all attributed to the *A. vaga* species complex according to morphology) were identified from outside Antarctica. Two cosmopolitan IEEs had European-North American distributions and one occurred in Europe, Africa and New Zealand. The phylogeny shows that the Antarctic endemic *A. grandis* consists of at least two morphologically indistinguishable entities (one of which was described above as *A. fontanetoi* sp. nov.). For three IEEs the morphology has not been described; therefore, these are listed as *A.* sp. 1–3. Finally, three IEEs that proved to be both genetically distinct and morphologically distinguishable are described above as *A. editae* sp. nov., *A. emsliei* sp. nov. and *A. coatsae* sp. nov. Molecular analysis confirmed that these species occur only in Antarctica, in spite of being previously confused with the cosmopolitan species *A. gracilis*, *A. vaga* s. str. and *A. barbata*. The integrity of *A. coatsae* sp. nov. as a single IEE was supported by the GMYC model, but not by the bPTP and 4× rule models. Both the GMYC and 4× rule, but not the bPTP model, confirmed the integrity of *A. fontanetoi* sp. nov. as a separate IEE.

The tree of *Philodina* consisted of 31 singletons and 42 IEEs identified by GMYC, mostly confirmed as IEEs by the 4× rule and bPTP (Fig. 17b). Nineteen singletons and ten IEEs contained sequences of Antarctic bdelloids. According to GMYC, 18 IEEs of *Philodina* had a European distribution, 17 were from North America and 4 from Asia, and 3 IEEs had cosmopolitan distributions. *Philodina* is the only one of the four investigated genera with cosmopolitan IEEs occurring in the Antarctic, those being *Ph.* sp. 4 (two sequences, from the Antarctic and the USA) and *Ph.* sp. 7 (25 sequences from the USA and one from Antarctica). Unfortunately, for both of these IEEs no data on morphology are available, and none of the sequences were obtained from vouchers of already known species. The remaining IEEs did not contain individuals from continents other than Antarctica. For six of them, listed here as *Ph.* sp. 1–6, no morphological data are available. *Philodina gregaria* appeared as one large pan-Antarctic IEE, three singletons and one IEE with atypical morphology, containing only two sequences. Two IEEs proved to be well distinguishable both morphologically and by the means of DNA taxonomy, both from *Ph. gregaria* and the morphologically similar non-Antarctic *Ph. acuticornis* Murray 1902, *Ph. flaviceps* Murray 1906, and *Ph. roseola* Ehrenberg, 1832. These two species are described above as the new Antarctic endemics: *Ph. shackletoni* sp. nov. and *Ph. dartnallis* sp. nov. The integrity of the *Ph. dartnallis* sp. nov. clade was supported by two of the three delimitation methods.

Putative species delimitation of *Habrotrocha* according to GMYC gave 22 IEEs and 1 singleton. Eleven IEEs and four singletons had strictly Antarctic

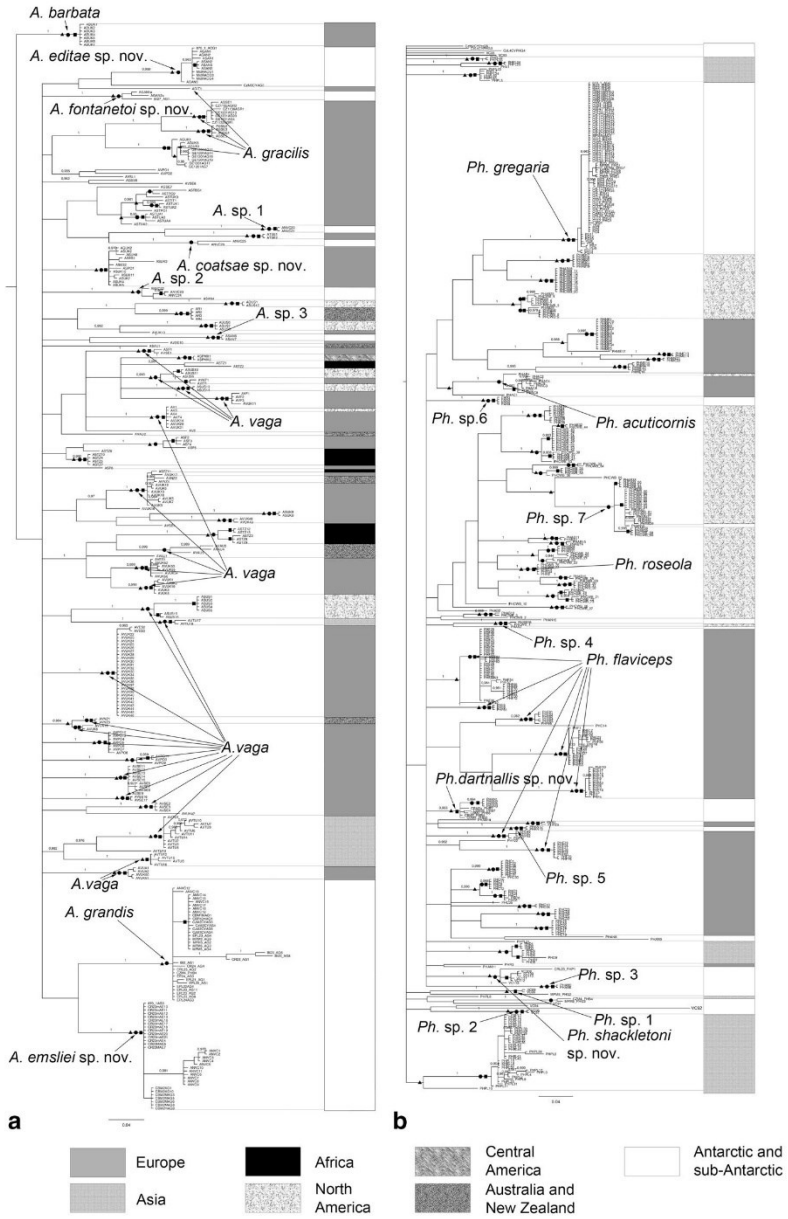


Fig. 17 Phylogenetic relationships in the genera *Adineta* (**a**) and *Philodina* (**b**). The consensus Bayesian trees of COX1 mtDNA data sets are shown on the *left*. Clades are marked as putative species delimited according to the 4× rule (*triangles*), GMYC (*circles*) and bPTP (*squares*). *Boxes* on the *right* show the distribution of samples across different continents. Individual labels on branches are given in larger resolution in the Supplementary file IV

distribution. Ten IEEs contained sequences from North America and one was from Europe (Fig. 18). No cosmopolitan IEEs were detected. For three IEEs (*H.* sp. 1, 2 and 3) morphological data are not available. Three IEEs are described above as *H. antarctica* sp. nov., *H. devetteri* sp. nov. and *H. vernadskii* sp. nov. based on both morphological and molecular differences from the similar non-Antarctic *H. constricta*, *H. crenata* s. lat., *H. elusa* s. lat. and *H. thienemanni* s. lat. *Habrotrocha* sp. 4 was identified as an IEE by the GMYC model, but not confirmed as a single entity by the 4× rule, being less than four times separated from both *H. antarctica* sp. nov. and *H. vernadskii* sp. nov. (which are genetically and morphologically sufficiently separate to be good species). *H.* sp. 4 and *H.* sp. 5 are morphologically identical to *H. devetteri* sp. nov., but were delimitated as separate IEEs by all three molecular delimitation methods. *H. angularis* has been confirmed as a separate species with a strictly Antarctic distribution.

Sixteen IEEs and 20 singletons were identified in *Macrotrachela* using GMYC, and this delimitation in most, but not all cases was confirmed by the two other approaches (Fig. 19). Four IEEs and three singletons were distributed in Antarctica, with eight IEEs identified from Europe, four from Asia, three from North America and one from Africa. No cosmopolitan IEEs were detected by any of the delimitation methods. For

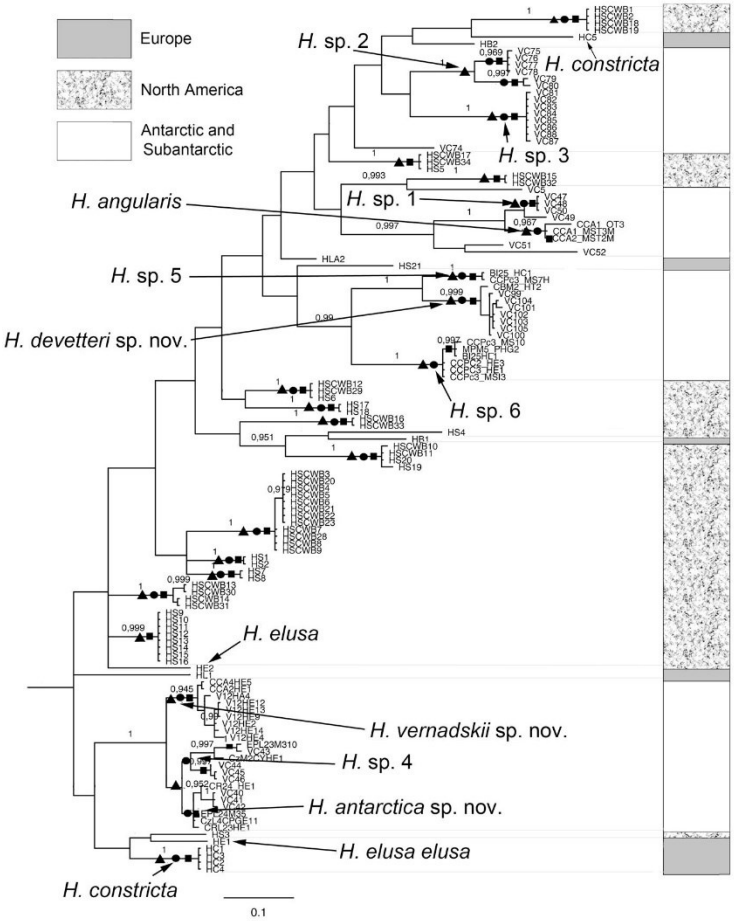
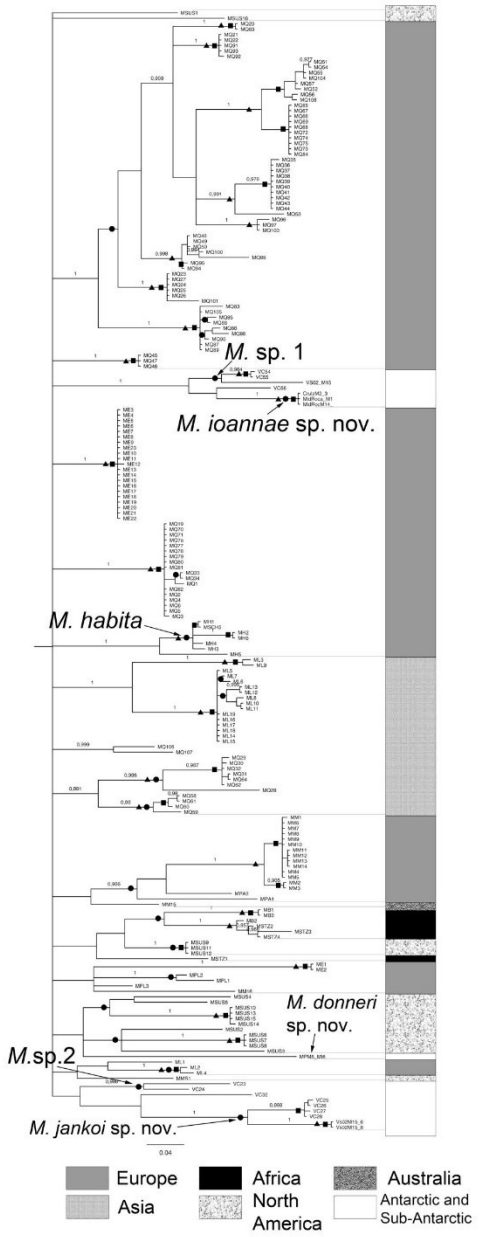


Fig. 18 Phylogenetic relationships in the genus *Habrotrocha* (consensus Bayesian tree, COX1 mt DNA data set). Putative species are delimitated according to the 4× rule (*triangles*), GMYC (*circles*) and bPTP (*squares*). Wider distributions of rotifers are shown as in boxes on the right. Individual labels on branches are given in larger resolution in Supplementary file IV



◀**Fig. 19** Phylogenetic relationships in the genus *Macrotrachela* (consensus Bayesian tree, COX1 mt DNA data set). Putative species are delimited according to the 4× rule (*triangles*), GMYC (*circles*) and bPTP (*squares*). Wider distributions of rotifers are shown as in the boxes on the right. Individual labels on branches are given in larger resolution in Supplementary file IV

one Antarctic IEE, *Macr.* sp. 2, no information is yet available on morphology. *Macrotrachela donneri* sp. nov., previously reported in Antarctica as *Macr.* cf *insolita* or *Macr.* cf *hewitti* (Donner, 1972) by Iakovenko & Tyshchenko (2006), was represented by a single sequence, which did not cluster with any non-Antarctic rotifers including the morphologically similar *Macr. habita*. For *Macr.* sp. 1 more morphological data are required. *Macrotrachela ioannae* sp. nov. and *Macr. jankoi* sp. nov. were confirmed by GMYC as good species occurring in Antarctic, but the bPTP approach revealed *Macr. jankoi* sp. nov. as two IEEs. The integrity of *Macr.* sp. 2, identified using GMYC, was not confirmed by the other two approaches.

The identification of IEEs based on our combined set of sequences, including both new sequences and the previously published data, was the same as presented in Velasco-Castrillón et al. (2014a) with the exception of three IEEs. According to the results of our analysis, the putative species Bd15 and Bd16 were identified as the single entity *H.* sp.1 by all three models. Bd31 and Bd32 were identified as *H.* sp. 2 by the 4× rule, but as separate entities by the GMYC and bPTP models. Bd5 and Bd6 were identified as *Macr.* sp. 2 according to GMYC, but this was not confirmed by the two other models. All the sequences published by Barraclough et al. (2007) and Fontaneto et al. (2008) were identified as members of *Adineta* (*A. editae* sp. nov., *A. fontanetoi* sp. nov., *A.* sp. 2, *A.* sp. 3). All 16 sequences published by Fontaneto et al. (2012) were confirmed as *Philodina* (*Ph.* sp. 3–13).

Biogeography

Of the 60 morphospecies found in the new material examined in this study, at least 17 can be considered true Antarctic and/or sub-Antarctic endemics, with 6 of these being already known and 11 newly recognized. A further ten morphospecies that closely resemble known and presumed cosmopolitan bdelloids, possessing only minor but consistent morphological differences from the original descriptions, require further detailed analysis. No molecular data are available for 13 morphospecies showing no discernible differences from known cosmopolitan

species, as well as for 20 identified only to the generic level, and these cannot currently be attributed with confidence to any classification. That gives at least 28 and possibly up to 45% endemicity (17 or up to 27 morphospecies out of 60) on the basis of classical taxonomy alone.

Based on molecular data obtained both from our new samples and GenBank, we identified 33 IEEs and 37 singletons from Antarctica and the sub-Antarctic using GMYC (largely confirmed by the two other approaches used), belonging to the genera *Adineta*, *Habrotrocha*, *Philodina* and *Macrotrachela*. Including a further five IEEs and eight singletons apparently representing other genera, a total of 38 IEEs and 45 singletons of Bdelloidea are now identified from this region. Only two of the IEEs occurring in Antarctica have been confirmed as having wider non-Antarctic distributions by molecular methods. At least 13 of the strictly Antarctic IEEs are clearly distinguishable by morphology, while at least four IEEs appear to represent cryptic species.

The molecular data obtained suggest there is a very high level of endemism among Antarctic bdelloids—36 out of 38 IEEs (95%). GMYC also identified considerable apparent endemism in the rotifer faunas (IEEs) of other major global regions: 55 of 59 found in Eurasia (93%), 65 of 70 (93%) in North America, and 3 of 4 (75%) in both Australia and New Zealand, and Africa. In the genus *Adineta*, 93.5% of the IEEs throughout the world were found to be endemic at the level of a continent (with no cosmopolitan IEEs found so far in Antarctica). In the genus *Philodina*, the percentage of endemic IEEs was lower (85%), with at least two cosmopolitan IEEs occurring in the Antarctic continent.

Sixteen IEEs were found only within continental Antarctic (5 representatives of *Adineta*, 4 of *Philodina* and 7 of *Habrotrocha*), three IEEs (2 of *Adineta* and 1 of *Macrotrachela*) were found only in the maritime Antarctic, and the distribution of 8 IEEs was either sub-Antarctic or unknown. Only six IEEs (1 of *Adineta*, 2 of *Habrotrocha* and 3 of *Philodina*), or 18% of their total number, occurred in both the maritime and continental regions of Antarctica.

Discussion

The hypothesis "Everything is everywhere, but the environment selects" (EiE hypothesis) was initially proposed by Beijerinck and Baas Becking in the early twentieth century (Baas Becking, 1934 in Willams, 2011) and recently gained a renewed interest of biogeographers. It presumes that organisms less than 1–2 mm in length tend to be cosmopolitan, having no "true endemics" because of the high dispersal capacities and large population sizes, and that their diversity is driven by ecological factors rather than historical processes (Hillebrand & Azovsky, 2001; Williams, 2011 and references therein). While this seems to be true for some microscopic organisms (Fenchel & Finlay, 2004), including some but not all bdelloid rotifers (Fontaneto et al., 2008), increasing number of studies are providing evidence that microbes can have distinct distribution patterns and show endemism on a global scale (Hughes Martiny et al., 2006; Garey et al., 2008; Ganter, 2011; Lacap et al., 2011). Some bdelloid mophospecies appear to be restricted at least to a particular continent, and the largest number of such taxa is found in Antarctica (Segers, 2007).

After a 100-year period of research, the Antarctic Bdelloidea still remain obscure and knowledge sparse in terms of their diversity, distribution and origin. The current study is only the fourth to report previously undescribed Antarctic bdelloid rotifers, with all 12 new taxa being currently known only from the continent. The study is the first to provide a robust combination of detailed, morphological, morphometric and molecular approaches, which are being utilized in the description of these new Antarctic taxa. This study is also the second to apply molecular approaches in describing the diversity and biogeography of Antarctic Bdelloidea carried out, like in Velasco-Castrillón et al. (2014a), through COX1 sequencing. To date, 15 morphospecies of 60 (25%) found in this study have been barcoded successfully. Of these, we have (1) described 12 species new to science using both classical and DNA taxonomy approaches, (2) revealed the presence of a number of cryptic species that are apparently morphologically identical to *A. grandis*, *H. antarctica* sp. nov. and *H. devetteri* sp. nov., (3) linked the molecular data provided by Velasco-Castrillón et al. (2014a) with morphospecies, and (4) provided evidence of generally very high levels of endemism of bdelloid rotifers in Antarctica, with the exception of the finding of two cosmopolitan species of *Philodina*, not identified in previous studies.

The difficulty of distinguishing bdelloid species based on morphology alone, due to their generally

highly conserved body morphology and structure, and ambiguity in defining specific characters have led to a prevailing misconception that the contemporary Antarctic fauna includes a large proportion of cosmopolitan bdelloids. Thus, Donner (1965, 1972a) considered minor differences in the size and shape of the bdelloid body and its appendages as only representing intraspecific variability. Previous studies have often attributed Antarctic specimens to species already known from elsewhere (mostly Europe) if most of the external morphological characters matched the original descriptions or they were identified through keys based on the latter. Our morphometric and molecular data support the conclusions of Fontaneto et al. (2007), who proved that careful morphometric measurement of hard parts of the mastax (trophi) can differentiate several morphologically distinct entities within one "classical" species, corresponding with IEEs determined by molecular analysis.

All three models applied here for the delimitation of IEEs based on molecular sequence data showed good correspondence with rotifer morphology. GMYC gave the best correspondence with the species-specific morphology, and in most cases the results were supported by two other approaches. However, the bPTP model, used similarly by Velasco-Castrillón et al. (2014a), tended to give finer subdivision of IEEs, creating an excessive number of entities unidentifiable at the morphological level. Our data confirmed the integrity of all but three of the IEEs identified by Velasco-Castrillón et al. (2014a) and also confirmed the attribution of some sequences to *Adineta* and *Philodina* as proposed by Barraclough et al. (2007) and Fontaneto et al. (2008, 2012).

Our data demonstrate that, in spite of their acknowledged high dispersal capacities, Bdelloidea have distinctive patterns of distribution on the global scale. It has been considered that rotifer species, including bdelloids, are generally widespread with endemism occurring at the continental level (Dumont, 1983; Ricci, 1987; Segers, 2007; Fontaneto et al., 2008). Fontaneto et al. (2008) found that, within the bdelloid genera *Adineta* and *Rotaria*, IEEs were widely distributed over Europe, some even occurring in different continents (*A. vaga* found in UK, Tanzania and New Zealand, see Fig. 17). According to our data, not only is the distribution of most bdelloid IEEs limited by continents, with the highest levels of endemic IEEs in Antarctica, but even within the Antarctic region there are substantial differences within local bdelloid faunas (maritime and continental Antarctica).

Conclusions

A striking feature of the data obtained in the current study is that of the extremely high levels of endemism to the Antarctic and sub-Antarctic region apparent in the bdelloid fauna. Clearly, consideration of the concept of endemism is itself limited by the quality and extent of the data available, from both the Antarctic and other regions. However, both the current study and that of Velasco-Castrillón et al. (2014a) are consistent in identifying (1) that considerably greater diversity in terms of divergence to the 'species level' is apparent in analyses of molecular (COX1) data than was the case in previous classical taxonomic studies of the group and (2) that Antarctic lineages are distinct from those of bdelloids from other continents available today in GenBank. In addition to the previous studies, we show that careful morphological analysis using morphometrics and SEM in many cases allows the detection of endemic Antarctic species of Bdelloidea even without molecular analysis.

Implicit in the assessment of considerable levels of endemism at the continental level is the conclusion that it is indicative of an extended history (long-term presence) allowing evolutionary divergence in situ in the Antarctic. This is consistent with a range of studies over the last 1 to 2 decades that have used both classical and molecular approaches to confirm high levels of endemism and long evolutionary histories in representatives of all the main terrestrial invertebrate groups occurring in Antarctica, including Tardigrada (Convey & McInnes, 2005), Nematoda (Andrássy, 1998; Maslen & Convey, 2006), Collembola (Greenslade, 1995; McGaughran et al., 2010; Torricelli et al., 2010), Acari (Pugh, 1993; Stevens & Hogg, 2006) and Diptera (Allegrucci et al., 2012). Wider reviews of this subject are provided by Convey et al. (2008) and Pugh & Convey (2008). Similar conclusions are increasingly being drawn from studies of some microbial groups (De Wever et al., 2009; Strunecký et al., 2012), most recently, mosses (Pisa et al., 2014).

The outcomes of the current study highlight the need for considerably greater survey efforts to be applied to groups of microscopic Antarctic fauna rich in cryptic species such as rotifers. Data obtained in the current

study suggest that at least some species of bdelloid rotifers are limited to particular parts of the Antarctic or sub-Antarctic. This again is consistent with recent findings in terrestrial biota (Convey et al. 2008; Pugh & Convey 2008; Velasco-Castrillón et al., 2014a) as well as the recent analysis of Terauds et al. (2012), which identified no less than 15 'Antarctic Conservation Biogeographic Regions' across the Antarctic continent alone. Thus, further targeted research among the bdelloid rotifers of Antarctica, integrating classical, morphometric and molecular biological approaches, should identify considerably greater levels of diversity and both continental and intracontinental regional endemism than are currently appreciated.

Acknowledgments We thank Dr. D. Fontaneto, Prof. W. H. De Smet and Prof. L. A. Kutikova for providing a number of poorly accessible literature sources and Dr. Ioanna Vaňková for her kind help and consultations on the Latin names for the new species. Prof. T. G. Barraclough is acknowledged for providing important suggestions on species delimitation methods and the code for PCA. Dr. V. N. Fursov is acknowledged for the help in imaging rotifers. We acknowledge the Centre for Polar Ecology of the University of South Bohemia, Polish Academy of Sciences, National Academy of Sciences of Ukraine, the Academy of Sciences of the Czech Republic, the National Antarctic Scientific Centre of Ukraine and the Centre "Animalia" at the Schmalhausen Institute of Zoology, Kiev, for the financial support and providing equipment and Raytheon Polar Services for logistical support. Funding also was provided by the Polish Ministry of Science and Higher Education (PMSHE) Program for Supporting International Mobility of Scientists and PMSHE grant nos. 2P04F00127, NN304069033 and NN305376438 (JS), the National Science Foundation project no. ANT 0739575 (JS), the Grant Agency of the Czech Academy of Sciences grant no. KJB600450903 (KJ, NI, EK), Czech Ministry of Education project no. LM2010009 (KJ), European Social Fund and the Czech Republic-supported project no. CZ.1.07/2.2.00/28.0190 (KJ), the Institute of Environmental Technologies, Ostrava, CZ.1.05/2.1.00/03.0100 supported by the Research and Development for Innovations Operational Program financed by Structural Funds of European Union and State Budget of the Czech Republic (ZĎ). PC is supported by the Natural Environmental Research Council core funding to the British Antarctic Survey's core 'Enviornmental Change and Evolution' program. This paper contributes to the SCAR 'State of the Antarctic Ecosystem' program.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

Adams, B. J., R. D. Bardgett, C. Ayres, D. H. Wall, J. Aislabie, S. Bamforth, R. Bargagli & S. C. Cary, 2006. Diversity and distribution of Victoria Land biota. Soil Biology and Biochemistry 38: 3003–3018.

Allegrucci, G., G. Carchini, P. Convey & V. Sbordoni, 2012. Evolutionary geographic relationships among chironomid midges from maritime Antarctic and sub-Antarctic islands. Biological Journal of the Linnean Society 106: 258–274.

Andrássy, I., 1998. Nematodes in the sixth continent. Journal of Nematode Systematics and Morphology 1: 107–186.

Barraclough, T. G., D. Fontaneto, C. Ricci & E. A. Herniou, 2007. Evidence for inefficient selection against deleterious mutations in cytochrome oxidase I of asexual bdelloid rotifers. Molecular Biology and Evolution 24: 1952–1962.

Bērziņš, B., 1987. Rotifer occurence in relation to pH. Hydrobiologia 147: 107–116.

Birky, C. W. & T. G. Barraclough, 2009. Asexual speciation. In Shön, I., K. Martens & P. Van Dijk (eds), Lost Sex: The evolutionary biology of parthenogenesis. Springer, Dordrecht: 201–216.

Birky, C. W., C. Wolf, H. Maughan, L. Herbertson & E. Henry, 2005. Speciation and selection without sex. Hydrobiologia 181: 29–45.

Birky, C. W., C. Ricci, G. Melone & D. Fontaneto, 2011. Integrating DNA and morphological taxonomy to describe diversity in poorly studied microscopic animals: new species of the genus *Abrochtha* Bryce, 1910 (Rotifera: Bdelloidea: Philodinavidae). Zoological Journal of the Linnean Society 161: 723–734.

Block, W., R. I. Lewis Smith & A. D. Kennedy, 2009. Strategies of survival and resource exploitation in the Antarctic fellfield ecosystem. Biological Reviews 84: 449–484.

Bryce, D., 1894. Further notes on Macrotrachellous Callidinae. Journal of the Quekett Microscopical Club 5: 436–455.

Cathey, D. D., B. C. Parker, G. M. Simmons, W. H. Vongue & M. R. Van Brunt, 1981. The microfauna of algal mats and artificial substrates in Southern Victoria Land lakes of Antarctica. Hydrobiologia 85: 3–15.

Convey, P., 1996. The influence of environmental characteristics on the life history attributes of Antarctic terrestrial biota. Biological Reviews 71: 191–225.

Convey, P., 2013. Antarctic ecosystems. In: Levin, S. A. (ed.) Encyclopedia of Biodiversity, Vol. 1, 2nd edition. Elsevier, San Diego: 179–188.

Convey, P. & S. J. McInnes, 2005. Exceptional, tardigrade dominated, ecosystems from Ellsworth Land, Antarctica. Ecology 86: 519–527.

Convey, P. & M. I. Stevens, 2007. Antarctic Biodiversity. Science 317: 1877–1878.

Convey, P., J. Gibson, C.-D. Hillenbrand, D. A. Hodgson, P. J. A. Pugh, J. L. Smellie & M. I. Stevens, 2008. Antarctic terrestrial life – challenging the history of the frozen continent? Biological Reviews 83: 103–117.

Convey, P., D. K. A. Barnes, H. Griffiths, S. Grant, K. Linse & D. N. Thomas, 2012. Biogeography and regional classifications of Antarctica. In Rogers, A. D., N. M. Johnston, E. Murphy & A. Clarke (eds), Antarctica: An Extreme Environment in a Changing World, Chapter 15. Blackwell, Oxford: 471–491.

Convey, P., S. L. Chown, A. Clarke, D. K. A. Barnes, V. Cummings, H. Ducklow, F. Frati, T. G. A. Green, S. Gordon, H. Griffiths, C. Howard-Williams, A. H. L. Huiskes, J. Laybourn-Parry, B. Lyons, A. McMinn, L.

S. Peck, A. Quesada, S. Schiaparelli & D. Wall, 2014. The spatial structure of Antarctic biodiversity. Ecological Monographs 84: 203–244.
Courtright, E. M., D. H. Wall & R. A. Virginia, 2001. Determining habitat suitability for soil invertebrates in an extreme environment: the McMurdo Dry Valleys, Antarctica. Antarctic Science 13: 9–17.
Crawley, M., 2007. The R Book. Wiley, Chichester.
Darriba, D., G. L. Taboada, R. Doallo & D. Posada, 2012. jModelTest 2: more models, new heuristics and parallel computing. Nature Methods 9: 772.
Dartnall, H. J. G., 1983. Rotifers of the Antarctic and Subantarctic. Hydrobiologia 104: 57–60.
Dartnall, H. J. G., 1992. The reproductive strategies of two Antartic rotifers. Journal of Zoology Zoological Society of London 227: 145–162.
Dartnall, H. J. G., 1995a. The rotifers of Heard Island: preliminary survey, with notes on other freshwater groups. Papers and Proceedings of the Royal of Tasmania 129: 7–15.
Dartnall, H. J. G., 1995b. Rotifers, and other aquatic invertebrates, from the Larsemann Hills, Antarctica. Papers and Proceedings of the Royal Society of Tasmania 129: 17–23.
Dartnall, H. J. G. & E. D. Hollowday, 1985. Antarctic rotifers. British Antarctic Survey Reports 100: 1–46.
Davis, R. C., 1981. Structure and function of two Antarctic terrestrial moss communities. Ecological Monographs 51: 125–143.
de Beauchamp, P., 1913. Rotifères. Deuxième Expédition Antartique Française, 1908–1910. Maison et C^{o}, Paris: 105-116.
de Beauchamp, P., 1940. Turbellariés et Rotifères. In Jeannel, R. (ed.), Croisière du Bougainville aux Iles Australes Françaises. Mémoires du Muséum National d'Histoire Naturelle, Nouvelle Série 14: 313–326.
De Smet, W. H., 1998. Preparation of rotifer trophi for light and scanning electronic microscopy. Hydrobiologia 387(883): 117–121.
De Smet, W. H. & E. A. Van Rompu, 1994. Rotifera and Tardigrada from some cryoconite holes of a Spitsbergen (Svalbard) glacier. Belgian Journal of Zoology 124: 27.
De Wever, A., F. Leliaert, E. Verleyen, P. Vanormelingen, K. Van der Gucht, D. A. Hodgson, K. Sabbe & W. Vyverman, 2009. Hidden levels of phylodiversity in Antarctic green algae: further evidence for the existence of glacial refugia. Proceedings of the Royal Society B 276: 3591–3599.
Donner, J., 1965. Ordnung Bdelloidea. (Rotatoria, Rädertiere). Bestimmungsbücher zur Bodenfauna Europas 6. Akademie Verlag, Berlin.
Donner, J., 1972a. Bericht über Funde von Rädertieren (Rotatoria) aus der Antarctis. Polskie Archiwum Hydrobiologii 19: 251–252.
Donner, J., 1972b. Die Rädertierbestände submerser Moose und weiterer Merotope im Bereich der Stauräume der Donau an der deutch-österreichischen Landesgrenze. Archiv für Hydrobiologie Suppl 44: 49–114.
Donner, J., 1980. Einige neue Forschungen über bdelloide Rotatorien, besonders in Böden. Revue d'Ecologie et de Biologie du Sol 17: 125–143.
Dougherty, E. C., 1964. Cultivation and nutrition of micrometazoa: I. The Antarctic rotifer *Philodina gregaria* Murray, 1910. Transactions of American Microscopical Society 53: 1–8.
Dougherty, E. C. & L. G. Harris, 1963. Antarctic Micrometazoa: fresh-water species in the McMurdo Sound Area. Science 140: 497–498.
Dumont, H. J., 1983. Biogeography of rotifers. Hydrobiologia 104: 19–30.
Everitt, D. A., 1981. An ecological study of an Antarctic freshwater pool with particular reference to Tardigrada and Rotifera. Hydrobiologia 83: 225–237.
Fenchel, T. & B. J. Finlay, 2004. The ubiquity of small species: patterns of local and global diversity. BioScience 54: 777–784.
Folmer, O., M. Black, W. Hoeh, R. Lutz & R. C. Vrijenhoek, 1994. DNA primers for amplifcation of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology 3: 294–299.
Fontaneto, D., E. A. Herniou, C. Boschetti, M. Caprioli, G. Melone, C. Ricci & T. G. Barraclough, 2007. Independently evolving species in asexual bdelloid rotifers. PLoS Biology 5: 914–921.
Fontaneto, D., T. G. Barraclough, K. Chen, C. Ricci & E. A. Herniou, 2008. Molecular evidence for broad-scale distributions in bdelloid rotifers: everything is not everywhere but most things are very widespread. Molecular Ecology 17: 3136–3146.
Fontaneto, D., C. Q. Tang, U. Obertegger, F. Leasi & T. G. Barraclough, 2012. Different diversification rates between sexual and asexual organisms. Journal of Evolutionary Biology 39: 262–270.
Fontaneto, D., N. Iakovenko & W. De Smet, 2015. Diversity gradients of rotifer species richness in Antarctica. Hydrobiologia. doi:10.1007/s10750-015-2258-5.
Fraser, C. I., A. Terauds, J. Smellie, P. Convey & S. L. Chown, 2014. Geothermal activity helps life survive ice ages. Proceedings of the National Academy of Sciences of the USA 111: 5634–5639.
Freckman, D. W. & R. A. Virginia, 1993. Extraction of nematodes from Dry Valley Antarctic soils. Polar Biology 13: 483-487.
Fujisawa, T. & T. G. Barraclough, 2013. Delimiting species using single-locus data and the generalized mixed Yule coalescent approach: a revised method and evaluation on simulated data sets. Systematic Biology 62: 707–724.
Ganter, P. F., 2011. Everything is not everywhere: the distribution of cactophilic yeast. In Fontaneto, D. (ed.), Biogeography of microscopic organisms. Cambridge University Press, Cambridge: 130–174.
Garey, J. R., S. J. McInnes & P. B. Nichols, 2008. Global diversity of tardigrades (Tardigrada) in freshwater. Hydrobiologia 595: 101–106.
Greenslade, P., 1995. Collembola from the Scotia Arc and Antarctic Peninsula including descriptions of two new species and notes on biogeography. Polskie Pismo Entomologiczne 64: 305–319.
Haigh, S. B., 1965. The bdelloid rotifers of New Zealand, part II. Journal of the Quekett Microscopical Club 30(7–13): 36–41.
Haigh, S. B., 1966. The bdelloid rotifers of New Zealand, Part III. Journal of the Quekett Microscopical Club 30: 193–201.

