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Taxonomy, diversity and ecology of freshwater diatom communities (Bacillariophyta) from moss habitats of Gough Island (southern Atlantic Ocean)

Taxonomie, diversita a ekologie společenstev sladkovodních rozsivek (Bacillariophyta) mechových habitatů ostrova Gough (jižní Atlantický oceán)

DIPLOMA THESIS

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Statement

I hereby state that I have completed this thesis by myself and that I have properly cited all literature and other information sources I have used. Neither this thesis nor its parts have been submitted to achieve any other academic title(s).

Prohlášení

Prohlašuji, že jsem závěrečnou práci zpracovala samostatně a že jsem uvedla všechny použité informační zdroje a literaturu. Tato práce ani její podstatná část nebyla předložena k získání jiného nebo stejného akademického titulu.

In Prague/ V Praze, 10. 8. 2016 Signature/Podpis

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ABSTRACT

The present study shows the results of a taxonomic and ecological survey on moss-inhabiting diatoms from Gough Island (40°21’ S, 9°53’ W). An analysis of more than 100 samples resulted in 141 identified diatom taxa, of which 21 were described by Carter (1966) and have not been reported elsewhere. Illustrative analyses of diatom communities from Tristan da Cunha and Inaccessible islands revealed an additional nine taxa, but an otherwise highly similar flora. The observed flora was highly disharmonic in its composition, represented by only a few diatom genera (*i.e. Eunotia, Pinnularia, Psammothidium* and *Chamaepinnularia*), a feature typical for oceanic islands. Also, few diatom species dominating the flora were unique to the Tristan da Cunha archipelago (Gough Island in particular) and possibly endemic, or cosmopolitan in distribution. Diatom taxa being typical for sub-Antarctic islands haven’t been observed in larger amount. This, together with the highly specific diatom flora of Gough Island, resulted in very low similarity values between Gough and the other islands of southern ocean. The closest to the Tristan da Cunha archipelago based on its flora was identified to be Ile Amsterdam from the south Indian Ocean, implying the influence of west-wind drift.

To investigate the diatom flora composition, a hierarchical cluster analysis based on Bray-Curtis distances identified five significant groups, and a PCA ordination allowed a further separation of the largest group into three sub-groups. The different assemblages were explained by differences in environmental variables, and an dbRDA analysis based on species and genera level count data found altitude and sea spray to explain most variation for both, followed by moisture at the genera level. Habitat types helped to identify ecological preferences unique for the (sub-)group’s assemblages but were of rather lower influence in general.

As separate papers, Chapter 3 and 4 present two studies (published or as a manuscript) handling critical sub-topics of this master thesis. **Chapter 3** provides a detailed description and taxonomic evaluation of *Eunotia linearis*, a diatom species identified as *Pseudoeunotia linearis* by Carter (1966). **Chapter 4** describes the complicated task of separation several species from a highly diversified (pseudo)cryptic *Frustulia crassinervia-saxonica* species complex present in the moss habitats of Gough Island.

Four appendices are attached, and present (**1**) an overview and taxonomical revision of diatom species identified after the historical publication of Carter (1966), (**2**) a list of diatom taxa observed and identified during this study from recent moss samples, (**3**) abstracts of posters which were presented at international meetings regarding the topic, and finally (**4**) a published study concerning diatom dispersal on the High Arctic archipelago of Spitsbergen.

KEYWORDS: Gough Island; Bacillariophyta; Moss-habitats; Taxonomy; Diversity; Ecology.

ABSTRAKT

Práce představuje výsledky taxonomické a ekologické analýzy mechy obývajících rozsivek z ostrova Gough (40°21’ S, 9°53’ W). Analýza provedená na více než 100 vzorcích odhalila 141 rozsivkových taxonů, ze kterých 21 bylo identifikováno na základě druhů popsaných již ve studii Johna Cartera z roku 1966, z nichž většina nebyla prezentována z jiných lokalit. Analýza rozsivkových společenstev z ostrovů Tristan da Cunha a Inaccessible provedena na ilustrativní bázi ukázala dalších devět taxonů, ale také velmi podobné složení rozsivkové flóry. Pozorovaná flóra byla ve značném nesouladu, kde společenstvu dominovalo pouze několik rodů (*Eunotia, Pinnularia, Psammothidium* a *Chamaepinnularia*), což je typické pro oceánské ostrovy. Také několik málo dominantních druhů bylo složeno z taxonů buď unikátních a potenciálně endemických pro celé souostroví, případně pouze pro ostrov Gough, anebo z taxonů spíše kosmopolitních ve svém rozšíření. Rozsivkové taxony typické pro sub-Antarktidu ostrovní flóře nedominovaly, což společně s vysokou specifitou rozsivkové flóry ostrova Gough ve výsledku ukázalo velmi nízké podobnostní hodnoty s ostatními ostrovy jižních oceánů. Na základě rozsivkové flóry se nejblíže souostroví Tristan da Cunha jeví ostrov Amsterdam z jižního Indického oceánu, naznačující vliv antarktického cirkumpolárního proudění.

K prozkoumání složení rozsivkové flóry, identifikovala shluková analýza na základě vzdáleností Bray-Curtis pět signifikantních skupin, kde dále analýza hlavních komponent (PCA) umožnila rozdělení té největší do třech podskupin. Výsledná seskupení byla vysvětlena rozdíly v environmentálních proměnných, a za pomoci dbRDA analýzi druhového a rodového složení byla jako nejvýznamnější identifikována rozdílná nadmořská výška společně s vlivem mořské vody, na úrovni rodů pak i rozdíly ve vlhkosti. Typy habitatů pomohly identifikovat ekologické preference unikátní pro jednotlivé skupiny vzorků, ale měly spíše menší vliv než jednotlivé parametry prostředí.

Kapitoly 3 a 4 předkládají kritická podtémata této diplomové práce formou jednotlivých článků (jedoho již publikovaného a druhého formou manuskriptu). **Kapitola 3** poskytuje detailní popis a taxonomické zhodnocení druhu *Eunotia linearis,* rozsivky identifikované dle popisu

Pseudoeunotia linearis ve studii Carter (1966). **Kapitola 4** se týká komplikované otázky oddělení několika druhů z velmi diversifikovaného (pseudo)kryptického druhového komplexu *Frustulia crassinervia-saxonica* obývající mechové habitaty ostrova Gough.

Součástí práce jsou čtyři přílohy, které obsahují (**1**) přehled a taxonomickou revizi druhů rozsivek identifikovaných na základě historické studie Johna Cartera z roku 1966, (**2**) list taxonů rozsivek pozorovaných a identifikovaných v průběhu této studie z recentních vzorků mechů, (**3**) abstrakty posterů prezentovaných na mezinárodních konferencí týkajících se tématu předkládané práce, a nakonec (**4**) publikovaný článek týkající se ekologické otázky šíření rozsivek na arktickém území Špicberků.

KLÍČOVÁ SLOVA: ostrov Gough; Bacillariophyta; Mechy; Taxonomie; Diversita; Ekologie.

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CHAPTER 1

GENERAL INTRODUCTION

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GENERAL INTRODUCTION

The locality of interest

The southern hemisphere is mostly covered by saltwater and is bordered by coastlines of the southern continents and the icy shores of Antarctica. Within this vast ocean, several archipelagos and small islands can be found hundreds to thousands of kilometres from any other landmasses. The Tristan da Cunha archipelago, including **Gough Island**, belongs among these, representing one of the most remote places in the world, but harbouring a unique, significantly diverse fauna and flora (Wace and Dickson 1965). Gough, along with another island from the archipelago, Inaccessible Island, were named UNESCO World Heritage Sites in 1995, and are protected as important biodiversity sites with one of the least disrupted ecosystems of its kind, supporting globally significant populations of seabirds, endemic plants, invertebrates and land birds (State of Conservation Report 2016).

The fauna of Gough Island includes two endemic species of almost flightless land birds (Gough moorhen *Gallinula comeri* Allen and Gough finch *Rowettia goughensis* Clarke) and almost the entire world's population of the Tristan albatross (*Diomedea dabbenena* Mathews, **Figure 1.1**) and Atlantic petrel (*Pterodroma incerta* Schlegel). The island's flora contains several endemic plant species (*e.g.,* Gough Brass Buttons *Cotula goughensis* R.N.R.Br., *Deschampsia robusta* Hubb, *D. wacei* Hubb, *Agrostis goughensis* Hubb) and some interesting ones are shared with Ile Amsterdam and St. Paul Island in south Indian Ocean (the Island tree *Phylica arborea* T.) and with St. Helena (Tussock Grass *Spartina arundinacea* (T.) Carmich) (Andrew 2007). Gough Island also hosts subantarctic fur seals (*Arctocephalus tropicalis* Gray) that breed there in great numbers, representing up to 50 % of the world's population (Bester 2006). Beaches and cliffs of Gough and Tristan da Cunha are also inhabited by recently split populations of Northern Rockhopper Penguin (*Eudyptes moseleyi*) which are restricted to Tristan da Cunha archipelago and Amsterdam and St Paul Islands (Indian Ocean), but apparently critically endangered nowadays (Cuthbert et al. 2009). The biggest threat to the avifauna on the island is formed by the introduced rodents (house mouse, *Mus musculus* L.).

Figure 1.1. *Gough finch* Rowettia goughensis *(upper), and Tristan albatross* Diomedea dabbenena *(below). Birds of Tristan albatross usually spend a few years practicing an elaborate ritualized mating display before deciding on a life partner. Photos of Christopher W. Jones (2015).*

These mice are the only terrestrial mammals on Gough Island where otherwise no large predators are found, and have a devastating impact on seabird populations, such as the critically endangered Tristan albatross. According to Cuthbert et al. (2016), the mice from Gough exhibit extreme features of the island syndrome, including their body mass being by 50 – 60 % greater than any other island mouse population, extremely high densities, and low seasonal variation in numbers.

The diversity, distribution and endemicity of the non-marine and non-vascular flora of Tristan da Cunha archipelago has been discussed previously (Vinšová 2014), accompanied by the introduction to the island biogeography in general, the site's origin, general settings, habitat types, climate and history highlights. From these main remarks and findings I will emphasize four: (i) the archipelago has a volcanic origin and has never been connected to the mainland – oceanic islands have higher rates of evolution and speciation that those of immigration, resulting in a higher number of endemic taxa but also in imbalanced floras en faunas; (ii) obvious from the native vascular plants, there are clear geographical affinities to the sub-Antarctic and Amsterdam-St. Paul island groups, with lichens, mosses, liverworts and hornworts more similar to South American floras, clearly illustrating the west-wind drift influence (clockwise circum-Antarctic) to the dispersion of the non-vascular flora; (iii) the endemism includes some 21 % of the non-vascular plants, mostly profound for the group of mosses; (iv) the most understudied were algal groups, and previous knowledge of diatoms from the Tristan da Cunha archipelago needs to be revised; a survey of the non-marine diatom flora is needed in order to extend the knowledge of diatom communities from sub-Antarctica and the Southern hemisphere, allowing further biogeographical comparisons that are well-needed in the light of ongoing research on the distribution of diatoms.

Diatoms (Bacillariophyta) and theories of microorganismal dispersion

Diatoms (Bacillariophyta) are unicellular algae inhabiting (semi-)terrestrial, marine and non-marine aquatic environments worldwide. Apart from their global significance to the carbon and silica cycles, they contribute widely to the global net primary production, accounting for between 20 – 25 % worldwide (Werner 1977) and at least 40 % in oceans (Mann 1999). Their unique structure consisting of a siliceous frustule (SiO_2) and its distinct morphological traits are often considered species-specific, and therefore form the basis for diatom identification. This enables scientists from all over the world to use diatoms in palaeoecological research, even after a long period of time, as their outer silica cell walls remain in sediment (Round et al. 1990; Smol and Stoermer 2010; Verleyen et al. 2009). It is believed that the distribution of diatoms is controlled mostly by physico-chemical characteristics of habitats, which is why they serve well as bio-indicators (Verleyen et al. 2009). The same, however, also confines them to only a limited number of environmental conditions, under which individual diatom species are able to survive and reproduce (Round, Crawford and Mann 1990; Toporowska et al. 2008).

Some authors suggested, and it was believed for a long time, that very small organisms are all easily dispersed due to their small size (Baas-Becking 1934) and are therefore ubiquitous, with no geographic isolation, and that only the environment selects, making speciation less common and local extinction virtually impossible (Fenchel and Clarke 1999; Finlay 2002; Fenchel and Finlay 2004; Martiny et al. 2006). This initiated a large taxonomical discussion for diatoms, as diatom species had mostly cosmopolitan distributions in the 1980s.

Antarctic diatom species were especially force-fitted to European species (Tyler 1996), but a later narrower species concept followed by an abrupt description of many new diatom species [according to Mann and Vanormelingen (2013), elevated the number of estimated diatom species to range between 30,000 and ca. 100,000]. Contrary to the ubiquity theory, several authors demonstrated the presence of clear biogeographical distributions within freshwater diatoms (Vyverman et al. 2007) and argued that the presence of diatoms is regulated by the same processes as macro-organisms (Vanormelingen et al. 2008), although possibly to a different degree (*see* also Astorga et al. 2012), with their distribution being constrained more by historical processes than determined by ecological tolerances (Kociolek and Spaulding 2000).

Diatoms proved themselves as well-designed agents usable for both ecological monitoring and palaeoecological studies, which can add a significant knowledge about past local and regional climate. The geographical position of Gough Island itself provides the only landmass in an otherwise vast ocean of saline water making it possible to study changes in past climate through multi-proxy palaeoecological studies. Related to questions of past climate, and microbial distribution and dispersion, the island furthermore presents a unique locality. Its remoteness together with a long history entirely without human influence (reviewed in Vinšová 2014) gives a solid base on which communities of microorganisms and their biogeographical affinities could be studied.

Species concepts in diatoms

In the presented study, I am using a morphological species concept to investigate diatom moss-inhabiting communities of Gough Island. Diatoms have been identified based on their morphology since their first discovery in the beginning of 18th century (Anonymous 1703), and this concept has been widely used since then as the (only) identification criteria (Mann 1999, 2010). Diatoms reproduce mainly by asexual mitotic division consequently causing a decrease in cell size following each of these splits, and subsequent sexual reproduction is essential to restore their maximum size (Round et al. 1990). This necessity of sexual reproduction allows the applicability of the biological species concept (Mayr 1942, 1946). However, the interspecific crossing barriers are not always strict even in morphologically and genetically distinct species, which complicates the usage of the biological species concept as delimitation (Vanormelingen et al. 2008; Geitler 1979). The (traditional) separation of new diatom species based on morphology is complicated by life cycle changes, plasticity of phenotypes, unstable taxonomy, as well as by the identification itself, as it is always subjective to a certain level (Mann 2010). Since the use of light microscopy (LM) is still the most practical way to screen the diversity of a sample, the discovery of new diatom species begins with observed morphological variation in assemblages (Mann 2010). The fine identification of species based on morphology can be confirmed and further elaborated using scanning electron microscope (SEM), allowing the observation of important ultrastructural details of the frustules that are essential in species delimitation. Unfortunately, some species have a lower morphological complexity, precluding them from possessing a distinct morphology, forming species complexes that are morphologically indistinguishable (van Oppen et al. 1996; Scharfen 2014; Verbruggen 2014). One method of finer identification of morphospecies involves geometric morphometry techniques (Adams et al. 2004; Zelditch et al. 2004), which can significantly better identify the boundaries of single morphospecies, in the complex morphological variation which could be visually underestimated otherwise. The method of geometric morphometry has been used to point out the hidden diversity of some traditional species [*e.g., Navicula cryptocephala* (Veselá et al. 2009)] and to distinguish new taxa from some of the others [*e.g., Pinnularia divergentissima* (Van de Vijver et al. 2013), *Sellaphora* (Mann et al. 2004)], suggesting that many traditional species actually represent species complexes. All of these (morphological) approaches were used in **Chapter 4**, where the morphological variability within the diatom genus *Frustulia* was found to be difficult to distinguish visually. Only the valve outlines showed some noticeable differences, making geometric morphometry techniques sufficiently adequate to separate several distinct morphotypes.

The narrowest species concept is however accessible thanks to the molecular genetic methods. Sequencing methods and DNA barcoding enables accurate delimitation of inter-/intraspecific boundaries and can also be used to reveal (pseudo)cryptic species diversity (Mann et al. 2010; Evans et al. 2007). With the ongoing boom of molecular studies, it is clear that many traditionally recognized morphospecies actually represent a high degree of cryptic species [*e.g., Achnanthidium minutissimum* (Potapova and Hamilton 2007), *Eunotia bilunaris* (Vanormelinger et al. 2013), *Gomphonema parvulum* (Abarca et al. 2014), *Hantzschia amphioxys* and *Pinnularia borealis* (Souffreau et al. 2013), and *Sellaphora pupula* (Mann et al. 2004 and 2008; Vanormelingen et al. 2013)] that could potentially have a crucial effect on ongoing discussions about biogeography, ecological monitoring and palaeocological reconstruction. It also could refine the ecological preferences of some taxa when within the same complex distinct environmental and biogeographical preferences are present (Poulíčková et al. 2008). However, describing new species after distinct lineages, regarded as the phylogenetic species concept, is being questioned due to problems regarding important marker choice, sufficient sampling and species monophyly (Alverson 2008). Another problem is the increasing number of sequences in repositories (*e.g.,* in GenBank) that are not linked to correct species names (Clerck et al. 2013) and are therefore unusable for practical application of diatoms (*e.g.,* for ecological monitoring) which relies on morphospecies. A potential replacement of the traditional approach could be DNA barcoding, if the method establishes itself as a fully functional one for the practical application of diatoms worldwide (Kollár 2016; Mann et al. 2010). It should be noted here that one cannot simply refrain from a certain level of subjectivity even when using modern molecular studies, as the decision of 'where to cut' is again upon us.

Newly delimited species following one or two concepts can be in direct conflict with the other(s) and vice versa. De Queiroz (2007) takes species as separately evolving metapopulation lineages, suggesting that a unified species concept should be adopted by scientists. According to this, one should accept all approaches as diverse properties that might (or not) be achieved by the metapopulation lineages during the speciation process in the near (or distinct) future, and search for the most of those properties while delimiting new species, so it can stand on a robust base that is likely to survive further testing, if happened (de Queiroz 2007; Williams and Reid 2009).

As was previously stated, several methods of delimiting new species are being used by diatomologists worldwide and using only morphology to delimit new species might not prove to be sufficient in the (near) future for certain groups. Morphology is, however, a character-based approach of great significance rather than any 'actual' or 'potential' breeding behavior patterns (Williams and Reid 2009). Diatoms have a long-lived history of being described after the character-based morphological approach, and it is still the most widely used approach for discovering diatom species diversity, and subsequently for describing of new species (Mann 2010). As species are basically artificial units for our use, drawing their limits regarding any species concept should principally be at disposal for answering one's research question. I emphasize here the need of being acquainted with distinct approaches that are being used, and similarly as de Queiroz (2007) and Williams and Reid (2009) suggests, search for the most when separating diatom species possibly new to science while following *any* method chosen, to the best of our knowledge and possibilities given. The morphological method is an accessible one, the only possible when working with old (or even historical) material, and well executable when the locality studied is remote and hard to reach, but at the same time of non-negligible importance and therefore worthwhile to study, such as in the case of Tristan da Cunha in general, and Gough Island in particular.

In the present thesis, historical drawings, together with LM and SEM photographs, and, for *Frustulia* genus, also geometric morphometry techniques were used, to access the diatom flora of the studied locality in the most precise way, of which morphology can only provide us with. Even if fresh material usable for molecular methods would be available from the studied locality, this would have to be linked with the diatom species historically described from the archipelago (Carter 1966; Preece et al. 1986) so it won't only be adding another 'dark taxon' of no proper species name (Clerck et al. 2013). This way, we can link new observations with those already been made historically (**Appendix 1**), unhide the true taxonomical position of some (Vinšová et al. 2016 – **Chapter 3**) and with the use of finer methods of morphological observations, also identify the possible hidden diversity of some species complexes (*see* **Chapter 4**).

Regional and local history of scientific surveys

The history of sub-Antarctic diatom studies dates back to the 19th century when Reinsch (1876) investigated the diatom flora of Kerguelen, followed by others, as reviewed in Van de Vijver and Beyens (1999). Diatoms in these early studies were generally identified as cosmopolitan, with no typical flora described for the sub-Antarctic region. In 1956, Mr. R. Wace collected two samples containing diatomaceous material from Gough Island during the Gough Island Scientific Survey, and in 1962, during an expedition organized by the Royal Society, Mr. Dickson collected ten samples from Tristan da Cunha Island (Carter 1966). Those were subsequently analyzed for diatoms by John Ripley Carter (1908-1993), an amateur diatomologist who mainly studied the British freshwater diatom flora. He made a contribution to the diatom taxonomy of not only a wide area in Scotland, but also the outer islands, prepared many thousands of slides from some 5.000 samples, and contributed widely to the knowledge of freshwater diatoms (Hartley 1994). John R. Carter analyzed the samples from the archipelago, and described numerous species as new to science, accompanied by small drawings. In total, 56 new species and formas from the Tristan da Cunha archipelago were described in Carter (1966) (*see* **Appendix 1**). However, several of these were not described validly according to the International Code of Botanical Nomenclature. For most new species holotypes were designated, but one species (*Pinnularia percepta*) was only described as a drawing. Some species were erroneously described as new should be considered as synonyms of previously described species (*e.g., Nitzschia rotula* is actually *N. terrestris*). Some were named identically with already existing species and got renamed later (*e.g., Eunotia johncarteri,* but named by Carter as *E. pectinoides*) and some have already been moved to different genus (*e.g. Achnanthes atalanta* is now accepted as *Psammothidium atalanta*).

These taxonomic and nomenclatorial problems, together with the general difficulties of identifying a diatom flora using old taxonomical publications, necessitated a revision prior to further analysis of recent diatom material. Williams and Reid (2002) listed all the species described by Carter, including those from the Tristan da Cunha archipelago, and made some nomenclatural changes in cases where necessary, which is, together with the revision, also listed in attached **Appendix 1**.

Although several of the original slides of Carter (1966) were preliminarily analyzed for species' presence compared to their original descriptions, species presented in this study, similar to Carter's, were also observed in new moss samples from Gough Island. For a formal comparison and altering the status of most, a proper formal morphological and taxonomical analysis is necessary. As an example, we revised one species described by Carter (1966) as *Pseudoeunotia linearis* (Vinšová et al. 2016). It was obvious from its morphological characteristics that the species did not belong to the (exclusively marine) genus *Pseudoeunotia* making therefore a transfer to the genus *Eunotia* necessary (Vinšová et al. 2016). This study is explained in **Chapter 3**. Other species probably also new to the science were observed in Carter's slides but left undescribed by him, and these would have to be analysed and eventually formally described, as was the case for *Orthoseira gremmenii* (Van de Vijver and Kopalová 2008).

Study area

Gough Island (40°21' S, 9°53' W) is a mountainous island, with most of the land above 400 m a.s.l., and the highest point being the Peak of Edinburg (910 m). The island is surrounded by orographic clouds and situated in the cool-temperate zone of the southern Atlantic Ocean (the so-called 'roaring forties', **Figure 1.2**) that influences the weather on Gough widely (Wace 1961). The coastline consists of steep sea cliffs and narrow boulder beaches.

Gough Island is almost uninhabited, apart from a small permanent crew (7 people) maintaining a South African weather station. Its climate is wet and cool with frequent gale-force winds, a mean annual temperature of 11,7°C (sea level), and 3000 mm of average annual precipitation. The main habitat types at sea level consist of tussock grasslands, fern bush, wet heathlands, peat bogs and alpine habitats (**Figure 1.3**) (Wace 1961; Wace and Dickson 1965; Andrew 2007).

As the island is one of the most remote and protected localities in the world (State of Conservation Report 2016), access permits are granted only to a limited number of persons (mainly scientists). As far as we know, no algologist has been present on the island. Gough Island is dominated by mosses, which inhabit broad gradients of environmental (*e.g.* moisture level) and physical (*e.g.* elevation) variables, and can be easily sampled in field for diatomaceous material.

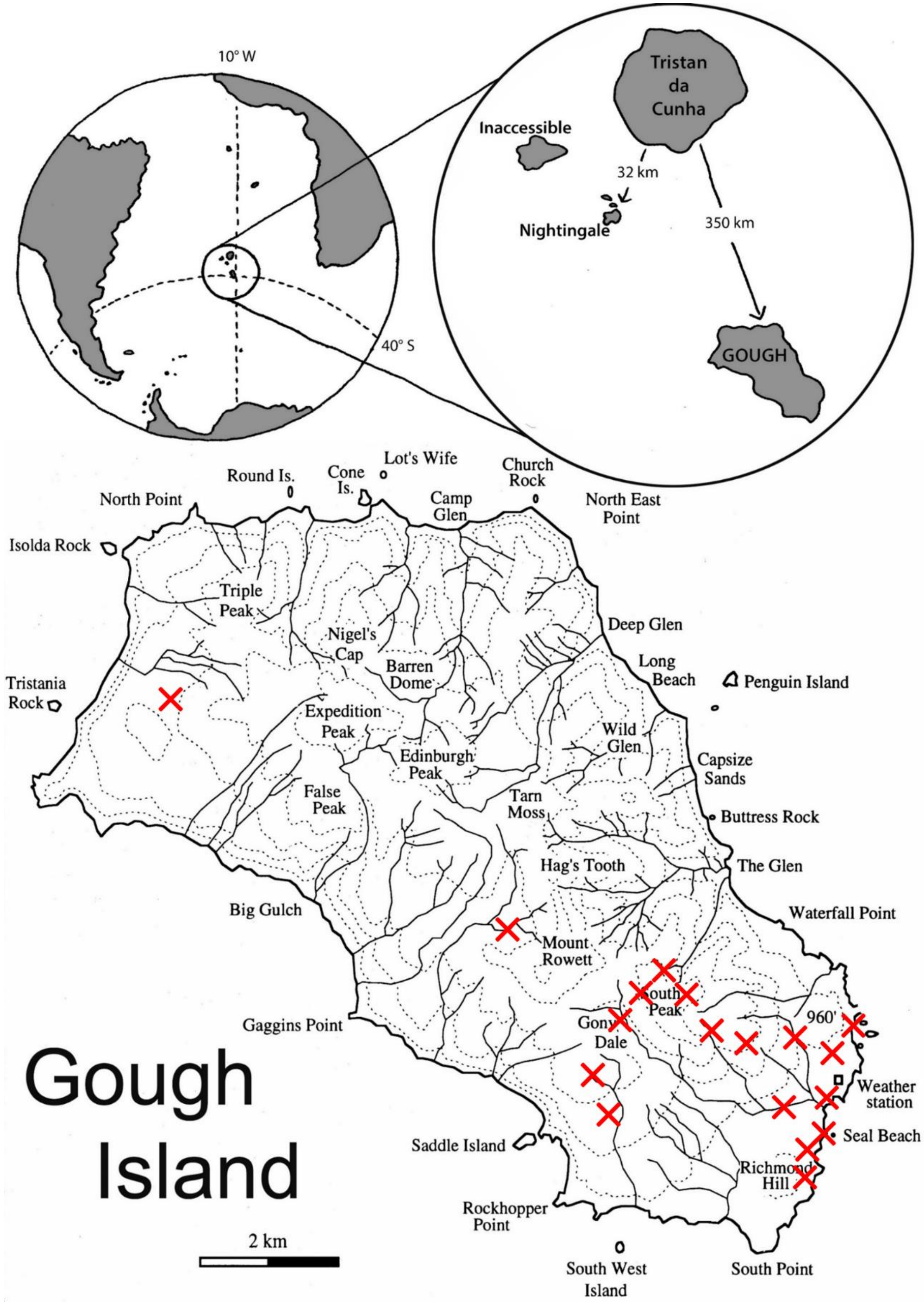

Figure 1.2. The South Atlantic Ocean with the Tristan da Cunha archipelago highlighted. Map of Gough Island with elevation levels, streams and some local geographical names. Red crosses ✕ indicate sites sampled and analysed for diatoms.

AIM AND OBJECTIVES

The presented thesis contributes to the general knowledge of diatom communities inhabiting various islands located in the Southern Hemisphere (Southern Oceans, sub-Antarctica and Maritime Antarctica). To be able to answer important and interesting questions about the diversity and structure of the island diatom flora, the dispersion of diatoms in between patchy lands of Southern Oceans, or even the evolution of diatoms in general, it was necessary to analyse the flora taxonomically first. Since the only publication describing the unique diatom flora of Gough Island was published 50 years ago (Carter 1966), a new morphological and taxonomical revision of the diatom flora was necessary prior to any further research. Using environmental parameters and diatom species distributions, the overall aim of this thesis is thus to improve our knowledge about one of the most remote islands in the world.

The present thesis focuses in particular on the following topics and questions:

- Taxonomy and diversity of the Gough Island diatom flora. Can the flora be identified using currently available literature? Is it possible to identify or link the observed diatom taxa with the taxa described in the historic publication of Carter (1966)? Is the diatom composition and species richness characteristic for a remote oceanic island? Are the observed species typically cosmopolitan, sub-Antarctic, or unique with regard to their biogeography?
- Ecological characterization of diatom communities inhabiting moss habitats of Gough Island. Do the diatom communities reflect specific environmental conditions? Are those thrived by any environmental gradient, and is it analogous with comparable localities? Can ecology cause a shift in a morphology of specific diatom species?
- Distribution of diatoms, and the similarity analysis of Gough Island and other islands of Southern Oceans. Are diatom moss-inhabiting communities similar to any of the others? Is it the distance, or rather the ‘on wind’ geographical position of a single island, what is more important in the distribution (and dispersal) of diatoms?

THESIS OUTLINE

GENERAL INTRODUCTION, MATERIAL & METHODS

Chapter 1 provides a brief introduction to the study area, on the overall importance of diatoms and a brief history of diatom studies on the Tristan da Cunha archipelago. Furthermore, the general material and methods used in the following chapters are discussed.

TAXONOMY, ECOLOGY & DISTRIBUTION RELATED TOPICS FORMING THE THESIS

Chapter 2 focuses on the general characteristics of moss-inhabiting diatom communities on Gough Island. First, species richness and diversity are discussed, followed by results concerning the diatom community structure and ecological characterization of the community. Environmental gradients are studied and the most important parameters are identified. Finally, a similarity analysis is performed to evaluate differences and similarities between Gough Island and different Southern Ocean islands to identify the biogeographical position of Gough Island.

Chapter 3 presents detailed analysis of the morphology and taxonomic position of an interesting diatom species, found in the moss material of Gough Island and identified based on the description in Carter (1966), the original material of which need to be analyzed. This chapter serves as an exemple for all further formal revisions of taxa described by Carter (1966) that were also found during this survey (and are included in **Appendix 1**).

Chapter 4 deals with species complex that is quite frequently found to be inhabiting moss habitats of Gough Island and that proved to have a quite difficult morphology. Several morphotypes were separated based on light and scanning electron microscopical observations, and their morphological variation in valve outline was furthermore analysed using modern geometric morphometry techniques. Furthermore, the extent to which the distribution of different morphotypes is defined by the environmental conditions was analyzed. At the end of this chapter, we briefly discuss whether these morphotypes might represent distinct species rather than simply ecotypes, and if they should be formally described as new to the science.

GENERAL DISCUSSION

In **Chapter 5**, the diversity and species composition of moss-habitats of Gough Island is discussed in the context of insularity and also compared on a regional scale. Chapter 5 further discusses the dispersal abilities of microorganisms and diatoms in particular, together with long-distance dispersal events and theories, and regional and local distribution patterns. The similarity analysis based on Sørensen-Dice index, comparing diatom moss-inhabiting communities of southern oceans islands, resulted in basic biogeographical patterns. Moreover, the overall environmental characterization of individual groups identified on Gough Island is given and discussed. Further, the current widely-discussed problem of species concepts in diatoms is summarized, and finally the apparent insular context of the observed diatom flora is shown. The beginning of **Chapter 5** summarizes and concludes the main results.

APPENDICES

Appendix 1 presents a taxonomic overview and a revision of diatom taxa observed in the recent material that were identified based on the historical publication of Carter (1966). Figures here present a unification of species using historical drawings together with pictures of modern light and scanning electron microscopy.

In **Appendix 2**, a list of all species observed and identified from the recent moss material is provided, together with authorities.

In **Appendix 3**, two abstracts of posters that were presented on international conferences during my master studies are attached. Both of them respectively concerns the topics which are studied in *Chapters 3* and *4* of the presented thesis.

Appendix 4 shows a paper published in my second year of masters, concerning similar ecological dispersal-related questions – in particular, to what extent adjacent habitats are responsible for structuring diatom assemblages? The locality studied in this paper is Spitsbergen (High Arctic); therefore, the paper is attached in the appendix and not included directly in the thesis.

GENERAL MATERIAL AND METHODS

Field sampling

Samples of mosses were gathered during the 2000 and 2004 summer seasons by Dr. Niek J.M. Gremmen[1], with a focus on the south part of Gough Island, and in 2006 Dr. J. Barendse[2] gathered a few moss samples from Inaccessible Island. Samples are now stored at the BR-collection, property of the Belgian federal government and given in permanent loan to the Botanic Garden Meise (Belgium). Several distinct habitats were identified and sampled, ranging from coastal cliffs and rockpools, across drainage channels and small rivers, to peat bogs, mires and peat slopes (**Figure 1.4**). Distinct habitats were sampled in order to have a representative set for the whole island, since some parts of the island are not easily accessible (especially the North gully and inland mountainous parts, **Figure 1.2**). Each habitat can be subdivided according to different main parameters. Environmental characterization of each site sampled was evaluated in field as in Van de Vijver et al. (2008). Briefly, five environmental parameters were measured for each site, including 'biotic influence' (0=none, 5=very heavy manuring and trampling), 'elevation level' (m a.s.l.), 'shelter' (1=very exposed, indicating in full sun light, 5=very sheltered), 'moisture' (1=dry, 6=water), and 'sea spray' (0=none, 5=very heavy, including inundation by waves), noted was also 'habitat' type which could be summarized as presented in **Figure 1.4**. Additional pH measurements and GPS coordinates for some localities were taken during the 2006 campaign, and we could therefore use them for a better image of the study locality and sites studied. Due to logistic constraints, it was unfortunately not possible to acquire more environmental measurements.

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[1] **Dr Niek J.M. Gremmen** – *Data-Analyse Ecologie, Hesselsstraat 11, 7981 CD Diever, The Netherlands*

[2] **Dr Jaco Barendse** – *Sustainability Research Unit/SANParks, Nelson Mandela Metropolitan University, Private Bag X6530, George 6530, South Africa*

Figure 1.3. *A view to the typical moss-dominated environment on Gough Island. Photos by Niek Gremmen (2000).*

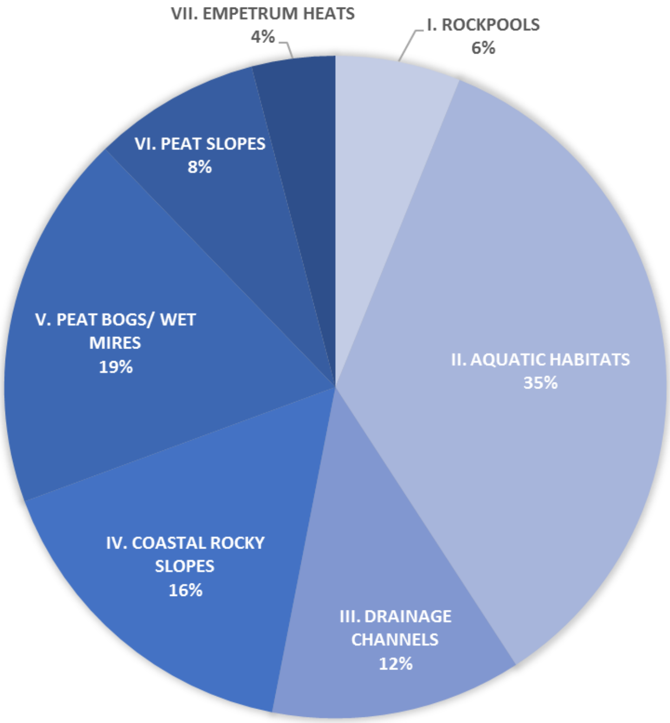

Figure 1.4. Distribution of samples over the major habitat types identified.

Slide preparation and enumeration

Mosses were prepared for light microscopy (LM) observation following the method described in Van der Werff (1955). Subsamples were cleaned by adding 37 % H_2O_2 and heating to 80 °C for about 1 h. The reaction was completed by addition of $KMnO_4$. Following digestion and centrifugation (3 x 10 minutes at 3700 x g), cleaned material was diluted with distilled water to avoid excessive concentrations of diatom valves on the slides. Cleaned diatom material was mounted in Naphrax®. Resulting permanent slides were analysed using an Olympus BX53 microscopes, equipped with differential interference contrast (Nomarski) equipped with UC30 Imaging System. For scanning electron microscopy (SEM), part of the oxidized suspension was filtered through a 1-µm Isopore™ polycarbonate membrane filter (Merck Millipore, Bedford, MA, USA). The stub was sputter-coated with a Gold–Palladium layer of 20 nm and studied in a ZEISS Ultra SEM microscope at 3 kV (Natural History Museum, London, UK) or a JEOL-JSM-7100 at 5 kV (Botanic Garden Meise, Belgium).

Almost 100 slides were scanned during a preliminary observation of the biodiversity. Some slides from the sample set were either empty or very low in valve number, and were therefore removed from further analysis. A subset of the remaining samples was chosen for community analysis in order to represent different types of habitat and to cover as much as possible altitudinal and environmental gradients. For each slide, at least 400 valves were identified and enumerated at random transects. In total, 49 slides were counted from Gough Island, with additional slides counted for Inaccessible (2) and Tristan da Cunha (1). The diatom taxa were identified as much as possible up to species level, variety or forma. When the taxonomic status of a taxon was uncertain, 'cf.' (*confer*: probably belongs to the species identified), 'aff.' (potentially new and undescribed species has affinities with a known species), or 'sp.' (species of genera given) were used.

For the identification of diatom species, mainly the following publications were consulted: Carter (1966); Preece et al. (1986); Cox (1995); Lange-Bertalot and Metzeltin (1996, 2001); Van de Vijver et al. (2000, 2002, 2008b, 2008c, 2013, 2014); Van de Vijver and Kopalová (2008); Poulíčková et al. (2010); Hofmann et al. (2011); Lange-Bertalot et al. (2011); Lowe et al. (2014); Sterken et al. (2015); Wetzel et al. (2015).

Relative abundances of taxa were calculated from diatom valve counts from each sample prior to further statistical analyses. Statistical analyses in the following chapters were performed using the **R** statistical environment (R Core Team 2016; RStudio Team 2015) or using the free statistical software **PAST** v.3.10 (Hammer et al. 2001).

CHAPTER 2

GENERAL ECOLOGY

The ecological part of the master thesis presents results in observed diversity and species richness, community structure, environmental characteristics and similarity analysis of diatom moss-inhabiting communities of Gough Island within the southern oceans islands.

METHODS

Statistical analyses

To evaluate to which extent our assemblage truly represents the total **species richness** of moss habitats on Gough Island, the Incidence Coverage-based Estimator of species richness (ICE – Lee and Chao 1994), the mean Chao2 richness estimator (Chao 1984; Colwell 2005), and second order Jackknife (Jack2 – Smith and Van Belle 1984), all non-parametric, were calculated using EstimateS program version 9.1 (Colwell 2013). To create a species accumulation curve, sites were ordered randomly and the species richness from the counts was estimated under 'Jack2' using *vegan* R package (Oksanen et al. 2016).

To reveal patterns in **species composition** among the samples, a distance matrix was calculated using the Bray-Curtis dissimilarity method, which exhibited the best fit for the dataset using the 'rank index' function in the *vegan* R package. From this, a hierarchical dendrogram was produced utilizing the 'average' clustering method (coefficient = 0.9). A distance cut-off value of 0.9 was set to reveal the main floral assemblages, resulting in five main groups. Significant differences among these five groups was tested using permutational multivariate analysis of variance (PERMANOVA, 999 permutations, $p < 0.05$). To gain more insight into the species composition patterns, the fifth (and largest) group was further split into three sub-groups under a lower cut-off value. To visualize these differences in diatom **flora structure** among samples clustering together in the dendrogram, samples were ordered after the calculated distance matrix, and an 'inkspot' plot was created using the *rioja* R package (Juggins 2015) using square-root transformed data of non-rare taxa (> 1.0 %) and plotted under the dendrogram. To better visualize the diatom taxa driving these groupings, a Principle Components Analysis (PCA) was performed using PAST v.3.10 (Hammer et al. 2001). For each of the five groups identified, α diversity (Shannon index, Buzas and Gibson's evenness (e^H/S) (Harper 1999)) was assessed using PAST software.

To detect **patterns in diatom species distribution as explained by environmental variables**, a distance-based Redundancy Analysis (dbRDA) was performed. This analysis carries out constrained ordinations using non-Euclidean distance measures, and analyses were performed using 'capscale' function of *vegan* R package under square-root transformed data and the Bray-Curtis dissimilarity method. Firstly, the correlation of all environmental variables was assessed before being added to the ordination with the 'vif.cca' function of *vegan* R package. The model was then performed using all independent (variance inflation factors < 3.0) environmental variables and plotted at both the species and genera levels. The significance of each term (environmental parameter), and of the full ordination model (999 permutations, $p < 0.05$) were both tested using a permutational analysis of variance (ANOVA). Significant differences within habitats incident to samples was tested using permutational multivariate analysis of variance (PERMANOVA, 999 permutations, $p < 0.05$).

Similarity analyses were performed to compare the moss-inhabiting diatom flora of Gough Island with those of other Southern Ocean islands. A symmetric similarity matrix was then calculated using the Sørensen-Dice similarity index formula:

$$d_{jk} = 2M / (2M + N)$$

where M is the number of taxa shared by the two sites, and N is the total number of taxa exclusively observed in each of the two sites (Dice 1945, or Sørensen 1948).

From the sub-Antarctica, South Georgia was included for the South Atlantic Province (Van de Vijver & Beyens 1997), Heard Island (Van de Vijver et al. 2004) and Prince Edward Islands (Van de Vijver et al. 2008) as representatives for the South Indian Province. Three representatives of the Maritime Antarctica were added for the comparison too, Livingston and James Ross Islands (Kopalová et al. 2014), together with King George Island (Vinocur and Maidana 2010). Two other islands from the home archipelago were added for comparison, the main island Tristan da Cunha and Inaccessible Island, although they only included one and two samples counted respectively, and the resulting comparisons should be taken only as illustrative rather than an actual evidence. Reviewed data of moss-inhabiting diatoms present on all the localities above were transformed to presence/absence data and analyzed. Localities are summarized in **Table 2.1** together with counting scores of each, if given.

To present the affinities within the analyzed islands, a cluster analysis was created utilizing the neighbor-joining method under the same similarity index. The neighbor-joining cluster analysis method finds pairs (neighbors) and minimize the total branch length, resulting in an unrooted dendrogram (Saitou and Nei 1987).

Table 2.1. Full list of localities used for the similarity analysis. Only taxa occurring in moss habitats were included in the analysis.

Abbre-viation	Island	Reference	Number of taxa	Counting scores, *ND* if unknown
GI	Gough	this thesis	141	94 % (ICE, Chao2), 82 % (Jack2)
INI	Inaccessible	this thesis - *illustrative*	25	*ND*
TDC	Tristan da Cunha	this thesis - *illustrative*	25	*ND*
LVI	Livingston	Kopalová et al. (2014)	123	87–89 % (ICE, Chao2)
JRI	James Ross	Kopalová et al. (2014)	69	62–69 % (ICE, Chao2)
HI	Heard	Van de Vijver et al. (2004)	178	*ND*
KGI	King George	Noga and Olech (2004); Vinocur and Maidana (2010)	160	*ND* *ND*
SGI	South Georgia	Van de Vijver and Beyens (1997)	93	*ND*
AI	Amsterdam	Chattová (2016) *unpublished results*	126	*ND*
MI	Marion	Van de Vijver, Gremmen and Smith 2008	175	~ 87 % (ICE, Chao2)
PEI	Prince Edward	Van de Vijver, Gremmen and Smith 2008	153	~ 87 % (ICE, Chao2)

RESULTS

Species composition and diversity

A total of 141 taxa belonging to 50 genera were observed during the survey of moss samples (**Appendix 2**). Seventeen of these taxa and one genera were found only during the preliminary scanning of the samples for assessing the flora diversity before counting. Those were usually represented by only a few valves in the whole assemblage (*i.e., Achnanthes muelleri*, *Halamphora incurvata*, *Navicula decissa* and *Pinnularia* cf. *seriata*) and were not included in the further statistical analyses. **Appendix 2** provides a full list of taxa observed in this study with associated authorship name, along with abbreviations used in figures. Of these, 38 taxa (three outside the counts) could not be identified to the species or lower taxonomic level. Many of the others were identified only after what Carter (1966) described as new to the science, but here more in-depth morphological identifications are necessary to clarify the taxonomic status for further studies, as illustrated by *Pseudoeunotia linearis* which was recently transferred to the genus *Eunotia* (Vinšová et al. 2016). Several other taxa belonging to genera *Chamaepinnularia, Eunotia*, *Frustulia*, *Humidophila*, *Melosira* and *Pinnularia* represent species most likely new to science and should be formally described in the future.

Using estimators of species richness, it is possible to indicate the theoretical total number of taxa present on the locality studied, and therefore project to which extent our assemblage represents the true biodiversity of the island's diatom moss-inhabiting flora. The expected total number of taxa in all samples is 133 (ICE & Chao2) or 152 (Jack2), suggesting that the sampling effort, and our counting protocol respectively, scored about 94 % (ICE, Chao2) or 82 % (Jack2) of all taxa present. The flattening of the species accumulation curve towards the end also indicates that a large part of the island's moss-inhabiting diatom flora has been collected (**Figure 2.1**).

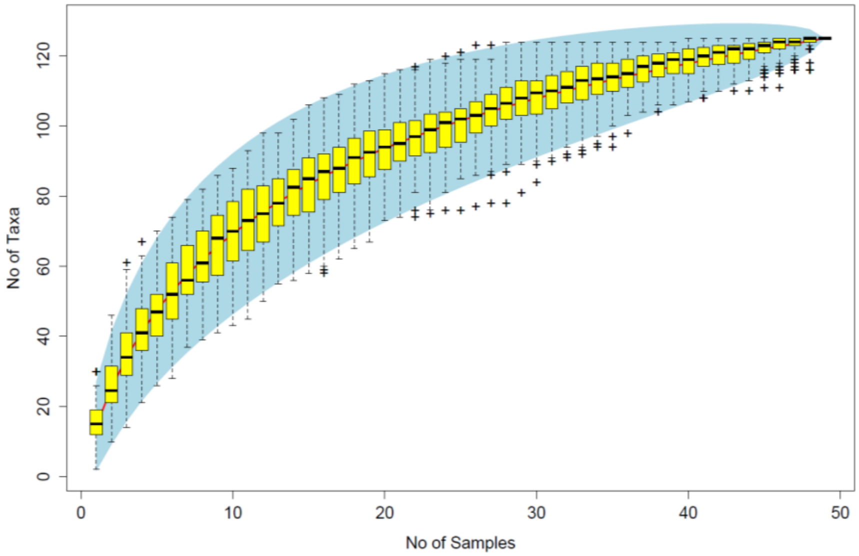

Figure 2.1. Species accumulation curve for the total sample set.

Species richness per sample ranged from 2 to 30 taxa, with a median value of 14. Out of 124 taxa identified during counting, 50 were found in one or two samples only, whereas the most common taxa were present in 33 (*Eunotia paludosa* var. *paludosa* Grunow group), 30 (both forms of *Eunotia johncarteri*), 24 (*Pinnularia posita*, *Psammothidium atalanta*) and 23 (*Eunotia johncarteri* forma thin, *Pinnularia restituta*) samples out of 49 in total. The eleven most abundant taxa (> 2.5 %) were composed of the most common ones listed, and of *Eunotia* cf. *fallax*, *Distrionella germanii*, *Eunotia* cf. *ambigua, Chamaepinnularia* cf*. begeri* and *Melosira* spX, accounting together whole 65 % of all valves counted.

Ten most dominant genera represented 91.7 % of all counted valves (**Figure 2.2**), illustrating the clear dominance of few genera only. Among the most taxon rich genera belonged *Pinnularia* with 17 taxa, *Eunotia* (16 taxa), *Navicula* (10 taxa), *Nitzschia* (9 taxa), and *Humidophila* (7 taxa), although the relative abundances of each genera revealed clear dominance of *Eunotia* (42 %), distantly followed by *Pinnularia* (14 %), *Psammothidium* (9 %), *Chamaepinnularia* (5 %), *Humidophila* (5 %) and the others (< 5 %) as is shown in **Figure 2.2.**

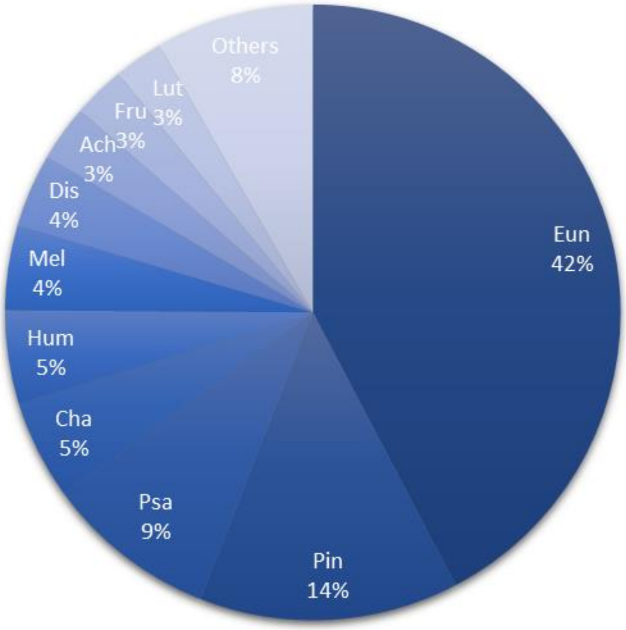

Figure 2.2. Composition of moss-inhabiting diatom community (genera level) based on abundance. Eunotia, Pinnularia, Psammothidium, Chamaepinnularia *and* Humidophila *representing genera of > 5 % (approximated). Abbreviations of the others:* Mel – Melosira, Dis – Distrionella, Ach – Achnanthes, Fru – Frustulia, Lut – Luticola.