Hillebrand, H. & A. I. Azovsky, 2001. Body size determines the strength of the latitudinal diversity gradient. Ecography 24: 251–256.

Martiny, J. B. H., B. J. Bohannan, J. H. Brown, R. K. Colwell, J. A. Fuhrman, J. L. Green, M. C. Horner-Devine, M. Kane, J. A. Krumins, C. R. Kuske, P. J. Morin, S. Naeem, L. Øvreås, A.-L. Reysenbach, V. H. Smith & J. T. Staley, 2006. Microbial biogeography: putting microorganisms on the map. Nature Reviews Microbiology 4: 102–112.

Iakovenko, N., 2004. Dynamika naselennia kolovertok pidstylky suboru v zymovyy period. In Ivanets, O. R. (ed.), Naukovi osnovy zberezennia biotychnoi riznomanitnosti. Tematychnyi zbirnyk v. 5. Liga-Press, Lviv: 279–284.

Iakovenko, N. S. & O. V. Tyshchenko, 2006. Rotifers (Rotifera) as a part of terrestrial bryophytic communities in Argentina Archipelago islands (Antarctic), Russia in the Antarctic. AASRI, St.-Petersburg: 229–230.

Iakovenko, N. S., E. Kašparová, M. Plewka & K. Janko, 2013. *Otostephanos* (Rotifera, Bdelloidea, Habrotrochidae) with the description of two new species. Systematics and Biodiversity 11: 477–494.

Jennings, P. G., 1976a. Ecological Studies on Antartic Tardigrades and Rotifers. Ph.D. Thesis, University of Leicester.

Jennings, P. G., 1976b. The Tardigrada of Signy Island, South Orkney Islands, with a note on Rotifera. BAS Bulletin 44: 1–25.

Jennings, P. G., 1979. The Signy Island terrestrial reference sites: population dynamics of Tardigrada and Rotifera. BAS Bulletin 47: 89–105.

Katoh, K., K. Misawa, K. Kuma & T. Miyata, 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Research 30: 3059–3066.

Koste, W., 1996a. Über die moosbewohnende Rotatorienfauna Madagaskars. Osnabrücker Naturwissenschaftliche Mitteilungen 22: 235–253.

Koste, W., 1996b. On soil Rotatoria from a lithotelma near Halali Lodge in Etosha National Park in N-Namibia, South Africa. Internationale Revue der gesamten Hydrobiologie und Hydrographie 81: 353–365.

Kutikova, L. A., 2005. Bdelloidnye kolovratki fauny Rossii. KMK Press, St. Peterburg.

Lacap, D. C., M. C. Y. Lau & S. B. Pointing, 2011. Biogeography of procaryotes. In Fontaneto, D. (ed.), Biogeography of microscopic organisms. Cambridge University Press, Cambridge: 35–42.

Maslen, N. R. & P. Convey, 2006. Nematode diversity and distribution in the southern maritime Antarctic – clues to history? Soil Biology and Biochemistry 38: 3141–3151.

McGaughran, A., I. D. Hogg & M. I. Stevens, 2008. Patterns of population genetic structure for springtails and mites in southern Victoria Land, Antarctica. Molecular Phylogenetics and Evolution 46: 606–618.

McGaughran, A., G. Torricelli, A. Carapelli, F. Frati, M. I. Stevens, P. Convey & I. D. Hogg, 2010. Contrasting phylogeographical patterns for springtails reflect different evolutionary histories between the Antarctic Peninsula and continental Antarctica. Journal of Biogeography 37: 103–119.

Milne, W., 1916. On the Bdelloid Rotifera of South Africa. Journal of the Quekett Microscopical Club 13(47–83): 149–184.

Murray, J., 1910. Antarctic Rotifera. British Antarctic. Expedition 1907–9(1): 41–65.

Murray, J., 1911. Bdelloid Rotifera of the South Africa. Annals Mededelingen of the van het Transvaal Museum 3: 1–19.

Nkem, J. N., D. N. Wall, R. A. Virginia, J. E. Barrett, E. J. Broos, D. L. Porazinska & B. J. Adams, 2006. Wind dispersal of soil invertebrates in the McMurdo Dry Valleys, Antarctica. Polar Biology 29: 346–352.

Örstan, A., 1992. Toxicity of acrylamide derivatives to embryos of the rotifer *Adineta vaga*. Bulletin of Environmental Contamination and Toxicology 48: 901–906.

Örstan, A., 1995. A new species of bdelloid rotifer from Sonora, Mexico. The Southwestern Naturalist 40: 255–258.

Peck, L. S., P. Convey & D. K. A. Barnes, 2006. Environmental constraints on life histories in Antarctic ecosystems: tempos, timings and predictability. Biological Reviews 81: 75–109.

Pisa, S., E. M. Biersma, P. Convey, J. Patiño, A. Vanderpoorten, O. Werner & R. M. Ros, 2014. The cosmopolitan moss *Bryum argenteum* in Antarctica: recent colonization or in situ survival? Polar Biology 37: 1469–1477.

Porazinska, D. L., D. H. Wall & R. A. Virginia, 2002. Invertebrates in ornithogenic soils on Ross Island. Antarctica. Polar Biol. 25: 569–574.

Porazinska, D. L., A. G. Fountain, T. H. Nylen, M. Tranter, R. A. Virginia & D. H. Wall, 2004. The Biodiversity and biogeochemistry of cryoconite holes from McMurdo Dry Valley glaciers, Antarctica. Arctic, Antarctic, and Alpine Research 36: 84–91.

Priddle, J. & H. J. G. Dartnall, 1978. The biology of an Antarctic aquatic moss community. Freshwater Biology 8: 469–480.

Pugh, P. J. A., 1993. A synonymic catalogue of the Acari from Antarctica, the sub-Antarctic Islands and the Southern Ocean. Journal of Natural History 27: 232–421.

Pugh, P. J. A. & P. Convey, 2008. Surviving out in the cold: Antarctic endemic invertebrates and their refugia. Journal of Biogeography 35: 2176–2186.

Rambaut, A., 2012. FigTree v.1.4.2. http://tree.bio.ed.ac.uk/software/figtree/.

Rambaut, A., M. A. Suchard, W. Xie, A. J. Drummond, 2013. Tracer v1.6. http://tree.bio.ed.ac.uk/software/tracer.

Ricci, C., 1987. Ecology of bdelloids: how to be successful. Hydrobiologia 147: 117–127.

Ricci, C., 2001. Dormancy patterns in rotifers. Hydrobiologia 446(447): 1–11.

Ricci, C. & M. Caprioli, 2005. Anhydrobiosis in bdelloid species, populations and individuals. Integrative and Comparative Biology 45: 750–763.

Ricci, C., G. Melone & E. Walsh, 2001. A carnivorous bdelloid rotifer, *Abrochtha carnivora* n. sp. Invertebrate Biology 120: 136–141.

Ricci, C., R. Shiel, D. Fontaneto & G. Melone, 2003. Bdelloid Rotifers Recorded from Australia with Description of *Philodinavus aussiensis* n.sp. Zoologischer Anzeiger 242: 241–248.

Richters, F., 1907. Die Fauna der Moosrasen des Gaussbergs und einiger südlicher Inseln. Deutsche Südpolar-Expedition 1901–1903(9): 259–302.

Richters, F., 1908. Moosbewohner. Schwedischen Südpolar. Expedition 1901–1903(4): 1–16.

Robeson, M. S., A. J. King, K. R. Freeman, C. W. Birky, A. P. Martin & S. K. Schmidt, 2011. Soil rotifer communities

are extremely diverse globally but spatially autocorrelated locally. Proceedings of National Academy of Sciences of the United States of America 108: 4406–4410.
Ronquist, F., M. Teslenko, P. van der Mark, D. L. Ayres, A. Darling, S. Höhna, B. Larget, L. Liu, M. A. Suchard & J. P. Huelsenbeck, 2012. MrBayes 3.2: efficient bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61: 539–542.
Ruttner-Kolisko, A. & E. Kronsteiner, 1979. Autokologie Parameter von Rotatorien aus extremen Biotopen. Jahresbericht Biologische Station Lunz 2: 111–114.
Sanderson, M. J., 2002. Estimating absolute rates of molecular evolution and divergence times: a penalized likelihood approach. Molecular Biology and Evolution 19: 101–109.
Segers, H., 2007. Annotated checklist of the rotifers (Phylum Rotifera), with the notes on nomenclature, taxonomy and distribution. Zootaxa 1564: 1–104.
Smykla, J., D. L. Porazinska, N. Iakovenko, K. Janko, W. M. Weiner, A. W. Niedbala & M. Drewnik, 2010. Studies on the Antarctic soil invertebrates: preliminary data on rotifers (Rotatoria) with notes on other taxa from Edmonson Point (Northern Victoria Land, Continental Antarctic). Acta Societatis Zoologicae Bohemicae 74: 135–140.
Smykla, J., B. Krzewicka, K. Wilk, S. D. Emslie & L. Śliwa, 2011. Additions to the lichen flora of Victoria Land, Antarctica. Polish Polar Research 32: 123–138.
Smykla, J., N. Iakovenko, M. Devetter & Ł. Kaczmarek, 2012. Diversity and distribution of tardigrades in soils of Edmonson Point (Northern Victoria Land, continental Antarctica). Czech Polar Reports 2: 61–70.
Smykla, J., M. Drewnik, E. Szarek-Gwiazda, Y. S. Hii, W. Knap & S. D. Emslie, 2015. Variation in the characteristics and development of soils at Edmonson Point due to abiotic and biotic factors, northern Victoria Land, Antarctica. Catena 132: 56–67.
Sohlenius, B. & S. Boström, 2005. The geographic distribution of metazoan microfauna on East Antarctic nunataks. Polar Biology 28: 439–448.
Sohlenius, B. & S. Boström, 2008. Species diversity and random distribution of microfauna in extremely isolated habitable patches on Antarctic nunataks. Polar Biology 31: 817–825.
Sohlenius, B., S. Boström & A. Hirschfelder, 1996. Distribution patterns of microfauna (nematodes, rotifers and tardigrades) on nunataks in Dronning Maud Land, East Antarctica. Polar Biology 16: 191–200.
Stevens, M. I. & I. D. Hogg, 2006. Contrasting levels of mitochondrial DNA variability between mites (Penthalodidae) and springtails (Hypogastruridae) from the Trans-Antarctic Mountains suggest long-term effects of glaciation and life history on substitution rates, and speciation processes. Soil Biology and Biochemistry 38: 3171–3180.
Stevens, M. I. S., F. Frati, A. McGaughran, G. Spinsanti & D. Hogg, 2006. Phylogeographic structure suggests multiple glacial refugia in northern Victoria Land for the endemic Antarctic springtail *Desoria klovstadi* (Collembola, Isotomidae). Zoologica Scripta 36: 201–212.
Strunecký, O., J. Elster & J. Komárek, 2012. Molecular clock evidence for survival of Antarctic cyanobacteria (Oscillatoriales, Phormidium autumnale) from Paleozoic times. Microbiol Ecology 82: 482–490.
Sudzuki, M., 1964. On the microfauna of the Antarctic region, I. Moss-water community at Langhovde. Japanese Antarctic Research Expedition Scientific Reports, Series E 19: 1–41.
Sudzuki, M., 1979. On the microfauna of the Antarctic region, III. Microbiota of the terrestrial interstices. Memoirs of National Institute of Polar Research (Tokyo). Special issue 11: 104–126.
Sudzuki, M., 1988. Comments on the antarctic Rotifera. Hydrobiologia 165: 89–96.
Tamura, K., G. Stecher, D. Peterson, A. Filipski & S. Kumar, 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. Molecular Biology and Evolution 30: 2725–2729.
Terauds, A., S. L. Chown, F. Morgan, H. J. Peat, D. Watts, H. Keys, P. Convey & D. M. Bergstrom, 2012. Conservation biogeography of the Antarctic. Diversity and Distributions 18: 726–741.
Torricelli, G., F. Frati, P. Convey, M. Telford & A. Carapelli, 2010. Population structure of *Friesea grisea* (Collembola, Neanuridae) in the Antarctic Peninsula and Victoria Land: evidence for local genetic differentiation of pre-Pleistocene origin. Antarctic Science 22: 757–765.
Velasco-Castrillón, A. & M. I. Stevens, 2014. Morphological and molecular diversity at a regional scale: a step closer to understanding Antarctic nematode biogeography. Soil Biology and Biochemistry 70: 272–284.
Velasco-Castrillón, A., T. J. Page, J. A. E. Gibson & M. I. Stevens, 2014a. Surprisingly high levels of biodiversity and endemism amongst Antarctic rotifers uncovered with mitochondrial DNA. Biodiversity. doi:10.1080/14888386.2014.930717.
Velasco-Castrillón, A., M. B. Schultz, F. Colombo, J. A. E. Gibson, K. A. Davies, A. D. Austin & M. I. Stevens, 2014b. Distribution and diversity of soil microfauna from East Antarctica: assessing the link between biotic and abiotic factors. PLoS One 9: e87529.
Velasco-Castrillón, A., J. A. E. Gibson & M. I. Stevens, 2014c. A review of current Antarctic limno-terrestrial microfauna. Polar Biology 37: 1517–1531.
Voigt, M., 1956–1957. Rotatoria: Die Rädertiere Mitteleuropas. Berlin-Nikolassee, Berlin.
Vyverman, W., E. Verleyen, A. Wilmotte, D. A. Hodgson, A. Willems, K. Peeters, B. Van de Vijver, A. De Wever, F. Leliaert & K. Sabbe, 2010. Evidence for widespread endemism among Antarctic micro-organisms. Polar Science 4: 103–113.
Williams, D. M., 2011. Historical biogeography, microbial endemism and the role of classification: everything is endemic. In Fontaneto, D. (ed.), Biogeography of microscopic organisms. Cambridge University Press, Cambridge: 11–31.
Zhang, J., P. Kapli, P. Pavlidis & A. Stamatakis, 2013. A general species delimitation method with applications to phylogenetic placements. Bioinformatics 15: 2869–2876.

Check for updates

Received: 7 November 2020 | Revised: 18 February 2021 | Accepted: 23 February 2021

DOI: 10.1111/jbi.14116

RESEARCH PAPER

Journal of Biogeography WILEY

Comparative phylogeography reveals consistently shallow genetic diversity in a mitochondrial marker in Antarctic bdelloid rotifers

Zeyneb Vildan Cakil[1,2] | Giuseppe Garlasché[1] | Nataliia Iakovenko[3,4,5] | Andrea Di Cesare[1] | Ester M. Eckert[1] | Roberto Guidetti[6] | Lina Hamdan[7] | Karel Janko[4,5] | Dzmitry Lukashanets[8] | Lorena Rebecchi[6] | Stefano Schiaparelli[9,10] | Tommaso Sforzi[1] | Eva Štefková Kašparová[5,11] | Alejandro Velasco-Castrillón[12] | Elizabeth J. Walsh[7] | Diego Fontaneto[1]

[1]National Research Council of Italy, Water Research Institute (CNR-IRSA), Molecular Ecology Group (MEG), Verbania Pallanza, Italy

[2]Department of Molecular Biology and Genetics, Middle East Technical University, Ankara, Turkey

[3]Department of Game Management and Wildlife Biology, Faculty of Forestry and Wood Sciences, Czech University of Life Sciences Prague, Prague, Czech Republic

[4]Department of Biology and Ecology, Faculty of Science, University of Ostrava, Ostrava, Czech Republic

[5]Laboratory of Fish Genetics, Institute of Animal Physiology and Genetics AS CR, Libĕchov, Czech Republic

[6]Department of Life Sciences, University of Modena and Reggio Emilia, Modena, Italy

[7]Department of Biological Sciences, University of Texas at El Paso, El Paso, TX, USA

[8]Research Institute, Klaipėda University, Klaipėda, Lithuania

[9]Department of Earth, Environmental and Life Science (DISTAV), University of Genoa, Genoa, Italy

[10]Italian National Antarctic Museum (MNA, Section of Genoa), University of Genoa, Genoa, Italy

[11]Department of Genetics and Breeding, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague, Prague, Czech Republic

[12]South Australian Museum, Adelaide, SA, Australia

Abstract

Aim: The long history of isolation of the Antarctic continent, coupled with the harsh ecological conditions of freezing temperatures, could affect the patterns of genetic diversity in the organisms living there. We aim (a) to test whether such pattern can be seen in a mitochondrial marker of bdelloid rotifers, a group of microscopic aquatic and limno-terrestrial animals and (b) to speculate on the potential mechanisms driving the pattern.

Location: Focus on Antarctica.

Taxon: Rotifera Bdelloidea.

Methods: We analysed different metrics of genetic diversity, also spatially explicit ones, including number of haplotypes, accumulation curves, genetic distances, time to the most recent common ancestor, number of independently evolving units from DNA taxonomy, strength of the correlation between geographical and genetic distances, population genetics neutrality and differentiation indices, potential historical processes, obtained from an extensive sample of cytochrome oxidase subunit I (COI) sequences obtained from bdelloid rotifers. We included 2242 individuals from 23 species in a comparison between Antarctic and non-Antarctic taxa, correcting for sample size directly in the analyses and then by confirming the results also using only a restricted dataset of nine well-sampled species.

Results: Antarctic species had consistently lower genetic diversity and potential younger relative age than non-Antarctic species, even if they were similar in sample size, geographical extent, neutrality and differentiation indices, and correlation between genetic and geographical distances.

Main conclusions: The extensive survey of genetic diversity in one mitochondrial marker in Antarctic bdelloids supports previous suggestions from other organisms

Zeyneb Vildan Cakil, Giuseppe Garlasché and Nataliia Iakovenko equally contributed.

 © 2021 John Wiley & Sons Ltd

Correspondence
Diego Fontaneto, National Research Council of Italy, Water Research Institute (CNR-IRSA), Molecular Ecology Group (MEG), Largo Tonolli 50, I-28922 Verbania Pallanza, Italy.
Email: diego.fontaneto@cnr.it

Funding information
Czech OP RDE; National Science Foundation, USA; PNRA, Italy

Editor: Ceridwen Fraser

that the origin and maintenance of terrestrial Antarctic fauna are different from those of other continents. Such differences could be speculated to be due, in the case of bdelloid rotifers, to the more recent origin of the species living there in comparison to non-Antarctic species.

KEYWORDS
diversification, endemism, meiofauna, polar regions, population genetics, Rotifera

1 | INTRODUCTION

Antarctica represents a unique region for biogeographical studies (Convey et al., 2008; Peat et al., 2007; Terauds et al., 2012): it is physically isolated from the other parts of the world and it has consistently harsh environmental conditions for life. Most global analyses of biodiversity gradients (Willig et al., 2003; Worm & Tittensor, 2018) assume a decrease in richness and diversity towards the poles (Hillebrand, 2004). Still, studies on the patterns and processes originating and maintaining diversity in Antarctica revealed radiations and high diversity, especially in the marine realm (Convey et al., 2014; Peck, 2018).

In marine Antarctic fauna, isolation and adaptation to cold temperatures led to novelties and diversification, such as the radiation of teleost fishes related to the evolution of antifreeze molecules (Chen et al., 2008; Matschiner et al., 2011), the loss of heat shock responses (Clark & Peck, 2009; Near et al., 2012) and evolutionary radiations in other groups of animals (Convey et al., 2014) leading to multiple species flocks (e.g. Bowen et al., 2020; Fassio et al., 2019). The degree of marine endemism is high, at all levels of taxonomic hierarchy from suborders in fish (Dornburg et al., 2017) to genera in amphipods (Verheye et al., 2017). At the species level, more than 75% of the taxa in some groups are endemic to Antarctica (Halanych & Mahon, 2018).

On the contrary, terrestrial fauna did not evolve new adaptations to desiccation and frost, but the animals and the other organisms currently living in Antarctica survived due to their already existing abilities (Convey, 1996), with more extensive and massive extinctions and fewer diversification events than in the marine environments (Convey et al., 2014; Zucconi et al., 2016). Most groups of animals went extinct in the terrestrial habitats of Antarctica: excluding the Antarctic Peninsula, just few soil arthropods (springtails and mites) and meiofauna (nematodes, rotifers and tardigrades) can be found (Pugh & Convey, 2008; Velasco-Castrillón et al., 2014), with no taxonomic groups higher than family endemic to the continent (Fontaneto et al., 2015; McInnes & Pugh, 1998). The terrestrial environments also host only two flowering plant species (Peat et al., 2007) and two native midge species (Contador et al., 2020), all endemic and restricted to part of the continent, the maritime Antarctic.

Selective extinction has been assumed to be a key driver of terrestrial biodiversity patterns in Antarctica (Convey et al., 2009). In terrestrial Antarctic systems, energy, mostly through solar irradiance intensity and thermal-related features, influencing availability of liquid water, is the most important physical driver of animal diversity (Baird et al., 2019; Chown et al., 2015; Convey et al., 2014). Even nowadays, during a warm interglacial period, terrestrial fauna in Antarctica can use a very minor part of the continent: only 0.3% of its surface is, at least seasonally, ice-free and with temperatures allowing animals to survive using liquid water (Convey et al., 2009). Thus, more than freezing temperatures per se, the limiting factor for survival of animals is the availability of suitable habitats, which are few, small and isolated. Terrestrial habitats are highly fragmented, interrupting migration and gene flow (Bergstrom & Chown, 1999; Chown & Convey, 2007; Convey et al., 2014). Biogeographical regionalisation is thus known to occur (Terauds et al., 2012; Verleyen et al., 2021), with genetic structure between populations and species even across relatively small areas (Carapelli et al., 2020; McGaughran et al., 2019; de Wouw et al., 2007).

The long-term isolation from the other Gondwanan faunas (Barnes et al., 2006; Hawes, 2015) limited the influence of immigration as a source of diversity (Guidetti et al., 2017). Ancient radiations and vicariance are known in terrestrial animals (e.g. Allegrucci et al., 2006; Chown & Convey, 2007; Maslen & Convey, 2006; Pertierra et al., 2020), with few taxa surviving in refugia during periods of intense cold (Fraser et al., 2012, 2014; Pugh & Convey, 2008). Expansion from refugia and survival through multiple Pleistocene glacial cycles is assumed to be the main mechanism through which Antarctic terrestrial fauna originated and is maintained (Cesari et al., 2016; McGaughran et al., 2010; Mortimer et al., 2011; Stevens et al., 2006).

The unique history of isolation, the extreme ecological conditions and patchiness of suitable habitats, in addition to low competition in the communities with few species, offer an intriguing model system to test general ideas on patterns and processes creating and maintaining biodiversity. Another peculiarity of Antarctic terrestrial animals is that they are all very small (e.g. mites, nematodes, rotifers, springtails, tardigrades), with biogeographical patterns that can be different between microscopic and macroscopic animals (Fontaneto, 2019; Heger et al., 2013). The main aim of this study is to provide further understanding on the evolutionary history of a group of Antarctic terrestrial animals, the bdelloid rotifers. Bdelloid rotifers are microscopic animals characterised by freezing and

desiccation resistance (Kaczmarek et al., 2019), by unclear asexuality (Vakhrusheva et al., 2020), and by unique genomic features (Nowell et al., 2018). They are ubiquitous in the ice-free areas of Antarctica (Garlasché et al., 2020). Definition and delimitation of species in asexual bdelloids, as in sexual animals, is traditionally based on morphological features and supported, when possible, by integrative approaches from phylogenetics, ecology, physiology, etc. (Fontaneto & Barraclough, 2015).

Bdelloids were among the first microscopic animals found and studied in Antarctica (Murray, 1910); even Shackleton noted their ability to withstand the harsh environmental conditions (Shackleton, 1909). Yet, desiccation and freezing tolerance, allowing passive dispersal in bdelloids, are not unique to Antarctic species (Kaczmarek et al., 2019). Thus, the biological drivers of spatial gradients in bdelloids could be similar between Antarctica and the rest of the world, given that no peculiar properties are currently known to occur in Antarctic bdelloids, and any difference between species in Antarctica and the rest of the world could be due to the uniqueness of Antarctic history and not to differential adaptations.

We address our aim of testing for the uniqueness of Antarctic bdelloids by comparing geographical patterns of genetic diversity in a set of species of Antarctic bdelloid rotifers with closely related species (belonging to the same genera or families) from other parts of the world. The main question we asked was whether the bdelloid species endemic to Antarctica had different diversification features compared to the species from the rest of the world, using various metrics to account for different facets of their evolutionary history.

2 | MATERIALS AND METHODS

2.1 | Sequence data collection

In total, 228 new sequences of 661 base pairs (bp) of the mitochondrial marker cytochrome c oxidase subunit I (COI) from Antarctic bdelloid rotifers were generated from single animals collected in the field. Animals were collected across the continent, mostly from Scott and Enderby areas and the Antarctic Peninsula (Appendix S1). DNA was extracted using Chelex or HotShot protocol (Montero-Pau et al., 2008) and the COI Folmer fragment was amplified using the HCO2198 and LCO1490 primers (Folmer et al., 1994), according to common protocols for rotifers (Gómez et al., 2002) (Table 1, Appendix S1). In all, 263 other previously published sequences from the same species were gathered from the literature, using GenBank (Sayers et al., 2019) and BOLD (Ratnasingham & Hebert, 2007) (Table 1, Appendix S1). The dataset included nine of the about 45 bdelloid morphological species known from Antarctica (Garlasché et al., 2020).

Given that Antarctic species are mostly endemic to the continent (Fontaneto et al., 2015; Garlasché et al., 2020; Iakovenko et al., 2015), the species to be used for comparison with Antarctic taxa were of the same genera (*Adineta* Hudson & Gosse, 1886 and *Philodina* Ehrenberg, 1830) or of the same family (Adinetidae and Philodinidae) of the Antarctic species. In all, 1587 sequences from species living outside Antarctica were gathered from published literature. We kept all the species with at least 15 sequences longer than 500 bp and that passed quality filtering controls, such as not having stop codons within the reading frame (Appendix S1). In addition, 164 sequences were obtained from *Philodina megalotrocha* in North America (Table 1, Appendix S1). None of the sequences retrieved from online repositories revealed evidence of misidentifications.

For each Antarctic COI sequence, we obtained the WGS84 geographical coordinates of the population of origin (Appendix S1). For the previously published sequences, such data were obtained from the archives of the authors of the sequences. For the non-Antarctic species, we gathered detailed geographical coordinates only for a subset of well-sampled species with at least 60 sequences.

Sequences were aligned using MAFFT (Katoh et al., 2019) and collapsed to haplotypes. A phylogenetic reconstruction between haplotypes was performed with Bayesian Inference using BEAST v2.5 (Bouckaert et al., 2019) through the CIPRES online portal (Miller et al., 2010) to obtain an ultrametric rooted tree, with terminals equidistant from the root and with edge lengths proportional to evolutionary rate and time, on a relative scale. Parameters in BEAST were set as default, except for GTR+I+G as evolutionary model (Debortoli et al., 2016), estimated random local clock, and 50,000,000 generations with a burnin of 20%.

2.2 | Metrics of genetic diversity

To understand the differences in genetic diversification between Antarctic and other species, we measured several metrics. Each metric was used to test hypotheses and to support inference on its effect or as a potential confounding factor in the statistical analyses.

The metrics we measured, as proxies of the overall genetic diversity, were as follows: (a) number of haplotypes, and rate of increase in the individual by haplotype accumulation curve; (b) maximum, mean and median genetic uncorrected distances and time to the most recent common ancestor in mutation-time units (MRCA); (c) number of potential independently evolving units within each species with different approaches from DNA taxonomy; (d) strength of the correlation between geographical and genetic distances between individuals; (e) population genetics neutrality and differentiation, through Tajima's D, Fu & Li's F and F_{ST} and (f) potential historical processes, through networks and Nested Clade Phylogeographical Analysis (NCPA). As additional confounding factors, we considered the total number of sequences for each species and the maximum and median geographical distances between the populations. Most metrics were obtained for all species, but the spatially explicit ones including geographical coordinates were obtained only for a subset of well-sampled species with more than 60 sequences, to provide reliable inference on spatial patterns. All data handling and analyses were performed in R v4.0.3 (R Core Team, 2020).

TABLE 1 Summary information for the 23 species used in the analyses, including area of origin (Antarctica or other), number of sequences, number of populations, proportion of sequences with known coordinates and maximum geographical distance in km between populations. Coordinates for non-Antarctic species were obtained only for those with at least 60 sequences; geographical distances were calculated only for the subset of nine well-sampled species with at least 60 sequences

Species	Origin	Sequences	Populations	Percentage with coordinates	Maximum geographical distance
Adineta editae Iakovenko, 2015	Antarctica	17	11	100.00	NA
Adineta emsliei Iakovenko et al., 2015	Antarctica	47	13	100.00	NA
Adineta gracilis Janson, 1893	Other	31	11	NA	NA
Adineta grandis Murray, 1910	Antarctica	90	33	100.00	2,930
Adineta vaga (Davis, 1873)	Other	203	41	89.66	19,274
Dissotrocha aculeata (Ehrenberg, 1830)	Other	25	19	NA	NA
Dissotrocha macrostyla (Ehrenberg, 1838)	Other	40	26	NA	NA
Habrotrocha antarctica Iakovenko et al., 2015	Antarctica	23	18	100.00	NA
Habrotrocha devetteri Iakovenko, 2015	Antarctica	22	9	100.00	NA
Macrotrachela ioannae Iakovenko, 2015	Antarctica	32	18	100.00	NA
Macrotrachela jankoi Iakovenko, 2015	Antarctica	17	8	100.00	NA
Macrotrachela multispinosa Thompson, 1892	Other	16	7	NA	NA
Macrotrachela quadricornifera Milne, 1886	Other	112	43	81.25	3,449
Philodina citrina Ehrenberg, 1830	other	62	17	98.39	3,720
Philodina dartnallis Iakovenko et al., 2015	Antarctica	15	7	100.00	NA
Philodina flaviceps Bryce, 1906	Other	86	15	100.00	52
Philodina gregaria Murray, 1910	Antarctica	228	58	100.00	3,593
Philodina megalotrocha Ehrenberg, 1832	Other	191	50	100.00	15,513
Philodina plena (Bryce, 1894)	Other	45	25	NA	NA
Pleuretra lineata Donner, 1962	Other	49	10	NA	NA
Rotaria rotatoria (Pallas, 1766)	Other	802	116	99.63	15,447
Rotaria sordida (Western, 1893)	Other	66	23	96.97	17,839
Rotaria tardigrada (Ehrenberg, 1830)	Other	23	16	NA	NA

The number of haplotypes for each species was used as a crude number and also as a proportion of the number of sequences. The cumulative frequency of occurrence of sequences per haplotype was obtained using a Coleman approximation, and the rate of increase in the curve was extrapolated as the minimum value of the first derivatives for each point of the curve using the R package 'vegan' v2.5-6 (Oksanen et al., 2019). In addition, we also compared the derivative at the minimum number of sequences for each species, namely 15; for the subset of well-sampled species, we also compared the values of the derivative at 60 sequences.