Community analysis

Based on different diatom structure among the samples, it was possible to divide them into five, statistically significant (F = 6.07, R^2 = 0.35 at $p < 0.001$), groups (**Figure 2.3 A**). The first three groups composed of only two, four and two samples respectively. The fourth group composed of 13 samples and the last group was composed out of the most, 28 samples. The fifth, widest, group was divided into three sub-groups for more detailed view. The 'inkspot' plot visualizes the flora samples structure and also dominant taxa for each group (**Figure 2.3 B**) (*see* **Appendix 2** for taxa abbreviations, and authorities).

The different groups can be characterized by a typical dominant diatom flora. **Group A** is characterized by *Luticola* sp1, and slightly by *Humidophila contenta* otherwise in low abundances throughout the assemblage. **Group B** is dominated by *Achnanthes okamurae* and *Planothidium pericavum*, with a slight effect of *H. nienta.* **Group C** is characterized by *Melosira* spX and *Eunotia* cf. *lecohui*, slightly also by *E. ambigua* and *Pinnularia restituta.* **Group D** is dominated clearly by *E. paludosa* var. *paludosa*, and only low influences of *E.* cf. *fallax* and *Chamaepinnularia* cf. *begeri* helped to separate this group. **Group E** is the most difficult one, representing few **sub-groups** (**E1**, **E2** and **E3**) of distinct dominants of lower separation values. This group could be characterized as group of *Distrionella germanii–Psammothidium atalanta–E.* spp–*Pinnularia restituta.* Both *P. atalanta* and *E. johncarteri* forma thin represents binding elements after their appearances throughout the group and abundances together.

A PCA ordination created to visualize the distribution of the samples explained 39.12 % of the variation of the species composition along Axis 1 and Axis 2 (**Figure 2.4**). The driving diatom taxa of each group is superimposed here with corresponding abbreviations and prolonged lines. Along Axis 1, the main gradual separation of **Group D is** driven by *E. paludosa* var. *paludosa*, whereas other groups vary little from each other. On the other side, Axis 2 explains the variation mainly among the other groups from group D, and especially notable here is the intra-group split of Group E into sub-groups identified, **E1+2** (lower left) from **E3** (upper left).

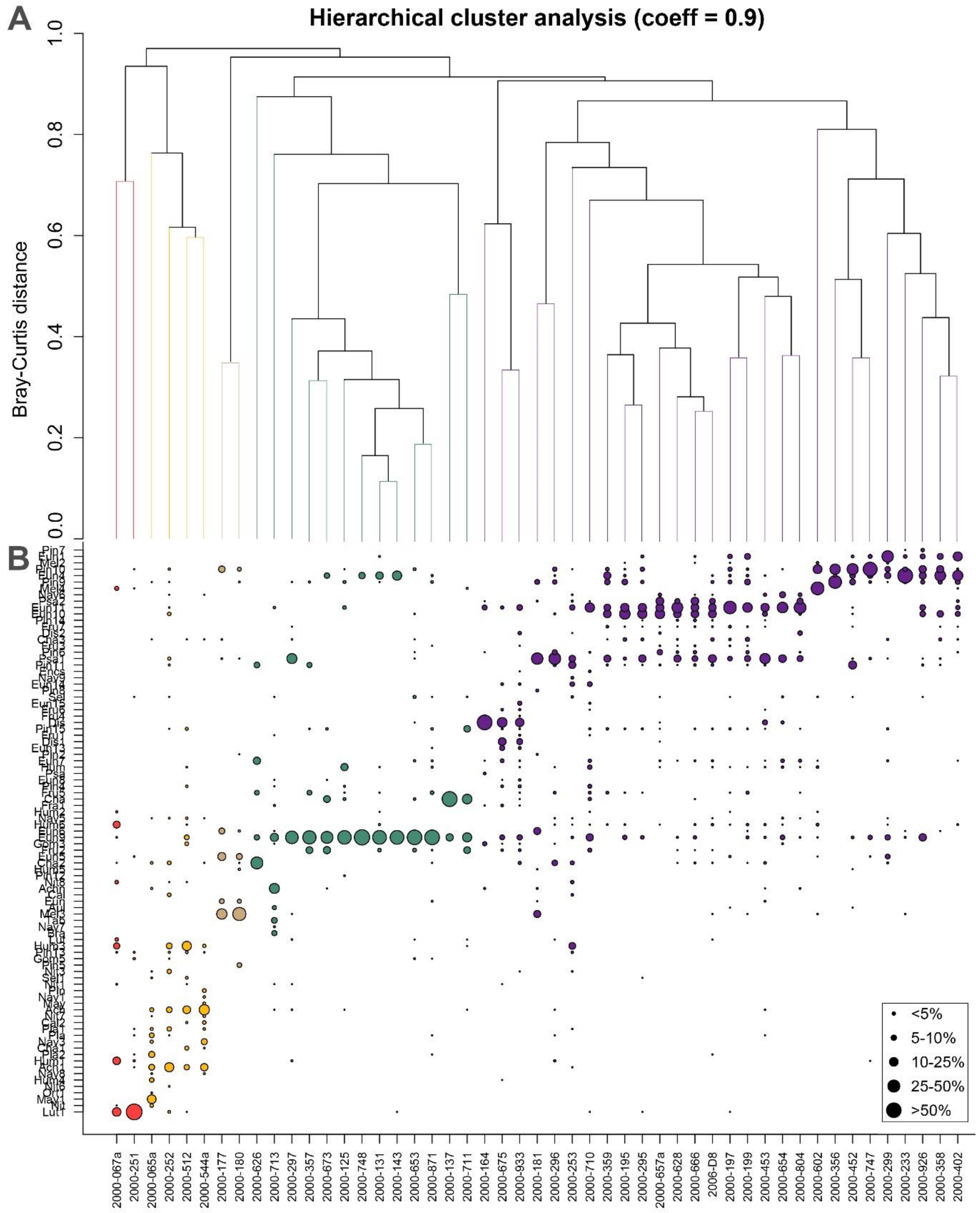

*Figure 2.3. Community analysis. (**A**) Hierarchical cluster dendrogram (Bray-Curtis distance using Average algorithm of transformation), five groups are indicated by different colors and those continues also in (**B**) an 'inkspot' plot, ordered according to the above diagram visualizing community structure and indicating dominant taxa per each group (abbreviations of species, see list in **Appendix 2**).*

Whereas sub-group E1 and E2 are driven by *E. johncarteri* and *P. atalanta,* it is *P. restituta* together with *E.* cf. *fallax* responsible for the conspicuous intra-group E split.

The PCA ordination model of samples clearly unionized first three groups identified, which variated only a little along both axes, visualizing clearly the flora structure distinctness of these three groups. **Groups A**, **B** and **C**, representing together eight samples only, and although they were identified as significantly distinct from each other, their diatom community structure reflects similar environmental factors, as will be discussed later.

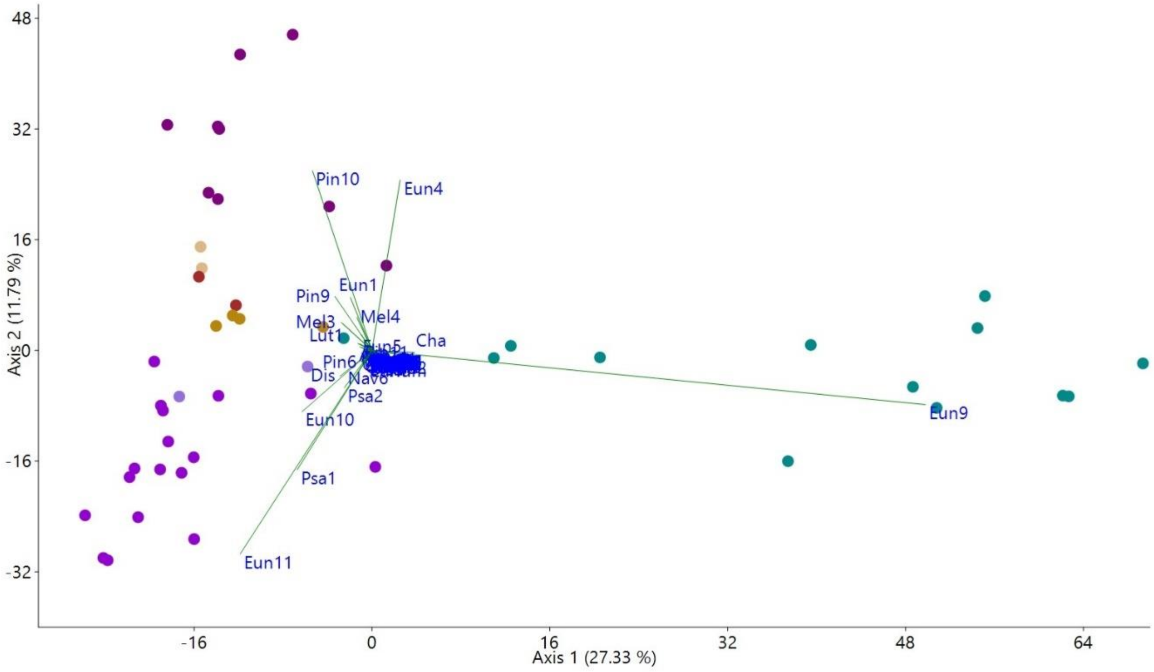

Figure 2.4. Principal component analysis (PCA). Five groups (and three sub-groups) are indicated by the similar colors. Note the restricted distribution of different groups and the split of Group E along Axis 2 (sub-groups E1+2 (lower left) and E3 (upper left)) following the two dominant taxa.

General characteristics of each group are summarized in **Table 2.2**, and their environmental characteristics are summarized by several boxplots with error bars in **Figures 2.5** and **2.6.**

Table 2.2. *The characteristics unified per each of five group (and three sub-groups) clustered. Sample, diversity, and environmental characteristics*

Each value: minimum and maximum (median in brackets)

	Group A	Group B	Group C	Group D	Group E	sub-groups:		
						1	2	3
Number of samples	2	4	2	13	28	2	17	9
Number of taxa in the counts	15	18–30 (22)	7–11 (9)	2–24 (11)	5–26 (14.5)	9–19 (14)	13–26 (19)	5–15 (11)
Shannon index (based on the counts)	1.48–1.84 (1.6)	2.74–2.98 (2.95)	1.45–1.71 (1.56)	1.93–2.10 (1.97)	2.99–3.08 (3.04)	1.48–1.89 (1.74)	2.79–2.92 (2.86)	2.12–2.26 (2.17)
Evenness index (based on the counts)	0.2–0.28 (0.19)	0.28–0.35 (0.32)	0.35–0.46 (0.39)	0.10–0.12 (0.11)	0.23–0.25 (0.24)	0.22–0.32 (0.25)	0.20–0.23 (0.21)	0.24–0.27 (0.25)
Altitude (m)	20–30 (25)	20–35 (20)	15	25–700 (500)	15–550 (90)	300–475 (388)	15–500 (90)	15–550 (90)
Shelter	2–4 (3)	2–4 (3)	5	1.5–5 (3)	2–5 (4)	2–4 (3)	2–5 (3.5)	3–4 (4)
Moisture	2	3–5 (4)	4.5	3–5.5 (4.5)	3–6 (5)	5	3–6 (5)	3–5.5 (4.5)
Sea spray	0.1–4 (2.05)	0.5–4 (1.5)	1	0	0–4 (0)	0	0–4 (0)	0–1 (0)
Biotic influence	0-1 (0.5)	0-0.1 (0)	0.1	0	0-1 (0)	0	0-1 (0)	0-1 (0)
Habitat types								
I. ROCKPOOLS	0	2	0	2	0	0	2	0
II. AQUATIC HABITATS	5	8	1	15	1	0	0	0
III. DRAINAGE CHANNELS	1	2	1	4	2	0	0	0
IV. COASTAL ROCKY SLOPES	1	1	0	2	1	2	2	1
V. PEAT BOGS/ WET MIRES	0	1	1	2	7	0	0	0
VI. PEAT SLOPES	2	1	0	3	1	0	0	0
VII. EMPETRUM HEATS	0	0	0	0	1	0	0	1
Habitat characteristics								
0 = soil	0	1	0	3	7	0	5	2
1 = rock	2	4	2	5	20	2	11	7
2 = coastal	2	3	2	1	7	3	3	1
3 = running water	0	0	0	2	16	0	11	5
4 = standing pools	0	1	0	0	1	0	1	0
5 = boggy areas/wetlands	0	0	0	5	2	0	1	1

Ecological analysis

The first dbRDA done on the species level, was highly significant (pseudo-$F = 2.25$, $p < 0.001$), with the first two axes ($p < 0.001$) accounting for 72.08 % of the constrained proportional variance (**Figure 2.7 A**). Each term (parameter) used was tested independently, which resulted for 'bio' ($F = 1.72$, $p < 0.05$), 'elevation' ($F = 4.91$, $p < 0.001$), 'shelter' ($F = 2.91$, $p < 0.01$), 'moisture' ($F = 2.0$, $p < 0.1$) and 'salt' ($F = 3.66$, $p < 0.001$).

On the diatom species level (**Figure 2.7 A**), the environmental variables of 'salt' and 'elevation' were strongly negatively correlated along both axes but more pronounced along Axis 1, indicating that these two represent samples of habitats significantly distinct. Mostly samples of groups A, B and C are highly responding to the parameter 'salt' (lower left), whereas group D highly corresponds along the parameters of 'elevation' (*see* also elevation range of (sub-)groups in **Figure 2.5**). Other important environmental variables having effect on the species distribution among different habitats is 'shelter', strongly negatively correlated with 'elevation' along Axis 1.

Another dbRDA was plotted with diatoms at the genera level, for assessing the general comparison of diatom flora community structure and its potentially distinct responses on each level. The resulted ordination analysis was also highly significant (pseudo-$F = 2.63$, $p < 0.001$), of Axis 1 ($p < 0.001$) and Axis 2 ($p < 0.05$) accounting together 80.84 % of the constrained accumulated proportional variance (**Figure 2.7 B**). Each term was tested for significance, resulting in 'elevation' ($F = 4.99$, $p < 0.001$), 'shelter' ($F = 2.49$, $p < 0.05$), 'salt' ($F = 5.82$, $p < 0.005$), 'moisture' ($F = 4.34$, $p < 0.001$) and 'bio' ($F = 1.79$, $p < 0.05$).

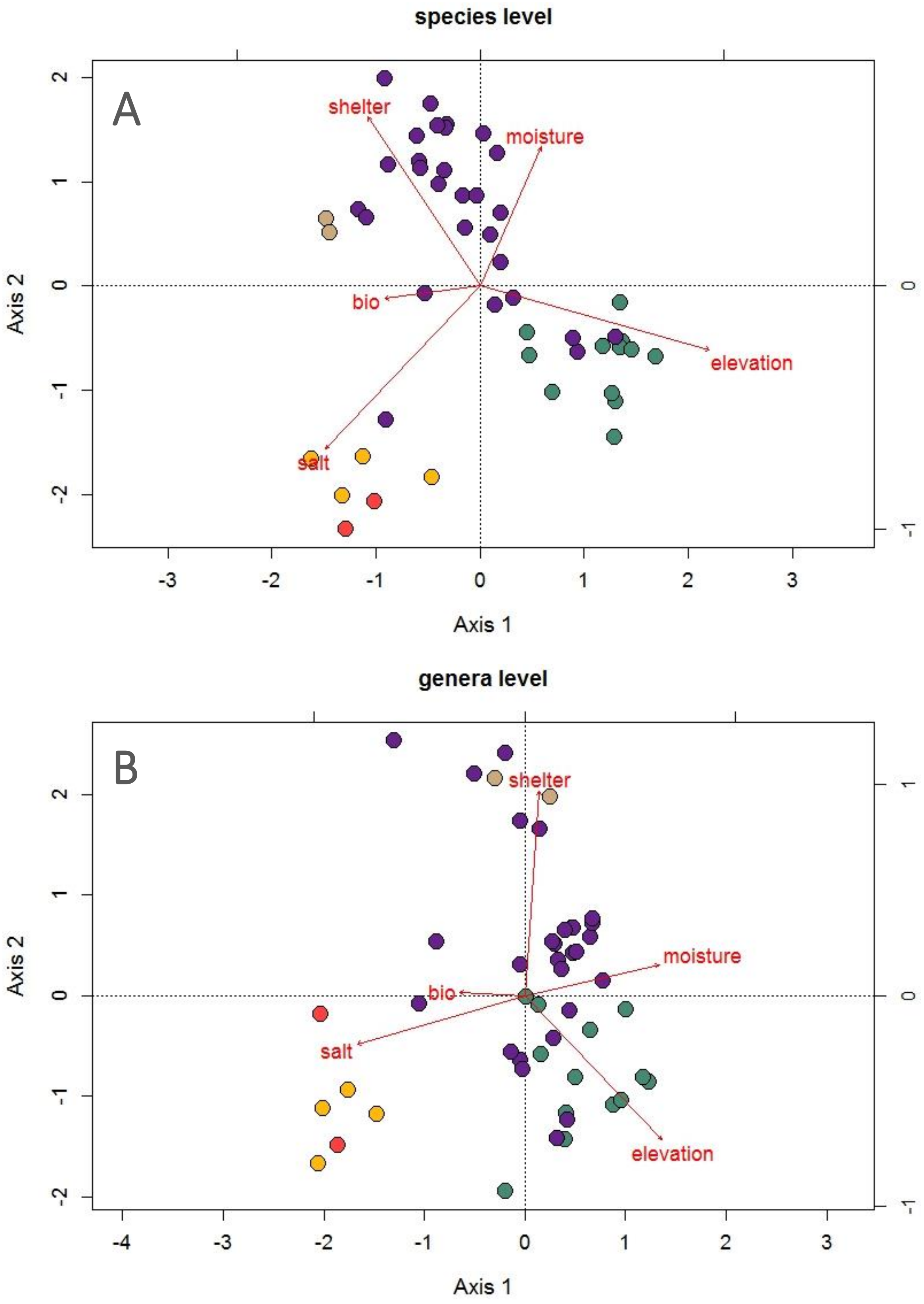

Figure 2.7. *(A) dbRDA showing diatom samples distributed along the measured environmental variables. The variables* ‘salt’ *and* ‘elevation’ *explained most of the variation. (B) The same analysis on diatom genera level, here, the most variation was explained, together with* ‘salt’ *and* ‘elevation’, *by the* ‘moisture’.

While ‘salt’ influenced the response of both species and genera levels generally the same, a different response can be seen on the genera for ‘shelter’ - ‘moisture’ - ‘elevation’ (**Figure 2.5 B**). Some of those from group E highly corresponds with ‘shelter’ (thus sheltered habitats), whereas the most of this group settles on more exposed and more moisture sites, with some being influenced by higher altitudes mostly. Interesting here is the strong correlation of group C with highly sheltered habitats on genera level, whereas regarding species, also a medium influence of ‘salt’ diverge the group in its direction (*see* also **Figure 2.6**).

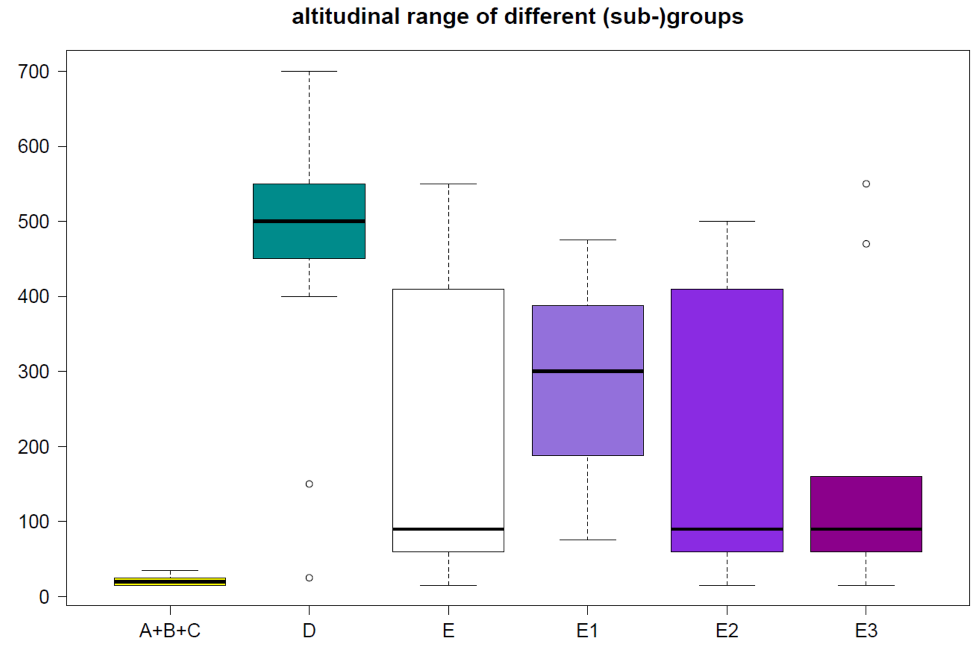

Figure 2.5. *Altitudinal range for each group and sub-group identified. The distribution of distinct groups along the altitudinal gradient follows the sea influence, notably here as the first three groups (A, B and C) merged on similar elevation. Note also the distinct overall range for sub-group E3.*

Habitat types were tested separately for the influence on community structure, resulting significantly ($F = 4.86$, $R^2 = 0.07$, $p < 0.001$) but apparently to a lower extent than was detected for groups’ influence ($R^2 = 0.35$, but *see* ‘community analysis’).

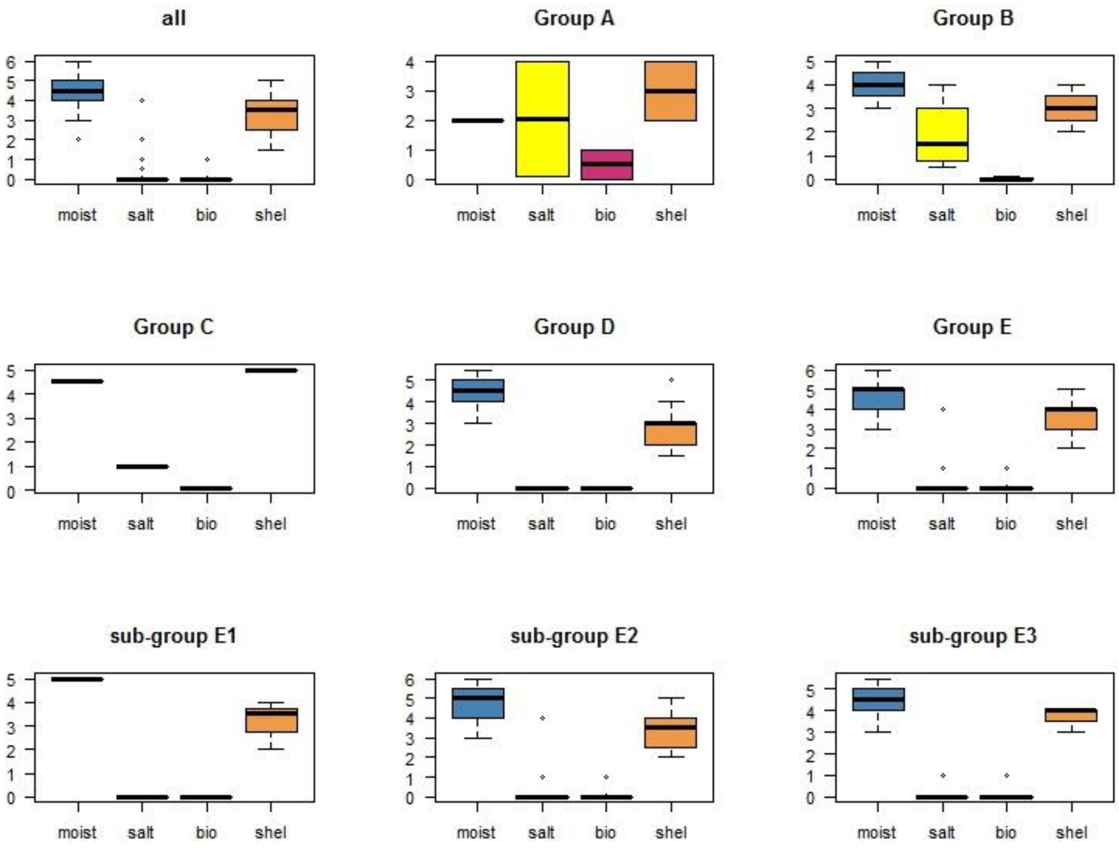

Figure 2.6. Environmental characteristics summarized for the whole dataset (first one) and for each group and sub-group identified. Explanation: moist – 'moisture', salt – sea influence, bio – biotic influence, shel – 'shelter'.

Similarity analysis

A similarity analysis was performed on the observed moss-inhabiting diatom flora from the other Southern Ocean islands. The resulting symmetric similarity matrix showed only a limited affinity of Gough Island to the other islands analyzed together (**Table 2.3**).

	GI	INI	TDC	LVI	JRI	HI	KGI	SGI	AI	MI	PEI
GI	--	0.31	0.22	0.13	0.10	0.21	0.11	0.11	0.21	0.19	0.20
INI			0.24	0.03	0.02	0.05	0.03	0.05	0.11	0.06	0.06
TDC				0.07	0.06	0.10	0.05	0.07	0.08	0.09	0.08
LVI					0.66	0.16	0.18	0.07	0.10	0.13	0.14
JRI						0.11	0.15	0.07	0.09	0.08	0.09
HI							0.21	0.17	0.23	0.61	0.60
KGI								0.17	0.11	0.17	0.17
SGI									0.12	0.16	0.18
AI										0.22	0.24
MI											0.80
PEI											

Table 2.3. Similarity analysis (Dice index) between the islands of southern oceans province, sub-Antarctica and Maritime Antarctica). GI – Gough Is., INI – Inaccessible Is., TDC – Tristan da Cunha Is., LVI – Livingston Is., JRI – James Ross Is., HI – Heard Is., KGI – King George Is., SGI – South Georgia Is., AI –Amsterdam Is., MI – Marion Is., PEI – Prince Edward Is.

To assess the general biogeographical position of Gough Island among the other islands analyzed in our similarity analysis, the neighbor-joining cluster model was performed (**Figure 2.8**). This analysis corresponded better to the real geographical positions compared to the other tree-making methods (*e.g.* unweighted-pair group method of analysis). Based on the nodes and the length of each line, the unrooted dendrogram grouped together three distinct biogeographical regions (indicated in dendrogram by red lines): (**1**) **Maritime Antarctica** (Livingston, James Ross, and King George Islands) and sub-Antarctic South Georgia Island, (**2**) **South Indian Province** (Marion and Prince Edward Islands), and more southwards positioned sub-Antarctic Heard Island; (**3**) **Cool-temperate zone**, islands of Tristan da Cunha archipelago and more separated Ile Amsterdam.

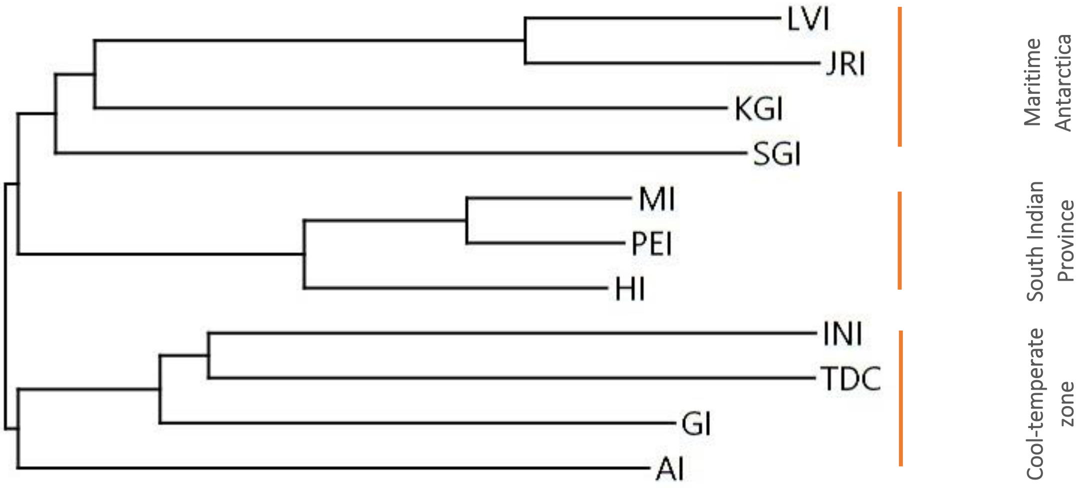

Figure 2.8. Neighbor-joining cluster analysis of Dice similarity among the analysed islands.

CHAPTER 3

Morphological observations on *Pseudoeunotia linearis* Carter (Bacillariophyta) and its transfer to the genus *Eunotia*.

The chapter presents an interesting moss-inhabiting species identified after what Carter (1966) described as new to the science, and from that time haven't been reported from elsewhere. We analyzed the species using light and electron scanning microscopy, provided it with morphometric measurements and based on its morphology, we proposed a transition of Pseudoeunotia linearis *Carter to the genus* Eunotia*. This paper presents an exemplar study of how should also the other species, found on Gough Island and identified after Carter's descriptions, be formally justified.*

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MORPHOLOGICAL OBSERVATIONS ON *PSEUDOEUNOTIA LINEARIS* CARTER (BACILLARIOPHYTA) AND ITS TRANSFER TO THE GENUS *EUNOTIA*

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ABSTRACT

During a survey of the moss-inhabiting diatom flora of Gough Island (Tristan da Cunha archipelago, southern Atlantic Ocean), a large population of *Pseudoeunotia linearis* Carter has been observed. A detailed morphological analysis has been made using light and scanning electron microscopy to reveal its ultrastructure. The results show a eunotioid structure with short raphe branches, uniseriate striae with irregularly placed rimmed areolae, and marginal linking spines surrounding the valve face. Based on these features, the position of *P. linearis* within the genus *Pseudoeunotia* cannot be confirmed and a transfer to the genus *Eunotia* is therefore suggested.

KEYWORDS: Bacillariophyta; *Eunotia*; Gough Island; morphology; *Pseudoeunotia*; taxonomy.

INTRODUCTION

Remote islands are often home to a remarkable unique and endemic diatom flora. This has been shown for the Hawaiian archipelago (Lowe and Sherwood 2010; Ripple and Kociolek 2013), New Caledonia (Moser, Lange-Bertalot and Metzeltin 1998) and Amsterdam Island (Van de Vijver et al. 2012). One of the most remote islands in the world is Gough Island, situated in the cool-temperate zone of the southern Atlantic Ocean. Despite its very interesting geographical position, its oceanic origin, and the number of interesting habitats, the freshwater and terrestrial diatom flora of Gough Island is very poorly known. Recently, a new survey of the moss-inhabiting diatom flora of Gough Island led to the publication of *Orthoseira gremmenii* Van de Vijver and Kopalová, a new *Orthoseira* species found north of the Gough Island weather station (Van de Vijver and Kopalová 2008).

The most complete floristic paper on the Tristan da Cunha and Gough Island diatom flora was published in 1966 by John Carter, who analysed 12 samples, of which only two were collected on Gough Island. In his 1966 paper, a very diverse and highly specific diatom flora was reported and a large number of new species were described, several of them from Gough Island, such as *Nitzschia serpentiformis* Carter or *Pinnularia posita* Carter. Apart from five newly described *Eunotia* species (*e.g., Eunotia morbida* Carter), two other eunotioid species were described but placed in the genus *Pseudoeunotia* Grunow in Van Heurck. Analysis of the drawings that illustrated the study in 1966, showed clear morphological differences between both taxa *Pseudoeunotia linearis* Carter and *Pseudoeunotia duplex* Carter). Whereas the latter shows a clear resemblance with *Pseudoeunotia doliolus* Grunow in Van Heurck, the typus generis of *Pseudoeunotia*, the other species (*P. linearis*) presents a completely different morphology. *Pseudoeunotia linearis* was described by Carter as '*Valvae lineares apicibus rotundatis, 10–35µ longae, 4µ latae. Striae transapicales irregulars circiter 11 in 10µ, minute punctatae'* (valves linear with rounded ends about 10–35 µm long and 4 µm wide. Striae irregular about 11 in 10 µm minutely punctate) (*see* Figure 3.46).

The discovery of a large population of this taxon during the recent Gough Island diatom survey, allowed for a better morphological characterization of this taxon and new insights in its taxonomical position. A transfer to the genus *Eunotia* is proposed.

STUDY SITE

Gough Island (40°21' S, 9°53' W) is a small uninhabited island located approximately midway between the southern tip of Africa and South America, at a distance of c.3000 km from the main large continents. The island belongs geopolitically to the United Kingdom Overseas Territory of Tristan da Cunha despite being located at approximately 300 km from the other islands of this group (Tristan da Cunha, Inaccessible Island and Nightingale Island). Gough Island measures c.6 km by 14 km resulting in a total surface of 65 km2. Large parts of the island are mountainous with most of the island above 400 m culminating at Edinburgh Peak with an altitude of 910 m above sea level (a.s.l.). The coastline consists of sea cliffs rising up to 300–450 m in height with narrow boulder beaches lacking any sheltered harbours. The southern end of Gough is the only area below 300 m. The sample investigated in this study was collected in the southern part of the island, not far from the South African weather station. The island is typically oceanic with a volcanic origin and has never been part of a continental landmass. Today, there are no signs of recent volcanic activity. Its climate is cool and wet, with a mean annual temperature (at sea level) of 11°C, a mean annual precipitation in excess of 3000 mm, and frequent gale-force winds. Four main types of vegetation can be found ranging from coastal tussock to fern bushes and peat bogs (Wace 1961; Cooper and Ryan 1994). There is no permanent habitation apart from a permanent weather station under South African lease.

MATERIALS AND METHODS

Although almost 100 samples were investigated from Gough Island, only a few large populations of *Pseudoeunotia linearis* Carter have been found. The largest population was chosen to be investigated in more detail for this study:

Gough 2000-177 (collection date 12/06/2000, leg. N.J.M. Gremmen) – Seal Beach (40°21'20" S, 9°52'58" W, 15 m a.s.l): moss carpet in tuffaceous deposits of a small cave; in steep slope leading up from the beach.

The sample was prepared for light microscopy (LM) observation following the method described in Van der Werff (1955). Small parts of the sample were cleaned by adding 37% H_2O_2 and heating to 80°C for about 1 h. The reaction was completed by addition of $KMnO_4$. Following digestion and centrifugation (3 × 10 minutes at 3700 g), cleaned material was diluted with distilled water to avoid excessive concentrations of diatom valves on the slides. Cleaned diatom material was mounted in Naphrax®. The resulting slide was analysed using an Olympus BX53 microscope, equipped with differential interference contrast (Nomarski) and the Olympus UC30 Imaging System. Sample and slide are stored at the BR-collection, property of the Belgian federal government and given in permanent loan to the Botanic Garden Meise (Belgium). For scanning electron microscopy (SEM), part of the oxidized suspension was filtered through a 1-µm Isopore™ polycarbonate membrane filter (Merck Millipore, Bedford, MA, USA). The stub was sputter-coated with a Gold–Palladium layer of 20 nm and studied in a ZEISS Ultra SEM microscope at 3 kV (Natural History Museum, London, UK). Diatom terminology follows Ross et al. (1979), Round, Crawford, and Mann (1990) and Lange-Bertalot, Bak, and Witkowski (2011).

For comparison, the type slide of *Pseudoeunotia linearis*, housed in the Natural History Museum, London (UK) (slide number BM77600) was analysed using LM. This sample was collected from the Gonydale Stream at an altitude of 1400 ft a.s.l. (427 m). Unfortunately, no raw or unmounted treated material was available for SEM analysis.

RESULTS

Light microscopy

Frustules in girdle view rectangular (Figures 3.1–3.4). Cells often connected (Figures 3.5, 3.6) forming chains of up to five frustules (Figures 3.1, 3.2). Valves only weakly dorsiventral (‘eunotioid’). Valves usually linear to linear–lanceolate with a weakly convex to straight dorsal margin. Ventral valve margin usually straight, although valves with slightly concave margin also observed. Marginal, broad, blunt spines surrounding the entire valve at the valve face–mantle junction. Apices never protracted, hardly differentiated, broadly rounded. Valve dimensions (n = 84): length 10.0–39.0 µm, width 2.0–4.0 µm. Terminal nodules close to the apices (Figures 3.7, 3.8, 3.10; *see* arrow). Distal raphe fissures impossible to resolve in LM. Striae widely and irregularly but very typically spaced, often not reaching the ventral valve margin, 8–15 in 10 µm and condensing near the apices to up to 17 in 10 µm. Mantle striae clearly visible in LM, both ventrally and dorsally (Figures 3.3–3.6). Areolae not resolvable in LM.

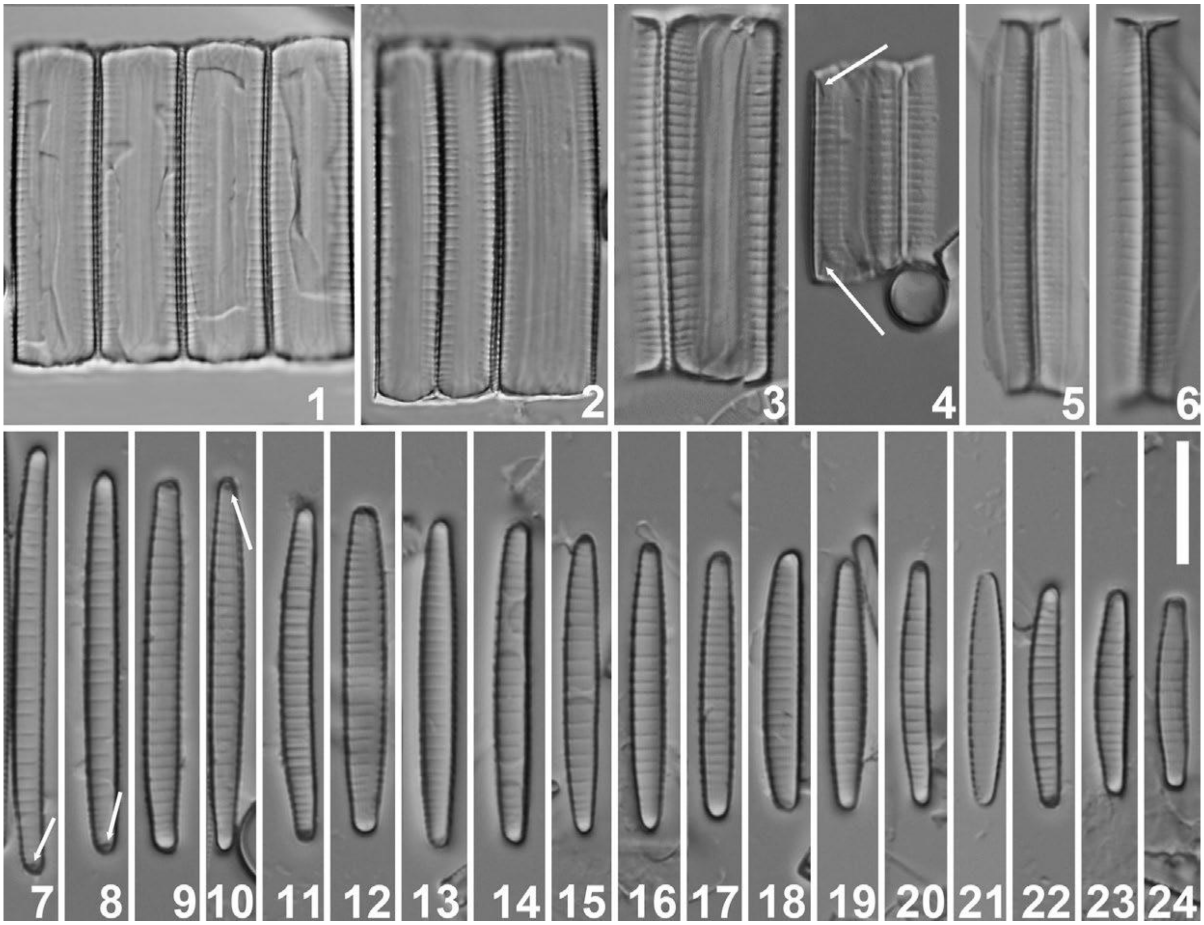


***Figure 3.2-3.24**. Light microscopical observations of* Pseudoeunotia linearis *Carter. All images taken from sample Gough 2000-177. (**1-2**) Two chains of resp. 4 and 3 frustules. (**3-6**) Girdle view of frustules and/or valves connected by spines. The arrow in Figure 4 indicates the raphe branches on the mantle. (**7-24**) Valve views showing the size diminution series. The arrows in Figures 7, 8 and 10 indicate the terminal nodule. Scale bar represents 10 µm.*

Scanning electron microscopy

Frustules connected by marginal linking spines (Figures 3.25, 3.27, 3.28). Spines irregularly formed, never acute (Figure 3.26). Girdle composed of a large number (up to 10) of open copulae (Figures 3.25, 3.28). Perforations on the copulae composed of irregularly scattered series of rounded pores (Figure 3.28). Valvocopula covered with small granules (Figure 3.28). Near the margin of the valvocopula, a series of narrow, often elongated but irregularly formed blisters present (Figure 3.28, arrows). Mantle quite deep, entirely covered with small granules (Figures 3.26, 3.29, 3.30). Occasionally, only one row of areolae around apices (Figures 3.28, 3.29).

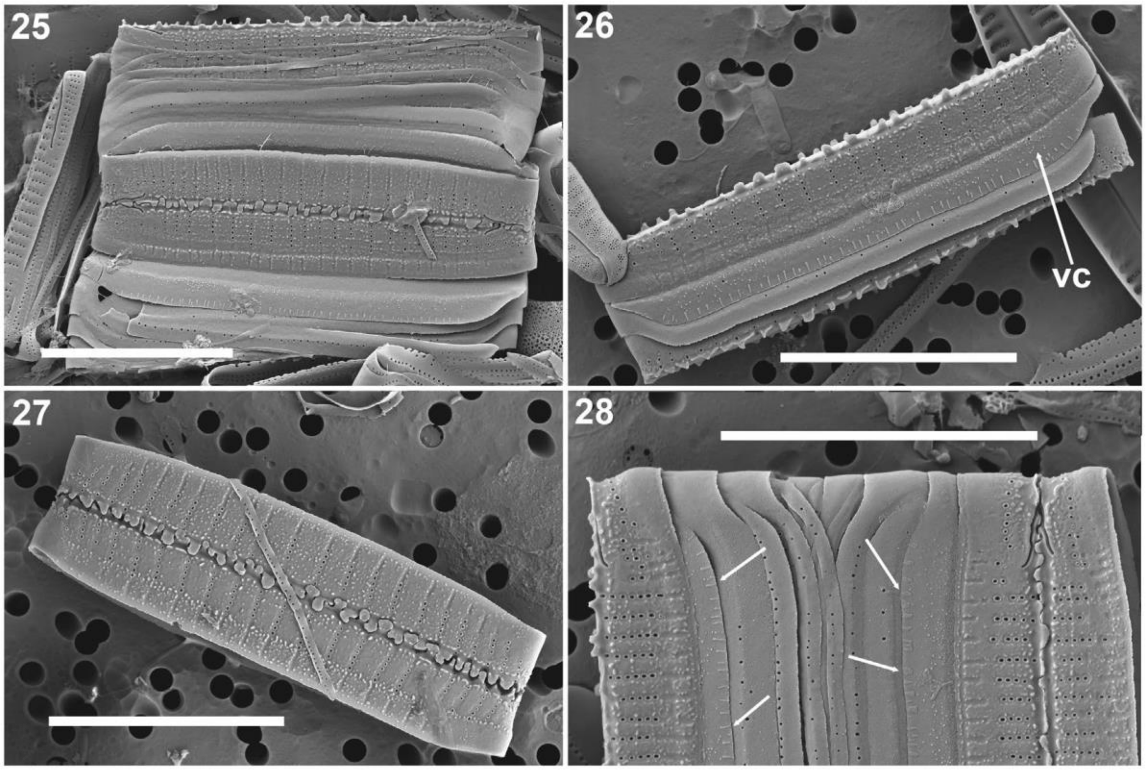


Figure 3.35–3.28. Scanning electron microscopical observations of Pseudoeunotia linearis *Carter. All images taken from sample Gough 2000-177. (**25**) External overview of an entire frustule linked to another valve. (**26**) External overview of an entire frustule showing the valvocopula (vc). (**27**) External view of two linked valves showing the row of irregular linking spines on both valve face/mantle junctions. (**28**) External detail of the apices of a frustule. The arrows indicate elongated blisters on the valvocopula. Note also the raphe branches on the mantle. Scale bar represents 10 µm.*

Valve face–mantle junction forming an almost 90° angle (Figures 3.29, 3.30). On the valve face–mantle junction, a series of broad, blunt, irregularly formed spines, entirely surrounding the valve face (Figures 3.29, 3.30, 3.31, 3.34, 3.35). Occasionally, spines replaced by a continuous marginal crest (Figures 3.32, 3.33). Striae uniseriate, composed of (often incomplete) series of rounded, rimmed areolae, located in a shallow groove (Figures 3.31–3.33). Areola density c.40 in 10 µm. Occasionally, areolae completely lacking (Figure 3.35). Areola series usually not reaching the ventral margin, continued only by a shallow groove (Figure 3.34). Near the apices, number of areolae per stria quite reduced or entirely lacking toward the raphe (Figures 3.32, 3.34, 3.36). Striae continuing on the dorsal mantle (Figures 3.31, 3.33) through marginal spines row (Figure 3.31), interrupted by a marginal crest when present (Figure 3.33).

On the ventral mantle, striae not clearly linked with those situated on valve face, separated from the valve face by marginal spines (Figures 3.29, 3.30), irregularly placed, denser and higher in number when compared with striae situated on valve face (Figures 3.29, 3.32, 3.35). Mantle striae likewise located in shallow grooves, the latter continuing up to the mantle edge (Figures 3.29, 3.30). Areolae often irregularly lacking in the mantle striae (Figure 3.30, arrows). Raphe branches short, situated at both apices, sometimes bordered by a shallow rim (Figure 3.34). Raphe continuing shortly onto the mantle, terminating almost parallel to the valve face–mantle junction (Figures 3.28, 3.29, 3.36). Distal part of the raphe usually curved following the apices, usually not reaching the dorsal margin (Figures 3.32, 3.33, 3.36). Valves with very short raphe, reduced to a small slit on the valve face, not continuing onto the mantle often observed (Figures 3.34, 3.35). Apical porefields absent (Figures 3.31–3.36). Rimoportulae never observed.

Internally, striae forming shallow, thin (incomplete) grooves (Figures 3.37–3.40). Areolae sometimes visible in the grooves (Figure 3.39). Raphe branches located on a very thin sternum, terminating distally onto small helictoglossae (Figures 3.39, 3.40). Rimoportulae absent (Figures 3.38–3.40).