DNA taxonomy was performed according to two methods. First, the Automated Barcoding Gap Discovery (ABGD; Puillandre et al., 2012) was applied on the alignments of all individuals for each species using its online tool (https://bioinfo.mnhn.fr/abi/public/abgd/) with default settings and choosing the initial partition as output. Second, the Generalised Mixed Yule Coalescent model (GMYC; Fujisawa & Barraclough, 2013) was applied on the ultrametric BEAST tree for each species. We used the R package 'splits' v1.0.19 (Ezard et al., 2017) for GMYC, reporting the most conservative solution within the equally likely solutions (within 2 AIC units).

Correlations between geographical and genetic distances, only for the subset of well-sampled species with more than 60 sequences, were performed with Mantel tests between distance matrices using the R package 'vegan'. The matrices of geographical distances were calculated according to the WGS84 ellipsoid with R package 'geosphere' v1.5.10 (Hijmans, 2019). The matrices of uncorrected genetic distances were obtained from the alignments of all individuals using the R package 'ape' v5.3 (Paradis & Schliep, 2018). The same package was also used to calculate the relative time to the most recent common ancestor (MRCA) for each species on a relative time scale from 0 for extant terminals to −1 for the root of the ultrametric BEAST tree. No time calibration was possible because no fossils exist (Tang et al., 2014).

We performed tests of neutrality from population genetics using Tajima's D for all species from the R packages 'pegas' v0.13 (Paradis, 2010). We did not correct the estimate for sample size (Schaeffer, 2002), as we were also interested in assessing the potential confounding effect of sample size on all our estimates through statistical models (see later). In addition, and only for the subset of well-sampled species with more than 60 sequences, we calculated Fu & Li's F statistics from the R packages 'PopGenome' v2.7.5

(Pfeifer et al., 2014). F_{ST} values were obtained using the R package 'strataG' v2.4.905 (Archer et al., 2017) at the population level.

Haplotype networks were obtained for each species using TCS (Clement et al., 2000) and phylogeographical inference on past demographic events that have shaped the genetic history of the populations of a species according to the rationale of NCPA (Templeton et al., 1995) was obtained using ANeCA (Panchal, 2007).

2.3 | Statistical analyses

We performed one statistical model for each response variable: for response variables that were numerical (e.g. mean genetic distance, relative crown age, etc.), we used linear models (LMs); for response variables measured as counts (e.g. number of haplotypes, number of taxonomic units, etc.), we used generalised linear models (GLMs) with quasi-Poisson error structure; for response variables represented by proportion data (e.g. number of haplotypes/number of sequences), we used GLMs with binomial error structure (Crawley, 2012).

The main explanatory variable we included in all the models was the geographical origin of the species, as a factor with two levels: Antarctica and the rest of the world. An additional explanatory variable included in all models was sample size, as number of sequences for each species, to account for its potential confounding effect. In addition, and only for the subset of well-sampled species with more than 60 sequences, we also included, as a third explanatory variable, the confounding effect of geographical extent (as the maximum distance between samples for each species). We did not include any phylogenetic confounding effect, given that all Antarctic species belonged to genera with species represented also in the non-Antarctic taxa included in the analyses.

For each analysis including multiple predictors, we extracted the adjusted R^2 values for each predictor using the R package 'rsq' v1.1 (Zhang, 2018). In addition, we also tested for pairwise correlations between metrics using Pearson correlation tests. Dataset and R scripts for the statistical analyses are available in GitHub: https://github.com/CNR-IRSA-MEG/metaR/tree/master/lib/ANTA_bdello.

3 | RESULTS

3.1 | The datasets

The dataset (Figure 1, Table 1) had two well-sampled Antarctic species: *Adineta grandis* with 90 sequences and *Philodina gregaria* (Figure 1a) with 228 sequences, gathered from different geographical areas of the continent (Figure 1d,j; Appendix S1). The areas with the highest number of known records for both species are in Victoria Land, along the shores of the Ross Sea, and in Princess Elizabeth Land, along the shores of Prydz Bay. We obtained samples from both areas, whereas we could not obtain any sequence from individuals from the Antarctic Peninsula and surrounding islands, where scattered records are known for the species (Figure 1d,j). The maximum geographical distance between samples was 2900 km for *A. grandis* and 3500 km for *P. gregaria* (Appendix S2). Other seven species from Antarctica had between 15 and 47 sequences (Table 1, Appendices S1 and S2).

The dataset for comparison with the rest of the world included 14 species (Figure 1, Table 1). Seven species were well sampled, with 62–802 sequences per species, across spatial extents ranging from 52 to 19,000 km, from one to four continents (Figure 1, Table 1). They were one species of the genus *Adineta*, *A. vaga*, three species of the genus *Philodina*, *P. citrina*, *P. flaviceps* and *P. megalotrocha*, together with three other species of the family Philodinidae, *Macrotrachela quadricornifera*, *Rotaria rotatoria* and *R. sordida*. Other seven species from the genus *Adineta* and the family Philodinidae were not so well sampled, with only 15–49 sequences per species.

Overall, 2242 sequences were included in the dataset, with 1965 having geographical coordinates (Appendices S1 and S2). The number of sequences per species was not different between Antarctic and non-Antarctic species (GLM: $t = 1.5$, $p = 0.138$; Figure 2a). The same scenario was supported when analysing only the subset of nine well-sampled species, with no differences in sample size between Antarctic and non-Antarctic species (GLM: $t = 0.0$, $p = 0.998$). For the well-sampled dataset, the maximum geographical distance between samples for each species was not different between being in Antarctica or not (LM: $t = 1.1$, $p = 0.298$; Figure 2b) and was not related to sample size ($t = 1.0$, $p = 0.368$).

3.2 | Uniqueness of Antarctic species

The number of haplotypes for each of the 23 species was affected by sample size, being related to the number of sequences (GLM: $t = 5.0$, $p < 0.0001$, $R^2 = 0.52$; Figure 2b) but was also lower in Antarctic than in non-Antarctic species ($t = 3.0$, $p = 0.007$, $R^2 = 0.26$). The relative proportion of number of haplotypes on the number of sequences was significantly lower in Antarctic than in non-Antarctic species (GLM: $z = 6.0$, $p < 0.0001$, $R^2 = 0.01$; Figure 2c) and also related to sample size ($t = -6.0$, $p < 0.0001$, $R^2 = 0.01$).

The rate of increase in the cumulative number of individuals for each haplotype was steep for all species (Figure S3.3 in Appendix S3), and not related to being in Antarctica or not (LM: $t = 1.5$, $p = 0.139$, $R^2 = 0.06$; Figure 2d), or to sample size ($t = -1.6$, $p = 0.111$, $R^2 = 0.08$).

The overall genetic diversity measured as uncorrected genetic distances between all pairs of individuals in a species, using the median values of each matrix per species, was significantly lower in Antarctic than in non-Antarctic species (LM: $t = 4.7$, $p = 0.0001$, $R^2 = 0.51$; Figure 2e) but not related to sample size ($t = 0.9$, $p = 0.342$, $R^2 = 0.01$). The mean, median and maximum values of uncorrected genetic distances for each species were highly correlated (Pearson's r values between 0.98 and 0.99, Figure S3.4). Genetic diversity, measured as the relative time to the most recent common ancestor (MRCA, grey and red circles in Figure 1b), was significantly lower in Antarctic than in non-Antarctic species (LM: $t = -5.6$, $p < 0.0001$,

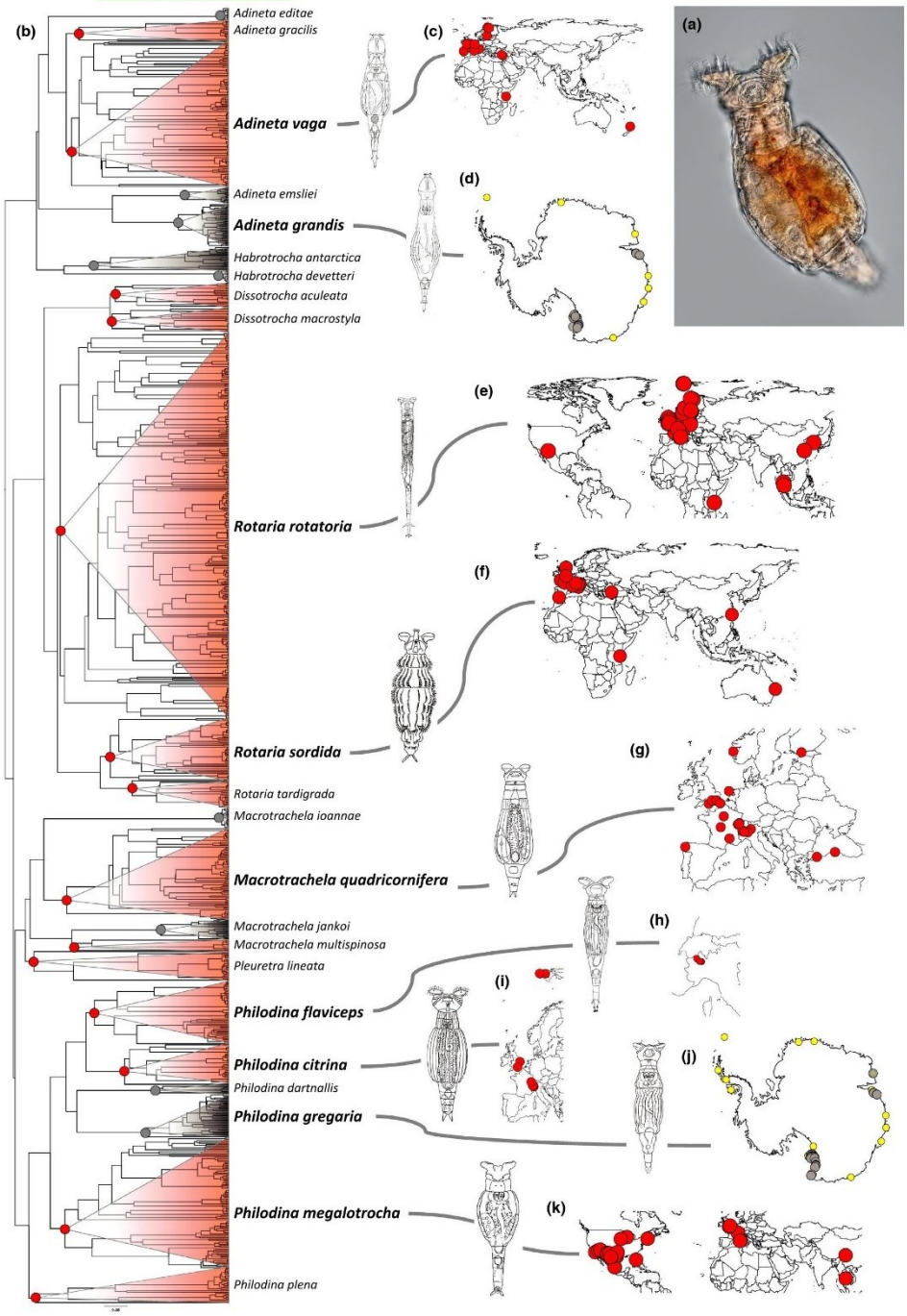

(a)
(b)
(c)
(d)
(e)
(f)
(g)
(h)
(i)
(j)
(k)
Adineta editae
Adineta gracilis
Adineta vaga
Adineta emsliei
Adineta grandis
Habrotrocha antarctica
Habrotrocha devetteri
Dissotrocha aculeata
Dissotrocha macrostyla
Rotaria rotatoria
Rotaria sordida
Rotaria tardigrada
Macrotrachela ioannae
Macrotrachela quadricornifera
Macrotrachela jankoi
Macrotrachela multispinosa
Pleuretra lineata
Philodina flaviceps
Philodina citrina
Philodina dartnallis
Philodina gregaria
Philodina megalotrocha
Philodina plena

FIGURE 1 The analysed dataset of COI sequences of bdelloid rotifers: (a) light microscopy photograph of *Philodina gregaria*, one of the species from Antarctica (photo courtesy of Michael Plewka, www.plingfactory.de); (b) BEAST phylogenetic reconstruction of the mutation/time calibrated relationships between all 1008 haplotypes (see Figure S3.1 in Appendix S3 for support values and tip labels) obtained from the 2242 COI sequences. Crown nodes of the most recent common ancestor (MRCA) are highlighted with circles, in grey for Antarctic and in red for non-Antarctic species, from which grey or red triangles show the diversification for each species. The names of the nine species in the subset of well-sampled species with at least 60 sequences are highlighted in bold and distribution maps are provided for them: (c) *Adineta vaga* from Africa, Asia, Europe and Oceania, (d) *Adineta grandis* from Antarctica, (e) *Rotaria rotatoria* from Africa, Asia, Europe and North America, (f) *Rotaria sordida* from Africa, Asia, Europe and Oceania, (g) *Macrotrachela quadricornifera* from Europe, (h) *Philodina flaviceps* from Northern Italy, (i) *Philodina citrina* from Europe, (j) *Philodina gregaria* from Antarctica and (k) *Philodina megalotrocha* from Asia, Europe and North America. Red and grey circles on maps mark the locations of the sequenced animals. Yellow circles (in d and j) mark all the currently known populations of the two well-sampled Antarctic species (data from Garlasché et al., 2020); for the almost cosmopolitan distribution of the non-Antarctic species, check Segers (2007). Maps are rectangular projections with longitude and latitude scales equivalent at the centre of the picture. Maps are not in scale. Maps of the other seven Antarctic species are reported in Figure S3.2

R^2 = 0.59; Figure 2f), and not affected by sample size (t = −1.1, p = 0.297, R^2 = 0.01).

The number of potential independently evolving units of DNA taxonomy within each species assessed with ABGD and GMYC was highly congruent (Appendix S2) and perfectly correlated (Pearson's r = 1.0, Figure S3.4): according to GMYC, very few of the Antarctic species revealed potential complexes of independently evolving units, whereas all the non-Antarctic species revealed a high level of hidden diversity. The number of taxonomic units from GMYC was significantly lower in Antarctic than in non-Antarctic species (GLM: t = 6.4, p < 0.0001, R^2 = 0.62; Figure 2g), even if also affected by biases in sample size (t = 4.3, p = 0.0003, R^2 = 0.46).

Values of Tajima's D were not different between Antarctic and non-Antarctic species (LM: t = −0.8, p = 0.429, R^2 = 0.02; Figure 2h), and they were not related to sample size (t = 1.2, p = 0.239, R^2 = 0.02).

Analysing only the nine well-sampled species (two of them from Antarctica) with more than 60 sequences, the results were overall qualitatively confirmed. The number of haplotypes for each species was affected by sample size, being related to the number of sequences (GLM: t = 4.0, p = 0.010, R^2 = 0.74), but not to differences between being in Antarctica or not (t = 1.9, p = 0.116, R^2 = 0.28), nor to spatial extent (t = 1.3, p = 0.240, R^2 = 0.13). The relative proportion of number of haplotypes on the number of sequences was confirmed to be significantly lower in Antarctic than in non-Antarctic species (GLM: z = 5.9, p < 0.0001) and still related to sample size (t = −4.8, p < 0.0001), but not to spatial extent (t = 0.3, p = 0.743).

The rate of increase in the cumulative number of individuals for each haplotype was significantly less steep in Antarctic than in non-Antarctic species (LM: t = 3.5, p = 0.017, R^2 = 0.65), and affected by sample size (t = −3.2, p = 0.023, R^2 = 0.61) but not by spatial extent (t = 1.6, p = 0.159, R^2 = 0.22).

The median value of uncorrected genetic distances between all individuals in a species was confirmed as significantly lower in Antarctic than in non-Antarctic species (LM: t = 9.3, p = 0.0002, R^2 = 0.93) but not related to sample size (t = 1.5, p = 0.204, R^2 = 0.16) or spatial extent (t = 0.3, p = 0.784, R^2 = 0.18). The relative time to the most recent common ancestor (MRCA) was confirmed as significantly lower in Antarctic than in non-Antarctic species (LM: t = −3.3, p = 0.021, R^2 = 0.63), and not affected by sample size (t = −1.4, p = 0.219, R^2 = 0.14) and by spatial extent (t = −0.3, p = 0.788, R^2 = 0.18).

The number of potential independently evolving units of DNA taxonomy according to GMYC was confirmed as significantly lower in Antarctic than in non-Antarctic species (GLM: t = 8.9, p = 0.0003, R^2 = 0.92), even if also affected by biases in sample size (t = 4.4, p = 0.007, R^2 = 0.81) and marginally by spatial extent (t = 3.0, p = 0.029, R^2 = 0.61).

The correlation between geographical and genetic distances between pairs of individuals was positive for all species, and significantly so from Mantel tests for all species except for *A. grandis* (Appendix S2), which had also the lowest correlation r value, 0.02; incongruously, the highest r value, 0.66, belonged to the other Antarctic species, *P. gregaria*. Correlation values were not different between Antarctic and non-Antarctic species (LM: t = −0.2, p = 0.860, R^2 = 0.19), neither were they related to sample size (t = 1.3, p = 0.254, R^2 = 0.10), nor with spatial extent (t = −1.0, p = 0.380, R^2 = 0.01).

None of the common metrics of population genetics we measured to assess neutrality and differentiation, namely Tajima's D (confirming the overall scenario), Fu & Li's F and F_{ST}, were different between Antarctic and non-Antarctic species, nor were they related to sample size or spatial extent (Table S3.1).

Regarding phylogeographical inference according to NCPA, the lowest and highest proportions of inconsistent results or failures to reject the null hypothesis were obtained for the two Antarctic species, 0.68 for *Philodina gregaria* and 0.94 for *Adineta grandis*, revealing no difference between Antarctic and non-Antarctic species in such proportions (GLM: z = 0.5, p = 0.592; Appendix S4), with no effect of sample size (z = −1.8, p = 0.077) or of spatial extent (z = 1.3, p = 0.194). Among the limited number of possible inference, the most common scenario was allopatric fragmentation, occurring in seven of the nine species; none of the inferred evolutionary histories was typical of Antarctic or of non-Antarctic species (Appendix S4).

4 | DISCUSSION

The main outcome of the analyses is that genetic patterns of diversity are confirmed to be different between Antarctic and non-Antarctic species, supporting a shallower genetic diversity in Antarctic than in non-Antarctic species for the mitochondrial marker COI. The

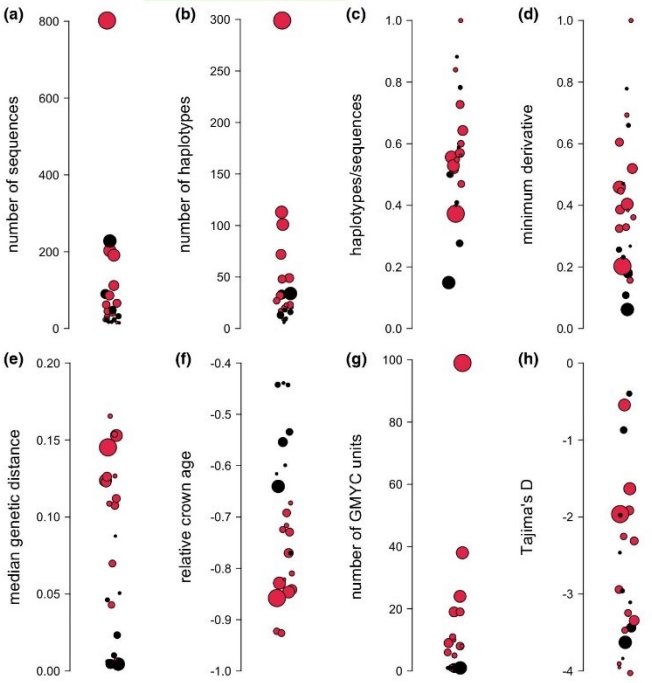


FIGURE 2 Values of the metrics of diversity for nine Antarctic (black circles) and 14 non-Antarctic species (red circles), with size of circles proportional to sample size: (a) number of sequences (as a scale for the size of the circles in the other plots), (b) number of haplotypes, (c) proportion of number of haplotypes on number of sequences, (d) rate of increase in the accumulation curve, (e) median values of the pairwise uncorrected genetic distances within species, (f) relative mutation time to the most recent common ancestor (MRCA) from the phylogeny in Figure 1b, (g) number of taxonomic units from a GMYC model, (h) Tajima's D

scenario occurs regardless of potential confounding factors such as differences in sample size or spatial extent, and are consistent across genera and families.

The first general inference is in support of the idea that Antarctica is a unique area. All the Antarctic species, except one, are endemic to the continent, whereas none of the non-Antarctic species is endemic to one continent only. Some of the sequences we analysed for the non-Antarctic species came from up to four continents. This scenario is not new, as it was previously suggested that biogeographical arenas for microscopic organisms with high capabilities for long-distance dispersal could work at large spatial scales (Heger et al., 2013; Lara et al., 2016). Yet, contrary to what is observed in other groups of Antarctic organisms that are endemic at higher taxonomic levels (Halanych & Mahon, 2018; McInnes & Pugh, 1998), the endemism of Antarctic bdelloids is restricted to the species level, with species belonging to cosmopolitan genera and families (Donner, 1965). Not all Antarctic species are strictly endemic: two of the sequences of one species, *M. ioannae*, came from the southern tip of South America, demonstrating the possibility for at least some bdelloids to cross the barrier between Antarctica and other continents (Iakovenko et al., 2015). Our knowledge on rotifer fauna in the Southern hemisphere is still very poor (Fontaneto et al., 2012), and it is not unlikely that the endemic status of other Antarctic species may be biased by our better knowledge for Antarctica than for other southern continents.

Another major idea about terrestrial diversity in Antarctica is that selective extinctions took place (Convey et al., 2014; Zucconi et al., 2016) with no new adaptations (Convey, 1996). The diversity of bdelloid rotifers is in agreement with selective extinction, given that species of limno-terrestrial, desiccation- and freezing-tolerant genera are mostly present (Iakovenko et al., 2015). Truly aquatic genera, such as *Rotaria*, mostly desiccation- and freezing-intolerant (Nowell et al., 2018), are under-represented, and almost only present in the sub-Antarctic islands (Garlasché et al., 2020). Yet, no study specifically tested the physiological adaptations of Antarctic bdelloids. In addition, there may be evidence of diversification happening also in Antarctica for bdelloids: *Adineta eimsliei* and *A. grandis* seem to be closely related taxa, separated from other species of the genus (Figure 1b), even if no reliable inference can be produced for this idea from sampling only five of the more than 20 known species of the genus (Donner, 1965; Iakovenko et al., 2015) and analysing only one mitochondrial marker. Another unusual feature of the two most common Antarctic bdelloids, *Adineta grandis* and *Philodina gregaria*, is that they share the feature of being the only species of their genera to be viviparous and not oviparous. This unique trait has been considered as a potential adaptation to the Antarctic environment (Iakovenko et al., 2015). Yet, no known advantages are related to viviparity in bdelloids. In other continents, viviparity is typical of aquatic genera, such as *Rotaria* and *Embata* (Donner, 1965). Thus, Antarctic bdelloid rotifers are at the moment ambiguous in supporting or contrasting the generality of the hypothesised processes of originating and maintaining terrestrial diversity in this unique and remote continent (Convey et al., 2014).

Regarding shallow genetic diversity in Antarctic species, the lower diversity and younger most recent common ancestor for

Antarctic bdelloids could be explained by a slower rate of evolution. This could be expected in polar taxa because of the low temperature (Gillooly et al., 2005; Martin & Palumbi, 1993) and potentially longer generation times (Convey et al., 2014). Yet, no evidence for such a slow-down of evolution has been found until now in any Antarctic group: no differences in the diversification rates in Antarctic taxa exist for marine animals such as molluscs (Crame & Clarke, 1997), crustaceans (Held, 2001), octopuses (Strugnell et al., 2012) or teleost fishes (Clarke & Johnston, 1996). In terrestrial animals, co-occurring Antarctic springtails and mites have different evolutionary rates (Stevens & Hogg, 2006), regardless of being in Antarctica, but coherent with the evolutionary rates within each of the two groups of organisms. Thus, the differences between Antarctic and non-Antarctic bdelloids are not to be explained by slower evolutionary rates, but by other processes.

Interestingly, none of the Antarctic species provided evidence of complexes of independently evolving units from COI, whereas some of the non-Antarctic species revealed tens of such potential cryptic taxa. Any method in DNA taxonomy based on the analysed barcoding mitochondrial COI marker (Hebert et al., 2003) can be affected by sample size and spatial extent (Bergsten et al., 2012). Yet, such confounding factors were explicitly included in our analyses and the results on the number of independently evolving units were consistent between the well-sampled and the overall datasets. No inference can be supported on the taxonomic reliability of the meaning of species delimitation based on only one marker in rotifers (Obertegger et al., 2018; Papakostas et al., 2016). All non-Antarctic species, recognised on morphology, hosted different cryptic species based on COI. If we would take for granted that the minimum, most conservative estimate of 'species' between ABGD and GMYC represented a taxonomic reality, number of individuals we would have for the resulting nine Antarctic 'species' would remain the same, from 15 to 228 (average = 54.4, median = 27.5). On the contrary, for the resulting over-inflated 240 non-Antarctic GMYC 'species' (Figure 2g) (excluding *P. plena*, the only species with no evidence of hidden diversity), the number of sequences per species would range from 2.5 to 16 (average 7.0, median = 6.2), making it impossible to perform any analysis of genetic diversity with our dataset. Given that most taxonomists of bdelloid rotifers work on both Antarctic and non-Antarctic species (e.g. Iakovenko et al., 2013 and Iakovenko et al., 2015), potential taxonomist bias cannot explain differences in species boundaries and levels of cryptic diversity between Antarctica and the rest of the world. What remains unexplained is the process leading to a perfect match between morphological and DNA taxonomy in Antarctica but not in the other parts of the world, making Antarctica a different evolutionary arena for the diversification of bdelloid rotifers.

Other metrics of genetic diversity did not differ between Antarctic and non-Antarctic (morphological) species. The negative values of Tajima's D could be related to recent selective sweep or population expansion after a recent bottleneck (Hartl & Clark, 2007), two processes that could be easily hypothesised for bdelloids in Antarctica during colder and harsher periods, with extreme reduction and fragmentation of suitable habitats (Convey et al., 2014; McGaughran et al., 2019). Yet, such negative values were recorded also in non-Antarctic species.

The correlation between geographical and genetic distances and the phylogeographical structures was significant and positive for Antarctic and non-Antarctic species, highlighting that microscopic animals such as bdelloids can show strong phylogeographical patterns (Fontaneto et al., 2008). Such patterns create a spatial structure for biogeographical regionalisation also in Antarctica (Terauds et al., 2012), notwithstanding the fact that most species are found across the whole continent (Garlasché et al., 2020; Iakovenko et al., 2015), apparently with no barriers to their distribution. One major problem in any inference on phylogeographical patterns in Antarctic bdelloids is that all the DNA sequence information that is available for the two best-sampled species come mostly from two areas, Victoria Land along the shores of the Ross Sea and Princess Elizabeth Land along the shores of Prydz Bay (Figure 1d,j). Thus, any significant correlation with geographical distances could be due to such bias in the origin of the samples, not supporting any evidence of isolation by distance but simply due to disjunct sampling areas; yet, the same haplotypes for each species were found in both areas.

All our inferences here are based on only a single mitochondrial marker, and could therefore be biased by any peculiarities of this marker; in future work, including additional markers could improve confidence in the inferences. Nevertheless, the general scenario of significant differences between Antarctic and non-Antarctic species is evident from this work. Furthermore, bdelloid rotifers are supposed ancient asexuals and their genomes should be under complete linkage (Judson & Normark, 1996), with overlapping patterns of diversification between markers (Schurko et al., 2009). No inconsistencies in evolutionary trajectories are known between mitochondrial and nuclear markers in *Adineta vaga*, the only morphological species for which the hypothesis of multi-locus phylogenetic congruence was explicitly tested (Debortoli et al., 2016; Wilson et al., 2018), and which is also one of the species we included in our analyses. The confounding effects of Horizontal Gene Transfer, a mechanisms that led to an estimate of about 10% of foreign genes currently occurring in bdelloid genomes (Nowell et al., 2018), and of the potential for recombination in bdelloids (Vakhrusheva et al., 2020) are still too rare to leave signatures in a comparative study of within-species diversification (Eyres et al., 2015).

5 | CONCLUDING REMARKS

Overall, our results reveal that within-species diversity of bdelloid rotifers in Antarctica is shallower and more recent. Apparently, Antarctic species are also more easily distinguishable, but potentially equally structured in space. Such differences with what happens in other parts of the world stands regardless of differences in sample

size and spatial extent. No reliable inference could be supported on the origin of limno-terrestrial bdelloid rotifers in Antarctica as dominated by extinctions without evolution of new adaptations and radiations or as dominated by adaptive evolution.

ACKNOWLEDGEMENTS

Funding was obtained through the Italian National Antarctic Research Program (PNRA, www.pnra.it) projects 2013/AZ1.13 to LR and PNRA16_00120-A1 (TNB-CODE) to SS and US National Science Foundation DEB 0516032 to EJW; sampling was partially supported by Neuron Endowment Fund to KJ; NI was supported by the Czech project EVA4.0 No. CZ.02.1.01/0.0/0.0/16_019/0000803 financed by OP RDE. We thank Michael Plewka for the photo of the Antarctic bdelloid, and all the logistic support we received to sample bdelloids in Antarctica. Sampling permits in Antarctica were obtained through the national reference contact points of the different countries of the authors, especially for samples from Antarctic Specially Protected Areas (ASPAs) and Antarctic Specially Managed Areas (ASMAs). No permits for Nagoya protocols were needed as the samples came from the national supervised areas by the authors, according to the Antarctic Treaty system. Collections from USA were taken under permits to EJW (Big Bend National Park, permit # BIBE-2006-SCI-0003; Guadalupe Mountains National Park, permit # GUMO-2009-SCI-0009; Carlsbad Caverns National Park, permit # CAVE-2008-SCI-0005; Balmorhea State Park, Texas Parks and Wildlife Department, permit # 02-04). Collections from México (Ojo de la Punta, Ojo de Santa Maria B, Ojo de en Medio, Poza Tortugas, and Manantial San Sebastian) were taken under a permit to Dr. Marcelo Silva Briano (permit # DGOPA/16216/281105-07984).

DATA AVAILABILITY STATEMENT

All newly generated sequences are available in GenBank, as listed in Appendix S1, and shared in Dryad: https://doi.org/10.5061/dryad.sj3tx964b; summary statistics are reported in Appendix S2; the alignment, the phylogenetic tree, the summary dataset of Appendix S2 and the R scripts for the statistical analyses are available on GitHub (https://github.com/CNR-IRSA-MEG/metaR/tree/master/lib/ANTA_bdello).

ORCID

Diego Fontaneto https://orcid.org/0000-0002-5770-0353

REFERENCES

Allegrucci, G., Carchini, G., Todisco, V., Convey, P., & Sbordoni, V. (2006). A molecular phylogeny of Antarctic Chironomidae and its implications for biogeographical history. *Polar Biology*, *29*, 320–326.

Archer, F. I., Adams, P. E., & Schneiders, B. B. (2017). strataG: An R package for manipulating, summarizing and analysing population genetic data. *Molecular Ecology Resources*, *17*, 5–11.

Baird, H. P., Janion-Scheepers, C., Stevens, M. I., Leihy, R. I., & Chown, S. L. (2019). The ecological biogeography of indigenous and introduced Antarctic springtails. *Journal of Biogeography*, *46*, 1959–1973.

Barnes, D. K. A., Hodgson, D. A., Convey, P., Allen, C. S., & Clarke, A. (2006). Incursion and excursion of Antarctic biota: Past, present and future. *Global Ecology and Biogeography*, *15*, 121–142.

Bergsten, J., Bilton, D. T., Fujisawa, T., Elliott, M., Monaghan, M. T., Balke, M., Hendrich, L., Geijer, J., Herrmann, J., Foster, G. N., Ribera, I., Nilsson, A. N., Barraclough, T. G., & Vogler, A. P. (2012). The effect of geographical scale of sampling on DNA barcoding. *Systematic Biology*, *61*, 851–869.

Bergstrom, D. M., & Chown, S. L. (1999). Life at the front: history, ecology and change on Southern Ocean islands. *Trends in Ecology and Evolution*, *14*, 472–477.