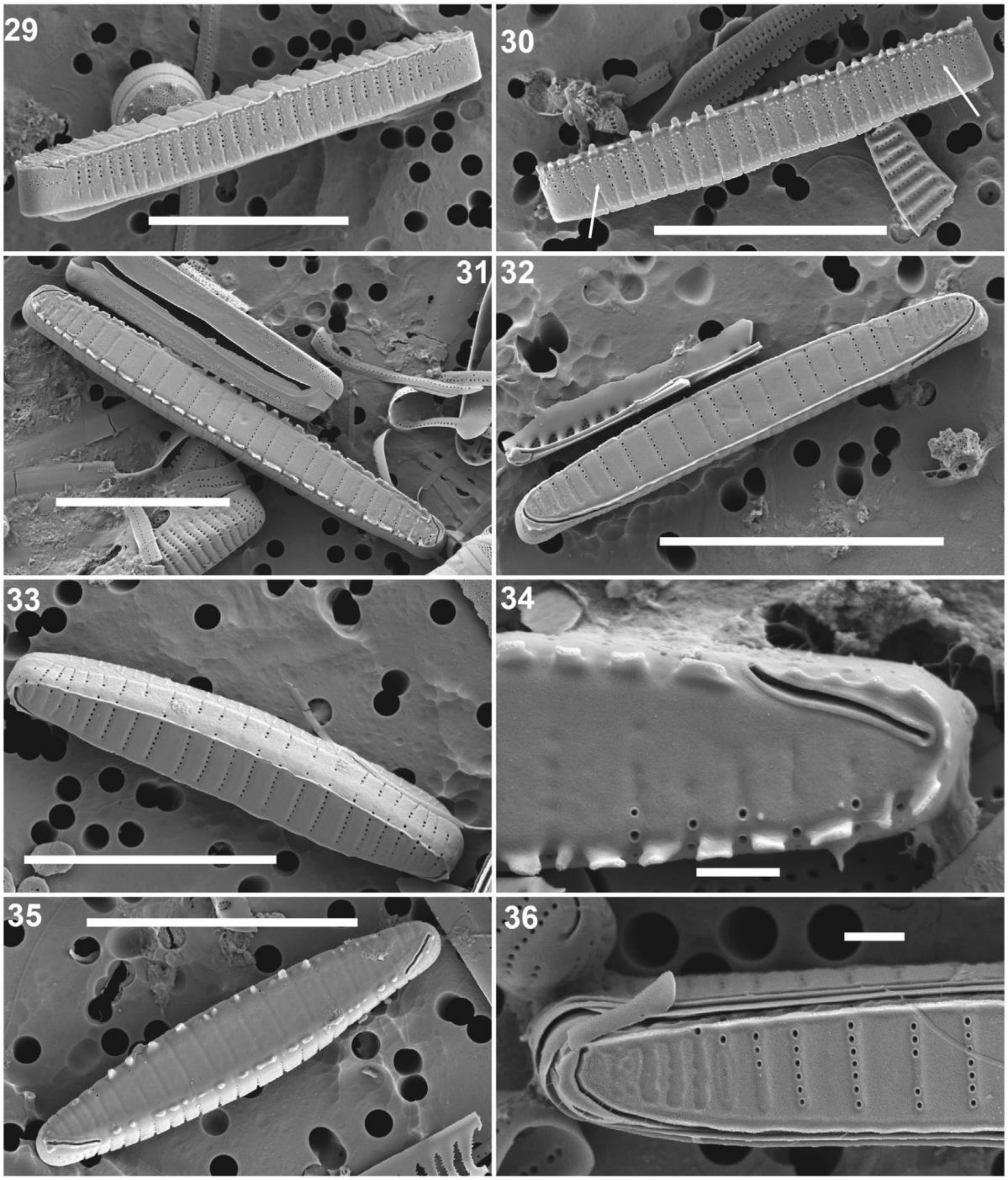


Figure 3.29–3.36. Scanning electron microscopical observations of Pseudoeunotia linearis *Carter. All images taken from sample Gough 2000-177. (**29**) External overview of a valve in oblique view showing the valve face/mantle junction. Note the presence of the marginal spines surrounding the valve face and the external raphe branches. (**30**) External overview of a valve in girdle view showing the mantle. The arrows indicate the lacking areolae. Note also the shallow grooves continuing to the mantle edge. (**31**) External view of an entire valve showing the marginal spines, the raphe branches and the stria structure. (**32**) External view of an entire valve showing a marginal crest (instead of spines), the raphe branches continuing on the mantle and the stria structure. (**33**) External view of an entire valve in oblique view showing the dorsal side with a marginal crest. (**34**) External detail of the valve apex showing the reduced number of areolae and shortened raphe slits on the valve face. Note the rimmed areolae. (**35**) External view of an entire valve lacking areolae in the striae and a reduced raphe slit. (**36**) External detail of the valve apex showing the raphe branches running from the valve mantle up to the dorsal side. Note the rimmed areolae. Scale bar represents 10 µm except for Figures 34 and 36 where scale bar = 1 µm.*

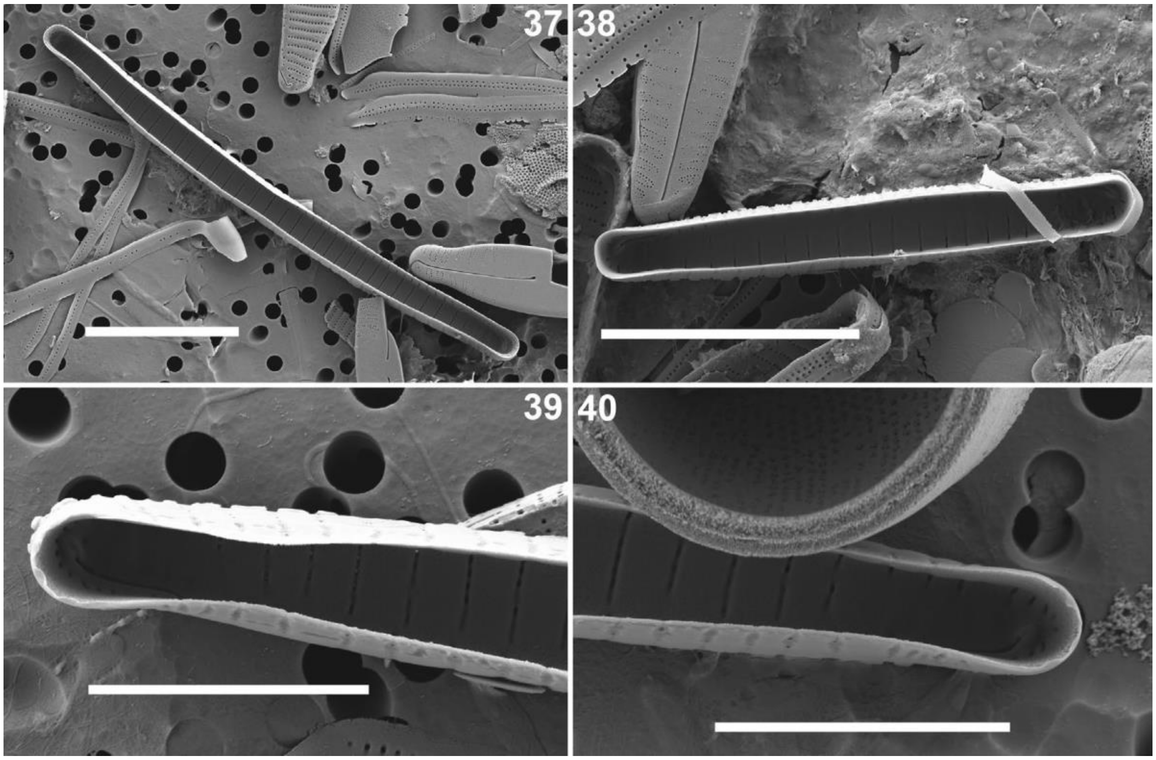
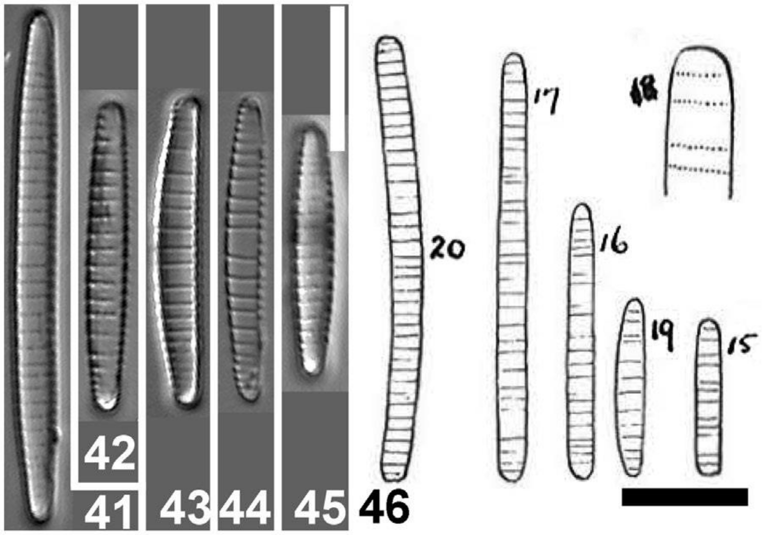


*Figure 3.47–3.40. Scanning electron microscopy observations of Pseudoeunotia linearis Carter. All images taken from sample Gough 2000-177. (**37**) Internal overview of an entire valve. (**38**) Internal overview of an entire valve. Note the presence of the shortened striae never reaching the ventral margin. (**39, 40**) Internal detail of the valve apices. Note the absence of rimoportulae and the presence of small helictoglossae. Scale bar represents 10 µm except for Figures 39 and 40 where scale bar = 5 µm.*

DISCUSSION

The observed population from Seal Beach clearly belongs to what Carter described in 1966 as *Pseudoeunotia linearis*. Light microscopy analysis of the type slide resulted in several observations (Figures 3.41–3.45). The absence of unmounted material prevented SEM observations but there are no morphological differences that can be noted between the type population and the Seal Beach population. The valves of the type population show a similar linear to slightly dorsiventral valve outline with irregularly distant striae, usually not reaching the ventral margin. Hence, we are confident that the SEM observations on the Seal Beach population can be used to obtain a better morphological knowledge of this taxon that will help to clarify its taxonomical position.



Figures 3.41–3.46. *Type material of* Pseudoeunotia linearis. *(**41–45**) Light microscopy observations of* P. linearis *Carter. All images taken from the type slide BM 77600 (Natural History Museum, London, UK). (**46**) Original drawing of* P. linearis *taken from Carter (1966).*

It is clear that *P. linearis* does not belong to the genus *Pseudoeunotia*. The latter genus was erected in 1881 by Grunow (in Van Heurck 1880–1883) to accommodate two species, despite being used already in 1862 and 1865 as a division for the genera *Synedra*, *Nitzschia* and *Ceratoneis* (Fourtanier and Kociolek 1999). *Pseudoeunotia hemicyclus* (Ehrenberg) Grunow was later transferred: first to *Amphicampa* by Hustedt (1959) and later by Patrick (in Patrick and Reimer 1966) to the newly erected genus *Semiorbis*. Medlin and Sims (1993) transferred the last remaining Grunow taxon, *P. doliolus* (Wallich) Grunow to the genus *Fragilariopsis*. Carter (1966) originally described two *Pseudoeunotia* taxa, *P. duplex* and *P. linearis*. The type slide for both *Pseudoeunotia* taxa is the same (BM 77600) but the analysis of this slide only revealed several specimens of the *P. linearis* and only half of a valve of *P. duplex*. Based on this half specimen, it is almost impossible to discuss the morphology of this species. However, the drawings in Carter (1966) and the single half valve that was observed, show a remarkable similarity with *P. doliolus*, suggesting a possible conspecificity. Medlin and Sims (1993) analysed in detail the morphology of *Pseudoeunotia* and concluded that there is no difference in valve ultrastructure between *Pseudoeunotia* and *Fragilariopsis* and suggested the inclusion of *P. doliolus* in *Fragilariopsis* despite the fact that the former has taxonomic priority. However, a formal request for the conservation of the name *Fragilariopsis* to replace *Pseudoeunotia* has never been made (Fourtanier and Kociolek 1999).

Whether *Fragilariopsis* or *Pseudoeunotia* is the correct name for the genus accommodating *P. doliolus* (and most likely also *P. duplex*) is of less importance in the present study since the morphology of *P. linearis* clearly differs from the genus description of *Pseudoeunotia* as discussed in Medlin and Sims (1993). *Pseudoeunotia/Fragilariopsis* belongs to the Bacillariales and is therefore closely related to, for example, *Nitzschia* and *Hantzschia*. Its main morphological features include the presence of an eccentric raphe with rather indistinct proximal raphe endings and lacking distal fissures, biseriate striae and the presence of siliceous ribs. Internally, the raphe is continuous (Koizumi and Yanagisawa 1990) and fibulae are present along the entire raphe course. All features are typically nitzschioid and have never been observed in *P. linearis*, which has typical 'eunotioid' characteristics.

Based on its features, *P. linearis* most likely belongs to the genus *Eunotia*, although the species presents some features that are rather unusual for the genus and only observed in a few species: the presence of marginal spines, the irregular stria/areola structure, the structure of the raphe and the formation of long chains. Only a few *Eunotia* species show the presence of marginal spines. The most typical one is *Eunotia denticulata* (Brebisson) Rabenhorst presenting several rows of small, acute spines on the dorsal margin of the valve face (Lange-Bertalot, Bak and Witkowski 2011). *Eunotia braendlei* Lange-Bertalot and Werum possesses short, blunt spines on the dorsal margin, facilitating chain formation (Werum and Lange-Bertalot 2004). Other taxa with spines include *Eunotia fennica* (Hustedt) Lange-Bertalot, *Eunotia noerpeliana* Metzeltin and Lange-Bertalot 1998 and *Eunotia diodon* Ehrenberg, all showing marginal acutely pointed dorsal spines. Marginal ventral spines have only been found in *Eunotia soleirolii* (Kutzing) Rabenhorst although in the latter spineless valves are also often observed. Most *Eunotia* taxa with spines use them to link specimens together in ribbon-like colonies, although not all colonies are formed by the use of spines. Some of the *P. linearis* valves showed a marginal crest instead of spines. A similar crest was observed in for instance *Eunotia fallax* A.Cleve (*see* Lange-Bertalot, Bak and Witkowski 2011, p. 535, Figures 8, 9 and 21 therein). An irregular striation pattern is observed in *Eunotia neofallax*, *Eunotia odebrechtiana* Metzeltin and Lange-Bertalot and *Eunotia parasiolii* Metzeltin and Lange-Bertalot, although none of them show the shallow grooves and typical rimmed structure of the areolae that characterize the striae of *P. linearis*. Many *Eunotia s*pecies show a grooved valve face with thickened transapical ribs bordering the striae but the shallow grooves lacking any bordering thickened ribs have never been observed (Metzeltin and Lange-Bertalot 1998, 2007; Furey, Lowe, and Johansen 2011; Lange-Bertalot, Bak, and Witkowski 2011). The fourth unusual feature constitutes the raphe structure of *P. linearis*. Although some valves display a normal eunotioid raphe, several valves only possess a short raphe slit on the valve face, bordered by thickened ribs. The absence of rimoportulae in *P. linearis* is also quite remarkable and although observed in several (mostly tropical) *Eunotia* species, it is uncommon for the genus *Eunotia* (Lange-Bertalot, Bak and Witkowski 2011).

Despite this combination of unusual features, *P. linearis* clearly belongs to the genus *Eunotia*. Other eunotioid genera such as *Actinella* (heterovalvarity, apical spines), *Perinotia* (stria structure) or *Peronia* (heterovalvarity, raphe structure) can be excluded as genera to accommodate *P. linearis* (Metzeltin and Lange-Bertalot 1998, 2007). A formal transfer of this species to the genus *Eunotia* is therefore necessary:

> *Eunotia linearis* (Carter) Vinšova, Kopalova and Van de Vijver *comb nov*.
> Basionym: *Pseudoeunotia linearis* Carter 1966 Nova Hedwigia 11, p. 479, pl. 9,
> Figures 15–19.

AKNOWLEDGEMENTS

This study was supported by the BELSPO CCAMBIO project to visit the National History Museum in London, UK. Dr Alex Ball and the staff of the IAC laboratory at the Natural History Museum are thanked for their help with the scanning electron microscopy. Dr Niek J.M. Gremmen collected the sample during fieldwork on Gough Island in 2000. Mrs Petra Vinšova benefitted from an Erasmus travel grant for her work in the Botanic Garden Meise and the University of Antwerp (Belgium).

CHAPTER 4

Morphological observations on the moss-inhabiting diatom genus *Frustulia* (Bacillariophyceae) from Gough Island (South Atlantic Ocean)

The chapter demonstrates the identification problems of one, morphologically difficult genus frequently observed in the moss material of Gough Island. Some of the diatom species observed in the assemblages showed a large variation in valve outline and morphological features, most likely implying that they represent actually a species complex composed. In the following pages, several morphotypes (or 'morphospecies') were delimited from the diatom genus Frustulia*, based on their visually distinct overall valve outline. Based on the counts, a distribution of these morphotypes most likely reflects an altitudinal gradient of Gough Island. It is, however, questionable whether these should be described as new species, or whether they represent a large morphological plasticity of distinct ecotypes.*

Unpublished manuscript (first draft, 2016)

MORPHOLOGICAL OBSERVATIONS ON THE MOSS-INHABITING DIATOM GENUS *FRUSTULIA* (BACILLARIOPHYCEAE) FROM GOUGH ISLAND (SOUTH ATLANTIC OCEAN).

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ABSTRACT

Several morphotypes (or 'morphospecies') of the diatom genus *Frustulia*, identified from moss habitats of Gough Island are discussed, based on their visually distinct valve outline following LM and SEM observations and morphometric analyses. Geometric morphometry techniques were used to analyze which morphological features determine the differences between the identified morphotypes. It is furthermore analyzed the distribution of these morphotypes in the diatom community structure, analyzed using a distance-based Redundancy analysis (dbRDA). The results show that elevation was the principal environmental variable determining the distribution of distinct morphotypes. The taxonomic identity of the delimited morphotypes is discussed whether these morphotypes could represent one phenotypically plastic species rather than several different species.

KEYWORDS: Bacillariophyta; *Frustulia*; Gough Island; geometric morphometry; SHERPA; taxonomy.

INTRODUCTION

Species from the diatom genus *Frustulia* Rabenhorst species are growing solitary or in mucilaginous tube colonies, in the benthos of many aquatic systems. *Frustulia* species are typically found in peat bogs, oligotrophic and acidic habitats with low conductivity values, and are considered sub-aerophytic or limno-terrestrial (Round et al. 1990; Lange-Bertalot and Jahn 2000; Rumrich et al. 2000; Lange-Bertalot 2001; Siver and Baskette 2004).

In 2000, Lange-Bertalot & Jahn revised several taxa from the genus *Frustulia* and concluded that several species were based erroneously on *Navicula rhomboides* Ehrenberg (Ehrenberg 1843). One year later, Lange-Bertalot (2001), proposed several new species to correct this taxonomical error: *F. krammeri* Lange-Bertalot & Metzeltin*, F. saxonica* Rabenhorst*, F. crassinervia* (Brébisson ex W.Smith) Lange-Bertalot & Krammer*, F. amphipleuroides* (Grunow) Cleve-Euler, and *F. erifuga* Lange-Bertalot & Krammer. In the last two decades, many new taxa have been described in the genus *Frustulia* (*e.g.* Rumrich et al. 2000; Van de Vijver et al. 2002; Van de Vijver 2002 and 2003; Siver and Baskette 2004; Lange-Bertalot and Sterrenburg 2004; Flower 2005; Van de Vijver and Gremmen 2006; Beier and Lange-Bertalot 2007; Siver et al. 2009; Graeff and Kociolek 2011; Blanco et al. 2012; Graeff et al. 2012; Urbánková et al. 2014).

Carter (1966) identified several *Frustulia* species from Tristan da Cunha: *Frustulia rhomboides* Rabh., *F. rhomboides* var. *saxonica* (Rabh.) De Toni, *F. vulgaris* Island and *Frustulia magaliesmontana* Chol., the latter also found on Gough Island. Gough Island is an ideal environment for some *Frustulia* spp., with an annual mean temperature between 11 and 15°C, dominated by mosses, with naturally acidic habitats (pH 4.1 – 5.5), peat bogs, wet peat slopes and mires. Based on their morphology, the populations on the island mostly resemble the *Frustulia crassinervia-saxonica* species complex (sensu Urbánková and Veselá 2013), but were treated as several different morphotypes within the complex, in order to obtain the most detailed image of the island's diatom diversity and community structure.

The representatives of the genus *Frustulia* on Gough Island show some resemblance to the traditional species *F. saxonica* and *F. crassinervia.* Unfortunately, these two have variable cell morphologies, overlap in some important morphological features and are therefore difficult to distinguish (Siver and Baskette 2004; Veselá et al. 2012). Even the documentation of these two common species is not consistent (Lange-Bertalot and Metzeltin 1996 and 2001) and therefore unclear when determined under the light microscope. It has been shown recently, that these two actually represents a species complex, *Frustulia crassinervia-saxonica* of several distinct lineages, of which only one could be considered cosmopolitan (Urbánková and Veselá 2013). Phylogenetic lineages of *F. crassinervia-saxonica* showed different patterns in geographic distribution among regions in Europe, but the frequencies of sequences within samples indicated that the distribution of common lineages most likely reflected different ecological preferences rather than dispersal restrictions (Veselá et al. 2012). According to Veselá et al. (2012) it is not possible to unambiguously determine the closely related lineages using only morphology. The valve shapes of monoclonal cultures representing different lineages substantially overlap.

In the presented study, several natural populations of *Frustulia* were analysed using geometric morphometry techniques. The identified morphotypes most likely belonged to the pseudo-cryptic *Frustulia crassinervia-saxonica* complex, but they seemed to be morphologically quite variable among the different sites of Gough Island. The preliminary characterization of ecological preferences of the different morphotypes' is presented and discussed, together with remarks on morphological similarities between the morphotypes and with some previously described species.

MATERIALS AND METHODS

Microscopy

A large number of *Frustulia* cells showing a distinct morphology were observed during the preliminary light microscopy (LM) scanning of slides obtained from moss samples. Most valves were photographed and basic morphometric data (width, length, striae number, width of central nodulus, width/length ratio) were measured. The Frustulia populations in samples 2000-164, 2000-195, but especially 2000-657A, 2000-673, 2000-933, were investigated using scanning electron microscope (SEM) in order to analyse the ultramorphological structures (areola shape and direction of striae, shape of the central nodulus and ribs, shape of helictoglossae). Different morphotypes recognized during the preliminary microscopical analysis, were also separated during the counting to evaluate the diatom community structure of the assemblages. However, during the counting of slides it proved quite difficult to identify them consistently as sometimes morphotypes appeared to show overlapping morphologies.

Morphometric data and SHERPA analysis

Random LM photos (n=114) of valves (already taken and visually identified to distinct morphotypes during the microscopical analysis) were analysed using the free software tool Shape Recognition, Processing and Analysis (SHERPA). SHERPA software was developed for the analysis of bright field microscopic images of diatom valves (Kloster, Kauer and Beszteri 2014) and in contrast with (semi-)landmarks based morphometric methods, SHERPA analyze valve shape not only via points along the outline using elliptic Fourier analysis (EFA) but also with heuristic shape descriptors. Number of valves analysed for each of morhotype were: morphotype V (n=23), morphotype H (n=10), morphotype V and morphotype N (both n=20) and morphotype E (n=54).

Firstly, SHERPA settings were re-configured to optimize uploaded images for segmentation methods. Uploaded images of *Frustulia* valves were obtained under differential interference contrast (DIC) which enhances contrast but the valve edges become either brighterer or darker (depending on the direction) than the background. The use of DIC shows advantages when a good resolution for striae is requested, but it is difficult for SHERPA to recognize and connect the complete valve. Because of this, several adjustments were made: (1) 'Distance to image border' was set to 0, so even valves really close to the border would be analyzed, (2) minimum 'Area' was set to 40, so even when very small parts of valves were detected only, they were analyzed, (3) 'Adaptive Thresholding' was used as the only method for segmentation as the others are either very sensitive to DIC relief artefacts ('RATS' and 'Canny Edge') or fail when white areas in the cropped images ('OTSU'), (4) the BlockSize value for Adaptive Thresholding has to be set out of range manually. The adjustments of the settings have been done in collaboration with Michael Kloster[3], the SHERPA software main developer.

Secondly, a full template set of *Frustulia* valves was prepared (some out of *F. saxonica* and *F. crassinervia* (images from the publication of Lange-Bertalot and Metzeltin 2001), with additional 10 templates prepared from our own material covering the variability) and uploaded to SHERPA prior to the image segmentation analysis, so only the valves best matching the templates chosen would be selected. After the image upload and setting adjustment, contour analysis automatically detected valves, or parts of valves, and draws contours (**Figure 4.1 A**). Every result was shown and manually reworked so the edge of valve was correctly identified (**Figure 4.1 B** with EFA points). While manually reworking, useful is to hide image using 'CTRL' (**Figure 4.1 C**). Resulted image is then overlaid by a contour and contour points for Elliptic Fourier analysis (**Figure 4.1 B + D**), which is also useful to check prior exporting the results. Exported object data evaluated by SHERPA contains 'Basic Morphology Data', 'Contour coordinates', 'Elliptic Fourier Descriptors', 'Coordinates of Contour Points Used for EFA' and 'Cropped Images of Contour' (or less) alongside with heuristic shape descriptors 'Rectangularity' (du Buf and Bayer 2002), 'Ellipticity' (Rosin 2003), 'Triangularity' (Rosin 2003), 'Roundness' (Russ 2011; all previous also described in Fischer and Bunke 2002), and 'Convexivity' (Russ 2011; Zunic and Rosin 2002), as referred in Kloster et al. 2014.

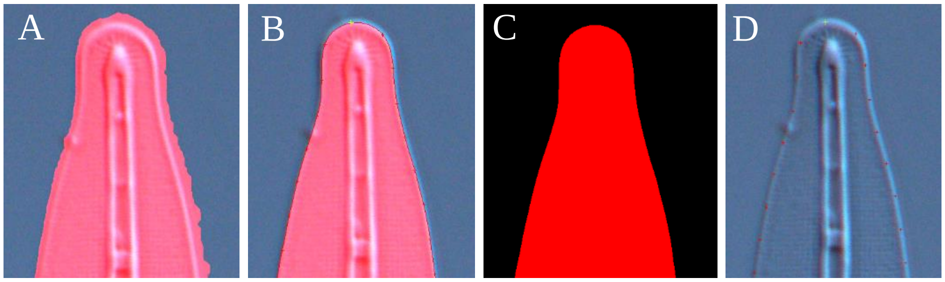

*Figure 4.5. Examples of image/valve contour workflow in SHERPA software. Images are being analyzed after the templates given, and contour is drawn along the valve edges when encountered (**A**). Reworked contour with contour points (**B**), countour only when the image is hidden (**C**), and contour points for Elliptic Fourier analysis (**D**).*

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Data analysis

Elliptic Fourier analysis (EFA) was performed in PAST ver. 3.10 (Hammer et al. 2001) using [x;y] coordinates exported in single EFA files from SHERPA software. The algorithm used in PAST is described by Ferson et al. (1985). The coordinates were transformed linearly to the major axis using generalized Procrustes analysis prior the shape analysis. Principal Components Analysis (PCA) of the EFA coefficients for the valve outlines were performed for each morphotype, with the visualization of the principal components as EFA deformations.