Bouckaert, R., Vaughan, T. G., Barido-Sottani, J., Duchêne, S., Fourment, M., Gavryushkina, A., & Drummond, A. J. (2019). BEAST 2.5: An advanced software platform for Bayesian evolutionary analysis. *PLoS Computational Biology*, *15*(4), e1006650.

Bowen, B. W., Forsman, Z. H., Whitney, J. L., Faucci, A., Hoban, M., Canfield, S. J., Johnston, E. C., Coleman, R. R., Copus, J. M., Vicente, J., & Toonen, R. J. (2020). Species radiations in the sea: What the flock? *Journal of Heredity*, *111*, 70–83.

Carapelli, A., Greenslade, P., Nardi, F., Leo, C., Convey, P., Frati, F., & Fanciulli, P. P. (2020). Evidence for cryptic diversity in the "pan-Antarctic" springtail *Friesea antarctica* and the description of two new species. *Insects*, *11*(3), 141.

Cesari, M., McInnes, S. J., Bertolani, R., Rebecchi, L., & Guidetti, R. (2016). Genetic diversity and biogeography of the south polar water bear *Acutuncus antarcticus* (Eutardigrada : Hypsibiidae) – Evidence that it is a truly pan-Antarctic species. *Invertebrate Systematics*, *30*, 635–649.

Chen, Z., Cheng, C.-h C., Zhang, J., Cao, L., Chen, L., Zhou, L., Jin, Y., Ye, H., Deng, C., Dai, Z., Xu, Q., Hu, P., Sun, S., Shen, Y., & Chen, L. (2008). Transcriptomic and genomic evolution under constant cold in Antarctic notothenioid fish. *Proceedings of the National Academy of Sciences of the United States of America*, *105*, 12944–12949.

Chown, S. L., Clarke, A., Fraser, C. I., Cary, S. C., Moon, K. L., & McGeoch, M. A. (2015). The changing form of Antarctic biodiversity. *Nature*, *522*, 431–438.

Chown, S. L., & Convey, P. (2007). Spatial and temporal variability across life's hierarchies in the terrestrial Antarctic. *Philosophical Transactions of the Royal Society of London, Series B, Biological Sciences*, *362*, 2307–2331.

Clark, M. S., & Peck, L. S. (2009). HSP70 heat shock proteins and environmental stress in Antarctic marine organisms: A mini-review. *Marine Genomics*, *1*, 11–18.

Clarke, A., & Johnston, I. A. (1996). Evolution and adaptive radiation of Antarctic fishes. *Trends in Ecology and Evolution*, *11*, 212–218.

Clement, M., Posada, D., & Crandall, K. A. (2000). TCS: A computer program to estimate gene genealogies. *Molecular Ecology*, *9*, 1657–1659.

Contador, T., Gañan, M., Bizama, G., Fuentes-Jaque, G., Morales, L., Rendoll, J., Simoes, F., Kennedy, J., Rozzi, R., & Convey, P. (2020). Assessing distribution shifts and ecophysiological characteristics of the only Antarctic winged midge under climate change scenarios. *Scientific Reports*, *10*, 9087.

Convey, P. (1996). The influence of environmental characteristics on life history attributes of Antarctic terrestrial biota. *Biological Reviews*, *71*, 191–225.

Convey, P., Chown, S. L., Clarke, A., Barnes, D. K. A., Bokhorst, S., Cummings, V., Ducklow, H. W., Frati, F., Green, T. G. A., Gordon, S., Griffiths, H. J., Howard-Williams, C., Huiskes, A. H. L., Laybourn-Parry, J., Lyons, W. B., McMinn, A., Morley, S. A., Peck, L. S., Quesada, A., … Wall, D. H. (2014). The spatial structure of Antarctic biodiversity. *Ecological Monographs*, *84*, 203–244.

Convey, P., Gibson, J. A., Hillenbrand, C. D., Hodgson, D. A., Pugh, P. J., Smellie, J. L., & Stevens, M. I. (2008). Antarctic terrestrial life–challenging the history of the frozen continent? *Biological Reviews*, *83*, 103–117.

Convey, P., Stevens, M. I., Hodgson, D. A., Smellie, J. L., Hillenbrand, C.-D., Barnes, D. K. A., Clarke, A., Pugh, P. J. A., Linse, K., & Cary, S. C. (2009). Exploring biological constraints on the glacial history of Antarctica. *Quaternary Science Reviews*, *28*, 3035–3048.

Crame, J. A., & Clarke, A. (1997). The historical component of marine taxonomic diversity gradients. In R. F. G. Ormond, J. D. Gage, & M. V. Angel (Eds.), *Marine biodiversity: Patterns and processes* (pp. 258–273). Cambridge University Press.

Crawley, M. J. (2012). *The R book*, 2nd ed. Wiley.

Debortoli, N., Li, X., Eyres, I., Fontaneto, D., Hespeels, B., Tang, C. Q., Flot, J.-F., & Van Doninck, K. (2016). Genetic exchange among bdelloid rotifers is more likely due to horizontal gene transfer than to meiotic sex. *Current Biology*, *26*, 723–732.

Donner, J. (1965). *Ordnung Bdelloidea (Rotatoria, Rädertiere)*. Akademie-Verlag.

Dornburg, A., Federman, S., Lamb, A. D., Jones, C. D., & Near, T. J. (2017). Cradles and museums of Antarctic teleost biodiversity. *Nature Ecology and Evolution*, *1*, 1379–1384.

Eyres, I., Boschetti, C., Crisp, A., Smith, T. P., Fontaneto, D., Tunnacliffe, A., & Barraclough, T. G. (2015). Horizontal gene transfer in bdelloid rotifers is ancient, ongoing and more frequent in species from desiccating habitats. *BMC Biology*, *13*, 1–17.

Ezard, T., Fujisawa, T., & Barraclough, T. G. (2017). splits: SPecies' LImits by threshold statistics. R package version 1.0-19/r52. Retrieved from https://R-Forge.R-project.org/projects/splits/

Fassio, G., Modica, M. V., Alvaro, M. C., Buge, B., Salvi, D., Oliverio, M., & Schiaparelli, S. (2019). An Antarctic flock under the Thorson's rule: Diversity and larval development of Antarctic Velutinidae (Mollusca: Gastropoda). *Molecular Phylogenetics and Evolution*, *132*, 1–13.

Folmer, O., Black, M., Hoeh, W., Lutz, R., & Vrijenhoek, R. (1994). DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, *3*, 294–299.

Fontaneto, D. (2019). Long-distance passive dispersal in microscopic aquatic animals. *Movement Ecology*, *7*(1), 10.

Fontaneto, D., Barbosa, A. M., Segers, H., & Pautasso, M. (2012). The 'rotiferologist' effect and other global correlates of species richness in monogonont rotifers. *Ecography*, *35*, 174–182.

Fontaneto, D., & Barraclough, T. G. (2015). Do species exist in asexuals? Theory and evidence from bdelloid rotifers. *Integrative and Comparative Biology*, *55*, 253–263.

Fontaneto, D., Barraclough, T. G., Chen, K., Ricci, C., & Herniou, E. A. (2008). Molecular evidence for broad-scale distributions in bdelloid rotifers: Everything is not everywhere but most things are very widespread. *Molecular Ecology*, *17*, 3136–3146.

Fontaneto, D., Iakovenko, N., & De Smet, W. H. (2015). Diversity gradients of rotifer species richness in Antarctica. *Hydrobiologia*, *761*, 235–248.

Fraser, C. I., Nikula, R., Ruzzante, D. E., & Waters, J. M. (2012). Poleward bound: Biological impacts of Southern Hemisphere glaciation. *Trends in Ecology & Evolution*, *27*, 462–471.

Fraser, C. I., Terauds, A., Smellie, J., Convey, P., & Chown, S. L. (2014). Geothermal activity helps life survive glacial cycles. *Proceedings of the National Academy of Sciences of the United States of America*, *111*, 5634–5639.

Fujisawa, T., & Barraclough, T. G. (2013). Delimiting species using single-locus data and the Generalized Mixed Yule Coalescent approach: A revised method and evaluation on simulated data sets. *Systematic Biology*, *62*, 707–724.

Garlasché, G., Karimullah, K., Iakovenko, N., Velasco-Castrillón, A., Janko, K., Guidetti, R., Rebecchi, L., Cecchetto, M., Schiaparelli, S., Jersabek, C. D., De Smet, W. H., & Fontaneto, D. (2020). A data set on the distribution of Rotifera in Antarctica. *Biogeographia*, *35*, 17–25.

Gillooly, J. F., Allen, A. P., West, G. B., & Brown, J. H. (2005). The rate of DNA evolution: effects of body size and temperature on the molecular clock. *Proceedings of the National Academy of Sciences of the United States of America*, *102*, 140–145.

Gómez, A., Serra, M., Carvalho, G. R., & Lunt, D. H. (2002). Speciation in ancient cryptic species complexes: Evidence from the molecular phylogeny of *Brachionus plicatilis* (Rotifera). *Evolution*, *56*, 1431–1444.

Guidetti, R., McInnes, S. J., Cesari, M., Rebecchi, L., & Rota-Stabelli, O. (2017). Evolutionary scenarios for the origin of an Antarctic tardigrade species based on molecular clock analyses and biogeographic data. *Contributions to Zoology*, *86*, 97–110.

Halanych, K. M., & Mahon, A. R. (2018). Challenging dogma concerning biogeographic patterns of Antarctica and the Southern Ocean. *Annual Review of Ecology, Evolution, and Systematics*, *49*, 355–378.

Hartl, D. L., & Clark, A. G. (2007). *Principles of population genetics*, 4th ed. Sinauer Associates.

Hawes, T. C. (2015). Antarctica's geological arks of life. *Journal of Biogeography*, *42*, 207–208.

Hebert, P. D., Cywinska, A., Ball, S. L., & Dewaard, J. R. (2003). Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *270*, 313–321.

Heger, T. J., Mitchell, E. A. D., & Leander, B. S. (2013). Holarctic phylogeography of the testate amoeba *Hyalosphenia papilio* (Amoebozoa: Arcellinida) reveals extensive genetic diversity explained more by environment than dispersal limitation. *Molecular Ecology*, *22*, 5172–5184.

Held, C. (2001). No evidence for slow-down of molecular substitution rates at subzero temperatures in Antarctic serolid isopods (Crustacea, Isopoda, Serolidae). *Polar Biology*, *24*, 497–501.

Hijmans, R. J. (2019). geosphere: Spherical trigonometry. R package version 1.5-10. Retrieved from https://CRAN.R-project.org/package=geosphere

Hillebrand, H. (2004). On the generality of the latitudinal diversity gradient. *American Naturalist*, *163*, 192–211.

Iakovenko, N. S., Kašparová, E., Plewka, M., & Janko, K. (2013). *Otostephanos* (Rotifera, Bdelloidea, Habrotrochidae) with the description of two new species. *Systematics and Biodiversity*, *11*, 477–494.

Iakovenko, N. S., Smykla, J., Convey, P., Kašparová, E., Kozeretska, I. A., Trokhymets, V., Dykyy, I., Plewka, M., Devetter, M., Durš, Z., & Janko, K. (2015). Antarctic bdelloid rotifers: Diversity, endemism and evolution. *Hydrobiologia*, *761*, 5–43.

Judson, O. P., & Normark, B. B. (1996). Ancient asexual scandals. *Trends in Ecology and Evolution*, *11*, 41–46.

Kaczmarek, Ł., Roszkowska, M., Fontaneto, D., Jezierska, M., Pietrzak, B., Wieczorek, R., Poprawa, I., Kosicki, J. Z., Karachitos, A., & Kmita, H. (2019). Staying young and fit? Ontogenetic and phylogenetic consequences of animal anhydrobiosis. *Journal of Zoology*, *309*, 1–11.

Katoh, K., Rozewicki, J., & Yamada, K. D. (2019). MAFFT online service: Multiple sequence alignment, interactive sequence choice and visualization. *Briefings in Bioinformatics*, *20*, 1160–1166.

Lara, E., Roussel-Delif, L., Fournier, B., Wilkinson, D. M., & Mitchell, E. A. D. (2016). Soil microorganisms behave like macroscopic organisms: Patterns in the global distribution of soil euglyphid testate amoebae. *Journal of Biogeography*, *43*, 520–532.

Martin, A. P., & Palumbi, S. R. (1993). Body size, metabolic rate, generation time and the molecular clock. *Proceedings of the National Academy of Sciences of the United States of America*, *90*, 4087–4091.

Maslen, N. R., & Convey, P. (2006). Nematode diversity and distribution in the southern maritime Antarctic—Clues to history? *Soil Biology and Biochemistry*, *38*, 3141–3151.

Matschiner, M., Hanel, R., & Salzburger, W. (2011). On the origin and trigger of the notothenioid adaptive radiation. *PLoS One*, *6*(4), e18911.

McGaughran, A., Terauds, A., Convey, P., & Fraser, C. I. (2019). Genome-wide SNP data reveal improved evidence for Antarctic glacial

refugia and dispersal of terrestrial invertebrates. *Molecular Ecology*, *28*, 4941–4957.

McGaughran, A., Torricelli, G., Carapelli, A., Frati, F., Stevens, M. I., Convey, P., & Hogg, I. D. (2010). Contrasting phylogeographical patterns for springtails reflect different evolutionary histories between the Antarctic Peninsula and continental Antarctica. *Journal of Biogeography*, *37*, 103–119.

McInnes, S. J., & Pugh, P. A. (1998). Biogeography of limno-terrestrial Tardigrada, with particular reference to the Antarctic fauna. *Journal of Biogeography*, *25*, 31–36.

Miller, M. A., Pfeiffer, W., & Schwartz, T. (2010). Creating the CIPRES Science Gateway for inference of large phylogenetic trees. Proceedings of the Gateway Computing Environments Workshop (GCE), New Orleans, LA, pp. 1–8.

Montero-Pau, J., Gómez, A., & Muñoz, J. (2008). Application of an inexpensive and high-throughput genomic DNA extraction method for the molecular ecology of zooplanktonic diapausing eggs. *Limnology and Oceanography: Methods*, *6*, 218–222.

Mortimer, E., Jansen van Vuuren, B., Lee, J. E., Marshall, D. J., Convey, P., & Chown, S. L. (2011). Mite dispersal among the Southern Ocean Islands and Antarctica before the last glacial maximum. *Proceedings of the Royal Society B: Biological Sciences*, *278*, 1247–1255.

Murray, J. (1910). Microscopic life at Cape Royds. British Antarctic Expedition, 1907–9, under the command of Sir E. H. Shackleton, C. V. O. In J. Murray (Ed.), *Reports on the Scientific Expeditions: Biology*, Vol. 1, Part 2 (pp. 17–22), pl. 1, 2, 4.

Near, T. J., Dornburg, A., Kuhn, K. L., Eastman, J. T., Pennington, J. N., Patarnello, T., Zane, L., Fernandez, D. A., & Jones, C. D. (2012). Ancient climate change, antifreeze, and the evolutionary diversification of Antarctic fishes. *Proceedings of the National Academy of Sciences of the United States of America*, *109*, 3434–3439.

Nowell, R. W., Almeida, P., Wilson, C. G., Smith, T. P., Fontaneto, D., Crisp, A., Micklem, G., Tunnacliffe, A., Boschetti, C., & Barraclough, T. G. (2018). Comparative genomics of bdelloid rotifers: Insights from desiccating and nondesiccating species. *PLoS Biology*, *16*, e2004830.

Obertegger, U., Cieplinski, A., Fontaneto, D., & Papakostas, S. (2018). Mitonuclear discordance as a confounding factor in the DNA taxonomy of monogonont rotifers. *Zoologica Scripta*, *47*, 122–132.

Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., & Wagner, H. (2019). vegan: Community ecology package. R package version 2.5-6. Retrieved from https://CRAN.R-project.org/package=vegan

Panchal, M. (2007). The automation of Nested Clade Phylogeographic Analysis. *Bioinformatics*, *23*, 509–510.

Papakostas, S., Michaloudi, E., Proios, K., Brehm, M., Verhage, L., Rota, J., Peña, C., Stamou, G., Pritchard, V. L., Fontaneto, D., & Declerck, S. A. J. (2016). Integrative taxonomy recognizes evolutionary units despite widespread mitonuclear discordance: Evidence from a rotifer cryptic species complex. *Systematic Biology*, *65*, 508–524.

Paradis, E. (2010). pegas: An R package for population genetics with an integrated-modular approach. *Bioinformatics*, *26*, 419–420.

Paradis, E., & Schliep, K. (2018). ape 5.0: An environment for modern phylogenetics and evolutionary analyses in R. *Bioinformatics*, *35*, 526–528.

Peat, H. J., Clarke, A., & Convey, P. (2007). Diversity and biogeography of the Antarctic flora. *Journal of Biogeography*, *34*, 132–146.

Peck, L. S. (2018). Antarctic marine biodiversity: Adaptations, environments and responses to change. In S. J. Hawkins, A. J. Evans, A. C. Dale, L. B. Firth, & I. P. Smith (Eds.), *Oceanography and marine biology: An annual review* (Vol. 56, pp. 105-236). Taylor and Francis.

Pertierra, L. R., Segovia, N. I., Noll, D., Martinez, P. A., Pliscoff, P., Barbosa, A., Aragón, P., Raya Rey, A., Pistorius, P., Trathan, P., Polanowski, A., Bonadonna, F., Le Bohec, C., Bi, K. E., Wang-Claypool, C. Y., González-Acuña, D., Dantas, G. P. M., Bowie, R. C. K., Poulin, E., & Vianna, J. A. (2020). Cryptic speciation in gentoo penguins is driven by geographic isolation and regional marine conditions: Unforeseen vulnerabilities to global change. *Diversity and Distributions*, *26*, 958–975.

Pfeifer, B., Wittelsburger, U., Ramos-Onsins, S. E., & Lercher, M. J. (2014). PopGenome: An efficient Swiss army knife for population genomic analyses in R. *Molecular Biology and Evolution*, *31*, 1929–1936.

Pugh, P. J. A., & Convey, P. (2008). Surviving out in the cold: Antarctic endemic invertebrates and their refugia. *Journal of Biogeography*, *35*, 2176–2186.

Puillandre, N., Lambert, A., Brouillet, S., & Achaz, G. (2012). ABGD, Automatic Barcode Gap Discovery for primary species delimitation. *Molecular Ecology*, *21*, 1864–1877.

R Core Team. (2020). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. Retrieved from https://www.R-project.org/

Ratnasingham, S., & Hebert, P. D. (2007). BOLD: The Barcode of Life Data System (http://www.barcodinglife.org). *Molecular Ecology Notes*, *7*, 355–364.

Sayers, E. W., Cavanaugh, M., Clark, K., Ostell, J., Pruitt, K. D., & Karsch-Mizrachi, I. (2019). GenBank. *Nucleic Acids Research*, *47*(D1), D94–D99.

Schaeffer, S. W. (2002). Molecular population genetics of sequence length diversity in the *Adh* region of *Drosophila pseudoobscura*. *Genetics Research*, *80*, 163–175.

Schurko, A. M., Neiman, M., & Logsdon, J. M. Jr (2009). Signs of sex: what we know and how we know it. *Trends in Ecology and Evolution*, *24*, 208–217.

Segers, H. (2007). Annotated checklist of the rotifers (Phylum Rotifera), with notes on nomenclature, taxonomy and distribution. *Zootaxa*, *1564*, 1–104.

Shackleton, E. H. (1909). Results of the British Antarctic Expedition, 1907–9. *The Geographical Journal*, *34*, 481–500.

Stevens, M. I., Greenslade, P., Hogg, I. D., & Sunnucks, P. (2006). Southern Hemisphere springtails: Could any have survived glaciation of Antarctica? *Molecular Biology and Evolution*, *23*, 874–882.

Stevens, M. I., & Hogg, I. A. (2006). Contrasting levels of mitochondrial DNA variability between mites (Penthalodidae) and springtails (Hypogastruridae) from the Trans-Antarctic Mountains suggest long-term effects of glaciation and life history on substitution rates, and speciation processes. *Soil Biology and Biochemistry*, *38*, 3171–3180.

Strugnell, J. M., Watts, P. C., Smith, P. J., & Allcock, A. L. (2012). Persistent genetic signatures of historic climatic events in an Antarctic octopus. *Molecular Ecology*, *21*, 2775–2787.

Tang, C. Q., Obertegger, U., Fontaneto, D., & Barraclough, T. G. (2014). Sexual species are separated by larger genetic gaps than asexual species in rotifers. *Evolution*, *68*, 2901–2916.

Templeton, A. R., Routman, E., & Phillips, C. A. (1995). Separating population structure from population history: A cladistic analysis of the geographical distribution of mitochondrial DNA haplotypes in the tiger salamander, *Ambystoma tigrinum*. *Genetics*, *140*, 767–782.

Terauds, A., Chown, S. L., Morgan, F., Peat, H. J., Watts, D., Keys, H., Convey, P., & Bergstrom, D. M. (2012). Conservation biogeography of the Antarctic. *Diversity and Distributions*, *18*, 726–741.

Vakhrusheva, O. A., Mnatsakanova, E. A., Galimov, Y. R., Neretina, T. V., Gerasimov, E. S., Naumenko, S. A., & Kondrashov, A. S. (2020). Genomic signatures of recombination in a natural population of the bdelloid rotifer *Adineta vaga*. *Nature Communications*, *11*, 6421.

Van de Wouw, M., van Dijk, P., & Huiskes, A. (2007). Regional genetic diversity patterns in Antarctic hairgrass (*Deschampsia antarctica* Desv.). *Journal of Biogeography*, *35*, 365–376.
Velasco-Castrillón, A., Schultz, M. B., Colombo, F., Gibson, J. A. E., Davies, K. A., Austin, A. D., & Stevens, M. I. (2014). Distribution and diversity of soil microfauna from East Antarctica: Assessing the link between biotic and abiotic factors. *PLoS One*, *9*, e87529.
Verheye, M. L., Backeljau, T., & d'Acoz, C. D. U. (2017). Locked in the ice-house: Evolution of an endemic *Epimeria* (Amphipoda, Crustacea) species flock on the Antarctic shelf. *Molecular Phylogenetics and Evolution*, *114*, 14–33.
Verleyen, E., Van de Vijver, B., Tytgat, B., Pinseel, E., Hodgson, D. A., Kopalová, K., Vyverman, W. (2021). Diatoms define a novel freshwater biogeography of the Antarctic. *Ecography*, in press. https://onlinelibrary.wiley.com/doi/full/10.1111/ecog.05374?af=R
Willig, M. R., Kaufman, D. M., & Stevens, R. D. (2003). Latitudinal gradients of biodiversity: Pattern, process, scale, and synthesis. *Annual Review of Ecology, Evolution, and Systematics*, *34*, 273–309.
Wilson, C. G., Nowell, R. W., & Barraclough, T. G. (2018). Cross-contamination explains "inter and intraspecific horizontal genetic transfers" between asexual bdelloid rotifers. *Current Biology*, *28*, 2436–2444.
Worm, B., & Tittensor, D. P. (2018). *A theory of global biodiversity (MPB-60)*, Vol. 60. Princeton University Press.
Zhang, D. (2018). rsq: R-Squared and related measures. R Package Version 1.1. Retrieved from https://CRAN.R-project.org/package=rsq
Zucconi, L., Onofri, S., Cecchini, C., Isola, D., Ripa, C., Fenice, M., Madonna, S., Reboleiro-Rivas, P., & Selbmann, L. (2016). Mapping the lithic colonization at the boundaries of life in Northern Victoria Land, Antarctica. *Polar Biology*, *39*, 91–102.

BIOSKETCH

All authors are interested in the patterns and processes in diversity of microscopic animals, especially in Antarctica.

Authors' contributions: ZVC, GG, NI, KJ, DL, SS, TS, ESK, AVC and DF conceived the study; NI, RG, LH, KJ, DL, SS, ESK, AVC, EJW and DF collected the data; ZVC, GG, NI, ADC, EME, LH, DL, TS, ESK and AVC performed work in the laboratory; ZVC, GG, NI, ADC, EME, RG, LH, KJ, DL, LR, SS, TS, ESK, AVC, EJW and DF analysed the data; DF wrote the first draft of the paper, with inputs from all other authors.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Cakil ZV, Garlasché G, Iakovenko N, et al. Comparative phylogeography reveals consistently shallow genetic diversity in a mitochondrial marker in Antarctic bdelloid rotifers. *J Biogeogr*. 2021;00:1–13. https://doi.org/10.1111/jbi.14116

7. Publication in preparation, bearing preliminary title: Southern Ocean: home for brave; Pelagic and deep sea communities have had bigger population size throw time.

The outcomes of the before-presented publication (Kašparová *et al.*, 2015) have inspired the hypothesis that population response to glaciation, and climatic change in general, may largely depend on ecological specialisation and lifestyle even in the very closely related species inhabiting the same environment. Namely, despite the Antarctic marine shelf experienced more or less vast ice sheet grows and retreats impacting the shelf communities as a whole (Thatje, Hillenbrand and Larter, 2005), most species had to survive glaciations „in situ (Janko *et al.*, 2011) and our data indicated that species restricted to a shallow shelf or benthic High and Sub-Antarctic areas might have been much more vulnerable to the recurrent ice sheet advances during the last glacial maxima than pelagic ones. However, our data supporting this view came from closely related vertebrates (Notothenioid fishes) and matured in the subsequent questions: how general is the validity of presented hypothesis, which types of ecological communities were less affected and why?

Accordingly, to address these questions and to test the generality of the hypothesis mentioned above about the role of lifestyle in determining the population resistance to glacial cycles, we extended the study by Kašparová *et al*. (2015) by adding newly obtained datasets from various organisms, diverging in their lifestyle inhabiting the Antarctic shelf. These included two sets of species newly sequenced in our laboratory: i.e. *Chaenodraco wilsoni*, the Notothenioid fish, and two population datasets of sea urchins *Sterechinus neumayeri* and *S. antarcticus*; preferentially inhabiting shallow and deep benthos along the Antarctic shelf, respectively. In addition, using NCBI, we downloaded 19 population datasets of various organisms comprising vertebrates (other published Notothenoid fishes) and common benthic invertebrates (Nemertea, Mollusca, Echinodermata and Arthropoda (Pycnogonida, Crustacea)).

There was done the comparative analysis of several diversity measures of mitochondrial DNA markers for all these datasets, either using cytochrome oxidase subunit I for Echinoderms, cytochrome *b* for fish, cytochrome oxidase II, NADH dehydrogenase I or D-

loop for datasets downloaded from NCBI. Genetic diversities, signals and timing of potential major population size changes were evaluated in all cases. The otcomes were then compared among the groups of species differing in their lifestyle. Specifically, species were divided according to their lifestyle into two categories.The shallow-bentic contrary to eurybathic along with pelagic and tested their vulnerability to climate change caused stess. In accordance with Thatje, Hillenbrand, Larter and Allcock, Strugnell (2005; 2012), we predicted that eurybathic species might have been protected from drastic climatic/glacial events by submerging into the deeper, colder and more stable water, where the iceberg/ice sheet scouring of the bottom debris causing avalanches would not play a significant role. Pelagic species could have profited from polar front northward shift and food concurrence reduction, thereby also avoiding the negative impact of glaciations. It contrasts with the second lifestyle cathegory, the shallow benthic species, which would have been most vulnerable to expanding ice sheet growing up to the shelf and thereby, their populations would have been more noticeably affected by severe bottlenecks. Consequently, eurybathic and pelagic species should bear a sign of an old population expansion with high haplotype diversity, whereas the shallow benthic species are predicted to bear signals of recent population expansion after the LGM bottleneck.

The data presented in this chapter haven't been published yet. However, I consider them highly relevant for being presented in this thesis as they nicely accomplish my long-term quest for generalizing patterns in Antarctic marine organisms.

7.1 ***Materials and methods:*** Sampling, data mining and species categorisation

This comparative study is based on three separated data source which corresponds to the successive hypothesis extension. First of all, the data comprise newly sampled benthic species of sea urchins differing in preferred depth distribution: (*Sterechinus neumayeri* (few newly sampled localities in addition to published study (Díaz *et al.*, 2018)) and *Sterechinus antarcticus* (entirely new data) along with one newly sampled species of fish (*Chaenodraco wilsoni*). Subsequentlly, 13 published fish species datasets from Kašparová *et al.*, (2015) and literature and NCBI database search for available population datasets resulted in addition of 14 published invertebrate and 7 fish species apart from Kašparová *et al.*, (2015)

(1) Samples of two echinoderm species were collected during the Polarstern cruise (ANTARKTIS, XXIII/8) lead in Antarctica in 2005-2006 and from the Vernacki base in 2012. They were collected by the bottom trawl and AGT in case of deep sea *Sterechinus antarcticus* and scuba diving for shallow shelf *Sterechinus neumayeri.* Individuals were sampled both at maritime Antarctic (Antarctic Peninsula) and High Antarctic regions (Weddell Sea, Terre Adéllie and Neumayer III Station) (Figure 1, Table 1).

(2) *Chaenodraco wilsoni* was sampled from Joinville Island and two specimens from South Shetlands during the same cruise (ANT-XXIII/8 with RV Polarstern in 2005-2006) (Figure 1, Table 1).

(3) Finally, the references to the datasets from Kašparová *et al.* (2015) and thouse available through NCBI and literature search are listed in Table 2 and References belonging to the Table 3.

All three source species (comprising newly sequenced, published by Kašparová *et al.* (2015) and GenBank database downloaded species) were divided into two categories according to their lifestyle characteristics. The first category comprises those living in water column; pelagic, then species, which can submerge to the deeper slope and are called eurybathic and lastly deep sea. The second category comprises benthic species, who are addicted by any developmental stage to shallow shelf, and so they are termed shallow-benthic. See the categorisation of lifestyle, Table 2.

Locality	GPS (South)	GPS (East)	Species	N	Data source
Terre Adélie	65.986389S	139.611389E	*S. neumaeyri*	15	D
Neumayer III	70.645078S	8.264133W	*S. neumaeyri*	11	SK
Weddel sea	71.933889S	47.998056W	*S. neumaeyri*	6	D
James Ross Island	64.314722ES	57.131389W	*S. neumaeyri*	14	D
Paradise Bay	64.851389S	62.91W	*S. neumaeyri*	10	D
Rothera Station	67.715278S	68.075W	*S. neumaeyri*	15/19	SK/D
Fildes Bay	62.208369S	58.966667W	*S. neumaeyri*	58	D
King George Island	61.825556S	58.587222W	*S. neumaeyri*	6	SK
Covadona Bay	63.366667S	58.15W	*S. neumaeyri*	40	D
Snow Hill	64.514333S	56.6705W	*S. neumaeyri*	3	SK
Larsen A	61.825556S	63.499997W	*S. neumaeyri*	9	SK
Larsen B	67.499997S	65.499997W	*S. neumaeyri*	4	SK
Vernadky	65.245833S	64.257778W	*S. neumaeyri*	15	SK
Joinville Island	62.5865S	54.7665W	*S. neumaeyri*	6	SK
O'Higgins Base	63.320258S	57.912775W	*S. neumaeyri*	5	D
Elephant I	61.431167S	56.118333W	*S. antarcticus*	5	SK
Larsen B	67.499997S	65.499997W	*S. antarcticus*	4	SK
Joinville Island	62.5865S	54.7665W	*S. antarcticus*	2	SK
Marguerite Bay	66.18 S	70,536 W	*S. antarcticus*	12	SK
Rothera Station	67.715278S	68.075W	*S. antarcticus*	3	SK
Weddel sea	71.933889S	47.998056W	*S. antarcticus*	3	SK
Terre Adélie	65.986389S	139.611389E	*S. antarcticus*	2	SK
South Shetlands	61.431167S	56.118333W	*Ch. wilsoni*	2	SK
Joinville Island	62.5865S	54.7665W	*Ch. wilsoni*	18	SK

Table 1: Sampling sites, their coordinates and numbers of specimens

N: nuber of specimens, Data source **D**: Díaz et al., 2018, **SK**: this study

As a distinct from Kašparová et al. (2015), here is omitted the intermediate category. The intermediate category comprised *Notothenia rossi* and Trematomus eulepidotus. *Notothenia rossi* is newly included in benthic species with respect to shelf spawning and juveniles grazing and laying eggs on the algae beads. Moreover, adults were seen in shallow water not beyond the shelf break (Kock, Pshenichnov and Devries, 2006). In spite of the fact that *Trematomu eulepidotus* eggs have been seen dwelling on sea sponge *Rosella nuda* (Kock, Pshenichnov and Devries, 2006; Young *et al.*, 2015) all the other developmental stages are

pelagic, moreover foraging in wather column and is capable of submerging. It is common in demersal water (having big eyes). It follows that it rightfully belongs to the pelagic group.

7.1.2 DNA extraction, PCR amplification

Total DNA from *Sterechinus antarcticus* and *Sterechinus newmayeri* was extracted either from the basal part of Echinoids spines or from blocks of muscles (comminator muscles) between latern hemi-pyramids by DNeasy Blood & Tissue Kit (Quiagen) according to the enclosed protocol.