A linear discriminant analysis (LDA) was used in order to reveal the influence of morphometric data exported after image contour analyses in SHERPA. Differences in morphotypes based on EFA were visualized using PCA scatter plots.

To test whether the morphotypes are significantly different from each other, groups were compared using Euclidean distances in non-parametric test of significant differences (ANOSIM) and Bonferroni corrected p-values. Basic morphometric data (width, height, width/height) and additional data and heuristic shape descriptors provided by SHERPA (area, perimeter, rectangularity, compactness, ellipticity, triangularity, roundness, convexity by perimeter, convexity by area) were tested for the differences.

An ordination distance-based redundancy analysis (dbRDA) was performed only with *Frustulia* spp. relative abundances in the samples, otherwise similarly as for the general ecology (**Chapter 2**), in order to reveal the influence of environmental parameters to the *Frustulia* spp. structure in the assemblage. The model, axes and each term were tested for significance using ANOVA. However, due to the lack of ecological data (such as pH or conductivity) the interpretation of these, must be taken with caution as significant variables might be actually correlated with some, possibly more important, unknown ones.

RESULTS

Ultrastructure of *Frustulia* morphotypes

Images obtained from scanning electron microscope (SEM) were used for ultramorphological analysis. Different morphotypes of *Frustulia* from various localities showed a high similarity with respect to important ultramorphological features typically used to distinguish species in the genus. In **Figure 4.2**, the main features are shown: Internally, (**Figure 4.2 A**) fusion of ribs with a linear-shaped helictoglossae; circumpolar striae, composed of only a few areolae at the apices are sometimes elongated; a thick hyaline zone (valve margin) always present at the end; (**Figure 4.2 B**) fusion of ribs in the valve center forming the central nodulus; rounded areolae, and straight and parallel striae, although often wavy near the central area. Externally, both apices show elongated areolae (**Figure 4.2 C**), and central area shows a reduction in the areola size but not in areola density; T-shaped raphe endings are present on both distal and proximal sides, often more curved in the valve center resembling an umbrella or mushroom hat.

Ultramorphological characters differed only slightly within identified morphotypes. Specific differences included the shape of areolae, being more elongated on the external valve view (morphotype H), or the stria direction that sometimes differ from parallel to slightly convergent (irregularly, but mostly in morphotype P). In general, morphotypes can be distinguished based on the overall valve outline, similarly as under the LM.

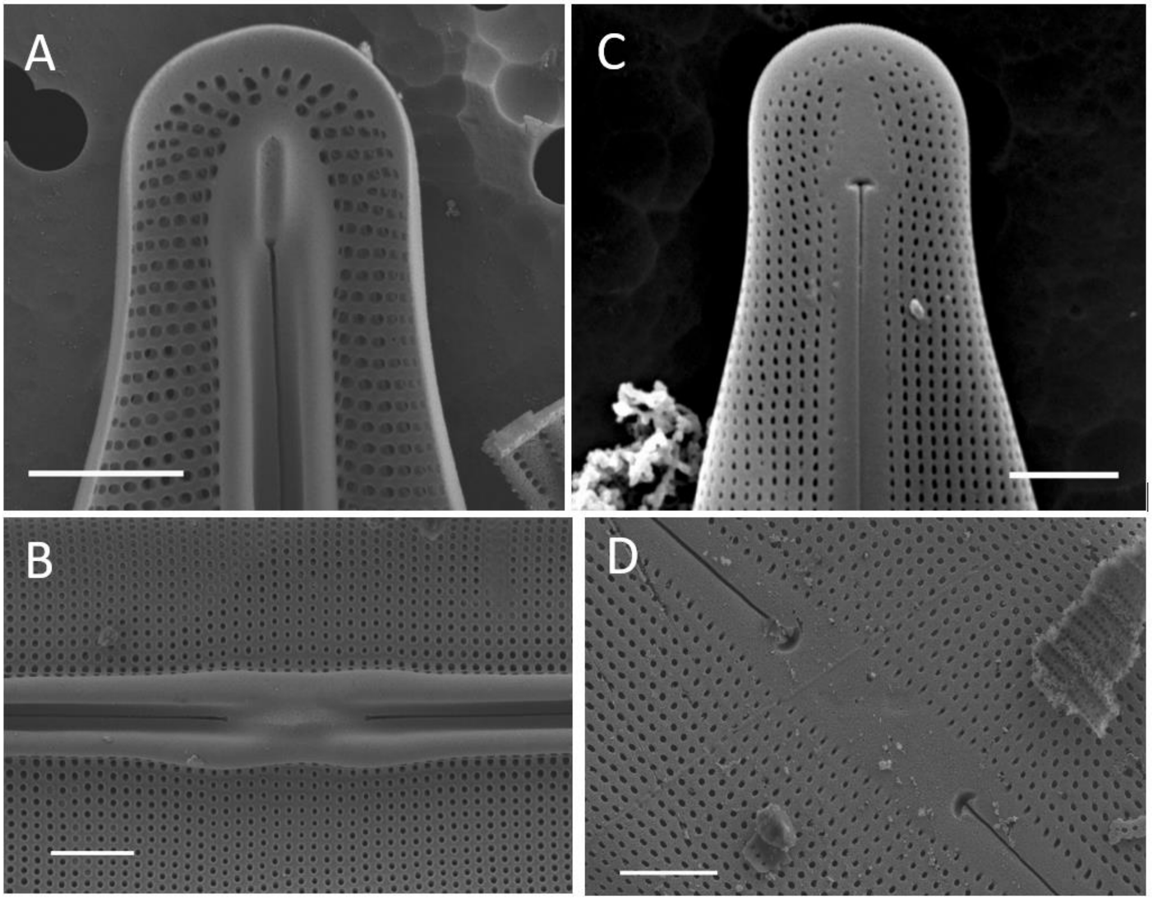

Figure 4.6. Important ultramorphological structures similar in Frustulia *morphotypes from moss habitats of Gough Island. (**A, C**) external and internal view of apices, linear-shaped helictoglossae, T-shaped raphe end; (**B, D**) internal and external view of valve central area, ribs fuse to form central nodulus (B), raphe T-shaped (umbrella like) endings are present on both, distal and proximal raphe endings. Scale bars = 2 µm.*

Overall mean valve outline of *Frustulia* from Gough Island

Several morphotypes could be delimited within the genus *Frustulia* inform the moss habitats of Gough Island. Objects, which were ordinated using EFA scores, were firstly colored in PCA according to morphotypes in order to reveal main morphological variations important to differences. PC 1 described the ‘overall size’ (as enlarging mostly) to be the most important when describing the variation in between groups, accounting for 99.95 % of variation. PC 2 described variation in ’valve width’, accounted for 0.04 % of the variation, and PC 3 with < 0.01 % of described variation in ‘necking’ (constriction before apices). PC 4 described the variation in margin undulation, with less than < 0.001 % of explained variation. All four important components in valve outline variation are shown in **Figure 4.3** (and **Figure 4.4**), and in respective figures of each morphotype in the following text.

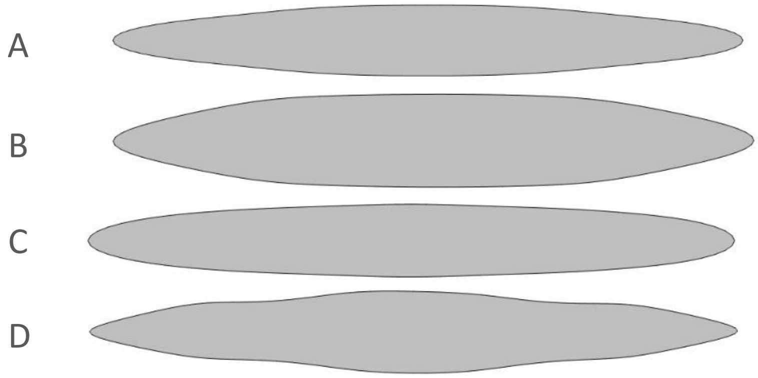

Figure 4.7. Visualized variation in four outline components in between groups, as described by PCA from the mean shape. (A) enlarging in size along PC 1 axis, (B) a negative position in the morphospace along the PC 2 axis describing variation in 'valve width', (C) *a negative position along the PC 3 axis describing parameter* 'necking', (D) *and a positive position along the PC 4 axis describing* 'margin undulation'. (Note that each image visualizes single morphological parameter after different PCs, while being exaggerated along axes (as described). Therefore, do not present the real shape of *Frustulia* valves.

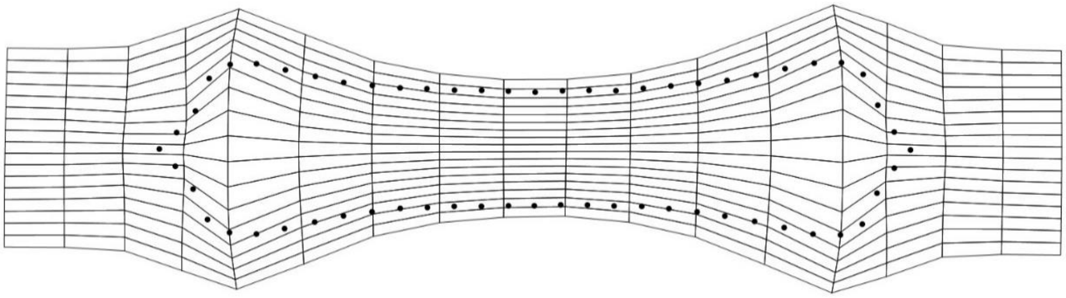

Figure 4.8. Necking. *The morphological parameter shown on deformation grid of relative warps (the parameter is located at the most prolonged lines) negatively exaggerated along PC 3 axis.*

Groups, as morphotypes delimited visually in LM analysis, were disregarded in the following step in order to reveal whether the components determining morphological differences in valve outline would remain similar or would differ. The PCA exhibited PC 1 'centroid size' to be the most important when describing the variation in the whole dataset, accounted for 92.25 % of the variation. PC 2 then described variation in 'necking' with 5.79 %, and PC 3 described variation in 'margin undulation', with 0.74 % of explained variation.

When compared to the previous between-groups ordination, the 'overall size' (or enlarging) here was not taken into account when describing important components of outline variation of the whole dataset, instead the 'valve width' was the most important one. The main ordination, however, remained the same, whether ‘size’ or ‘valve width’ was plotted against ‘necking’, implicating the high correlation of both components, and therefore similar results of both analyses were produced. The structuring of *Frustulia* valve morphology following the parameters ‘valve width’ and ‘necking’ are visualized in the below scatter plot (**Figure 4.5**).

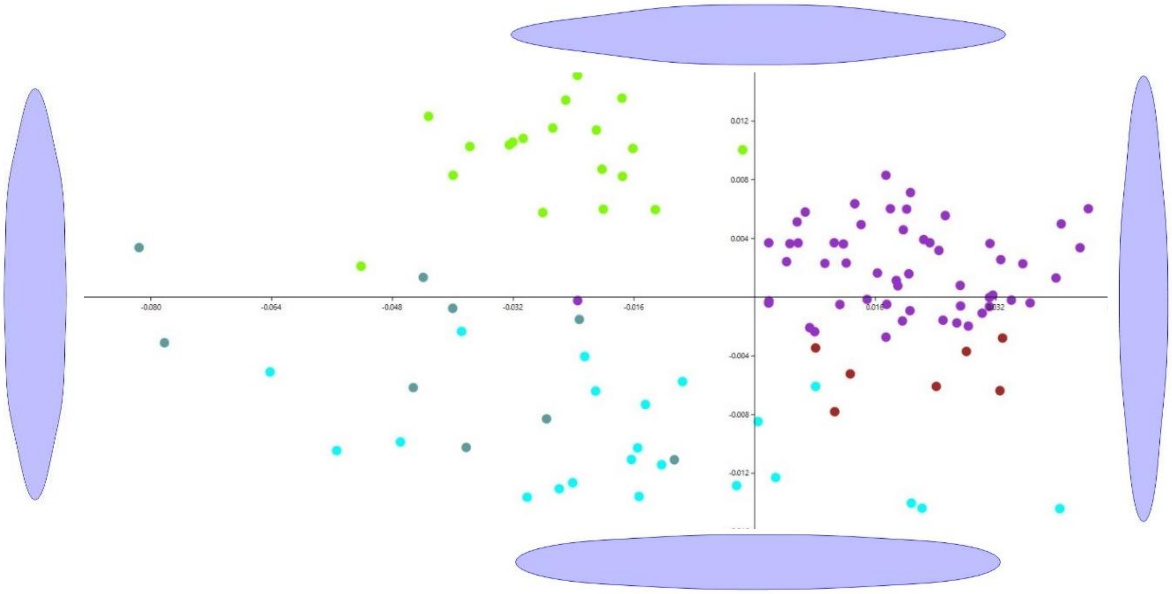

Figure 4.9. *Scatter plot of PCA based on mean EFA coordinates. Groups were disregarded and ordinated along PC 1 and PC 3 axes. Axis 1 describes the variation of valves in the* ‘valve width’, *Axis 3 describes the morphological feature* ‘necking’ (both features described by the exaggerated visualizations along the specified axes). *Morphotypes (groups) are identified in different colors:* ● morph. N, ● morph. E, ● morph. P, ● morph. V, ● morph. H.

Analysis of morphometric data

After the contour analysis (and reworking) in SHERPA software, the basic morphometric data (width, height, width/height ratio) along with the additional variables and heuristic shape descriptors counted from the valve contour (area, perimeter, rectangularity, compactness, ellipticity, triangularity, roundness, convexity by area and convexity by perimeter) were exported and analyzed in PAST.

Linear discriminant analysis (LDA) separated morphotypes quite well, although only morphotype E and morphotype N were clearly set apart along both axes, whereas morphotypes H, V and P showed partial overlapping (**Figure 4.6**). Axis 1 and Axis 2 explained together 86.30 % of variance, and the confusion matrix showed 92.98 % (Jackknifed) of valves to be correctly identified. The similar position of morphotypes H, V and P along both axes implicates unclear morphological separation of these three.

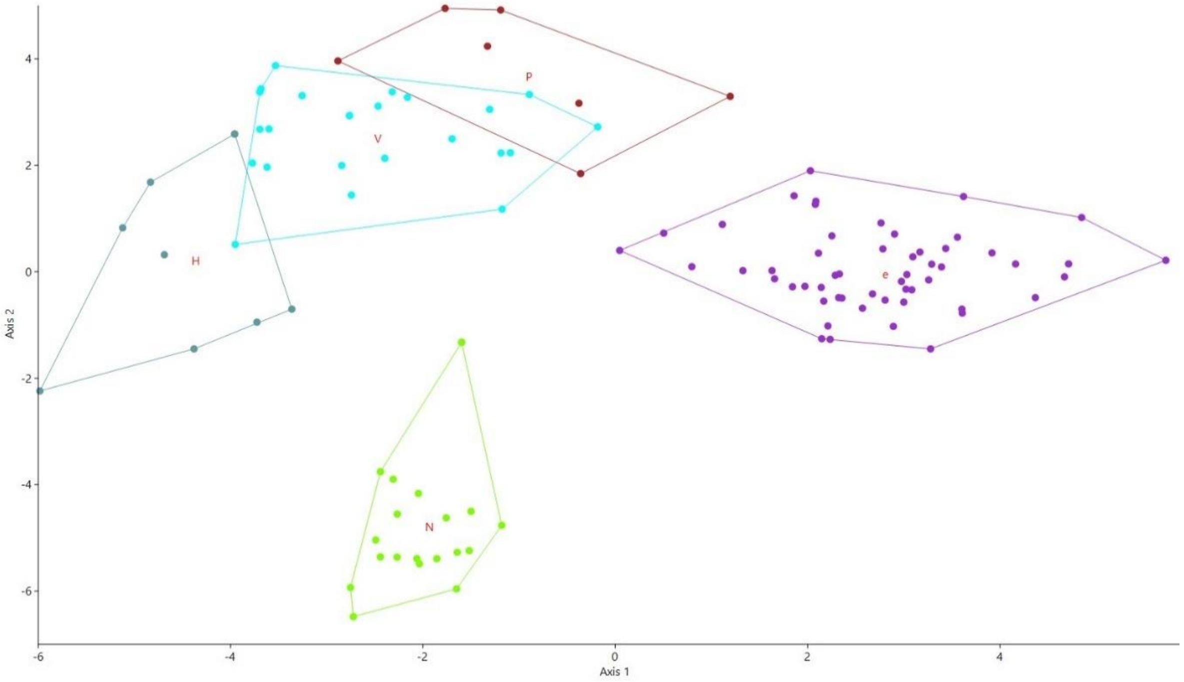

Figure 4.10. LDA of *<u>all morphometric data</u>* *as described in the text. Morphotypes E and N are clearly separated, whereas the remaining three showing partial overlapping. Morphotypes (groups) are identified by letters and different colors:* ● morph. N, ● morph. E, ● morph. P, ● morph. V, ● morph. H.

The analysis of differences between morphotypes was run on classic morphometric data (width, height, width/height), and on additional morphometric variables exported from SHERPA (area, perimeter, rectangularity, compactness, ellipticity, triangularity, roundness, convexity by perimeter, convexity by area). The results are summarized in a pairwise comparison matrix with Bonferroni-corrected p-values (**Table 4.1**), with each parameter also in **Figure 4.7**.

Table 4.1. One-way ANOSIM pairwise matrix with Bonferroni-corrected p-values describing whether morphotypes are significantly different. P-values of classic morphometric data only are given in between brackets if different from the values obtained after the analysis of all morphometric data together.

	E	V	P	N	H
V	*n.s.* (0.002)				
P	0.001 (0.005)	0.001			
N	0.001	0.001	0.001		
H	0.001	0.023 (0.033)	0.002 (0.001)	0.023 (0.016)	

Summarized, all morphotypes were significantly different when only ‘width’, ‘height’ and ‘width/height ratio’ was tested. When also the others were taken into account together with the previous, only morphotypes E and V were not significantly different. Although these two were identified as quite similar according to the SHERPA’s additional morphometric data, they are, however, very different in the overall valve shape (*see* description of each).

The most pronounced significant differences are ‘Rectangularity’, ‘Ellipticity’, ‘Triangularity’ and ‘Roundness’ different (according to ANOSIM with $p < 0.001$; but *see* also **Figure 4.7**). The morphotypes are however very similar in the values ‘Area’, ‘Perimeter’ and in ‘Convexity by perimeter’ which override the others when tested together. Otherwise, morphotypes V and H on one side and morphotypes E, N and P on the other, can be separated from each other by ‘Rectangularity’ and ‘Ellipticity’. Morphotype V (**Figure 4.8 b**) was identified as the most elliptic (and at least triangular) one, similarly as morphotype H (**Figure 4.8 e**). Morphotype P (**Figure 4.8 c**), being the biggest morphotype, has the highest values encountered for basic morphometric data and also for the ‘Convexity by Area’, but has very similar ‘Convexity by Perimeter’ with regard to morphotype E (**Figure 4.8 a**). These two morphotypes have also similarly low values of ‘Roundness’ and ‘Compactness’, whereas the others are characterized by higher values. Morphotype N (**Figure 4.8 d**) is less convex in both area and perimeter, than the other morphotypes, but can be characterized together with morphotype E by higher ‘Triangularity’ and lower ‘Rectangularity’ and ‘Ellipticity’. These two are however distinct in ‘Compactness’ and ‘Roundness’ (E being less in both), similarly as morphotypes P and H (P being less in both). Morphotype N has been identified by far as the smallest one in basic morphometric data and in ‘Area’.

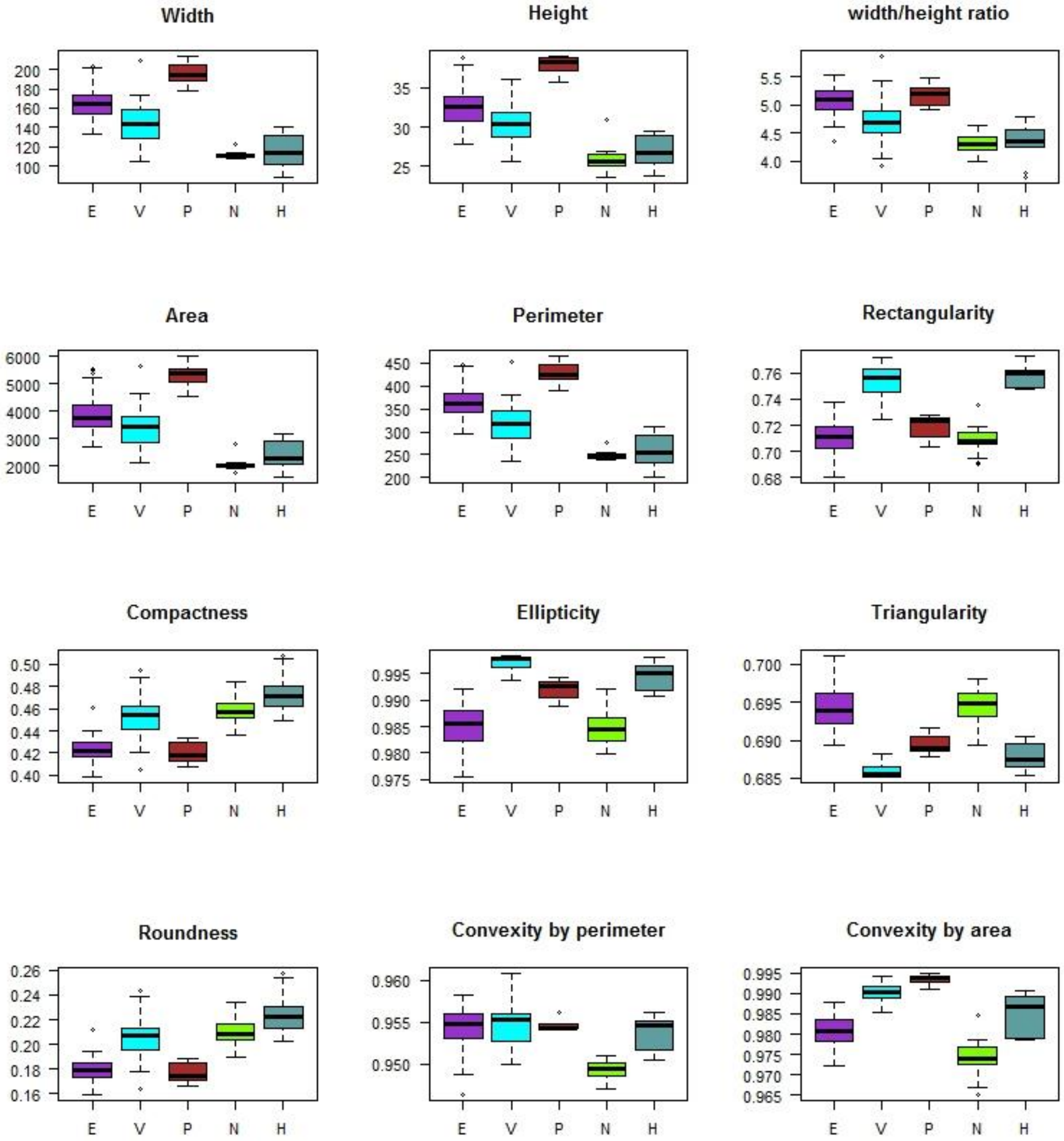

Figure 4.11. Ranges with medians of accounted morphometric data and heuristic shape descriptors per each morphotype.