A Fragment of mitochondrial cytochrome oxidase subunit I*,* COI gene, was amplified by nested PCR using primers described by Lee (Lee *et al.*, 2004) and newly developed primers LCOI 1523_E (5´- GCT AGC ACA ACC AGG CTC TC -3´), RCOI_E (5´-AGT GGC GGC AGT AAA GTA CG-3´). Polymerase chain reaction comprised 10 pm of each primer, 12.5 µl of PPP master mix with Mg^{+II} and approximately 10 ng of genomic DNA in total volume of 25 µl. The thermocycler conditions were by Díaz (Díaz *et al.*, 2011). Sequences were aligned by CLUSTALX software implemented in BioEdit Sequence Alignment Editor. *Chaenodraco wilsoni* mitochondrial cytochrome *b* (cyt *b*) was amplified in accordance with Kašparová et al. (2015). Species sequences were downloaded from GenBank database according to the published literature; see Table 2 for the source publication.

7.1.3 Genetic diversity and population structure

For all three data sorces thre were performed several population genetic diversity tests. Because inferrences of demographic history may be affected by hidden population structure, the alignments were divided with respect to published/analysed (see AMOVA tests below) population structuration, to published clades structuration and lastly to biogeographic provinces (Table 2). There have been recognised three biogeographic provinces in the Antarctic marine sector: 1: West Antarctic Peninsula with Sub-Antarctic islands, 2: East coast of Antarctic Peninsula with Weddell Sea and 3: East Antarctic with Ross Sea (Damerau *et al.*, 2012; Dömel *et al.*, 2017; Caccavo *et al.*, 2018; Vernet *et al.*, 2019; Schiavon *et al.*, 2021). It is

needed to admit the inconsistency in the Northern- west Antarctic peninsula region (Caccavo *et al.*, 2018; Reeve *et al.*, 2019).

Each newly sequenced alignment, just like alignments of already published species, were characterised by number of sequnces (N), lengh of the mitochondrial fragment (L), number of segregating sites (S), nucletode diversity (Pi) , number of haplotypes (h) and haplotype diversity (Hd) estimated with the program DnaSP ver. 6.12.03 (Librado and Rozas, 2009).

It was Analysis of Molecular Variance (AMOVA) implemented in Arlequin ver 3.5.2.2 (Excoffier, Laval and Schneider, 2007) that tests for geographic subdivision using the best fitting model of sequence evolution used for *Sterechinus neumayeri* newly sequenced localities and *S. antarcticus*. AMOVA estimates the level of genetic structuration within populations, between populations within a region (in all species sampled at least at two sites) and between regions. For published species used in this study, the information of genetic subdivision was taken rom the source publication (see Table 3 depicting population connectivity between locations).

The genealogical relationships (median-joining networks) among COI haplotypes of the entire dataset *of Sterechinus antarcticus* and unpublished particular locality datasets (Vernadsky, Neumayer III, Rothera) of *Sterechinus neumayeri* were made in PopArt 1.7 (Leigh and Bryant, 2015). Species bearing star-like haplotype networks are considered to have undergone a recent demographic expansion; selective sweep was detected in neither study (Kašparová *et al.*, 2015; Díaz *et al.*, 2018).

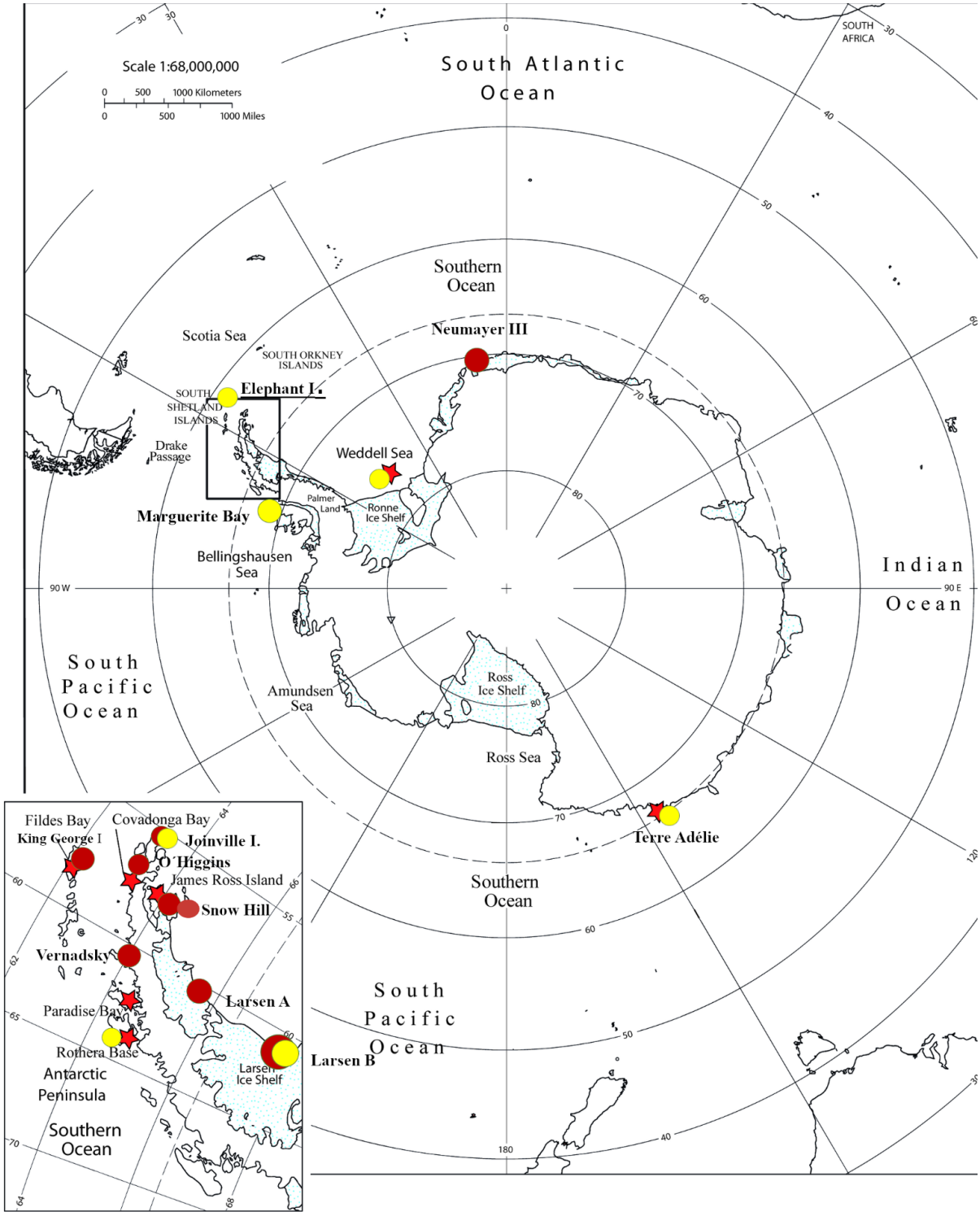


Figure 1:

Sterechinus neumayeri localities published by Díaz et.al. (2018) are depicted by asterisk, newly sampled novel localities of *Sterechinus neumayeri* are depicted by brawn circles; newly sampled localities of *Sterechinus antarcticus* are depicted by yellow circles.

7.1.4 Demographic inference

The neutrality tests Tajima's D and Fu's Fs (Tajima, 1989; Fu, 1997), evaluating whether the population deviated from mutation-drift equilibrium were performed in DnaSP ver. 6.12.03 (Librado and Rozas, 2009) with 1000 coalescent simulations.

Follow up the tests of population stability included the estimated time of the major demographic events by fitting models of sudden population expansion. Firstly, the mismatch distribution model was used for the timing of the population expansion that offered time of population expansion in mutational units per generation time for one locus (τ). Assuming the validity of the formula $t = \tau/2\mu$ (where μ equals the substitution rate per locus, rather than per site), the timing of expansion was calculated both without (in mutational units per sites **(t)**) and with several distinct substitution rates. There were used absolute timings a, with the fish mutational rate **(t (exp)[Kya; fish mutational rate]),** b, with the species-specific published mutational rate **(t (exp)[Kya; published mutational rate])**, and lastly, after ten fold correction **(t (exp)[Kya; published mutational rate]/10)** proposed by Ho (2011). All analyses were done in DnaSP ver. 6.12.03 (Librado and Rozas, 2009). In species, the substitution rate of which was not published, there was used the close relative´s one. Just one example, a close relative of *Chaenodraco wilsoni* is *Chaenocephalus aceratus* (Near *et al.*, 2012), the evolution time of whom has been used (Damerau *et al.*, 2014).

For literature searched species, the genetic structuration of which was detected, there were measured demographic parametrs in all possible localities to confirm/deny the effect of refugee to species population specific expansion timing (Table 2). To depict the relation of population size changes timings to biogeographic regions, there were used species populations from particular biogeographic zone to be compared.

Secondly, past population dynamics throw time implemented in BEAST version 1.8.0. (Drummond *et al.*, 2012) to date and evaluate past population grow range for *Sterechinus antarcticus* were estimated Two independent Extended Bayesian stepwise Skyline plot runs, of 60 Mega generations, with 10% burn-in removal, with HKY sequence evolution model (previously estimated by IQ-TREE software (Trifinopoulos *et al.*, 2016)), with strict clock

normally distributed mutation rate (used the mean of published mutation rates; 6,15 % per Mya after the correction of 10% time dependence of molecular rate at the population level (Ho *et al.*, 2005)) were merged in Tracer v.1.0.1. All runs had a number of independent samples from the marginal posterior distribution (ESS) over 200.

7.2 ***Results***

7.2.1 Genetic diversity

Two newly sequenced taxa (*Sterechinus antarcticus* and *Chaenodraco wilsoni*) and newly sequenced localities for *Sterechinus neumayeri* were analysed. There was detected neither stop codon nor insertion/deletion in 560 bp length of *Sterechinus antarcticus* COI gene, within 496 bp COI gene of *Sterechinus neumayeri* from newly sequenced localities and finally from 398 bp of *Ch. wilsoni* cyt *b* (Table 4). *Sterechinus neumayeri´s* King George, Joinville Island, O´ Higgins and Larsen B sampling sites did not contain any polymorphism.
Although AMOVA detected no population structuration for *Sterechinus antarcticus* (FST= 0.17755, P value= 0,075; FSC= 0.16935, P value= 0,077; FCT= 0.00987, P value= 0.58) we made genetic diversity demographic metrics firstly together in one analysis and secondly for particular localities to see if the trend is changing despite the small sample size. Congruently with Díaz et al. (2018) we have not found any sign of population structuration in *Sterechinus neumayeri* (FST= -0.04199, P value=0,484; FSC= 0.01276, P value= 0,356; FCT= -0.05546, P value= 0.924), neither.
Both *Ch. wilsoni* cyt *b* sequences from South Shetland Islands belongs to main Joinville Island haplotype, which means there is no conspicious need to make AMOVA (Figure 2). The highest haplotype and nucleotide diversity of *S. neumayeri* had East Antarctic Neumayer Station III then Rothera Station, Weddell Sea and lastly Larsen B (Table 4); Díaz (Díaz *et al.*, 2018) published the highest diversity at Rothera Station as well.
The highest genetic diversity indexes (Hd, π) for *Sterechinus antarcticus* localities were at Weddell Sea, Larsen B and Elephant Island, respectively (Table 4).
The overall genetic diversity indexes Hd, π are 3.5 and 2.2 times higher for *S. antarcticus* than for *S. neumayeri* from newly sampled localities (Table 4). *Chaenodraco wilsoni* Hd and π was 0.001 and 0.363 which is more than two times less than *Sterechinus antarcticus* (Table 4).

Consequently, comparative analysis was done on 36 already published species (Table 5), belonging to 52 molecularly recognised clades/subclades (Table 2) along with the sea urchin *Sterechinus neumayeri* (new localities), next *S. antarcticus* and *Chaenodraco wilsoni*. Values from the table 2 has been used to visualize the suspectited trend of higher genetic diversity of species belonging to deep sea or thouse ones pelagic as distinct from the bentics and shallow shelf species with lower genetic diversity (Figure 3 a and b).
On the contrary, if we plot the relation of biogeographic zones (1, 2, 3) to genetic diversity (Hd, π) (Table 2) regardless the lifestyle, there is ambiguity in interpretation. There is visible higher nucleotide diversity in Eastern Antarctic and Ross sea region than in the West antarctica. The Weddell Sea region comprising the Eastern part of Antarctic Penninsula has both diversity indexes of the lowest value (Figure 4 a, b).

7.2.2 Demographic inference

Congruently, despite the fact that AMOVA has detected no population structuration we made separate demography metrics for each locality and as well for the whole dataset in case of *Sterechinus neumayeri* and *S. antarcticus*. The median joining haplotype network did detect in neither locality star-like shape for *Sterechinus antarcticus* and showed not centralized haplotype network bearing numerous mutation steps from the central haplotype (Figure 5 a). Contrary to *S. antarcticus* and in line with Díaz et al. (2018), *Sterechinus neumayeri* bears star-like pattern for newly sequenced localities i. e. Rothera and Neumayer Stations III (Figure 5 b) and for those published (Díaz *et al.*, 2018).
Although *Chaenodraco wilsoni* ´s haplotype network does not show a classic star-like pattern (Figure 2), the Mismatch distribution showed an L-shaped slope and Tajima´s D and Fu´FS metrics were negative; even though the later non-significant.
The *Sterechinus antarcticus* Tajima´s D and Fu´F_S indexes were not in all cases negative (Table 4) and were non-significant which is concordant with the complex haplotype network. Similarly, the mismatch distributions did not followed L-shaped curve (Figure 5a). This is

discordant with *Sterechinus neumayeri*, showing significant deviation from mutation-drift equilibrium, having Tajima's D and Fu's F_S indices negative and significant apart from Larsen A, which is insignificant (Table 4). Díaz *et al*. (2018) showed the insignificant values as well for Paradise Bay and James Ross Island. In contradiction, Mismatch distribution showed L-shaped slope for Larsen A and Rothera station, but not for Neumayeri Station III (Figure 5b).

Bayesian skyline plot of *Sterechinus antarcticus* across all sampled locations confirmed not significant deviation from mutation drift equilibrium by population size throw time measurement (Figure 6), which detected 0,1 Ne * generation time population size grouth, just negligible, insignificant recent rise with high uncertainty. Chaenodraco wilsoni EBSP could not reach the result, because data did not bear enough polymorphism.
Already published Bayesian skyline plots for this study analysed species (references in Table 5) showed consistently more recent demographic expansions for shallow benthic species. The onset of population expansion for shalow benthic species is in line with Pleistocene temperature transition (Damerau *et al.*, 2014). The only exception is *Pareledone turqueti*, which might have been sampled from its refuge (Strugnell *et al.*, 2012)

All published, GenBank database downloaded species, (Table 2, 4) did not displayed the significant and negative Tajima's D and Fu's F_S indices even thow being benthic (*Chaenodraco aceratus, Chionodraco myersi, Chionodraco rastrospinosus*). Nevertheless, Damerau et al. (2014) found population expansion by Bayesian skyline plot approach for *Ch. aceratus* on two mitochondrial markers. *Chaenodraco hamatus* has significant devation from the mutation drift equilibrium tested by Tajima's D and Fu's F_S just for Ross Sea region (Patarnello *et al.*, 2003).
Figure 3 shows population genetic metrics and their values from the Table 5 for all analysed species in relation to lifestyle characteristics. There are two groups: shalow benhic and pelagic species which comprise as well eurybathic species. There was compared expansion time in mutational units and then the expansion time multiplied by published mutation rate and with ten fold correction. (Table 2, 4). The most ancient expansion have *Parborlasia corrugatus, Trematomus newnesi* and *Euphausia superba;* all of which are pelagic or deep sea species. Shallow shelf *Gobionotothen giberifrons* along *with Trematomus hansoni* and *Lepidonotothen nudifrons* belong to the oldest dated expansion fish species (Table 2). Lastly,

the expansion time is shown with the same mutation rate. It was chosen the fish mutational time published by Janko et al. (2007) It is obvious that for *Lepidonotothen larseni* was the mutation rate ill-chosen. (Table 2, 4).

We can comprehensively conclude that benthic species possess lower genetic diversity and more recent population expansions irrespective of the mutational time beeing used. (Table 2, 5; Figure 3 c, d). Naturally, using the mutational rate appropriate for the particular species makes the assumptions more precise eg. (González-Wevar, David and Poulin, 2011; Strugnell *et al.*, 2012).

The relationship of population expansion timing to biogeographic provincy (1, 2, 3) regardless of lifestyle of all species and localities (Table 2) is more informative than in case of genetic diversity indexes. Congruently, both expansion timings dating (Figure 4c and d) shows the older populations expansions in the East Antarctica along with the Ross Sea region. Thre is the unity of the existence of the yangest populations in the Weddel Sea region.

Species	Data source	locus	organism	Locality	BP	Life	N	L	S	π*10³	h	Hd*10	Fu's Fs statistic	Tajimas D	τ	t *10³	tO	t P	t P/10
Aethotaxis mitopteryx	Kašparová et al,2015	cytb	Osteichthyes	EI	2	1	33	553	31	4,78	19	8,69	-14,642***	- 2,42273***	1,347	1,22	248,55	248,55	**24,86**
Astrotoma agassizii clade 1	Jossart 2019	COII	Echinoderma	ANT, SG, RS, SR,W antarctic cont	123	1	138	602	28	1,5	34	6,18	−31,56***	- 2,4***	0,894	0,74	151,54	12,07	**1,21**
Astrotoma agassizii clade 1_subclade a	Jossart 2019	COII	Echinoderma	ADE,AMU,WED, ROSS	23	1	20	602	8	1,33	9	6,53	- 8,005***	-2,17464***	0,8	0,66	135,60	10,80	**1,08**
Astrotoma agassizii clade 1_subclade b	Jossart 2019	COII	Echinoderma	ADE,AMU,WED, ROSS, SG	123	1	110	602	21	1,05	22	4,84	-31,240***	-2,42572***	0,63	0,52	106,79	8,51	**0,85**
Astrotoma agassizii clade 5	Jossart 2019	COII	Echinoderma	SG, SR	1	1	37	602	16	2,61	15	8,39	−10.93***	−1.93***	1,571	1,30	266,29	21,22	**2,12**
Colossendeis megalonyx clade A	Dietz et al., 2015	COI	Pycnogonida	EI	2	1	93	545	24	1,51	23	5,66	-30,301***	-2,50212***	0,586	0,54	109,72	46,75	**4,67**
Colossendeis megalonyx clade D1	Dietz et al., 2015	COI	Pycnogonida	EWS, SO, SSh, TA, EI, WANT	123	1	92	413	27	4,32	30	7,63	-30,59***	-2,1047***	0,348	0,42	85,98	36,64	**3,66**
Colossendeis megalonyx clade E1	Dietz et al., 2015	COI	Pycnogonida	BI, TA, SSh	13	1	26	545	16	7,99	8	8	0,258 NS	0,13429 NS	0,736	0,68	137,80	58,72	**5,87**
Colossendeis megalonyx clade I	Dietz et al., 2015	COI	pycnogonida	WANT, SO, SSh, EWS	12	1	60	484	14	6,81	12	7,54	0,842 NS	0,28771 NS	1,384	1,43	291,79	124,33	**12,43**
Colossendeis megalonyx clade A	Dietz et al., 2015	COI	Pycnogonida	SG	1	1	16	545	7	3,93	6	8,08	-0,505NS	0,05363NS	1,333	1,22	249,58	106,34	**10,63**
Colossendeis megalonyx clade A	Dietz et al., 2015	COI	Pycnogonida	SSH, EI , SO	12	1	141	545	33	1,82	31	5,7	-42,21***	-2,48969***	0,232	0,21	43,44	18,51	**1,85**
Euphausia superba	Bortolotto et al., 2011	ND1	Crustacea	WS, RS, ANT, SAI	123	1	641	154	45	13,937	124	8,561	-26,28904**	- 1,79083***	2,5	8,12	1656,51	381,07	**38,11**
Chionodraco hamatus	Patarnello, 2003	D-loop	Osteichthyes	TA	3	1	22	249	7	5,72	8	8,18	-3,226 NS	-0,8266 NS	1,424	2,86	583,56	92,24	**9,22**
Limacina antarctica	Sromek et al., 2015	COII	Mollusca	DP	1	1	60	559	57	6,1	47	9,67	−26.198***	−2.454***	3,486	3,12	636,34	129,92	**12,99**
Nematocarcinus lanceopes	Raupach et al., 2010	COI	Crustacea	SS, SA, ANT, EWS, ERS	123	1	184	657	43	5,48	54	8,92	-32,887***	-1,65762***	2,549	1,94	395,89	96,99	**9,70**
Nymphon australe	Membrives_2017	COI	pycnogonida	ANT, EA, WS	13	1	364	554	59	6,57	85	9,18	−95,972***	−1,842**	4,691	4,23	864,03	368,15	**36,82**
Nymphon australe	Membrives_2017	COI	Pycnogonida	EA	3	1	227	554	39	4,96	50	8,57	NA	NA	3,432	3,10	632,14	269,35	**26,93**
Nymphon australe	Membrives,2017	COI	Pycnogonida	ANT	1	1	95	554	29	5,35	29	7,89	-17,882***	-1,51174*	1,777	1,60	327,30	139,46	**13,95**
Nymphon australe	Membrives_2017	COI	Pycnogonida	SS, SG	1	1	13	554	5	2,75	6	7,69	NA	NA	1,312	1,18	241,66	102,97	**10,30**
Nymphon australe	Membrives_2017	COI	Pycnogonida	WS	3	1	29	554	8	1,33	7	4,29	NA	NA	0,943	0,85	173,69	74,01	**7,40**
Odonaster validus clade I	Janosik, 2010	COI	Echinoderma	ANT, SAI, SR	13	1	67	570	38	4,36	27	8,5	-21,506***	-2,33122***	0,93	0,82	166,49	13,26	**1,33**
Pagothenia borchgrevinki	Janko et al., 2007	cytb	Osteichthyes	CH, CA	3	1	38	578	16	3,55	20	8,411	- 19,04 **	- 1,95**	1,94	1,68	342,49	342,49	**34,25**
Pallenopsis patagonica clade C	Domel, 2017	COI	Pycnogonida	EANT, WS	2	1	17	426		0,83	3	2,28	-0,963 NS	-1,70573*	NA	NA	NA	NA	**NA**
Pallenopsis patagonica clade D	Domel, 2017	COI	Pycnogonida	Tip of ANT	1	1	17	426	8	3,31	8	7,79	-4,021**	-1,4267 NS	1,412	1,66	338,22	144,11	**14,41**
Pallenopsis patagonica clade F	Domel, 2017	COI	Pycnogonida	EANT, WS	2	1	17	426	2	1,62	3	6,03	0,42027 NS	0,283 NS	0,691	0,81	165,52	70,52	**7,05**
Parborlasia corrugatus	Thornhill et al., 2008	COI	Nemertea	ANT	1	1	73	599	18	1,8	14	6,91	-9,52***	-2,10628**	0,269	0,22	45,82	3433,35	**343,33**
Pleuragramma antarcticum	Zane et al., 2006	D-loop	Osteichthyes	EWS, KG, EI, TNB	123	1	256	210	NA	8,374	110	8,96	not available	−2,389**	1,449882	3,45	704,51	111,36	**11,14**
Promachocrinus kerguelensis	Wilson 2007	COII	Echinoderma	BS	1	1	22	623	12	5,07	7	7,71	0,378 NS	-0,14477 NS	1,316	1,06	215,55	17,17	**1,72**
Promachocrinus kerguelensis	Wilson 2007	cytb	Echinoderma	BS	1	1	22	663	8	3,87	6	7,19	0,624 NS	0,55726 NS	1,215	0,92	187,00	14,90	**1,49**
Promachocrinus kerguelensis	Wilson 2007	cytb	Echinoderma	BI	1	1	37	663	6	0,89	7	5,18	-4,489 **	-1,60904*	0,586	0,44	90,19	7,19	**0,72**
Promachocrinus kerguelensis	Wilson 2007	COII	Echinoderma	BI	1	1	37	623	3	0,26	4	1,58	-3,743***	-1,72176***	0,162	0,13	26,53	2,11	**0,21**
Sterechinus antarcticus	SK	COI	Echinoderma	WS	2	1	3	570	4	4,68	3	10	-0,341 NS	NA	2,667	2,34	477,44	380,40	**38,04**
Sterechinus antarcticus	SK 2021	COI	Echinoderma	LB	2	1	4	570	5	4,68	3	8,33	0,556 NS	-0,21249 NS	1,117	0,98	199,96	159,32	**15,93**
Sterechinus antarcticus	SK 2020	COI	Echinoderma	EI	2	1	5	570	3	3,16	2	6	2,429 NS	1,57274 NS	1,025	0,90	183,49	146,20	**14,62**
Sterechinus antarcticus	SK	COI	Echinoderma	All anta	123	1	31	570	9	2,99	9	7,03	-2,752NS	-0,75256 NS	0,772	0,68	138,20	110,11	**11,01**
Sterechinus antarcticus	SK	COII	Echinoderma	Rothera	1	1	3	570	1	1,17	NA	NA	0,201 NS	NA	0,667	0,59	119,41	95,14	**9,51**
Sterechinus antarcticus	SK	COII	Echinoderma	Maguerite Bay	1	1	12	570	6	2,48	4	4,55	0,121 NS	-1,10898 NS	NA	NA	NA	NA	**NA**
Trematomus eulepidotus	Kašparová et al, 2015	cytb	Osteichthyes	ANT, SO	1	1	35	399	20	7,56	21	8,64	- 15, 249***	- 2,29808***	1,174	1,47	300,24	300,24	**30,02**
Trematomus newnesi	Janko et al,, 2007	cytb	Osteichthyes	SS, CH,TA	31	1	36	483	15	6,87	13	9,065	-7,81*	-0,83 NS	2,15	2,23	454,22	454,22	**45,42**
Pareledone turqueti	2012 Strugnel	COI	Mollusca	SR	1	2	48	654	5	1,2	8	6,13	- 4,06 *	NA	0,912	0,70	142,30	183,00	**18,30**
Abatus agassizii	Díaz et al., 2012	COII	Echinoderma	SSh	1	2	27	799	4	296	5	2,8	-3,024 ***	-1.888***	NA	NA	NA	10,00	1,00
Doris kerguelensis clade 24	Wilson et al,, 2009)	COI	Mollusca	BS	1	2	19	627	14	3,81	11	8,89	-5,377**	-1,66524**	1,84	1,47	299,45	58,69	**5,87**
Doris kerguelensis clade 29	Wilson et al,, 2009)	COI	Mollusca	BS	1	2	34	627	24	4,92	19	8,79	-10,972***	-1,64303**	1,858	1,48	302,38	59,27	**5,93**
Doris kerguelensis clade 29_sub_1	Wilson et al,, 2009)	COI	Mollusca	BS	1	2	8	627	8	3,96	6	9,29	-0,97349NS	-2,086NS	2,464	1,96	401,00	78,60	**7,86**
Doris kerguelensis clade 29_sub_2	Wilson et al,, 2009)	COI	Mollusca	BS	1	2	26	627	18	2,96	14	8,25	-10,069***	-2,15124***	1,421	1,13	231,26	45,33	**4,53**
Gobionotothen giberifrons	Kašparová et al,, 2015	cytb	Osteichthyes	ANT, SSH, EI	1	2	23	640	17	3,05	15	8,58	-12,746***	- 2,085 **	1,953	1,53	311,38	311,38	**31,14**
Gobionotothen giberifrons	Kašparová et al, 2015	cytb	Osteichthyes	ANT	1	2	23	640	17	3,05	15	8,58	- 12,746**	- 2,08520***	1,953	1,53	311,38	311,38	**31,14**
Gymnodraco acuticeps	Kašparová et al,, 2015	cytb	Osteichthyes	SS, JI, EI	1	2	29	732	6	0,66	6	3,2	-4,152*	-1,97778***	0,12	0,08	16,73	16,73	**1,67**
Chaenocephalus aceratus	Damerau, 2012	D loop	Osteichthyes	ANT	1	2	36	355	3	1,31	3	3,98	0,04 NS	-0,79487 NS	0,467	0,66	134,23	44,14	**4,41**
Chaenodraco wilsoni	SK	cytb	Osteichthyes	ANT	1	2	20	398	3	1	4	3,63	-2,135*	- 1,44071 *	0,389	0,49	99,73	99,73	**9,97**
Champsocephallus gunari	Damerau, 2012	D loop	Osteichthyes	EI, ANT,SOI, SSH	1	2	64	337	8	1,59	9	4,3	-7,062 ***	-1,78118 **	0,537	0,80	162,60	25,70	**2,57**
Chionodraco hamatus	Patarnello, 2003	D-loop	Osteichthyes	RS	3	2	9	248	6	6,05	6	8,33	-2,978**	-1,39844*	1,5	3,02	617,18	97,55	**9,76**
Chionodraco hamatus	Patarnello, 2003	D-loop	Osteichthyes	RS	3	2	29	249	10	5,86	11	8,28	-6,286***	-1,37614*	1,448	2,91	593,39	93,79	**9,38**
Chionodraco hamatus	Patenello, 2003	D-loop	Osteichthyes	WS	2	2	14	249	5	4,6	5	5,93	-1,322 NS	-0,9698 NS	0,73	1,47	299,16	47,29	**4,73**
Chionodraco myersi	Patarnello, 2003	D-loop	Osteichthyes	RS	3	2	51	249	5	3,12	6	6,05	- 1,875 NS	-0,33788NS	0,756	1,52	309,81	48,97	**4,90**
Chionodraco rastrospinosus	Patenello, 2003	D-loop	Osteichthyes	EI, SSh, SO	1	2	63	249	6	3,73	7	6,76	-1,957NS	-0,6604 NS	0,928	NA	NA	60,11	**6,01**
Chionodraco rastrospinosus	Damerau, 2012	D-loop	Osteichthyes	ANT,SO	1	2	30	323	5	3,64	8	7,7	-3,274**	-0,18393 NS	1,1777	1,82	372,05	58,81	**5,88**
Chorismus antarcticus	Raupach 2010	COI	Crustacea	SSh, ANT, EWS, ERS	123	2	179	657	23	2,09	23	6,39	-16,996***	: -1,86620**	1,07	0,81	166,19	40,72	**4,07**
Lepidonotothen larseni	Damerau, 2014	cytb	Osteichthyes	SO, SG,BI	1	2	79	647	NA	3	31	7,78	-48,969***	-2,12603**	3,178	2,46	501,21	30,70	**3,07**
Lepidonotothen nudifrons	Kašparová et al,, 2015	cytb	Osteichthyes	ANT	1	2	40	463	11	2,01	11	6,33	-8,233***	- 1,97088 **	0,906	0,98	199,67	199,67	**19,97**
Lepidonotothen squamifrons	Damerau, 2012	cytb	Osteichthyes	ANT	1	2	26	708	9	1,28	9	6,25	-6,335***	-1,98099**	0,905	0,64	130,43	130,43	**13,04**
Margarella antarctica	González-Wevar et al,	cytb	Mollusca		1	2	90	396	5	0,5	6	1,9	- 6.203**	- 1.73	NA	NA	NA	NA	**NA**
Nacella concinna	González-Wevar et al,	COI	Mollusca	ANT	1	2	160	663	18	1,28	16	6,3	- 12, 38***	- 1,77*	0,975	0,74	150,06	73,53	**7,35**
Notothenia rossi	Kašparová et al, 2015	cytb	Osteichthyes	EI, SSH	1	2	30	723	10	1,26	11	6,8	-9,332***	- 2,02488**	0,91	0,63	128,43	128,43	**12,84**
Pagetopsis macropterus	Kašparová et al,, 2015	cytb	Osteichthyes	ANT	1	2	**25**	687	6	0,91	7	4,87	-4,992***	- 1,82397**	0,62	0,45	92,09	92,09	**9,21**
Sterechinu neumayeri	SK	COI	Echinoderma	Neumayer III	3	2	11	496	4	1,47	5	6,18	-2,908 *	-1,71166*	0,727	0,73	149,56	119,16	**11,92**
Sterechinu neumayeri	SK/Diaz	COI	Echinoderma	Rothera	1	2	34	496	7	0,94	7	3,73	-5,637***	-2,08681***	0,467	0,47	96,07	76,55	**7,65**
Sterechinu neumayeri	Díaz et al., 2018	COI	Echinoderma	WS	2	2	19	931	2	0,72	3	6	-0,858*	-1,13197 NS	0,667	0,36	73,11	58,25	**5,82**
Sterechinu neumayeri	SK	COI	Echinoderma	Vernadsky	1	2	15	496	2	0,54	3	2,57	-1,49051**	-1,49051**	0,267	0,27	54,93	43,76	**4,38**
Sterechinu neumayeri	SK/Diaz	COI	Echinoderma	Neumayer III, TA	3	2	26	496	10	1,55	8	4,74	-5,538***	-2,31196***	0,241	0,24	49,58	39,50	**3,95**
Sterechinu neumayeri	SK	COI	Echinoderma	LA	2	2	9	496	1	0,45	2	2,22	-0,263 NS	-1,08823 NS	0,222	0,22	45,67	36,39	**3,64**
Sterechinu neumayeri	Díaz et al., 2018	COI	Echinoderma	AL	3	2	15	931	8	1,15	5	4,76	-2.769**	-2.086**	0,206	0,11	22,58	17,99	**1,80**
Sterechinu neumayeri	Díaz et al., 2018	COI	Echinoderma	PB	1	2	10	931	1	0,21	2	2	-0,339 NS	-1,11173 NS	0,2	0,11	21,92	17,47	**1,75**
Sterechinu neumayeri	Díaz et al., 2018	COI	Echinoderma	James Ross Island	2	2	14	931	1	0,15	2	1,43	-0,879 NS	-1,15524NS	0,143	0,08	15,67	12,49	**1,25**
Sterechinu neumayeri	Díaz et al., 2018	COI	Echinoderma	FB	1	2	58	931	1	0,04	2	0,34	-1,761**	-1,08751***	0,034	0,02	3,73	2,97	**0,30**
Trematomus bernacchi	Janko et al, 2007	cytb	Osteichthyes	SS, CR, TA, CH	13	2	61	468	9	1,29	10	4,82	- 7,7**	- 1,8*	0,631	0,67	137,58	137,58	**13,76**
Trematomus hansoni	Janko et al,, 2007	cytb	Osteichthyes	CH, SG, ANT, TA, SO, CS, CR	13	2	76	378	20	4,21	16	8,9	-8,681**	- 1,83377**	0,89	1,18	240,25	240,25	**24,03**
Trematomus nicolai	Kašparová et al,, 2015	cytb	Osteichthyes	CH, ANT, SO,SG	13	2	34	357	2	0,36	3	1,16	-2,516*	- 1,4996*	0,118	0,17	33,73	33,73	**3,37**
Trematomus penneli (Janko, 2007)	Janko et al,, 2007	cytb	Osteichthyes	RS, TA	3	2	49	500	2	0,25	3	1,321	- 2,29**	-1,31 NS	0,121	0,12	24,69	24,69	**2,47**

Table 2 : Demographic compatative tests of all species and clades

Life: Lifestyle: 1— eurybathic and/or pelagic**,** 2— shallow-benthic**, Locality symbols denote**: WS— Weddell Sea, EWS—East Weddell Sea, BS—Bransfield Strait, SR—Shag Rock, RS—Ross Sea, TA— Terre Adélie, CH— Cape Hallett, SO—South Orkney, SG—South Georgia, EI—Elephant Island, SS—South Shetlands, SSd—South Sandwich, JI—Joinville Island, CR—Cape Roberts, TNB—Terra Nova Bay, Rothera—Rothera research station, CS—Casey, CA—Cape Armitage, BI— Bouvetøya Island, ANT—Antarctic penninsula, All—all sampled localities, DP— Drake Passage, WANT—West Antarctic, SAI—Sub Antarctic Islands, **Data source**: source publications the genetic diversity and population indexes or locus sequences were gained, **N**: number of sequnces, **L**: lengh of the mitochondrial fragment, **S**: number of segregating sites, **π**: nucletode diversity, **locus**: amplified locus, **h**: number of haplotypes, **BP:** Biogeographic province **Life:** Lifestyle, **Hd**: haplotype diversity, **τ**: estimate of τ, **t**: time of expansion in mutational units, **tO**: t (exp)[Kya; Osteichthyes mutational rate]: time of expansion in Kya with used Osteichthyes mutational time,**t P:** t (exp)[Kya; published mutational rate] time of expansion in Kya with used published mutational time, **T P/10:** t (exp)[Kya; published mutational rate]/10: expansion time after 10 fold correction, significance of population dynamic statistics: * P< 0,05, **P< 0,01, *** P<0,001, NS: not significant

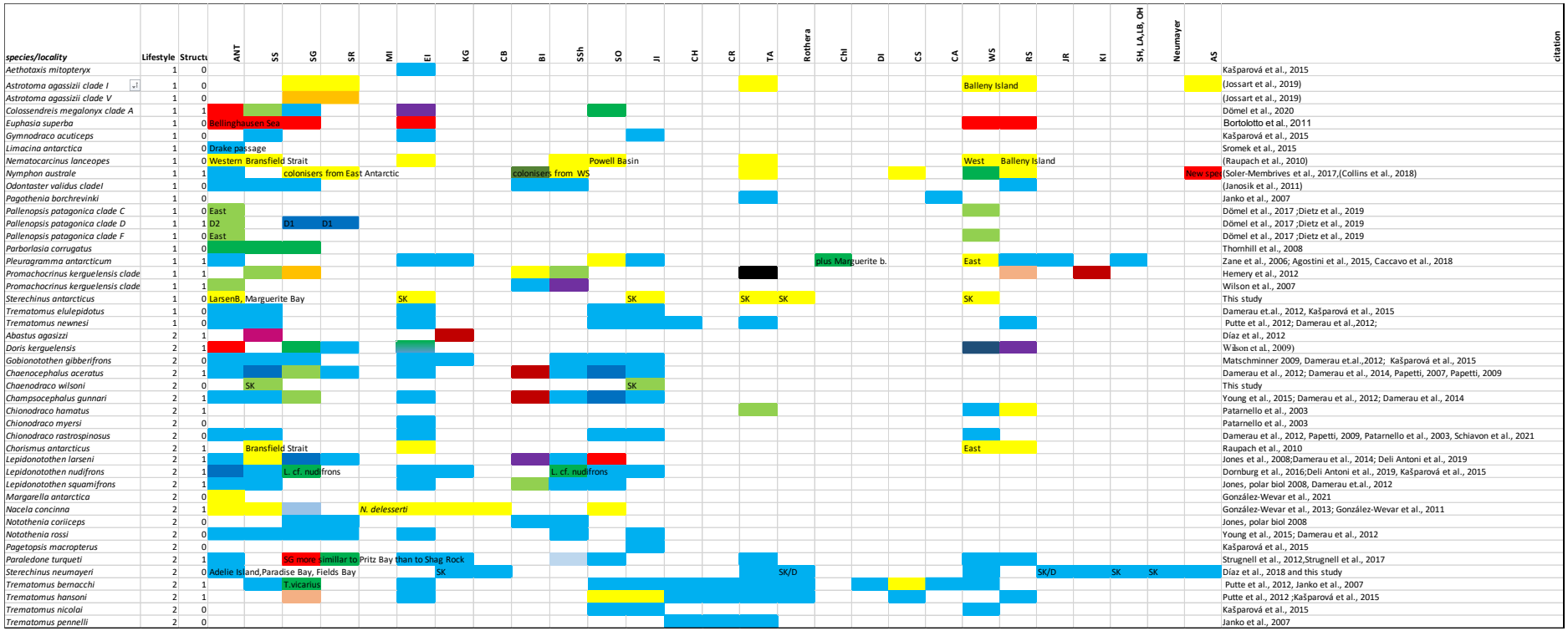


Table 3: Interpopulation gene flow between localitis

depicted by the same colour, different colours suggest form of isolation. Structure: 1: there is structure, 0: So far, no published genetic structuration, MI—Marion Island, WS— Weddell Sea, BS— Bransfield Strait, SR—Shag Rock, RS—Ross Sea, TA— Terre Adélie, CH— Cape Hallett, SO—South Orkney, SG—South Georgia, EI—Elephant Island, SS—South Shetlands, SSd—South Sandwich, JI—Joinville Island, CR—Cape

Roberts, TNB—Terra Nova Bay, Rothera—Rothera research station, CS—Casey, CA—Cape Armitage, BI—Bouvetøya Island, ANT—Antarctic penninsula, WANT—West Antarctic, CB— Covadona Bay, AS—Amundsen Sea, ChI— Charcot Island, DI— Deception Island

Citation: source publication see the references within the reference part A

Species	DS	locus	Locality	Life style	N	L	S	π * 103	h	Hd	Fu's Fs statis	Tajimas D	τ	t *104	tO	t P	t P/10
Sterechinus antarcticus	SK	COI	Elephant Island	1	5	570	3	3,16	2	0,6	2,43 NS	1,57 NS	1,03	8,99	183,49	146,20	**14,62**
Sterechinus antarcticus	SK	COI	Larsen B	1	4	570	5	4,68	3	0,833	0,56 NS	-0,21 NS	1,12	9,80	199,96	159,32	**15,93**
Sterechinus antarcticus	SK	COI	Rothera	1	3	570	1	1,17			0,20 NS	NA	0,67	5,85	119,41	95,14	**9,51**
Sterechinus antarcticus	SK	COI	Marguerite Bay	1	12	570	6	2,48	4	0,455	0,12 NS	-1,11 NS	0,00	0,00	0,00	0,00	**0,00**
Sterechinus antarcticus	SK	COI	Weddell Sea	1	3	570	4	4,68	3	1	-0,34 NS	NA	2,67	23,39	477,44	380,40	**38,04**
Sterechinus antarcticus	**SK**	**COI**	**all anta**	**1**	**31**	**570**	**9**	**2,99**	**9**	**0,703**	**-2,75 NS**	**-0,75 NS**	**0,77**	**6,77**	**138,20**	**110,11**	**11,01**
Sterechinu neumayeri	SK	COI	Neumayer III	2	11	496	4	1,47	5	0,618	-2,91 *	-1,71*	0,73	7,33	149,56	119,16	**11,92**
Sterechinu neumayeri	SK/D	COI	Rothera	2	34	496	7	0,94	7	0,373	-5,64***	-2,09 ***	0,47	4,71	96,07	76,55	**7,65**
Sterechinu neumayeri	SK	COI	Larsen A	2	9	496	1	0,45	2	0,222	-0,26 NS	-1,08 NS	0,22	2,24	45,67	36,39	**3,64**
Sterechinu neumayeri	SK	COI	Vernadsky	2	15	496	2	0,54	3	0,257	-1,49 **	-1,49**	0,27	2,69	54,93	43,76	**4,38**
Sterechinu neumayeri	**SK**	**COI**	**all anta**	**2**	**69**	**496**	**13**	**0,87**	**13**	**0,32**	**-16,71 *** **	**-2,45*** **	**0,08**	**0,80**	**16,25**	**12,95**	**1,29**
Chaenodraco wilsoni	**SK**	**cyt*b***	**South Shatlands**	**2**	**20**	**398**	**3**	**1**	**4**	**0,363**	**-2,14***	**- 1,44 NS**	**0,39**	**4,89**	**99,73**	**45,25**	**4,52**

Table 4: Demographic test of newly sequenced and analysed species

Life: Lifestyle: 1 — eurybathic and/or pelagic, 2 — shallow-benthic, DS: data source SK — this study, D — Díaz et al., 2018, N: number of sequnces, L: lengh of the mitochondrial fragment, S: number of segregating sites, π: nucletode diversity, h: number of haplotypes, Hd: haplotype diversity, τ: estimate of τ, t: time of expansion in mutational units, tO: t (exp)[Kya; Osteichthyes mutational rate]: time of expansion in Kya with used Osteichthyes mutational time,t P: t (exp)[Kya; published mutational rate] time of expansion in Kya with used published mutational time, T P/10: t (exp)[Kya; published mutational rate]/10: expansion time after 10 fold correction, significance of population dynamic statistics: * P< 0,05, **P< 0,01, *** P<0,001, NS: not significant

Species	Source publication	locus	organism	Locality	Life	N	L	S	Pi * 10	Hd	h	Fu's Fs	Tajima's D	τ	t *10^3	tO	t P	t P/10
Euphausia superba	Bortolotto et al., 2011	*ND1*	Crustacea	WS, RS, ANT	1	641	154	45	1,39	0,8561	124	-26,28904**	- 1,79083***	2,5	8,12	1656,51	381,07	**38,11**
Nematocarcinus lanceopes	Raupach et al., 2010	*COI*	Crustacea	SSd, ANT, EWS, ERS	1	184	657	43	0,55	0,892	54	: -32,887***	-1,65762***	2,549	1,94	395,89	96,99	**9,699**
Sterechinus antarcticus	SK	*COI*	Echinodermata	All	1	31	570	9	0,3	0,703	9	-2,752NS	-0,75256 NS	0,772	0,68	138,20	110,11	**11,01**
Astrotoma agassizii clade 1	Jossart 2019	*COI*	Echinodermata	SG, RS, SR, WANT	1	138	602	28	0,15	0,618	34	–31,56***	- 2,4***	0,894	0,74	151,54	21,21	**2,121**
Promachocrinus kerguelensis clade A	Wilson 2007	*COI*	Echinodermata	BS	1	22	623	12	0,51	0,771	7	0,378 NS	-0,14477 NS	1,316	1,06	215,55	30,18	**3,018**
Odonaster validus clade I	Janosik, 2010	*COI*	Echinodermata	ANTP, SAI, RS	1	67	570	38	0,44	0,85	27	-21,506***	-2,33122***	0,93	0,82	166,49	23,31	**2,331**
Aethotaxis mitopteryx	Kašparová et al,, 2015	*cytb*	Osteichthyes	EI	1	33	553	31	0,48	0,869	19	-14,642***	- 2,42273***	1,347	1,22	248,55	148,52	**14,85**
Pagothenia borchgrevinki	Janko et al,, 2007	*cytb*	Osteichthyes	CA,TA	1	38	578	16	0,36	0,8411	20	- 19,04 **	- 1,95**	1,94	1,68	342,49	166,16	**16,62**
Pleuragramma antarcticum	Zane et al., 2006	*D-loop*	Osteichthyes	EWS, KG, EI, TNB	1	256	210		0,84	0,896	110	not available	–2,389**	1,4499	3,45	704,51	111,36	**11,14**
Trematomus eulepidotus	Kašparová et al,, 2015	*cytb*	Osteichthyes	ANT, SO	1	35	399	20	0,76	0,864	21	- 15, 249***	- 2,29808***	1,174	1,47	300,24	179,41	**17,94**
Trematomus newnesi	Janko et al,, 2007	*cytb*	Osteichthyes	SS, CH,TA	1	36	483	15	0,69	0,9065	13	-7,81*	-0,83 NS	2,15	2,23	454,22	300,77	**30,08**
Limacina antarctica	Sromek et al., 2015	*COI*	Mollusca	DP	1	60	559	57	0,61	0,967	47	–26.198***	–2.454***	3,486	3,12	636,34	129,92	**12,99**
Parborlasia corrugatus	Thornhill et al., 2008	*COI*	Nemertea	ANT, SAI	1	73	599	18	0,18	0,691	14	-9,52***	-2,10628**	0,269	0,22	45,82	3433,35	**343,3**
Colossendeis megalonyx clade A	Dietz et al., 2015	*COI*	Pycnogonida	SG	1	16	545	7	0,39	0,808	6	-0,505NS	0,05363NS	1,333	1,22	249,58	106,34	**10,63**
Nymphon australe	Membrives_2017	*COI*	Pycnogonida	SSd, SG	1	13	554	5	0,28	0,769	6	- 0,779 NS	- 1,874 NS	1,312	1,18	241,66	102,97	**10,3**
Pallenopsis patagonica clade D	Domel, 2017	*COI*	Pycnogonida	Tip of ANT	1	17	426	8	0,33	0,779	8	: -4,021**	-1,4267 NS	1,412	1,66	338,22	144,11	**14,41**
Nacella concinna	González-Wevar et al., 2011	*COI*	Mollusca	ANT	2	160	663	18	0,13	0,63	16	- 12, 38***	- 1,77*	0,975	0,74	150,06	73,53	**7,353**
Chorismus antarcticus	Raupach 2010	*COI*	Crustacea	SSd, ANT, EWS, ERS	2	179	657	23	0,21	0,639	23	: -16,996***	: -1,86620**	1,07	0,81	166,19	40,72	**4,072**
Sterechinu neumayeri	Díaz et al., 2018	*COI*	Echinodermata	All	2	69	496	13	0,09	0,32	13	-16,705***	-2,4534***	0,079	0,08	16,25	12,95	**1,295**
Gobionotothen giberifrons	Kašparová et al,, 2015	*cytb*	Osteichthyes	ANTP SSH, EI	2	23	640	17	0,31	0,858	15	-12,746***	- 2,085 **	1,953	1,53	311,38	160,61	**16,06**
Gymnodraco acuticeps	Kašparová et al,, 2015	*cytb*	Osteichthyes	SS, JI, EI	2	29	732	6	0,07	0,32	6	-4,152*	-1,97778***	0,12	0,08	16,73	10,00	**1**
Chaenocephalus aceratus	Damerau, 2012	*D-loop*	Osteichthyes	ANT	2	36	355	3	0,13	0,398	3	0,04 NS	-0,79487 NS	0,467	0,66	134,23	44,14	**4,414**
Chaenodraco wilsoni	SK	*cytb*	Osteichthyes	ANT	2	20	398	3	0,1	0,363	4	-2,135*	- 1,44071 *	0,389	0,49	99,73	45,25	**4,525**
Champsocephallus gunari	Damerau, 2012	*D-loop*	Osteichthyes	EI, AP, SO, SS	2	64	337	8	0,16	0,43	9	-7,062 ***	-1,78118 **	0,537	0,80	162,60	43,30	**4,33**
Chionodraco hamatus	Patarnello, 2003	*D-loop*	Osteichthyes	RS	2	29	249	10	0,59	0,828	11	-6,286***	-1,37614*	1,448	2,91	593,39	93,79	**9,379**
Chionodraco myersi	Patarnello, 2003	*D-loop*	Osteichthyes	RS	2	51	249	5	0,31	0,605	6	- 1,875 NS	-0,33788NS	0,756	1,52	309,81	48,97	**4,897**
Chionodraco rastrospinosus,	Patenello, 2003	*D-loop*	Osteichthyes	EI	2	63	249	6	0,37	0,676	7	-1,957NS	-0,6604 NS	0,928	1,86	380,30	60,11	**6,011**
Lepidonotothen larseni	Damerau, 2014	*cytb*	Osteichthyes	SO, SG,BI	2	79	647		0,3	0,778	31	-48,969***	-2,12603**	3,178	2,46	501,21	30,70	**3,07**
Lepidonotothen nudifrons	Kašparová et al,, 2015	*cytb*	Osteichthyes	ANT	2	40	463	11	0,2	0,633	11	-8,233***	- 1,97088 **	0,906	0,98	199,67	160,39	**16,04**
Lepidonotothen squamifrons	Damerau, 2012	*cytb*	Osteichthyes	AP	2	26	708	9	0,13	0,625	9	-6,335***	-1,98099**	0,905	0,64	130,43	7,99	**0,799**
Notothenia rossi	Kašparová et al,, 2015	*cytb*	Osteichthyes	EI, SS	2	30	723	10	0,13	0,68	11	-9,332***	- 2,02488**	0,91	0,63	128,43	31,47	**3,147**
Pagetopsis macropterus	Kašparová et al,, 2015	*cytb*	Osteichthyes	ANT	2	**25**	687	6	0,09	0,487	7	-4,992***	- 1,82397**	0,62	0,45	92,09	48,52	**4,852**
Trematomus bernacchi	Janko et al,, 2007	*cytb*	Osteichthyes	SS, CR, TA, CH	2	61	468	9	0,13	0,482	10	- 7,7**	- 1,8*	0,631	0,67	137,58	83,23	**8,323**
Trematomus hansoni	Janko et al,, 2007	*cytb*	Osteichthyes	SAI	2	76	378	20	0,42	0,89	16	-8,681**	- 1,83377**	0,89	1,18	240,25	135,32	**13,53**
Trematomus nicolai	Kašparová et al,, 2015	*cytb*	Osteichthyes	CH, ANT, SO, SG	2	34	357	2	0,04	0,116	3	-2,516*	- 1,4996*	0,118	0,17	33,73	18,36	**1,836**
Trematomus penneli	Janko et al,, 2007	*cytb*	Osteichthyes	RS, TA	2	49	500	2	0,03	0,1321	3	- 2,29**	-1,31 NS	0,121	0,12	24,69	10,61	**1,061**
Pareledone turqueti	2012 Strugnel	*COI*	Mollusca	SR	2	48	654	5	0,12	0,613	8	- 4,06 *		0,912	0,70	142,30	183,00	**18,3**
Doris kerguelensis clade 29	Wilson et al., 2009	*COI*	Mollusca	BS	2	34	627	24	0,49	0,879	19	-10,972***	-1,64303**	1,858	1,48	302,38	59,27	**5,927**

Table 5: Demographic comparative tests:

Life: Lifestyle: 1— eurybathic and/or pelagic**,** 2— shallow-benthic**, Locality symbols denote**: MI— Marion Island, WS— Weddell Sea, EWS— East Weddell Sea, BS— Bransfield Strait, SR—Shag Rock, RS—Ross Sea, TA— Terre Adélie, CH— Cape Hallett, SO—South Orkney, SG—South Georgia, EI—Elephant Island, SS—South Shetlands, SSd—South Sandwich, JI—Joinville Island, CR—Cape Roberts, TNB—Terra Nova Bay, Rothera—Rothera research station, CS—Casey, CA—Cape Armitage, BI— Bouvetøya Island, ANT—Antarctic penninsula, All—all sampled localities, DP— Drake Passage, WANT—West Antarctic, SAI—Sub Antarctic Islands, **Source publication**: source publications the genetic diversity and population indexes or locus sequences were gained, **N**: number of sequnces, **L**: lengh of the mitochondrial fragment, **S**: number of segregating sites, **π**: nucletode diversity, **h**: number of haplotypes, **Hd**: haplotype diversity, **τ**: estimate of τ, **t**: time of expansion in mutational units, **tO**: t (exp)[Kya; Osteichthyes mutational rate]: time of expansion in Kya with used Osteichthyes mutational time,**t P:** t (exp)[Kya; published mutational rate] time of expansion in Kya with used published mutational time, **T P/10:** t (exp)[Kya; published mutational rate]/10: expansion time after 10 fold correction, significance of population dynamic statistics: * P< 0,05, **P< 0,01, *** P<0,001, NS: not significant

Chaenodraco wilsoni

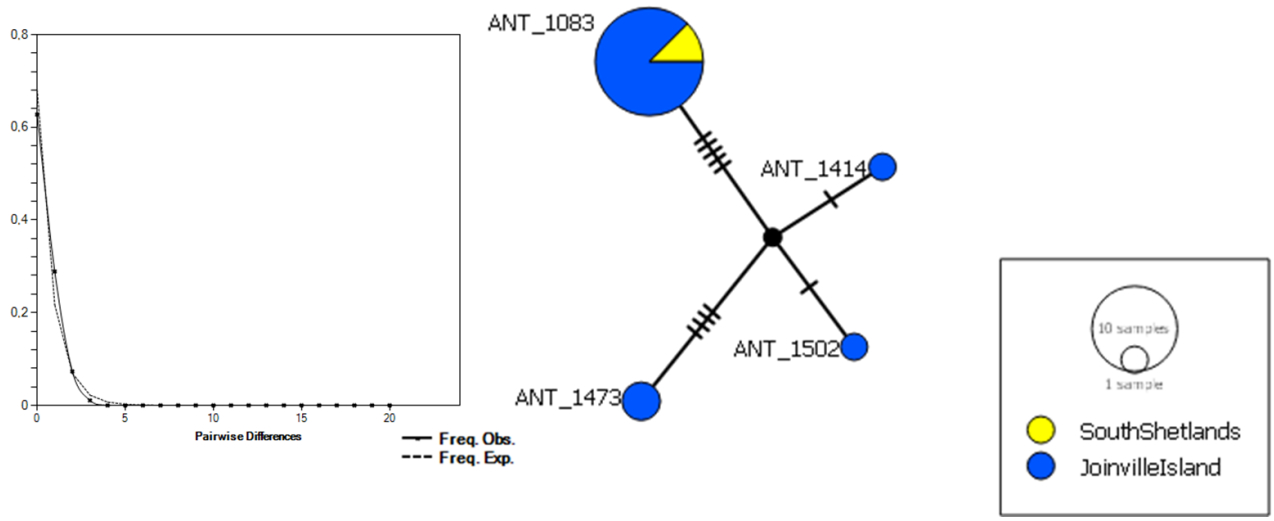


Figure 2 : Mismatch distribution curve of *Chaenodraco wilsoni* and its haplotype network. All hapllotypes are Joinville Island haplotypes

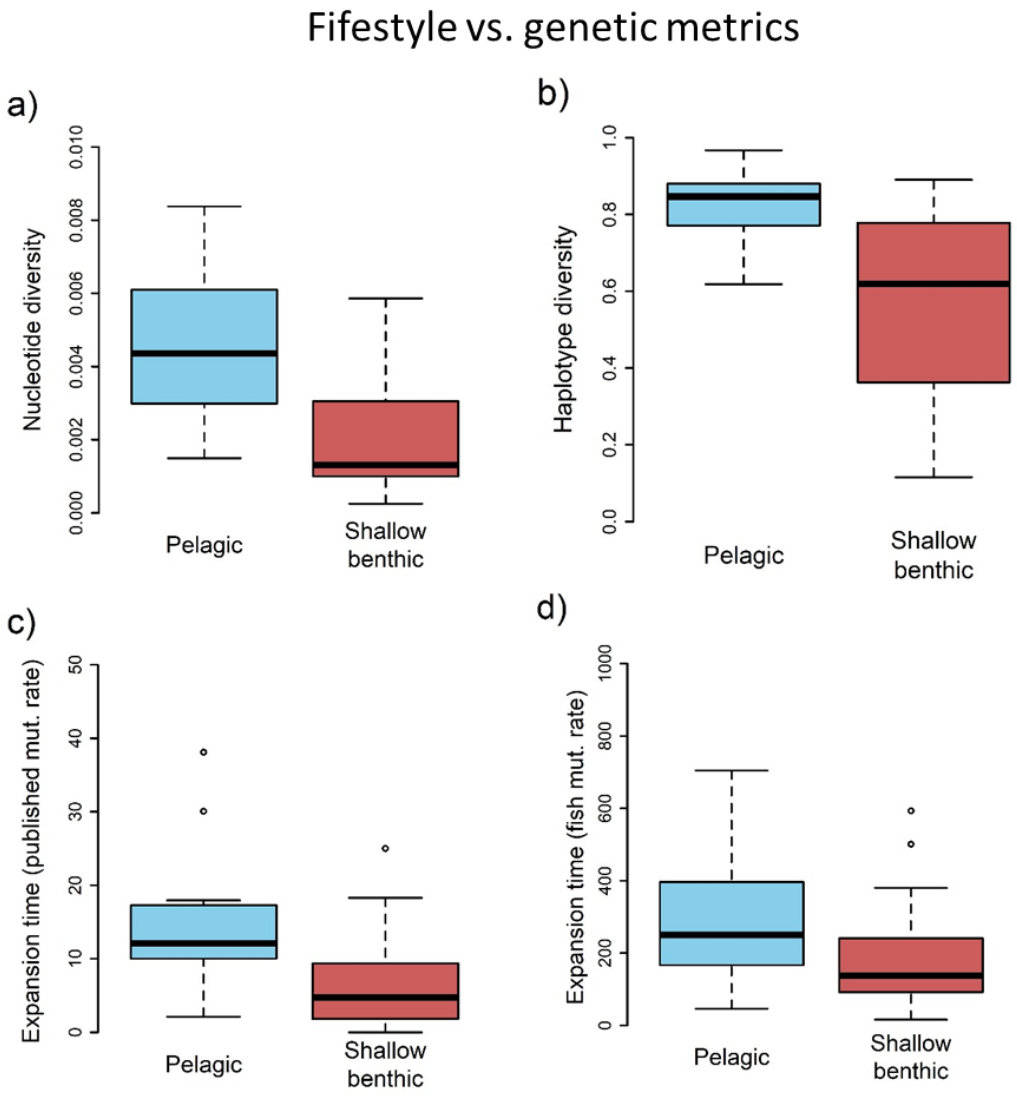


Figure 3: The effect of lifestyle to genetic metrics

All panels compares relation of lifestyle to population genetic metrics (shallow benthic vs. pelagic, where belons as well eurybathic species) a, nucleotide diversity; b, haplotype diversity; c, expansion time counted with published mutation rate; d, the expansion time shown with the „fish“ mutation time published by Janko (2007).

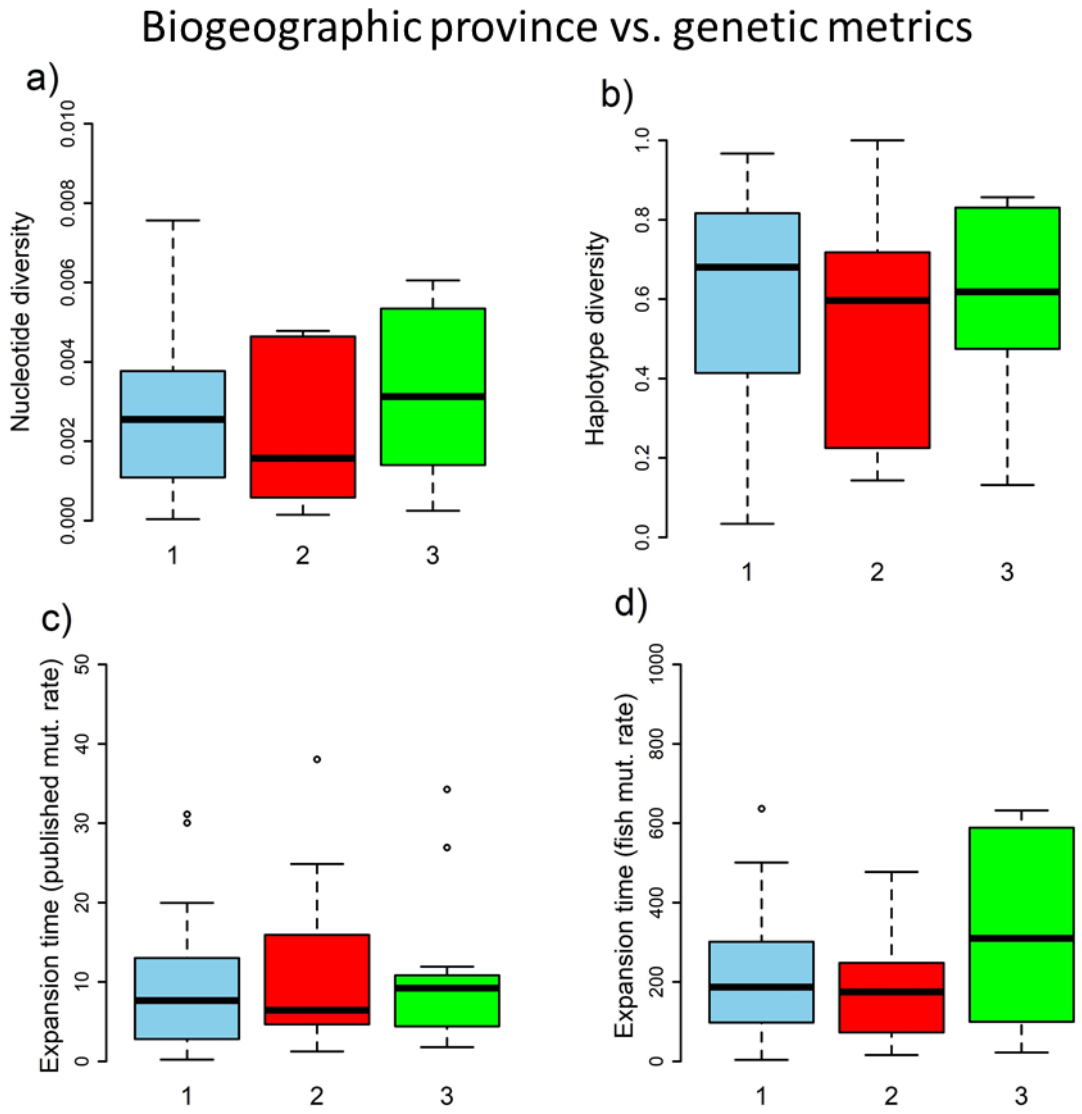


Figure 4: The effect of bioeographic zone (1, 2, 3) to genetic metrics

a, nucleotide fiversity: π; **b,** Haplotype diversity: Hd. Figure panels **c,** and **d,** shows expansion time counted with published mutation rate and the expansion time shown with the „fish" mutation time published by Janko (2007), respectively; in relation to biogeographic province.