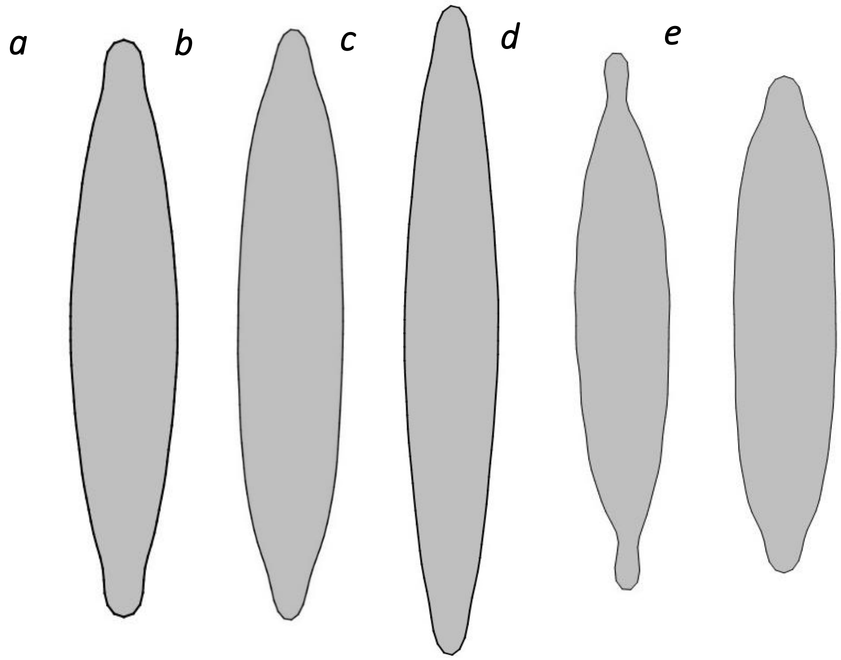

*Figure 4.8. PCAs of overall mean shapes of different morphotypes EFA. (**a**) morphotype E: PC 1 explained 47.63 % of variance, PC 2 46.74 %. (**b**) morphotype V: PC 1 explained 81.99 %, and PC 2 15.04 %. (**c**) morphotype P: PC 1 explained 74.99 %, PC 2 20.19 %, and PC 3 2.55 %. (**d**) morphotype N: PC 1 explained 53.01 %, PC 2 37.90 %, and PC 3 3.9 % (image exaggerated). (**e**) morphotype H: PC 1 explained 80.64 % of variance, PC 2 15.65 %, and PC 3 ('necking') 2.03 %. Axes were equaled.*

Morphotypes of *Frustulia*

In the following text, each morphotype is delimited based on its unique morpho-characters. Images of light microscope (LM) were used for the formal morphological description and subsequently also for analyses in SHERPA software. Images of scanning electron microscope (SEM) were used for ultramorphological observations. EFA coordinates obtained from SHERPA software were further used in different principal component analysis (PCA) to ordinate the mean valve shape outline particularly (in **Figure 4.8**), as it seemed to be the most characterizing parameter for possible application on Gough's *Frustulia* population.

Frustulia spE (morphotype E) (Figs 4.8a, 4.9, 4.10)

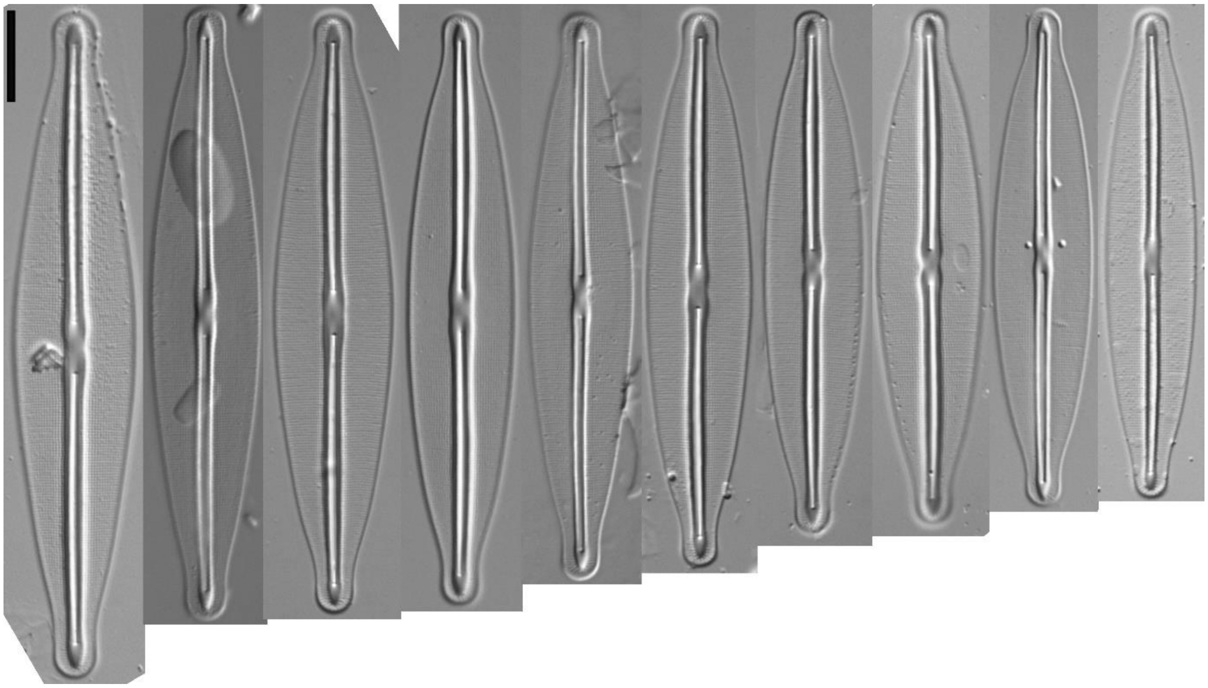

Figure 4.9. Morphotype E. Light micrographs showing the range of variability within the main population. Scale bar = 10 µm.

Main population locality: Gough Island, narrow rock channel on west side of South Peak, from dense moss turf in drainage line; wet; medium sheltered; 650 m above sea level. Sample nr 2000-673; coll. date 16.09.2000, leg. N.J.M. Gremmen.

Description: Valves elliptic-lanceolate (smaller forms) to rhombic-lanceolate (larger forms) with constricted, subcapitate apices. Valve margins sometimes undulated right before the apices rig, although most valves never undulated. Valve dimensions (n=54): length (45)56–78(80) µm, width 10–14 µm, central nodulus 4.2–5.5. Longitudinal ribs and raphe structure similar to *Frustulia saxonica*, almost straight with conspicuous constricted central nodule. Longitudinal ribs fused near the apices forming a weakly developed protruding porte-crayon. Striae parallel near the central area, only rarely subparallel towards the poles but radiate at the apices, 33–36 in 10 µm.

Remarks: *Frustulia* spE clearly belongs to the *F. saxonica* species complex, and most closely fits to what was described as F. saxonica morphotype II (L: 37–70 µm, B: 10.5–13 µm, in Lange-Bertalot 2001). It is generally less robust in valve morphology than the *F. saxonica* species complex, although smaller forms can be very similar. Although quite similar in length, *F.* spE is broader, with more parallel and finer striae (*F. saxonica* 29–32 in 10 µm, in Lange-Bertalot 2001; 30 in 10 µm in Lange-Bertalot & Metzeltin 1996, 2001). *Frustulia* spE most resemble to what was described as *F.* cf. *saxonica* (L: 34–63, B: 9–12.5, stria density similar) in Graeff et al. (2012) although with slightly larger valve dimensions and with more constricted apices and less pronounced porte-crayon structure. *Frustulia* spE can be mostly diffearated from both, *F.* cf. saxonica and *F. saxonica* by the (ultra)structure of the apices, and generally narrower overall valve outline (less rhombic). *Frustulia* spE is less rhombic than *F. rexii* Graeff & Kociolek (Graeff et al. 2012). Striae of *F.* spE are radiate at the apices whereas those of *F. inculta* Siver, Pelczar & Hamilton and *F. krammeri* Lange-Bertalot & Metzeltin are not. *Frustulia* spE has also much less distinct porte-crayon (if noticed) than *F. inculta, F. krammerii, or F. rexii*. *Frustulia* spE is narrower than *F. quadrisinuata* Lange-Bertalot (Lange-Bertalot 1996: L 55–75 µm, B 14–16 µm) with less undulate margins and more protracted apices. *F.* sp. E has similar striae density as *F. crassinervia* (Lange-Bertalot 2001: L 30–55 µm, B 8–12 µm), but is bigger in length, with much less undulate margins and more constricted central nodule. *Frustulia pangaeopsis* Lange-Bertalot, *F. pangea* Metzeltin & Lange-Bertalot and *F. bahlsii* Edlund & Brant (Edlund and Brant 1997: L 98–193 µm, B 24–33 µm) are much bigger than *F.* spE, with coarser striation. *Frustulia bahlsii* has much rounder helictoglossae than *F.* spE, which resemble *F. curvata* Kulichova & Urbankova (Urbankova et al. 2014: L 52–62 µm, B 11–12 µm, striae 28–30 in 10 µm) by its size range and longitudinal ribs, but is narrower, with finer striation and somehow more constricted apices (especially bigger forms of *F.* spE). *Frustulia* spE has coarser striae than *F. lebouvieri* Van de Vijver & Gremmen (Van de Vijver and Gremmen 2006: L 40–75 µm, B 11–15 µm, striae 32–38 in 10 µm) having also more protracted helictoglossae and less round overall shape. *Frustulia lacus-templi* Cejudo-Figueiras, Blanco & Alvarez-Blanco is smaller (Blanco et al. 2012: L 34.6–57.3 µm, B 8.5–10.4 µm, striae 34–36 in 10 µm) than *F.* spE, with more restricter terminal nodule.

Associated floras- *Frustulia* spE co-occurred on sites together with high abundances of *Chamaepinnularia* cf. *begeri, Eunotia paludosa* var. *paludosa* group, *Pinnularia vapila,* but also with *P. sistassa.* This morphotype was frequently found on Gough Island, mostly in smaller forms but also the bigger, very elegant forms were not rare. In within genus, mostly have been found together with *F.* spN but can be differentiated from this one visually by being bigger in length, having less constricted and less rounded apices, being less rounded in overall appearance and also conspicuous in spE are the longitudinal ribs, being strong and constricted in the central fuse.

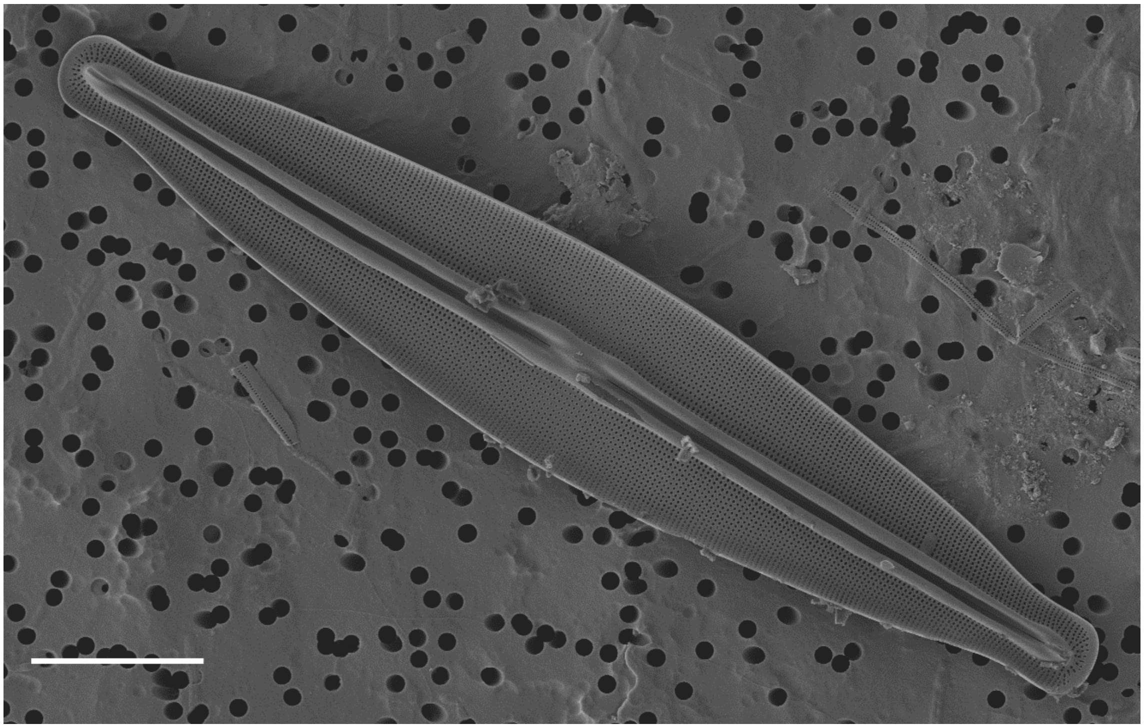

Figure 4.10. Frustulia *spE internally. Scale bar represent 10 µm.*

Frustulia spV (morphotype V) (Figs 4.8b, 4.11, 4.12)

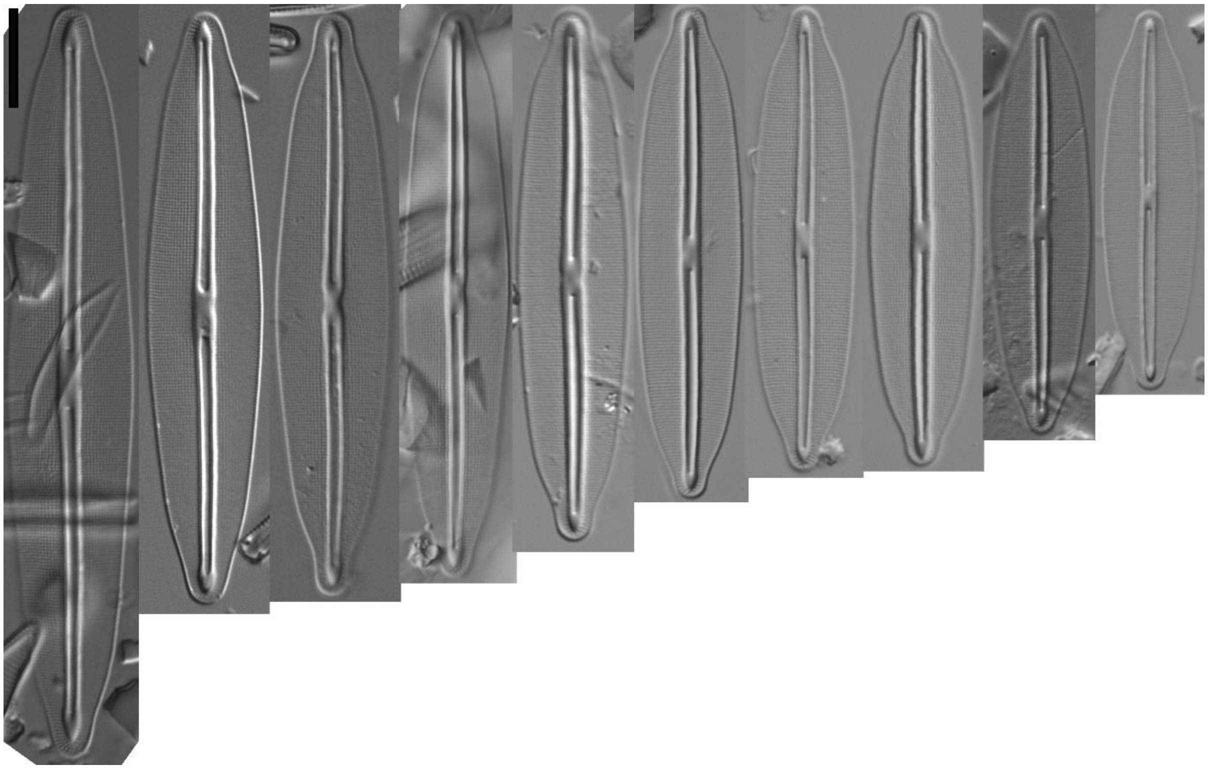

Figure 4.11. Morphotype V. Light micrographs showing the range of variability within the main population. Scale bar = 10 µm.

Main population locality: Gough Island, Golden Higway river (south of Tafelkop), in *Bryum* cushion on rocks in river bed, 10 cm above water level; very wet; sheltered; 150 m above sea level. Sample nr 2000-666; coll. date 16.09.2000, leg. N.J.M. Gremmen.

Description: Valves linear-lanceolate with slightly triundulate margins and protracted, slightly rostrate apices. Valve dimensions (n=23): length 38–75 µm, width 10–14 µm, and central nodulus 3–6. Longitudinal ribs almost straight, only slightly constricted at the central nodule, resembling ribs of *Frustulia crassinervia* (sensu Lange-Bertalot 2001). Longitudinal ribs fused with the helictoglossae although not discernible in LM, oval in smaller forms. Striae parallel entirely radiate at the apices, 31–33 in 10 µm.

Remarks: *Frustulia* spV is not rhomboid and is much smaller in size compared to *F. saxonica, F. rexii, F. krammeri* or *F. inculta,* it is also much more parallel with less conspicuous margins undulation than *F. crassinervia. Frustulia* spV has comparable narrow apices as *F. lebouvieri* and is similar in size, but *F. lebouvieri* is never triundulate and is generally much rounder. *Frustulia* spV can be easily recognized from *F. pulchra* by its smaller size (Van de Vijver et al. 2002: L 69–116 µm, B 14–21 µm, striae 27–28 in 10 µm), more constricted central nodule and much less distinct porte-crayon structure by which also differs from *F. subantarctica* which has bigger length range (Van de Vijver et al. 2002: L 50–100 µm)

Associated floras: *Frustulia* spV co-occurred on sites together with high abundances of *Eunotia pectinoides* (both forms)*, E.* cf. *fallax, Pinnularia posita* and *Psammothidium atalanta.* This morphotype was frequently found together with *F.* spN and *F.* spH. from which it can be differentiated visually by more undulated margins, and mostly by narrowly protracted apices. *Fr.* spV was not that abundant as morphotypes E or N, but have been frequently found throughout the samples.

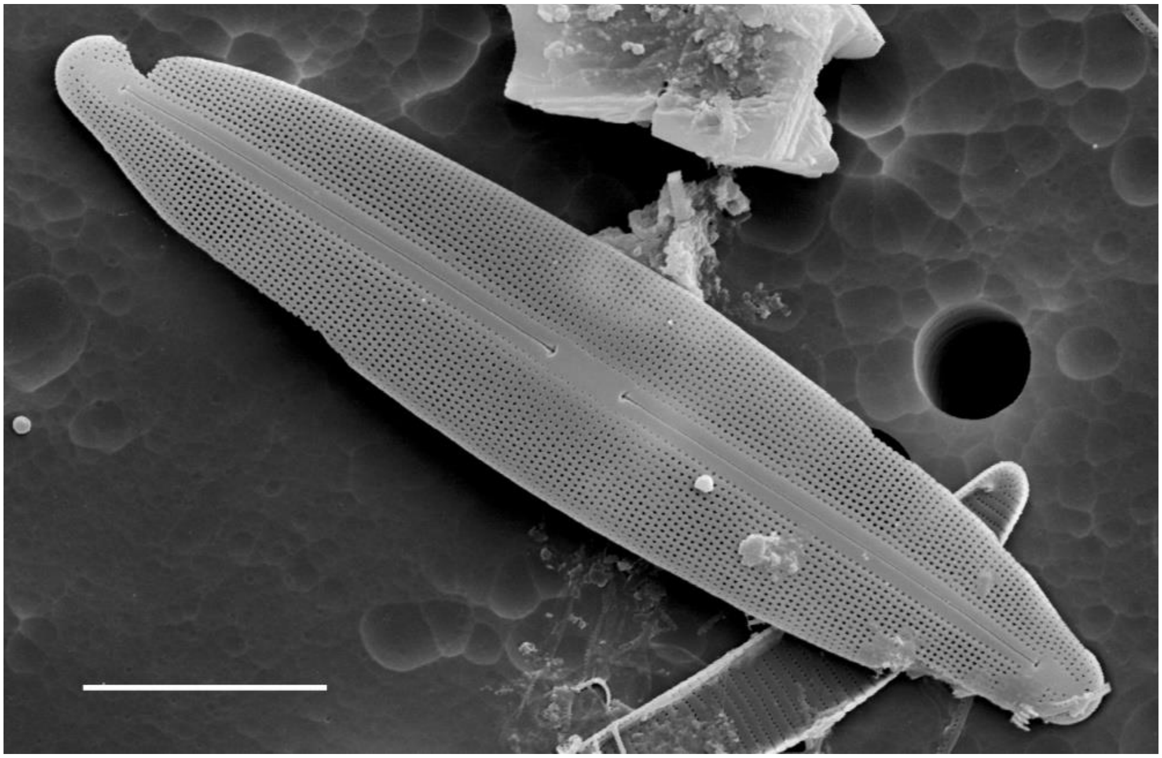

Figure 4.12. Frustulia *spV externally. Scale bar represent 10 µm.*

Frustulia spP (morphotype P) (Figs 4.8c, 4.13, 4.14)

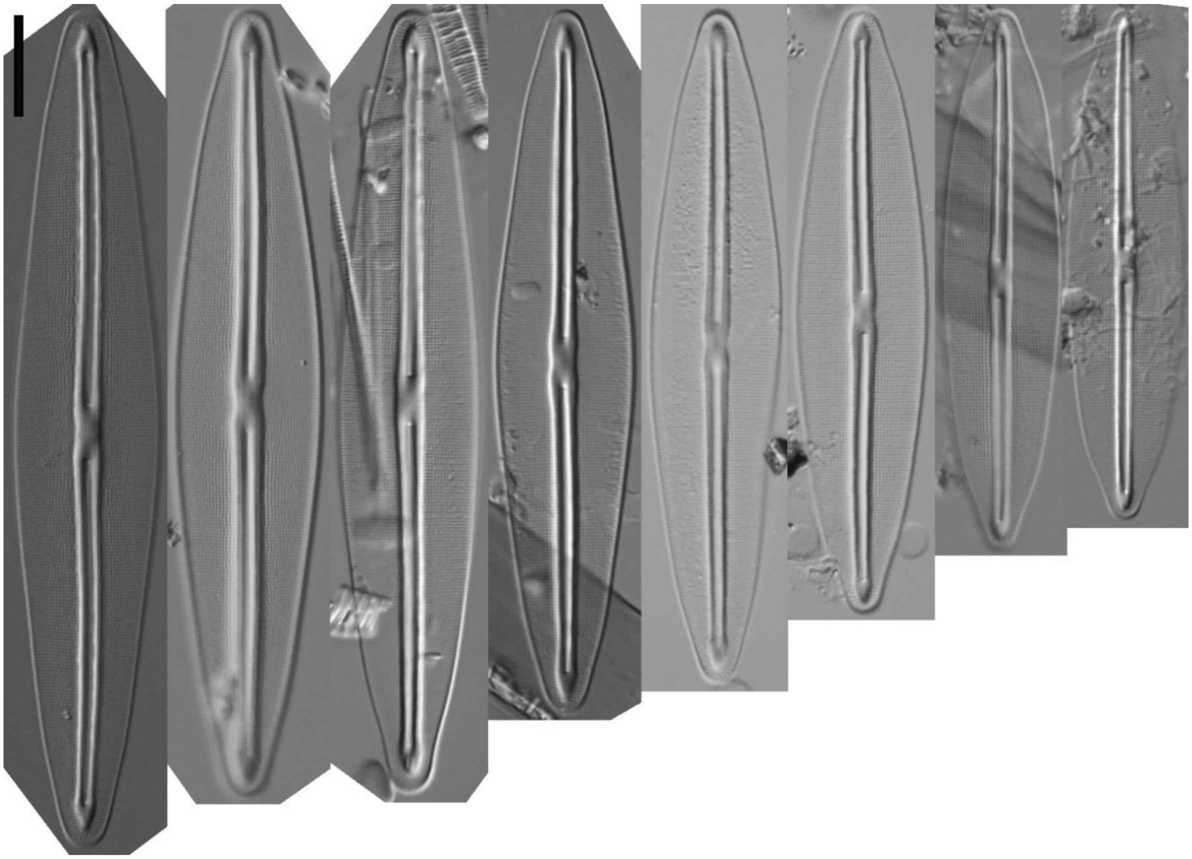

Figure 4.13. Morphotype 'P'. Light micrographs showing the range of variability within the main population. Scale bar = 10 µm.

Main population locality: Gough Island, North facing slope of Tafelkop, moss and hepatics mat on shallow peat overlying mineral soil in sloping mire; very wet; very exposed; 475 m above sea level. Sample nr 2000-675; coll. date 16.09.2000, leg. N.J.M.Gremmen.

Description: Valves rhomboid to broadly-lanceolate (small forms) with moderately triundulate margins and slightly protracted, rostrate apices. Valve dimensions (n=12): length 50–84 µm, width 11.5–15 µm, central nodulus 3.8–6. Longitudinal ribs almost straight, slightly constricted at the central nodule. Longitudinal ribs and the helictoglossae fused at the apices forming a distinct porte-crayon structure, especially visible in larger forms. Striae parallel throughout becoming slightly convergent towards the apices and entirely radiate at the apices, 31–33 in 10 µm.

Remarks: Due to its rhombic valve outline, *Frustulia* spP resembles other rhombic *Frustulia* species such as *F. saxonica, F. rexii, F. krammeri* and *F. inculta,* but can be differentiated by moderately undulate margins, less constricted apices and less protracted helictoglossae. This morphospecies has also radiate striate around the apices. *Frustulia* spP is smaller than *F. krammeri,* and narrower than *F. inculta,* with the valve end being more 'pointy' than has *F. saxonica. Frustulia* spP has similarly undulate margins as *F. quadrisinuata* and presents a similar size range, but has less constricted and less rounded apices. Moreover, its striation pattern is finer. The largest two valves of *F. lebouvieri* in Van de Vijver and Gremmen (2006) (L 40–75 µm, B 11–15 µm, striae 32–38 in 10 µm) highly resemble the smaller valves of *F.* spP. Although *F.* spP is coarser in striation, with more 'pointy' apices and probably also not that round in overall shape, main populations of both should be compared to observe if *F.* spP does not represent only the smaller size range of *F. lebouvieri*. *Frustulia subantarctica* has very similar valve measurements (Van de Vijver et al. 2002: L 50–100 µm, B 11–13 µm, str 33 in 10 µm) but *F.* spP has much less distinct porte-crayon structure which never protrudes from helictoglossae and more constricted central nodule. *Frustulia* spP is distinct from *F.* spE by more narrowly protracted non-constricted apices, less rounded at the ends, visually has much more undulated margin. Smaller forms of *F.* spP apparently overlap with the morphology of *F.* spV and are hard to distinguish.