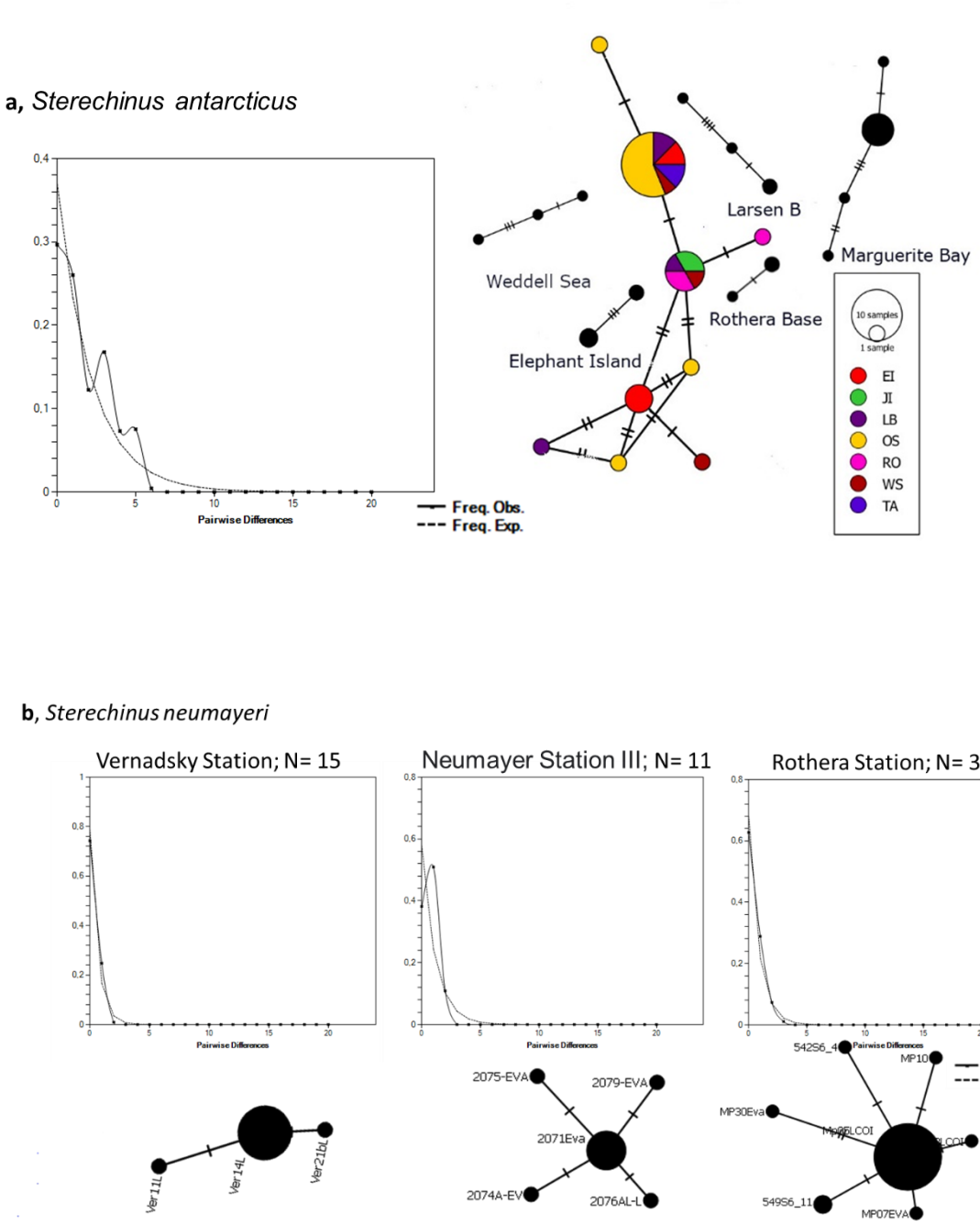


Figure 5:

The Mismatch distribtion of *Sterechinus antarcticus* is depicted in left panel of **a**, section and its median- joining haplotype network are right. Locations of *Sterechinus antarcticus* are depicted by colours and abbreviations: EI: Elephant Island, JI: Joinvile Island, LB: Larsen, OS: Marguerite Bay, RO: Rothera Base, WS: Neumayer Station III, TA: Terre Adéllie. Black

haplotype networks shows the situation in particular locality. **b,** *Sterechinus neumayeri* Vernadsky, Neumayer Station III and Rothera Station sampling localities mismatch distribution is upper panel, and lower panel shows median- joining haplotype networks.

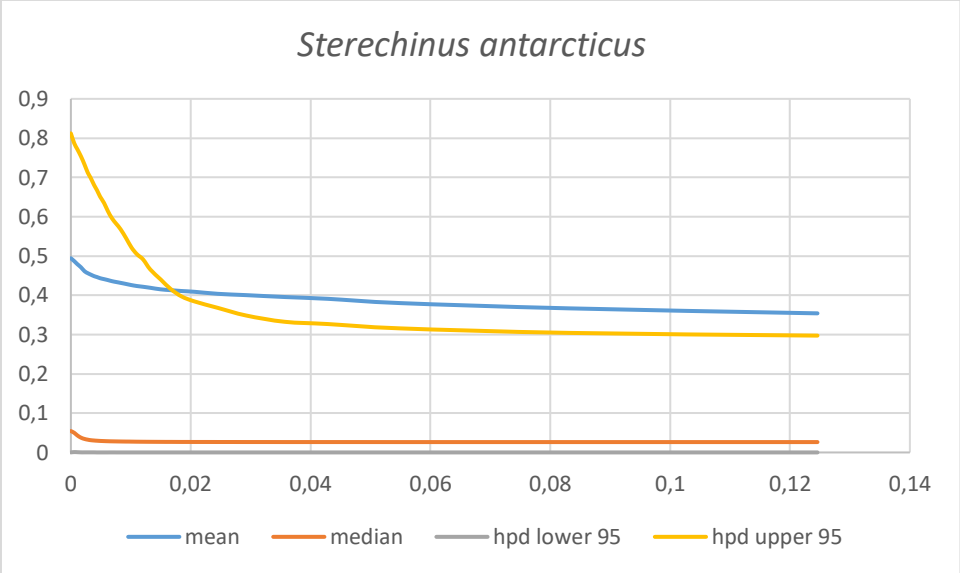


Figure 6:

Demographic trends of effective population size (Ne) constructed by EBSP for Sterechinus antarcticus. The vertical axis shows the estimated log of Neμ and the horizontal axis represents time in Mya; the time is zero at present.

7.3 ***Conclussion:***

Our comparative analysis clearly demonstrated that populations of shallow benthic species, irrespective of their taxonomical belonging, harbour significantly lower genetic diversity in their mitochondrial genes than the group covering eurybathic and pelagic species. We also noted that shallow benthic organisms usually contained a strong signal of recent population expansions, which was not generally present in pelagic and eurybathic species.

Even though the dating of such historical expansion either in mutation rate or in absolute dating may be spurious due to various confounding factors like usage of non-homologous mtDNA regions or species-specific nature of molecular clocks; caused e.g. by loss of polymorphism by purification selection, drift and saturation reducing the long-term substitution rate (Ho *et al.*, 2005; Grant *et al.*, 2012), we might still consider the dating of major population change as a meaningful contribution to general debate.

It is supported by the fact that, when a published species specific mutation rate for particular species was available, the applied dating fitted to the geological events,(e.g. (Damerau *et al.*, 2014; Kašparová *et al.*, 2015)). Our results are thus in accordance with Hoareau´s (2016) premise of the demographic transition method comparing expansion dates with ecological evidence. It follows that the presented comparative study brings a comprehensive and conceivable perspective to use the lifestyle as a determinant of the stress effect, as the LGM certainly was, on species population size.

Furthermore, our data are consistent with the hypothesis that pelagic or eurybathic species had more resources and possibly fewer competitors during the climate transition to major glaciations, such as the LGM, which resulted in their population boom. On the contrary, shallow shelf benthic species underwent considerable bottleneck given that advancing grounded glaciers destroyed their habitats on the shelf and slope, possibly also multiplied by falling debris and stones. They consequently underwent relatively recent population expansions, estimated onset of which roughly coincides with temperature rise after the LGM, which also depend on the locality (Anderson *et al.*, 2002) (Table 2).

It was tempting to find out if the species phylogeography might be correlated with the lifestyle (Table 3); under the inspiration by Van de Putte *et al.* (2012). I would conclude that even if we consider all biological aspects of the particular species, the situation is complex in analysis. By studying the references from Table 3, it is evident that it is necessary to consider molecular markers, sampling area, marine currents and sea topography barriers to explain species-specific gene flow and phylogeography. For illustration, even though *Pleuragramma antarctica* is whole pelagic, EST- microsatellites reviled Joinville Island and West Antarctic Peninsula as a different population to East Antarctic peninsula coast (Agostini et al., 2015, Caccavo et al., 2018).

In particular, besides earlier mentioned hypothesis we also must take into consideration the fact that the grounded ice scoured the shelf along with reduced available habitat and limited species to isolated refugee or polynyas, the ice free primary productivity places. There is an evidence that East Antarctic ice sheet did not reached the shelf in all the coastline. The south-western Ross Sea, Prydz Bay and deeper parts of the Bransfield Basin (between the north-western Antarctic Peninsula and the South Shetland Islands) were likely free of grounded ice (Anderson *et al.*, 2002). In fact, the older populations size change detection in Eastern Antarctic (Table 2, Figure 4) is congruent with the fact that there were more available habitats for marine fauna during the last glaciation. This result can just outline the situation since there are not many species sampled simultaneosly in thouse three biogeographic regions. But, if we compare the available benthic species which have good sampling vs. pelagic and eurybathic (deep sea) we can see similar pattern.

Representative sampling was published for deep sea Sea spider*, Nymphon australe*, data of which suggested refugia in Eastern Antarctic and Antarctic Peninsula with more ancient expansion compared to localities where species underwent either bottleneck and/or colonisation dated earlier by subsequent expansion (Weddell Sea and Antarctic Islands) (Soler-Membrives *et al.*, 2017). Other eurybathic intervebrate, *Sterechinus antarcticus*, has the oldest population expansion in Weddell Sea, but unfortunatelly we have no data from the East Antarctic (This study). The shallow benthic *Sterechinus neumayeri* has the oldest population expansion in the most Eastern locality, the Neumayer Station III (Table 2).

On the other hand, numerous refuges either from paleontological monitoring (Anderson *et al.*, 2002) or from population genetic history (Lau *et al.*, 2020) are deduced. It is evidence of primary production though the LGM in both the Ross Sea (Brambati *et al*. 2002) and Amundsen Sea (Thatje *et al.*, 2008; Clarke and Crame, 2010) or Antarctic penninsula region (Wilson *et al.*, 2007; Dietz *et al.*, 2015b; Dömel *et al.*, 2017; Soler-Membrives *et al.*, 2017).

To conclude, recent advances in our understanding of hydrography and physical-biological interactions provide a contemporary life-history framework for assessing the genetic structure and population history. Our data also demonstrate that the species lifestyle brings the common pattern explaining species' demographic reaction to past climatic changes and physical stress associated with glaciation periods.

7.4 ***References belonging to the table 3:***

Agostini, C. *et al.* (2015) 'Genetic differentiation in the ice-dependent fish Pleuragramma antarctica along the Antarctic Peninsula', *Journal of Biogeography*, 42(6), pp. 1103–1113. doi:10.1111/jbi.12497.

Batta-Lona, P.G. *et al.* (2011) 'Population genetic variation of the Southern Ocean krill, Euphausia superba, in the Western Antarctic Peninsula region based on mitochondrial single nucleotide polymorphisms (SNPs)', *Deep Sea Research Part II: Topical Studies in Oceanography*, 58(13), pp. 1652–1661. doi:10.1016/j.dsr2.2010.11.017.

Caccavo, J.A. *et al.* (2018) 'Along-shelf connectivity and circumpolar gene flow in Antarctic silverfish (Pleuragramma antarctica)', *Scientific Reports*, 8(1), p. 17856. doi:10.1038/s41598-018-36030-x.

Damerau, M. *et al.* (2012) 'Comparative population genetics of seven notothenioid fish species reveals high levels of gene flow along ocean currents in the southern Scotia Arc, Antarctica', *Polar biology*, 45(7), pp. 1073–1086. doi:10.1007/s00300-012-1155-x.

Damerau, M. *et al.* (2014) 'Population divergences despite long pelagic larval stages: lessons from crocodile icefishes (Channichthyidae)', *Molecular Ecology*, 23(2), pp. 284–299. doi:10.1111/mec.12612.

Deli Antoni, M.Y. *et al.* (2019) 'Comparative populational study of Lepidonotothen larseni and L. nudifrons (Teleostei: Nototheniidae) from the Antarctic Peninsula and the South Shetland Islands, Antarctica', *Polar Biology*, 42(8), pp. 1537–1547. doi:10.1007/s00300-019-02540-1.

Díaz, A. *et al.* (2012) 'Restricted geographic distribution and low genetic diversity of the brooding sea urchin Abatus agassizii (Spatangoidea: Schizasteridae) in the South Shetland Islands: A bridgehead population before the spread to the northern Antarctic Peninsula?', *Revista chilena de historia natural*, 85, pp. 457–468.

Díaz, A. *et al.* (2018) 'Genetic structure and demographic inference of the regular sea urchin Sterechinus neumayeri (Meissner, 1900) in the Southern Ocean: The role of the last glaciation', *PLOS ONE*, 13(6), p. e0197611. doi:10.1371/journal.pone.0197611.

Dietz, L. *et al.* (2019) 'Phylogenomics of the longitarsal Colossendeidae: The evolutionary history of an Antarctic sea spider radiation', *Molecular Phylogenetics and Evolution*, 136, pp. 206–214. doi:10.1016/j.ympev.2019.04.017.

Dömel, J.S. *et al.* (2017) 'Nuclear and Mitochondrial Gene Data Support Recent Radiation within the Sea Spider Species Complex Pallenopsis patagonica', *Frontiers in Ecology and Evolution*, 4. doi:10.3389/fevo.2016.00139.

Dömel, J.S. *et al.* (2020) 'Analyzing drivers of speciation in the Southern Ocean using the sea spider species complex Colossendeis megalonyx as a test case', *Polar Biology*, 43(4), pp. 319–342. doi:10.1007/s00300-020-02636-z.

Dornburg, A. *et al.* (2016) 'Cryptic species diversity in sub-Antarctic islands: A case study of Lepidonotothen', *Molecular Phylogenetics and Evolution*, 104, pp. 32–43. doi:10.1016/j.ympev.2016.07.013.

González-Wevar, C.A. *et al.* (2021) 'Contrasting biogeographical patterns in Margarella (Gastropoda: Calliostomatidae: Margarellinae) across the Antarctic Polar Front', *Molecular Phylogenetics and Evolution*, 156, p. 107039. doi:10.1016/j.ympev.2020.107039.

González-Wevar, C.A., David, B. and Poulin, E. (2011) 'Phylogeography and demographic inference in Nacella (Patinigera) concinna (Strebel, 1908) in the western Antarctic Peninsula', *Deep Sea Research Part II: Topical Studies in Oceanography*, 58(1), pp. 220–229. doi:10.1016/j.dsr2.2010.05.026.

Hoffman, J.I. *et al.* (2011) 'Effects of brooding and broadcasting reproductive modes on the population genetic structure of two Antarctic gastropod molluscs', *Marine Biology*, 158(2), pp. 287–296. doi:10.1007/s00227-010-1558-6.

Janko, K. *et al.* (2007) 'Did glacial advances during the Pleistocene influence differently the demographic histories of benthic and pelagic Antarctic shelf fishes? – Inferences from intraspecific mitochondrial and nuclear DNA sequence diversity', *BMC Evolutionary Biology*, 7(1), p. 220. doi:10.1186/1471-2148-7-220.

Janosik, A.M., Mahon, A.R. and Halanych, K.M. (2011) 'Evolutionary history of Southern Ocean Odontaster sea star species (Odontasteridae; Asteroidea)', *Polar Biology*, 34(4), pp. 575–586. doi:10.1007/s00300-010-0916-7.

Jones, C.D. *et al.* (2008) 'Diversity, relative abundance, new locality records and population structure of Antarctic demersal fishes from the northern Scotia Arc islands and Bouvetøya', *Polar Biology*, 31(12), pp. 1481–1497. doi:10.1007/s00300-008-0489-x.

Jossart, Q., Sands, C.J. and Sewell, M.A. (2019) 'Dwarf brooder versus giant broadcaster: combining genetic and reproductive data to unravel cryptic diversity in an Antarctic brittle star', *Heredity*, 123(5), pp. 622–633. doi:10.1038/s41437-019-0228-9.

Kašparová, E. *et al.* (2015) 'Lifestyle and Ice: The Relationship between Ecological Specialization and Response to Pleistocene Climate Change', *PLOS ONE*, 10(11), p. e0138766. doi:10.1371/journal.pone.0138766.

Lau, S.C.Y. *et al.* (2020) 'Detecting glacial refugia in the Southern Ocean', *Ecography*, 43(11), pp. 1639–1656. doi:https://doi.org/10.1111/ecog.04951.

Papetti, C. *et al.* (2007) 'Microsatellite analysis reveals genetic differentiation between year-classes in the icefish Chaenocephalus aceratus at South Shetlands and Elephant Island', *Polar Biology*, 30(12), p. 1605. doi:10.1007/s00300-007-0325-8.

Papetti, C. *et al.* (2012) 'Population genetic structure and gene flow patterns between populations of the Antarctic icefish Chionodraco rastrospinosus', *Journal of Biogeography*, 39(7), pp. 1361–1372. doi:10.1111/j.1365-2699.2011.02682.x.

Patarnello, T. *et al.* (2003) 'Phylogeography of the Chionodraco genus (Perciformes, Channichthydae) in the Southern Ocean', *Molecular Phylogenetics and Evolution*, 28(3), pp. 420–429. doi:10.1016/S1055-7903(03)00124-6.

Raupach, M.J. *et al.* (2010) 'Genetic homogeneity and circum-Antarctic distribution of two benthic shrimp species in the Southern Ocean, Chorismus antarcticus and Nematocarcinus lanceopes', *Marine Biology*, 157(8), pp. 1783–1797. doi:10.1007/s00227-010-1451-3.

Schiavon, L. *et al.* (2021) 'Species distribution, hybridization and connectivity in the genus Chionodraco: Unveiling unknown icefish diversity in antarctica', *Diversity and Distributions*, 27(5), pp. 766–783. doi:https://doi.org/10.1111/ddi.13249.

Soler-Membrives, A. *et al.* (2017) 'Genetic signature of Last Glacial Maximum regional refugia in a circum-Antarctic sea spider', *Royal Society Open Science*, 4(10). doi:10.1098/rsos.170615.

Sromek, L., Lasota, R. and Wolowicz, M. (2015) 'Impact of glaciations on genetic diversity of pelagic mollusks: Antarctic Limacina antarctica and Arctic Limacina helicina', *Marine Ecology Progress Series*, 525, pp. 143–152. doi:10.3354/meps11237.

Strugnell, J.M. *et al.* (2012) 'Persistent genetic signatures of historic climatic events in an Antarctic octopus', *Molecular Ecology*, 21(11), pp. 2775–2787. doi:10.1111/j.1365-294X.2012.05572.x.

Thornhill, D.J. *et al.* (2008) 'Open-ocean barriers to dispersal: a test case with the Antarctic Polar Front and the ribbon worm Parborlasia corrugatus (Nemertea: Lineidae)', *Molecular Ecology*, 17(23), pp. 5104–5117. doi:10.1111/j.1365-294X.2008.03970.x.

Van de Putte, A.P. *et al.* (2012) 'Comparative phylogeography of three trematomid fishes reveals contrasting genetic structure patterns in benthic and pelagic species', *Marine Genomics*, 8, pp. 23–34. doi:10.1016/j.margen.2012.05.002.

Wilson, N.G., Schrödl, M. and Halanych, K.M. (2009) 'Ocean barriers and glaciation: evidence for explosive radiation of mitochondrial lineages in the Antarctic sea slug Doris kerguelenensis (Mollusca, Nudibranchia)', *Molecular Ecology*, 18(5), pp. 965–984. doi:10.1111/j.1365-294X.2008.04071.x.

Young, E.F. *et al.* (2015) 'Oceanography and life history predict contrasting genetic population structure in two Antarctic fish species', *Evolutionary Applications*, 8(5), pp. 486–509. doi:10.1111/eva.12259.

Zane, L. et al. (2006) 'Demographic history and population structure of the Antarctic silverfish Pleuragramma antarcticum', Molecular Ecology, 15(14), pp. 4499–4511. doi:10.1111/j.1365-294X.2006.03105.

8. Summary and discussion

Even though there are still detectable marks of“ ancient” mild climate supporting flourishing life (Mörs, Reguero and Vasilyan, 2020) in the Antarctic, the Eocene climate transitions and consequent Circum-Antarctic current formation have trapped the Antarctic at its current southern location isolated by wind and ocean currents from the rest of the world. The isolation of the Antarctic continent and the Antarctic marine realm both by sharp temperature drop in the ocean and currents, then the remoteness from the other continents permitted to evolve amazing unique flora and fauna.

8.1 *The terrestrial Antarctic*

“Even though at first glance, the Polar Regions seems to be intuitively poorer than tropic regions, but on analysis, the obviousness tends to disappear. If we can have one or two species of large family adapted to the rigors of Arctic existence, why cannot we have more?” (Hutchinson, 1959). Those paraphrased words of the president of Zoology in Yale, the“ father of modern ecology” even throw speaking about the Northern polar region, demonstrate the situation coming obvious by the use of the next millennium technics; the nature of adaptation and the relationship between evolution and diversity (Clarke and Crame, 2010).

The Antarctic is no more considered the depauperate wasteland**.** A lot of cryptic species and specialised organisms have developed with their intrinsic mechanisms to protect against or avoid freezing (Convey *et al.*, 2008) **(*Publication V, IV).***

Just as the Notothenioids dominate and are endemic for the Antarctic marine realm (Near *et al.*, 2012), the Rotifers dominate the Antarctic terrestrial microorganisms.

The wind is driving abiotic force not only in the water realm but also determines the ice and snow accumulation in the terrestrial realm. Rotifers have the possibility to travel around all the continent. Species evolved special structure, and we see the correlation between genetic and geographic distance (Fontaneto, Iakovenko and De Smet, 2015; Kagoshima *et al.*, 2019; McGaughran *et al.*, 2019) and ***Publication V, VI.*** We may thus postulate that it is wind and

possibly also birds that mediate the transport (personal communication with Diego Fontaneto).

Yet everything, it is admittedly hard to be settled due to katabatic winds, and moreover, propagules has to be transported to the“ islands” of ice free terrestrial habitats (Hawes *et al.*, 2007). Such species thus possess patchy distribution, hundreds of km from each other (Kagoshima *et al.*, 2019; McGaughran *et al.*, 2019) **(*Publication V)***, resembling islands in the ice which are isolated by frozen water. The patchiness is confirmed by long diversification times of micro-arthropods, living just on Victoria Land, separated by Drygalski Ice Tongue in multi-million years (Collins *et al.*, 2019). The patchineess is caused by intraregional isolation and differentiation in abundancy and diversity due to food availability, fluid water availability, substratum or sediment structure hardness, light, iceberg scour, oceanographic fronts, depth, temperature, isolation, geomorphology, seasonality, and gyres; mostly explained in more details earlier (Eastman, 1993; Smykla *et al.*, 2018).

It follows that despite the micro-invertebrates are considered to be cosmopolitan, they have a higher endemicity rate in the Antarctic than is in any other continent ***(Publication V, VI).***

Indeed, the ultimate appreciation of the phenomenon is complicated by the fact that to date, many studies considered endemism on genus level, or even higher, while it is the species level, which is mostly relevant, as we show in ***Publication VI.*** *W*e asked as well if there is any phylogenetical and ecological difference in Antarctic vs. non- Antarctic species. If we speculate on potential mechanisms driving the evolution pattern we can conclude that, it is Bdelloids that confirm scenario of limno-terestrial high Antarctic species persistence. Bdelloids, who survived the selective extinction in the Antarctica are better adapted to extreme freezing conditions by desiccation and freezing tolerance. Other adaptation could be the viviparousness of *Philodina gregaria* and *Adineta grandis* or asexuality. Contrary, Aquatic Rotaria are almost all freezing intolerant thus have their southernmost existence on Subantarctic Islands (Garlasché *et al.*, 2020). *Adinetas emslei* and *Adineta grandis* could speciate in Antarctica.

In line with the fact that rotifers have the centrum of distribution in the mild climate, being scarce in tropics and common in Polar Regions, the age of their existence differ over the

continents as well. Firstly, the insight of long term even ancient Antarctic Bdelloids in situ existence has been supported by the high endemicity rate **(Publication V)** and relatedness to the dating of Antarctic invertebrates (Convey *et al.*, 2008). In deeper insight, we discovered younger and less diverse genera in the Antarctic continent compared to the others continents. The Antarctic Bdelloids have shallower genetic diversity, younger evolution time spent in the Antarctic continent contrary to older most recent common ancestors of non-Antarctic species ***(Publication VI).***

8.2 *The marine Antarctic*

The Southern ocean evolution is admittedlly driven by the cold and cyclic glaciations. The most recent and the most serious glaciation was the last glacial maximum approximately 20 Kya ago. Wayne Sousa defined disturbance as" a discrete, punctuated killing, displacement, or damaging of one or more individuals (or colonies) that despite massive disturbance levels and slow recolonization potential, directly or indirectly creates an opportunity for new individuals (or colonies) to become established" (Sousa 1985, p. 356) as highlighted Barnes and Conlan (Barnes and Conlan, 2007).

The most common and endemic species in the Antarctic marine shelf are Notothenioid fish. in James Ross Island (***Publication II***).

Antarctic marine phylogeography is being studied besides invertebrates (Hemery *et al.*, 2012; Strugnell *et al.*, 2012; Dömel, Convey and Leese, 2015; Dömel *et al.*, 2017; Jossart, Sands and Sewell, 2019) in terms of closely related species in fish (Damerau *et al.*, 2012) ***(Publication I, IV).*** There is a lively debate on where larval dispersal contributes to population structure and how is it influenced by the length of the pelagic period (***Publication I***) or adult stage (Damerau *et al.*, 2012, 2014; Deli Antoni *et al.*, 2019). Similarly, the brooding vs. broadcasting effect on the genetic structure has been studied eg. in limpets (Hoffman *et al.*, 2011). In Asteroids, brooders have more apparent spatial structure than broadcasters (Moreau *et al.*, 2019). Seastar study with negligible differences of reproduction mode accent the effect of geomorphology and currents, put simply the physical environment (Jossart,

Sands and Sewell, 2019), to population structure. It follows that having more detailed description of physical environemnt with respect of species-specific patterns, we are always closer to explain population structure.

We advocate the view that the lifestyle as a whole comprising characteristics including the eggs care, clutch substrate or type of larvae along with the adult bathymetric range and foraging is a prominent parameter determining the population response to glaciation/deglaciation effects ***Publication IV*** and ***Publication VII in prep.***
More specifically, taxa with low haplotype diversity and congruently with recent population expansion show considerable intraspecific genetic structuration (i.e., due to limited gene flow leading to future isolation). Those holobenthic, benthic or shallow-benthic mait have dispersal obstructions (Convey, 1996) and inhabit habitats physically damaged by the grounded ice sheet or search for their niche. Partition can cause depth, temperature, sea fluxes, and seafloor relief (geography).

On the contrary, species with higher genetic diversity, resulted in an old population expansions, exhibited broad gene flow, limited speciation potential and lack intraspecific structuration (pelagic species, or those benthic that can travel on the continental slope; eurybathic). Of course, our categorisation was rather crude, and it has to be made with caution and more characteristics of lifestyle should be considered (the ability to escape the growing grounded ice sheet) ***Publication IV*** and ***Publication VII in prep.*** Because ***Publication VII in prep*** was comparing the data from most of GenBank database available even unrelated species, we can add one example from Arthropoda. Contrary to expectation, the brooder, *Nymphon australe,* the most common sea spider is able to walk the seafloor caring his babies. This probably explains its regionally differentiated population. But the walking seems to mediate the gene flow, and so there was not found cryptic species e.g. (Mahon, Arango and Halanych, 2008; Arango, Soler-Membrives and Miller, 2011). Moeover, it was able to find refugees during glacial periods. The expansion time is rather older (36 – 7,5 Kya depending on the locality) congruently to other benthic or eurybathic analysed species **(*Publication VII in prep).***

9. Conclusion

My PhD thesis considerably contributes to a change of the traditional view of polar species distribution from circumpolar ***(Publication I, IV, and Publication VII in prep.)*** or cosmopolitan (i. e.“ everything is everywhere” in (Williams, 2011) for microorganisms), to the appreciation of the high degree of endemicity**,** high degree of patchiness in both diversity and abundance even within a small geographic region. This may have considerable consequences for endemism in most studied invertebrates **(*Publication V, VI).“*** Everything is everywhere, but the environment selects'' presumes that environmental processes rather than historical processes drive the diversity. It agrees with (Shmakova *et al.*, 2021), and it is actually concordant with the outcome that the last glacial maximum more harmed the benthic species than the pelagic (eurybathic and deep-sea); ***(Publication IV, Publication VII in prep)***.

It follows that the situation on the terrestrial and marine environment has a basis in common. The harsh Antarctic conditions formed the species distribution and abundances over the cyclical glaciations, and species that had adapted (AFGP in fish) or did not extinct (Rotifera) had been forced to escape the ice formation (the growing grounded ice sheet for benthic fish, the growing ice sheet itself for rotifers).

The thesis brings a comprehensive view of polar region evolution in both environments, marine and terrestrial, by a comparative approach made up of dozens of species.

Please, allow me a personal note. During my beginnings of the polar studies, in 2008, the Antarctic was meant to be the natural laboratory, due to its functional isolation, stable climate and endemic fauna with its in situ evolution. Today, after more than a dozen of years, the insight is going to be in question and more power is devoted to the monitoring of the fate of invaders from the North and remarkably growing temperature consequences with the looming impact in the Antarctic Peninsula region. I feel a big change in people thinking about global climate change. But are we willing to change our culture? What are we willing to give up (Deppeler and Davidson, 2017)? Is it really necessary and wise to build up Ocean protections in situ?

10. References

Allcock, A.L. and Strugnell, J.M. (2012) 'Southern Ocean diversity: new paradigms from molecular ecology', *Trends in Ecology & Evolution*, 27(9), pp. 520–528. doi:10.1016/j.tree.2012.05.009.

ALLEGRUCCI, G. *et al.* (2012) 'Evolutionary geographic relationships among orthocladine chironomid midges from maritime Antarctic and sub-Antarctic islands', *Biological Journal of the Linnean Society*, 106(2), pp. 258–274. doi:10.1111/j.1095-8312.2012.01864.x.

Anderson, J.B. *et al.* (2002) 'The Antarctic Ice Sheet during the Last Glacial Maximum and its subsequent retreat history: a review', *Quaternary Science Reviews*, 21(1), pp. 49–70. doi:10.1016/S0277-3791(01)00083-X.

Arango, C.P., Soler-Membrives, A. and Miller, K.J. (2011) 'Genetic differentiation in the circum—Antarctic sea spider Nymphon australe (Pycnogonida; Nymphonidae)', *Deep Sea Research Part II: Topical Studies in Oceanography*, 58(1–2), pp. 212–219. doi:10.1016/j.dsr2.2010.05.019.

Aronson, R.B. *et al.* (2007) 'Climate Change and Invasibility of the Antarctic Benthos', *Annual Review of Ecology, Evolution, and Systematics*, 38(1), pp. 129–154. doi:10.1146/annurev.ecolsys.38.091206.095525.

Aronson, R.B. and Blake, D.B. (2001) 'Global Climate Change and the Origin of Modern Benthic Communities in Antarctica', *Integrative and Comparative Biology*, 41(1), pp. 27–39. doi:10.1093/icb/41.1.27.

Barnes, D.K.A. and Conlan, K.E. (2007) 'Disturbance, colonization and development of Antarctic benthic communities', *Philosophical Transactions of the Royal Society B: Biological Sciences*, 362(1477), pp. 11–38. doi:10.1098/rstb.2006.1951.

Barnes, D.K.A. and Griffiths, H.J. (2008) 'Biodiversity and biogeography of southern temperate and polar bryozoans', *Global Ecology and Biogeography*, 17(1), pp. 84–99. doi:https://doi.org/10.1111/j.1466-8238.2007.00342.x.

Barnes, D.K.A., Griffiths, H.J. and Kaiser, S. (2009) 'Geographic range shift responses to climate change by Antarctic benthos: where we should look', *Marine Ecology Progress Series*, 393, pp. 13–26. doi:10.3354/meps08246.

Barnes, D.K.A. and Peck, L.S. (2008) 'Vulnerability of Antarctic shelf biodiversity to predicted regional warming', *Climate Research*, 37(2/3), pp. 149–163.

Brenner, M. *et al.* (2001) 'The role of iceberg scours in niche separation within the Antarctic fish genus Trematomus', *Polar Biology*, 24(7), pp. 502–507. doi:10.1007/s003000100246.

Briggs, J.C. (2003) 'Marine centres of origin as evolutionary engines', *Journal of Biogeography*, 30(1), pp. 1–18. doi:10.1046/j.1365-2699.2003.00810.x.

Briggs, J.C. and Bowen, B.W. (2012) 'A realignment of marine biogeographic provinces with particular reference to fish distributions', *Journal of Biogeography*, 39(1), pp. 12–30. doi:10.1111/j.1365-2699.2011.02613.x.

Caccavo, J.A. *et al.* (2018) 'Along-shelf connectivity and circumpolar gene flow in Antarctic silverfish (Pleuragramma antarctica)', *Scientific Reports*, 8(1), p. 17856. doi:10.1038/s41598-018-36030-x.

Cakil, Z.V. *et al.* (2021) 'Comparative phylogeography reveals consistently shallow genetic diversity in a mitochondrial marker in Antarctic bdelloid rotifers', *Journal of Biogeography*, 48(7), pp. 1797–1809. doi:10.1111/jbi.14116.

Carrero, D. *et al.* (2019) 'Differential mechanisms of tolerance to extreme environmental conditions in tardigrades', *Scientific Reports*, 9(1), p. 14938. doi:10.1038/s41598-019-51471-8.

Chen, Z. *et al.* (2008) 'Transcriptomic and genomic evolution under constant cold in Antarctic notothenioid fish', *Proceedings of the National Academy of Sciences*, 105(35), pp. 12944–12949. doi:10.1073/pnas.0802432105.

Chown, S.L. and Convey, P. (2007) 'Spatial and temporal variability across life's hierarchies in the terrestrial Antarctic', *Philosophical Transactions of the Royal Society B: Biological Sciences*, 362(1488), pp. 2307–2331. doi:10.1098/rstb.2006.1949.

Clark, M.S. *et al.* (2018) 'Life in the intertidal: Cellular responses, methylation and epigenetics', *Functional Ecology*, 32(8), pp. 1982–1994. doi:https://doi.org/10.1111/1365-2435.13077.

Clarke, A. and Crame, J.A. (2010) 'Evolutionary dynamics at high latitudes: speciation and extinction in polar marine faunas', *Philosophical Transactions of the Royal Society B: Biological Sciences*, 365(1558), pp. 3655–3666. doi:10.1098/rstb.2010.0270.

Colleoni, F. *et al.* (2018) 'Spatio-temporal variability of processes across Antarctic ice-bed–ocean interfaces', *Nature Communications*, 9(1), pp. 1–14. doi:10.1038/s41467-018-04583-0.

Collins, G.E. *et al.* (2019) 'Spatial and Temporal Scales Matter When Assessing the Species and Genetic Diversity of Springtails (Collembola) in Antarctica', *Frontiers in Ecology and Evolution*, 7. doi:10.3389/fevo.2019.00076.

Convey, P. (1996) 'The Influence of Environmental Characteristics on Life History Attributes of Antarctic Terrestrial Biota', *Biological Reviews*, 71(2), pp. 191–225. doi:https://doi.org/10.1111/j.1469-185X.1996.tb00747.x.

Convey, P. *et al.* (2008) 'Antarctic terrestrial life – challenging the history of the frozen continent?', *Biological Reviews*, 83(2), pp. 103–117. doi:https://doi.org/10.1111/j.1469-185X.2008.00034.x.

Convey, P. and McInnes, S.J. (2005) 'Exceptional Tardigrade-Dominated Ecosystems in Ellsworth Land, Antarctica', *Ecology*, 86(2), pp. 519–527. doi:https://doi.org/10.1890/04-0684.

Convey, P. and Stevens, M.I. (2007a) 'Antarctic Biodiversity', *Science*, 317(5846), pp. 1877–1878. doi:10.1126/science.1147261.

Convey, P. and Stevens, M.I. (2007b) 'Antarctic Biodiversity', *Science*, 317(5846), pp. 1877–1878. doi:10.1126/science.1147261.

Crame, J.A. (2018) 'Key stages in the evolution of the Antarctic marine fauna', *Journal of Biogeography*, 45(5), pp. 986–994. doi:10.1111/jbi.13208.

Cristini, L. *et al.* (2012) 'Influence of the opening of the Drake Passage on the Cenozoic Antarctic Ice Sheet: A modeling approach', *Palaeogeography, Palaeoclimatology, Palaeoecology*, 339–341, pp. 66–73. doi:10.1016/j.palaeo.2012.04.023.

Damerau, M. *et al.* (2012) 'Comparative population genetics of seven notothenioid fish species reveals high levels of gene flow along ocean currents in the southern Scotia Arc, Antarctica', *Polar biology*, 45(7), pp. 1073–1086. doi:10.1007/s00300-012-1155-x.

Damerau, M. *et al.* (2014) 'Population divergences despite long pelagic larval stages: lessons from crocodile icefishes (Channichthyidae)', *Molecular Ecology*, 23(2), pp. 284–299. doi:10.1111/mec.12612.

Dayton, P.K. (1989) 'Interdecadal Variation in an Antarctic Sponge and Its Predators from Oceanographic Climate Shifts', *Science*, 245(4925), pp. 1484–1486. doi:10.1126/science.245.4925.1484.

Debortoli, N., Laender, F.D. and Doninck, K.V. (2018) 'Immigration from the metacommunity affects bdelloid rotifer community dynamics most', *bioRxiv*, p. 450627. doi:10.1101/450627.

Deli Antoni, M.Y. *et al.* (2019) 'Comparative populational study of Lepidonotothen larseni and L. nudifrons (Teleostei: Nototheniidae) from the Antarctic Peninsula and the South Shetland Islands, Antarctica', *Polar Biology*, 42(8), pp. 1537–1547. doi:10.1007/s00300-019-02540-1.

Deppeler, S.L. and Davidson, A.T. (2017) 'Southern Ocean Phytoplankton in a Changing Climate', *Frontiers in Marine Science*, 4. doi:10.3389/fmars.2017.00040.

DeVries, A.L. and Wohlschlag, D.E. (1969) 'Freezing resistance in some Antarctic fishes', *Science (New York, N.Y.)*, 163(3871), pp. 1073–1075.

Díaz, A. *et al.* (2011) 'Evolutionary pathways among shallow and deep-sea echinoids of the genus Sterechinus in the Southern Ocean', *Deep Sea Research Part II: Topical Studies in Oceanography*, 58(1), pp. 205–211. doi:10.1016/j.dsr2.2010.10.012.

Díaz, A. *et al.* (2018) 'Genetic structure and demographic inference of the regular sea urchin Sterechinus neumayeri (Meissner, 1900) in the Southern Ocean: The role of the last glaciation', *PLOS ONE*, 13(6), p. e0197611. doi:10.1371/journal.pone.0197611.

Dietz, L. *et al.* (2015a) 'Regional differentiation and extensive hybridization between mitochondrial clades of the Southern Ocean giant sea spider Colossendeis megalonyx', *Open Science*, 2(7), p. 140424. doi:10.1098/rsos.140424.

Dietz, L. *et al.* (2015b) 'Regional differentiation and extensive hybridization between mitochondrial clades of the Southern Ocean giant sea spider Colossendeis megalonyx', *Royal Society Open Science*, 2(7), p. 140424. doi:10.1098/rsos.140424.

Diez, M.J. and Lovrich, G.A. (2010) 'Reproductive biology of the crab Halicarcinus planatus (Brachyura, Hymenosomatidae) in sub-Antarctic waters', *Polar Biology*, 33(3), pp. 389–401. doi:10.1007/s00300-009-0716-0.

Dömel, J.S. *et al.* (2017) 'Nuclear and Mitochondrial Gene Data Support Recent Radiation within the Sea Spider Species Complex Pallenopsis patagonica', *Frontiers in Ecology and Evolution*, 4. doi:10.3389/fevo.2016.00139.

Dömel, J.S. *et al.* (2019) 'Combining morphological and genomic evidence to resolve species diversity and study speciation processes of the Pallenopsis patagonica (Pycnogonida) species complex', *Frontiers in Zoology*, 16(1), p. 36. doi:10.1186/s12983-019-0316-y.

Dömel, J.S., Convey, P. and Leese, F. (2015) 'Genetic Data Support Independent Glacial Refugia and Open Ocean Barriers to Dispersal for the Southern Ocean Sea Spider Austropallene Cornigera ()', *Journal of Crustacean Biology*, 35(4), pp. 480–490. doi:10.1163/1937240X-00002351.

Donner, J. (1965) *Ordnung Bdelloidea (Rotatoria, Rädertiere)*. Berlin: Akademie-Verlag.

Drummond, A.J. *et al.* (2012) 'Bayesian phylogenetics with BEAUti and the BEAST 1.7', *Molecular Biology and Evolution*, 29(8), pp. 1969–1973. doi:10.1093/molbev/mss075.

Eastman, J.T. (1993) *Antarctic Fish Biology: Evolution in a Unique Environment*. 1st Edition. San Diego: Academic Press.

Excoffier, L., Laval, G. and Schneider, S. (2007) 'Arlequin (version 3.0): An integrated software package for population genetics data analysis', *Evolutionary Bioinformatics Online*, 1, pp. 47–50.

Eyres, I. *et al.* (2015) 'Horizontal gene transfer in bdelloid rotifers is ancient, ongoing and more frequent in species from desiccating habitats', *BMC Biology*, 13(1), p. 90. doi:10.1186/s12915-015-0202-9.

Flot, J.-F. *et al.* (2013) 'Genomic evidence for ameiotic evolution in the bdelloid rotifer Adineta vaga', *Nature*, 500(7463), pp. 453–457. doi:10.1038/nature12326.

Flot, J.-F. *et al.* (2016) 'Response to Signorovitch et al.', *Current Biology*, 26(16), p. R755. doi:10.1016/j.cub.2016.06.052.

Fontaneto, D., Iakovenko, N. and De Smet, W.H. (2015) 'Diversity gradients of rotifer species richness in Antarctica', *Hydrobiologia*, 761(1), pp. 235–248. doi:10.1007/s10750-015-2258-5.

Frederich, M., Sartoris, F. and Pörtner, H.-O. (2001) 'Distribution patterns of decapod crustaceans in polar areas: a result of magnesium regulation?', *Polar Biology*, 24(10), pp. 719–723. doi:10.1007/s003000100270.

Fu, Y.-X. (1997) 'Statistical Tests of Neutrality of Mutations Against Population Growth, Hitchhiking and Background Selection', *Genetics*, 147(2), pp. 915–925.

Garlasché, G. *et al.* (2020) 'A data set on the distribution of Rotifera in Antarctica', *Biogeographia – The Journal of Integrative Biogeography*, 35(0). doi:10.21426/B635044786.

González-Wevar, C.A. *et al.* (2021) 'Contrasting biogeographical patterns in Margarella (Gastropoda: Calliostomatidae: Margarellinae) across the Antarctic Polar Front', *Molecular Phylogenetics and Evolution*, 156, p. 107039. doi:10.1016/j.ympev.2020.107039.

González-Wevar, C.A., David, B. and Poulin, E. (2011) 'Phylogeography and demographic inference in Nacella (Patinigera) concinna (Strebel, 1908) in the western Antarctic Peninsula', *Deep Sea Research Part II: Topical Studies in Oceanography*, 58(1), pp. 220–229. doi:10.1016/j.dsr2.2010.05.026.

Grant, W.S. *et al.* (2012) 'Limits of Bayesian skyline plot analysis of mtDNA sequences to infer historical demographies in Pacific herring (and other species)', *Molecular Phylogenetics and Evolution*, 65(1), pp. 203–212. doi:10.1016/j.ympev.2012.06.006.

Griffiths, H.J. (2010) 'Antarctic Marine Biodiversity – What Do We Know About the Distribution of Life in the Southern Ocean?', *PLOS ONE*, 5(8), p. e11683. doi:10.1371/journal.pone.0011683.

Griffiths, H.J. *et al.* (2021) 'Breaking All the Rules: The First Recorded Hard Substrate Sessile Benthic Community Far Beneath an Antarctic Ice Shelf', *Frontiers in Marine Science*, 8. doi:10.3389/fmars.2021.642040.

Halanych, K.M. and Mahon, A.R. (2018) 'Challenging Dogma Concerning Biogeographic Patterns of Antarctica and the Southern Ocean', *Annual Review of Ecology, Evolution, and Systematics*, 49(1), pp. 355–378. doi:10.1146/annurev-ecolsys-121415-032139.

Hashimoto, T. *et al.* (2016) 'Extremotolerant tardigrade genome and improved radiotolerance of human cultured cells by tardigrade-unique protein', *Nature Communications*, 7(1), p. 12808. doi:10.1038/ncomms12808.

Hawes, N.A. *et al.* (2018) 'Effects of temperature and salinity stress on DNA methylation in a highly invasive marine invertebrate, the colonial ascidian Didemnum vexillum', *PeerJ*, 6, p. e5003. doi:10.7717/peerj.5003.

Hawes, T.C. *et al.* (2007) 'Aerial dispersal of springtails on the Antarctic Peninsula: implications for local distribution and demography', *Antarctic Science*, 19(1), pp. 3–10. doi:10.1017/S0954102007000028.

Hawes, T.C., Marshall, C.J. and Wharton, D.A. (2011) 'Antifreeze proteins in the Antarctic springtail, Gressittacantha terranova', *Journal of Comparative Physiology. B, Biochemical, Systemic, and Environmental Physiology*, 181(6), pp. 713–719. doi:10.1007/s00360-011-0564-4.

Hemery, L.G. *et al.* (2012) 'Comprehensive sampling reveals circumpolarity and sympatry in seven mitochondrial lineages of the Southern Ocean crinoid species Promachocrinus kerguelensis (Echinodermata)', *Molecular Ecology*, 21(10), pp. 2502–2518. doi:10.1111/j.1365-294X.2012.05512.x.

Hengherr, S. *et al.* (2009) 'Freeze tolerance, supercooling points and ice formation: comparative studies on the subzero temperature survival of limno-terrestrial tardigrades', *The Journal of Experimental Biology*, 212(Pt 6), pp. 802–807. doi:10.1242/jeb.025973.

Hespeels, B. *et al.* (2020) 'Iron Ladies – How Desiccated Asexual Rotifer Adineta vaga Deal With X-Rays and Heavy Ions?', *Frontiers in Microbiology*, 11. doi:10.3389/fmicb.2020.01792.

Hillebrand, H. (2004) 'On the generality of the latitudinal diversity gradient', *The American Naturalist*, 163(2), pp. 192–211. doi:10.1086/381004.

Ho, S.Y.W. *et al.* (2005) 'Time dependency of molecular rate estimates and systematic overestimation of recent divergence times', *Molecular Biology and Evolution*, 22(7), pp. 1561–1568. doi:10.1093/molbev/msi145.

Ho, S.Y.W. *et al.* (2011) 'Time-dependent rates of molecular evolution', *Molecular Ecology*, 20(15), pp. 3087–3101. doi:10.1111/j.1365-294X.2011.05178.x.

Hoareau, T.B. (2016) 'Late Glacial Demographic Expansion Motivates a Clock Overhaul for Population Genetics', *Systematic Biology*, 65(3), pp. 449–464. doi:10.1093/sysbio/syv120.

Hoban, S. (2018) ‘Integrative conservation genetics: Prioritizing populations using climate predictions, adaptive potential and habitat connectivity’, *Molecular Ecology Resources*, 18(1), pp. 14–17. doi:10.1111/1755-0998.12752.

Hoffman, J.I. *et al.* (2011) ‘Effects of brooding and broadcasting reproductive modes on the population genetic structure of two Antarctic gastropod molluscs’, *Marine Biology*, 158(2), pp. 287–296. doi:10.1007/s00227-010-1558-6.

Hofmann, G.E. *et al.* (2000) ‘Heat-shock protein expression is absent in the antarctic fish Trematomus bernacchii (family Nototheniidae)’, *Journal of Experimental Biology*, 203(15), pp. 2331–2339.

Hughes, K.A. *et al.* (2019) ‘Human-mediated dispersal of terrestrial species between Antarctic biogeographic regions: A preliminary risk assessment’, *Journal of Environmental Management*, 232, pp. 73–89. doi:10.1016/j.jenvman.2018.10.095.

Hutchinson, G.E. (1959) ‘Homage to Santa Rosalia or Why Are There So Many Kinds of Animals?’, *The American Naturalist*, 93(870), pp. 145–159.

Iakovenko, N.S. *et al.* (2013) ‘Otostephanos (Rotifera, Bdelloidea, Habrotrochidae) with the description of two new species’, *Systematics and Biodiversity*, 11(4), pp. 477–494. doi:10.1080/14772000.2013.857737.

Iakovenko, N.S. *et al.* (2015) ‘Antarctic bdelloid rotifers: diversity, endemism and evolution’, *Hydrobiologia*, 761(1), pp. 5–43. doi:10.1007/s10750-015-2463-2.

Janko, K. *et al.* (2007) ‘Did glacial advances during the Pleistocene influence differently the demographic histories of benthic and pelagic Antarctic shelf fishes? – Inferences from intraspecific mitochondrial and nuclear DNA sequence diversity’, *BMC Evolutionary Biology*, 7(1), p. 220. doi:10.1186/1471-2148-7-220.

Janko, K. *et al.* (2011) ‘Multilocus analyses of an Antarctic fish species flock (Teleostei, Notothenioidei, Trematominae): phylogenetic approach and test of the early-radiation event’, *Molecular Phylogenetics and Evolution*, 60(3), pp. 305–316. doi:10.1016/j.ympev.2011.03.008.

Jossart, Q., Sands, C.J. and Sewell, M.A. (2019) ‘Dwarf brooder versus giant broadcaster: combining genetic and reproductive data to unravel cryptic diversity in an Antarctic brittle star’, *Heredity*, 123(5), pp. 622–633. doi:10.1038/s41437-019-0228-9.

Kagoshima, H. *et al.* (2019) ‘Integrated taxonomy combining morphological and molecular biological analyses of soil nematodes from maritime Antarctica’, *Polar Biology*, 42(5), pp. 877–887. doi:10.1007/s00300-019-02482-8.

Kamilari, M. *et al.* (2019) 'Comparative transcriptomics suggest unique molecular adaptations within tardigrade lineages', *BMC genomics*, 20(1), p. 607. doi:10.1186/s12864-019-5912-x.

Kašparová, E. *et al.* (2015) 'Lifestyle and Ice: The Relationship between Ecological Specialization and Response to Pleistocene Climate Change', *PLOS ONE*, 10(11), p. e0138766. doi:10.1371/journal.pone.0138766.

Keller, G., Macleod, N. and Barrera, E. (1992) 'Eocene-Oligocene faunal turnover in planktic foraminifera, and Antarctic glaciation', *Unknown Journal*, pp. 218–246.

Klages, M. *et al.* (1994) 'Stone crabs close to the Antarctic continent - Lithodes murrayi Henderson, 1888 (Crustacea; Decapoda; Anomura) off Peter I Island (68 51'S, 90 51'W)', *Polar biology* [Preprint]. Available at: https://epic.awi.de/id/eprint/1669/ (Accessed: 10 December 2020).

Kock, K.-H., Pshenichnov, L.K. and Devries, A.L. (2006) 'Evidence for egg brooding and parental care in icefish and other notothenioids in the Southern Ocean', *Antarctic Science*, 18(2), pp. 223–227. doi:10.1017/S0954102006000265.

Kopp, R.E. *et al.* (2005) 'The Paleoproterozoic snowball Earth: A climate disaster triggered by the evolution of oxygenic photosynthesis', *Proceedings of the National Academy of Sciences*, 102(32), pp. 11131–11136. doi:10.1073/pnas.0504878102.

Lau, S.C.Y. *et al.* (2020) 'Detecting glacial refugia in the Southern Ocean', *Ecography*, 43(11), pp. 1639–1656. doi:https://doi.org/10.1111/ecog.04951.

Lee, J.E. and Chown, S.L. (2011) 'Quantification of intra-regional propagule movements in the Antarctic', *Antarctic Science*, 23(4), pp. 337–342. doi:10.1017/S0954102011000198.

Lee, Y.-H. *et al.* (2004) 'Molecular phylogeny and divergence time of the Antarctic sea urchin (Sterechinus neumayeri) in relation to the South American sea urchins', *Antarctic Science*, 16(1), pp. 29–36. doi:10.1017/S0954102004001786.

Leigh, J.W. and Bryant, D. (2015) 'popart: full-feature software for haplotype network construction', *Methods in Ecology and Evolution*, 6(9), pp. 1110–1116. doi:10.1111/2041-210X.12410.

Lewis, P.N., Riddle, M.J. and Smith, S.D.A. (2005) 'Assisted passage or passive drift: a comparison of alternative transport mechanisms for non-indigenous coastal species into the Southern Ocean', *Antarctic Science*, 17(2), pp. 183–191. doi:10.1017/S0954102005002580.

Librado, P. and Rozas, J. (2009) 'DnaSP v5: a software for comprehensive analysis of DNA polymorphism data', *Bioinformatics*, 25(11), pp. 1451–1452. doi:10.1093/bioinformatics/btp187.

Linse, K. *et al.* (2006) 'Biodiversity and biogeography of Antarctic and sub-Antarctic mollusca', *Deep Sea Research Part II: Topical Studies in Oceanography*, 53(8), pp. 985–1008. doi:10.1016/j.dsr2.2006.05.003.

Mahon, A.R., Arango, C.P. and Halanych, K.M. (2008) 'Genetic diversity of Nymphon (Arthropoda: Pycnogonida: Nymphonidae) along the Antarctic Peninsula with a focus on Nymphon australe Hodgson 1902', *Marine Biology*, 155(3), p. 315. doi:10.1007/s00227-008-1029-5.

Mašová, Š. and Kašparová, E. (2012) *Preliminary report on fish diversity at the Prince Gustav Channel, the northern part of the James Ross Island, Antarctica*, *Masarykova univerzita*. Available at: https://www.muni.cz/vyzkum/publikace/1095935 (Accessed: 12 October 2018).

Matschiner, M., Hanel, R. and Salzburger, W. (2011) 'On the Origin and Trigger of the Notothenioid Adaptive Radiation', *PLOS ONE*, 6(4), p. e18911. doi:10.1371/journal.pone.0018911.

McCarthy, A.H. *et al.* (2019) 'Antarctica: The final frontier for marine biological invasions', *Global Change Biology*, 25(7), pp. 2221–2241. doi:10.1111/gcb.14600.

McGaughran, A. *et al.* (2010) 'Contrasting phylogeographical patterns for springtails reflect different evolutionary histories between the Antarctic Peninsula and continental Antarctica', *Journal of Biogeography*, 37(1), pp. 103–119. doi:https://doi.org/10.1111/j.1365-2699.2009.02178.x.

McGaughran, A. *et al.* (2019) 'Genome-wide SNP data reveal improved evidence for Antarctic glacial refugia and dispersal of terrestrial invertebrates', *Molecular Ecology*, 28(22), pp. 4941–4957. doi:https://doi.org/10.1111/mec.15269.

Mercado Casares, B., Soto Àngel, J.J. and Peña Cantero, Á.L. (2017) 'Towards a better understanding of Southern Ocean biogeography: new evidence from benthic hydroids', *Polar Biology*, 40(10), pp. 1975–1988. doi:10.1007/s00300-017-2113-4.

Molina, E., Gonzalvo, C. and Keller, G. (1993) 'The Eocene-Oligocene planktic foraminiferal transition: extinctions, impacts and hiatuses', *Geological Magazine*, 130(4), pp. 483–499. doi:10.1017/S0016756800020550.

Moreau, C. *et al.* (2019) 'Is reproductive strategy a key factor in understanding the evolutionary history of Southern Ocean Asteroidea (Echinodermata)?', *Ecology and Evolution*, 9(15), pp. 8465–8478. doi:10.1002/ece3.5280.

Mörs, T., Reguero, M. and Vasilyan, D. (2020) 'First fossil frog from Antarctica: implications for Eocene high latitude climate conditions and Gondwanan cosmopolitanism of Australobatrachia', *Scientific Reports*, 10(1), p. 5051. doi:10.1038/s41598-020-61973-5.

Near, T.J. *et al.* (2012) 'Ancient climate change, antifreeze, and the evolutionary diversification of Antarctic fishes', *Proceedings of the National Academy of Sciences of the United States of America*, 109(9), pp. 3434–3439. doi:10.1073/pnas.1115169109.

Near, T.J. *et al.* (2015) 'Identification of the notothenioid sister lineage illuminates the biogeographic history of an Antarctic adaptive radiation', *BMC Evolutionary Biology*, 15(1), p. 109. doi:10.1186/s12862-015-0362-9.

Nowell, R.W. *et al.* (2018) 'Comparative genomics of bdelloid rotifers: Insights from desiccating and nondesiccating species', *PLOS Biology*, 16(4), p. e2004830. doi:10.1371/journal.pbio.2004830.

O.B.E, R.H.L.S.K.C.P.C. (1999) *The Heart of the Antarctic: Being the Story of the British Antarctic Expedition, 1907-1909*. New York: Carroll & Graf.

Patarnello, T. *et al.* (2003) 'Phylogeography of the Chionodraco genus (Perciformes, Channichthydae) in the Southern Ocean', *Molecular Phylogenetics and Evolution*, 28(3), pp. 420–429. doi:10.1016/S1055-7903(03)00124-6.

Patarnello, T. *et al.* (2011) 'How will fish that evolved at constant sub-zero temperatures cope with global warming? Notothenioids as a case study', *BioEssays: News and Reviews in Molecular, Cellular and Developmental Biology*, 33(4), pp. 260–268. doi:10.1002/bies.201000124.

Peck, L.S. (2018) 'Chapter 3 Antarctic Marine Biodiversity : Adaptations, Environments and Responses to Change', *Oceanography and Marine Biology* [Preprint]. doi:10.1201/9780429454455.

Pierrat, B. *et al.* (2013) 'Comparative biogeography of echinoids, bivalves and gastropods from the Southern Ocean', *Journal of Biogeography*, 40(7), pp. 1374–1385. doi:10.1111/jbi.12088.

Potocka, M. and Krzemińska, E. (2018) 'Trichocera maculipennis (Diptera)—an invasive species in Maritime Antarctica', *PeerJ*, 6, p. e5408. doi:10.7717/peerj.5408.

Pugh, P.J.A. and Convey, P. (2008) 'Surviving out in the cold: Antarctic endemic invertebrates and their refugia', *Journal of Biogeography*, 35(12), pp. 2176–2186. doi:https://doi.org/10.1111/j.1365-2699.2008.01953.x.

Raupach, M.J. *et al.* (2010) 'Genetic homogeneity and circum-Antarctic distribution of two benthic shrimp species of the Southern Ocean, Chorismus antarcticus and Nematocarcinus lanceopes', *Marine Biology*, 157(8), pp. 1783–1797. doi:10.1007/s00227-010-1451-3.

Reeve, K.A. *et al.* (2019) 'Horizontal circulation and volume transports in the Weddell Gyre derived from Argo float data', *Progress in Oceanography*, 175, pp. 263–283. doi:10.1016/j.pocean.2019.04.006.

Reguero, M.A. *et al.* (2014) 'Final Gondwana breakup: The Paleogene South American native ungulates and the demise of the South America–Antarctica land connection', *Global and Planetary Change*, 123, pp. 400–413. doi:10.1016/j.gloplacha.2014.07.016.

Rintoul, S.R. and Sokolov, S. (2001) 'Baroclinic transport variability of the Antarctic Circumpolar Current south of Australia (WOCE repeat section SR3)', *Journal of Geophysical Research: Oceans*, 106(C2), pp. 2815–2832. doi:https://doi.org/10.1029/2000JC900107.

Rivas-Martínez, S. *et al.* (2021) 'Biogeographical and bioclimatic outline of Antarctica', *Plant Biosystems - An International Journal Dealing with all Aspects of Plant Biology*, 155(1), pp. 5–15. doi:10.1080/11263504.2020.1840455.

Sabovljević, M. and Frahm, J.-P. (2011) 'Genetic diversity of the relict moss Rhytidium rugosum (Hypnales) in Europe inferred from the ITS region (nrDNA)', *Biologia*, 66(1), pp. 42–49. doi:10.2478/s11756-010-0129-0.

Schiavon, L. *et al.* (2021) 'Species distribution, hybridization and connectivity in the genus Chionodraco: Unveiling unknown icefish diversity in antarctica', *Diversity and Distributions*, 27(5), pp. 766–783. doi:https://doi.org/10.1111/ddi.13249.

Shtarkman, Y.M. *et al.* (2013) 'Subglacial Lake Vostok (Antarctica) Accretion Ice Contains a Diverse Set of Sequences from Aquatic, Marine and Sediment-Inhabiting Bacteria and Eukarya', *PLOS ONE*, 8(7), p. e67221. doi:10.1371/journal.pone.0067221.

Signorovitch, A. *et al.* (2015) 'Allele Sharing and Evidence for Sexuality in a Mitochondrial Clade of Bdelloid Rotifers', *Genetics*, 200(2), pp. 581–590. doi:10.1534/genetics.115.176719.

Smykla, J. *et al.* (2018) 'Geochemical and biotic factors influencing the diversity and distribution of soil microfauna across ice-free coastal habitats in Victoria Land, Antarctica'. doi:10.1016/J.SOILBIO.2017.10.028.

Soler-Membrives, A. *et al.* (2017) 'Genetic signature of Last Glacial Maximum regional refugia in a circum-Antarctic sea spider', *Royal Society Open Science*, 4(10). doi:10.1098/rsos.170615.

Stevens, M.I. *et al.* (2006) 'Southern Hemisphere Springtails: Could Any Have Survived Glaciation of Antarctica?', *Molecular Biology and Evolution*, 23(5), pp. 874–882. doi:10.1093/molbev/msj073.

Strugnell, J.M. *et al.* (2012) 'Persistent genetic signatures of historic climatic events in an Antarctic octopus', *Molecular Ecology*, 21(11), pp. 2775–2787. doi:https://doi.org/10.1111/j.1365-294X.2012.05572.x.

Tajima, F. (1989) 'Statistical method for testing the neutral mutation hypothesis by DNA polymorphism.', *Genetics*, 123(3), pp. 585–595.

Tavares, M. and Melo, G. a. S.D. (2004) 'Discovery of the first known benthic invasive species in the Southern Ocean: the North Atlantic spider crab Hyas araneus found in the Antarctic Peninsula', *Antarctic Science*, 16(2), pp. 129–131. doi:10.1017/S0954102004001877.

Thatje, S. *et al.* (2008) 'Life Hung by a Thread: Endurance of Antarctic Fauna in Glacial Periods', *Ecology*, 89(3), pp. 682–692. doi:10.1890/07-0498.1.

Thatje, S. and Arntz, W. (2004) 'Antarctic reptant decapods: more than a myth?', *Polar Biology:*, 27, pp. 195–201.

Thatje, S., Hillenbrand, C.-D. and Larter, R. (2005) 'On the origin of Antarctic marine benthic community structure', *Trends in Ecology & Evolution*, 20(10), pp. 534–540. doi:10.1016/j.tree.2005.07.010.

Thatje, S. and Lörz, A.N. (2005) 'First record of lithodid crabs from Antarctic waters off the Balleny Islands', *Polar Biology:*, 28, pp. 334–337.

Thornhill, D.J. *et al.* (2008) 'Open-ocean barriers to dispersal: a test case with the Antarctic Polar Front and the ribbon worm Parborlasia corrugatus (Nemertea: Lineidae)', *Molecular Ecology*, 17(23), pp. 5104–5117. doi:10.1111/j.1365-294X.2008.03970.x.

Trifinopoulos, J. *et al.* (2016) 'W-IQ-TREE: a fast online phylogenetic tool for maximum likelihood analysis', *Nucleic Acids Research*, 44(W1), pp. W232-235. doi:10.1093/nar/gkw256.

Velasco-Castrillón, A. *et al.* (2014) 'Surprisingly high levels of biodiversity and endemism amongst Antarctic rotifers uncovered with mitochondrial DNA', *Biodiversity*, 15(2–3), pp. 130–142. doi:10.1080/14888386.2014.930717.

Vernet, M. *et al.* (2019) 'The Weddell Gyre, Southern Ocean: Present Knowledge and Future Challenges', *Reviews of Geophysics*, 57(3), pp. 623–708. doi:https://doi.org/10.1029/2018RG000604.

Welch, D., Ricci, C. and Meselson, M. (2009) 'Bdelloid Rotifers: Progress in Understanding the Success of an Evolutionary Scandal', in *Lost Sex: The Evolutionary Biology of Parthenogenesis*, pp. 259–279. doi:10.1007/978-90-481-2770-2_13.

Williams, D. (2011) 'Historical biogeography, microbial endemism and the role of classification: Everything is endemic', in, pp. 11–32. doi:10.1017/CBO9780511974878.003.

Wilson, N.G. *et al.* (2007) 'Multiple lineages and absence of panmixia in the" circumpolar" crinoid Promachocrinus kerguelensis from the Atlantic sector of Antarctica', *Marine Biology*, 152(4), pp. 895–904. doi:10.1007/s00227-007-0742-9.

Wolovick, M.J. and Moore, J.C. (2018) 'Stopping the flood: could we use targeted geoengineering to mitigate sea level rise?', *The Cryosphere*, 12(9), pp. 2955–2967. doi:https://doi.org/10.5194/tc-12-2955-2018.

Young, E.F. *et al.* (2015) 'Oceanography and life history predict contrasting genetic population structure in two Antarctic fish species', *Evolutionary Applications*, 8(5), pp. 486–509. doi:10.1111/eva.12259.