Associated floras: *Frustulia* spP co-occurred on sites together with high abundances of *Distrionella germanii, D. germanii* var. *acostata, Eunotia paludosa* var. *paludosa, E. pectinoides* [forma thin], *E.* sp2 and *E.* sp5. This morphotype was frequently found together with *F.* spE, less with *F. saxonica* and *F.* spL. *Frustulia* spP is conspicuous morphotype, but was very infrequently found in low numbers only.

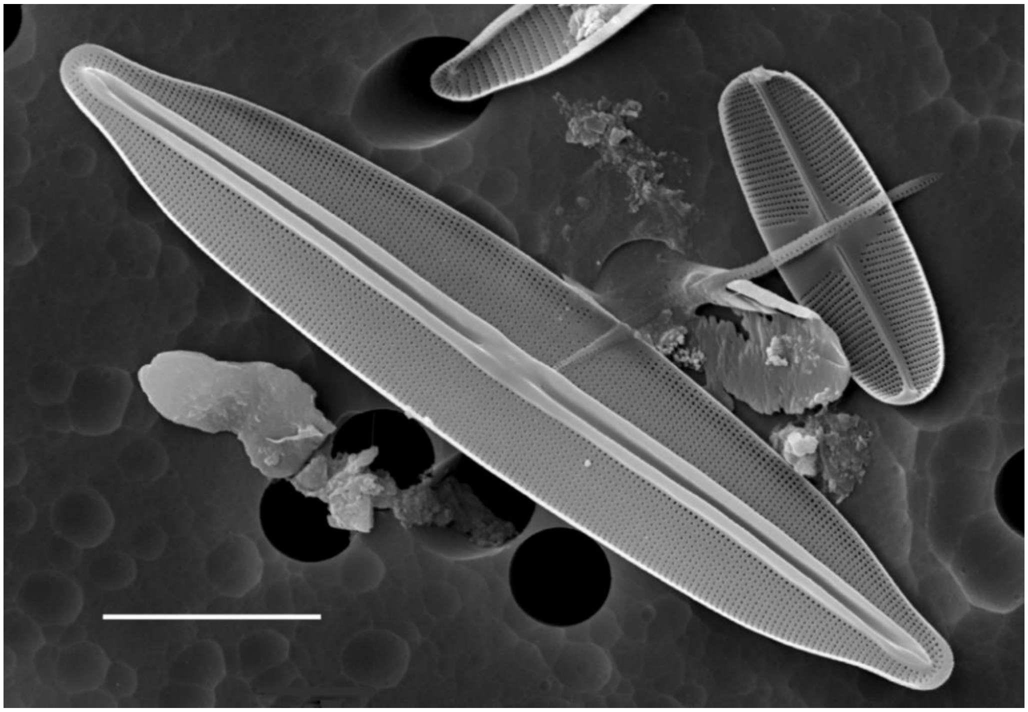

Figure 4.14. Frustulia *spP internally. Scale bar represent 10 µm.*

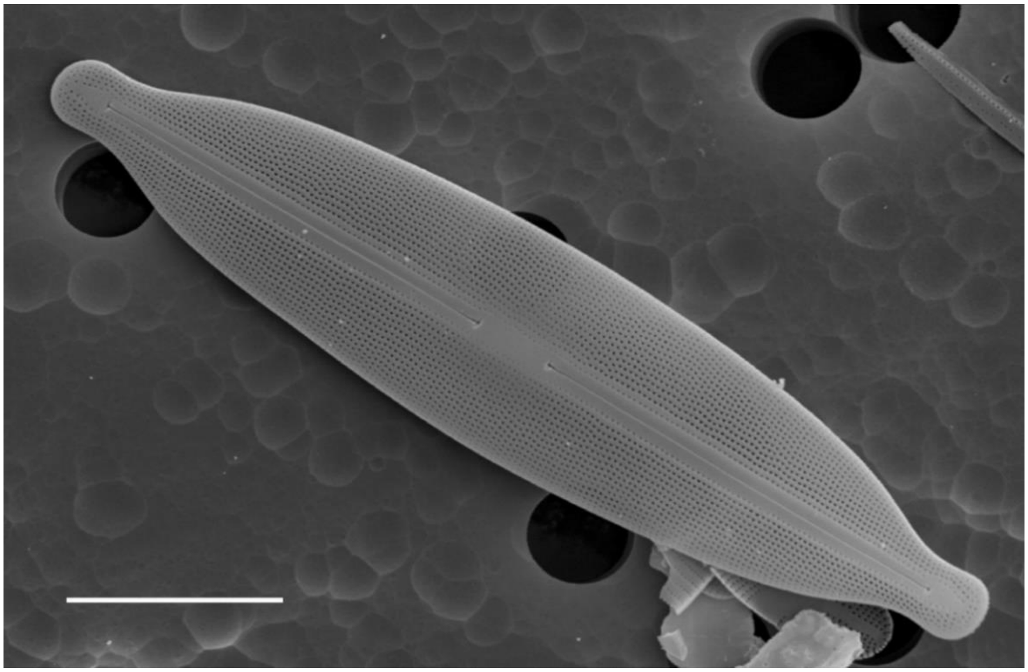

Figure 4.16. Frustulia *spN externally (below). Scale bar represent 10 µm.*

Frustulia spN (morphotype N) (Figs 4.8d, 4.15, 4.16)

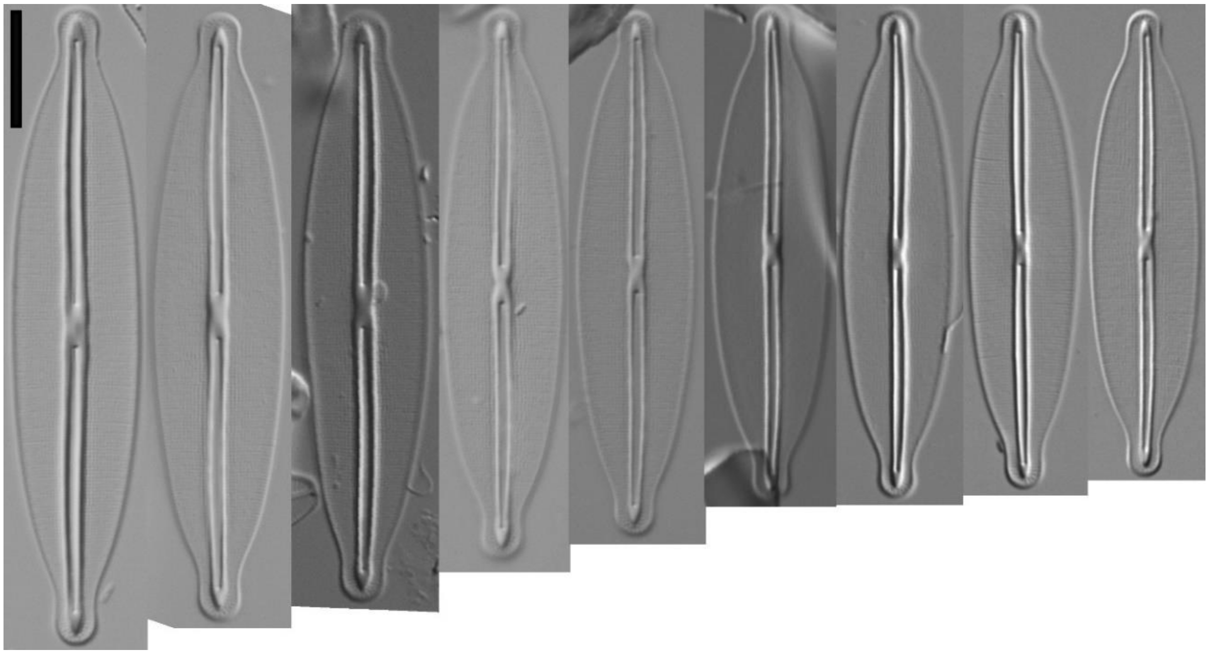

Figure 4.15. Morphotype 'N'. Light micrographs showing the range of variability within the main population. Scale bar = 10 µm.

Main population locality: Gough Island, Golden Higway river (south of Tafelkop), in *Bryum* cushion on rocks in river bed, 10 cm above water level; very wet; sheltered; 150 m above sea level. Sample nr 2000-666; coll. date 16.09.2000, leg. N.J.M. Gremmen.

Description: Valves linear-lanceolate with narrowly rounded, protracted and constricted apices. Valve dimensions (n= 20): Length (39)42–(41)54 µm, width (9)10–11.3 µm, central nodulus 3–4.3. Longitudinal ribs almost straight but more undulate than in the other morphotypes, only weakly constricted at the central nodule. Longitudinal ribs and helictoglossae fused at the apices. Striae parallel, entirely radiate around the apices, 36–37 in 10 µm.

Differential diagnosis: *Frustulia* spN resembles *F. crassinervia* by its narrowly rounded and moderately protracted apices, also by the similar size and striation. *Frustulia* spN has, however, very linear round valve shape without margins triundulation. *Frustulia* spN is smaller than *F. lebouvieri* with more protracted apices and also slightly smaller than *F. curvata* with a finer striation pattern. *Frustulia* spN is smaller than *F.* spE and differs also by a much rounder appearance with more and differently constricted apices. *Frustulia* spN has lighter structure of longitudinal ribs than *F.* spE with a less constricted central fusion and very small helictoglossae.

Associated floras: *Frustulia* spN co-occurred on sites together with high abundances of *Chamaepinnularia* sp1, *Eunotia paludosa* var. *paludosa,* and *Pinnularia sitassa*. This morphotype was frequently found together with *F.* spE and *F.* spV. *Frustulia* spN was frequently found with medium abundances.

Frustulia spH (morphotype H) (Figs 4.8e, 4.17, 4.18)

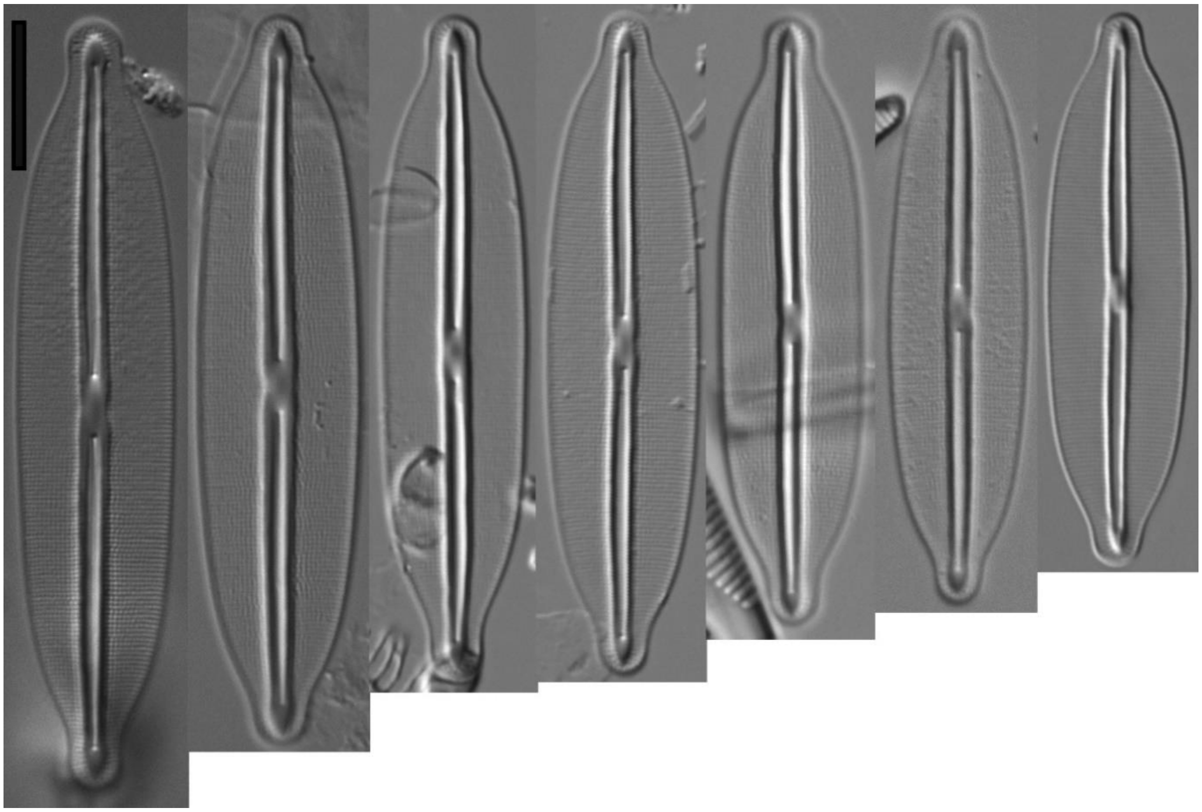

Figure 4.127. Morphotype 'H'. Light micrographs showing the range of variability within the main population. Scale bar = 10 µm.

Main population locality: yet no main population, very limited in numbers – images obtained from several samples.

Description: Valves elliptic-linear with parallel, sometimes very slightly undulate, margins and clearly protracted, rostrate, broadly rounded apices. Valve dimensions (n=7): length 37–52 µm, width 9.7–11.3 µm, central nodulus 3–4.3 µm. Longitudinal ribs almost straight, only slightly constricted at the central nodulus. Longitudinal ribs fused with helictoglossae at the apices, pointy, but not conspicuous in LM. Striae parallel to convergent towards the apices, entirely circumradiate around helictoglossae, 32–34 in 10 µm.

Remarks: *Frustulia* spH generally differs by its parallel margins. They can, however, present some undulation in the middle similarly to *F.* spV. The apices of *F.* spH can be either narrowly protracted or constricted similarly to *F.* spN. It is really questionable whether this morphotype really represents a different taxon or just possess some morphological differences with *F.* spN and *F.* spV. It was infrequently found in very small abundances and only together with the others.

Associated floras: *Frustulia* spH co-occurred on sites together with high abundances of *Eunotia* cf. *ambigua, E. pectinoides* (both forms), *Pinnularia posita, P. restituta, Psammothidium atalanta* and *P. investians*. This morphotype was found in higher abundances only when also *F.* spV was present.

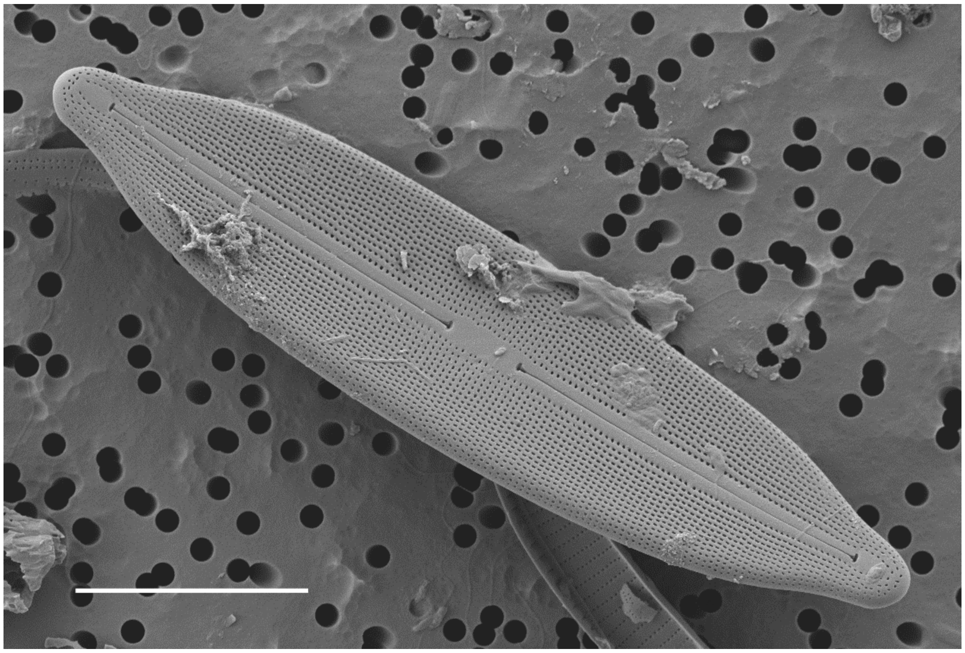

Figure 4.18. Frustulia *spH externally. Scale bar represent 10µm.*

Frustulia on Gough Island

Representatives of *Frustulia* morphotypes were unevenly distributed among the island's moss dominated habitats. Morphotypes most resembling the traditionally recognized species *F. crassinervia* and *F. saxonica* (sensu Lange-Bertalot and Metzeltin 2001) have been reported separately during the community analysis, but these valves appeared to be extremely rare. The widely distributed *Frustulia vulgaris* (Thwaites) De Toni has also been found, but only in very low abundances on Gough and Tristan da Cunha Island, whereas other morphologically diverse and distinct specimens belonging to *Frustulia* were observed within the assemblages. The most common morphotypes in the sample counts were *F.* spE (53 % of all *Frustulia* valves counted), followed by *F.* spN (27 %) and *F.* spV (9 %). Other morphotypes and species were observed and occurred in at least one sample with a relative abundance of more than 1 %, except *F. crassinervia* which was extremely rare (**Figure 4.19**). The most frequently found were *Frustulia* spN and *F.* spV (in 14 out of 49 samples), followed by also frequent *F.* spE (12/14), and *F.* spH (7/49). *F. crassinervia* and *F.* spL were extremely rare, both found in only one sample respectively.

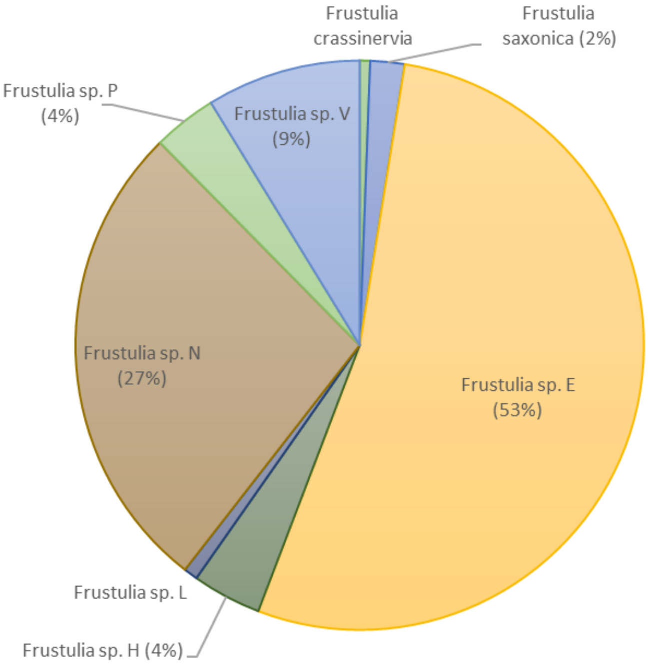

Figure 4.19. Proportional representation of Frustulia *spp. as counted in samples (from relative abundances).*

The typical habitat of most *Frustulia* species is oligotrophic and acidic such as peat bogs (sensu Lange-Bertalot 2001). *Frustulia* species on Gough have been found mostly in aquatic and moist habitats, and less common in moss cushions on rocks in small rivers. The highest numbers of *Frustulia* valves have been encountered in samples restricted to drainage channels (or gullies), from moss cushions found on wet peat soil or stones, on altitudinal higher sites (median 450 m a.s.l.), and at the same time medium exposed and highly moistened. The highest relative abundances of *Frustulia* valves were found in samples 2000-357 (25.40 % of relative abundance in sample), being 150 m a.s.l., and only medium wet. A general ecological characteristic that could be applied on the distribution of this genus on Gough Island is that it has been found restricted to inland habitats, with no sea spray influence. An ordination dbRDA performed in order to identify the environmental variables with possible influence to the distribution of *Frustulia* spp. in samples (pseudo-$F = 2.25$, $p < 0.01$; **Figure 4.20**) revealed that only the parameter 'elevation' was of a significant influence ($F = 4.92$, $p < 0.001$). The other environmental variables ('moisture', 'shelter' and 'bio') were insignificant, and the sea spray ('salt') was totally outside the *Frustulia* genus.

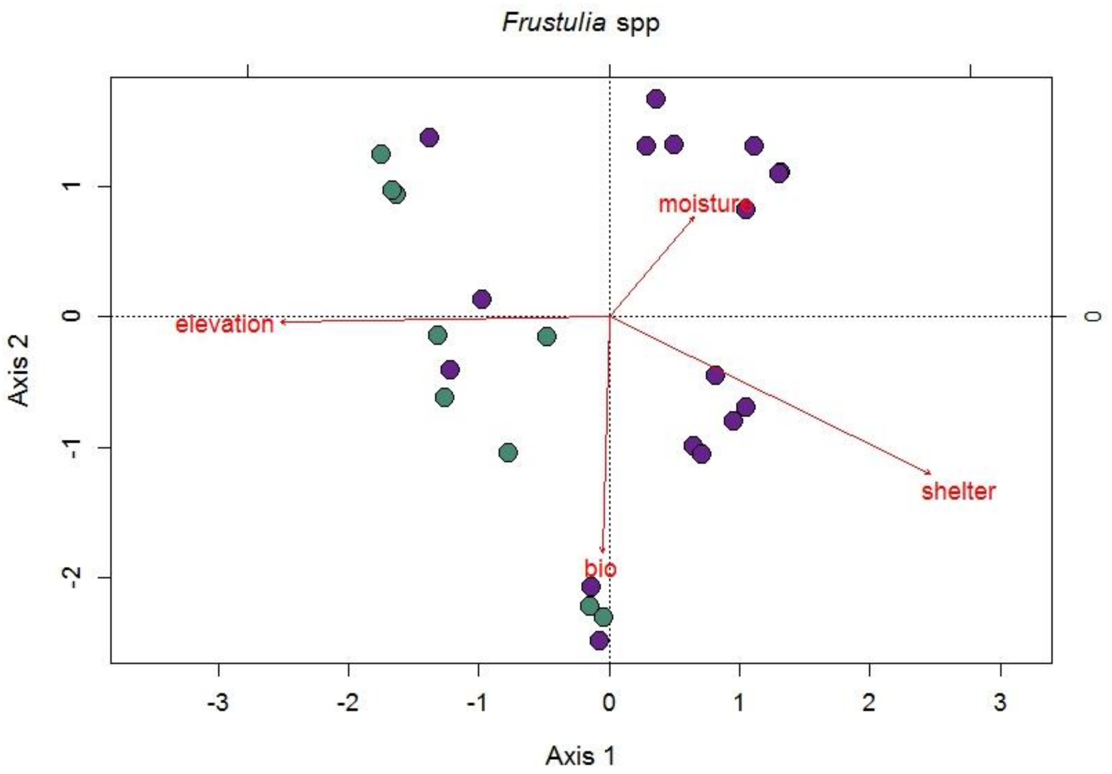

Figure 4.20. dbRDA of samples restricted to only Frustulia *spp. relative abundances, and given environmental parameters.* (elevation = increasing altitude, moisture = moisture level, bio = biotic influence, shelter = sheltered habitats) *Elevation was identified as the only significant variable (F = 4.92, p < 0.001). Axes 1 and 2 were both significant (p < 0.001 and p < 0.01), describing together 38.51 % of proportional variance. The different colors represent different groups as clustered in the overall community analysis with all the species (*see *Chapter 2: green = group D, purple = group E).*

Apparently one sample (2000-933) was taken on a site of seepage water reach; however this one was extremely extraordinary in the shape variation of *Frustulia* species. Although it was the most species rich sample for *Frustulia*, these exhibited some morphological deviations with generally larger valve dimensions than was typical for them (*e.g. Frustulia* spL (n=4; length 86–100 µm, width 16–17 µm) which was observed only in this sample). Another sample rich in *Frustulia* spp. was 2000-710, also quite high in relative abundance (~ 10 %), also from drainage gully and high altitude (450 m), obtained from *Sphagnum* sp. From the ecological point of view, *Frustulia* spN, *F.* spV and *F.* spE reflected mostly the altitudinal gradient of Gough Island, with *F.* spE being most abundant at higher altitudes, and on the other side with *F.* spV, which rarely exceeded 150 altitudinal meters. *F.* spN was quite well distributed below 500 m a.s.l., with a median on ~ 250 m (*see* **Figure 4.21**). This species' preference in distribution according to the altitudinal gradient had a similar effect for the environmental parameter 'shelter', with those of higher elevations being more exposed (*F.* spE) than those of lower elevations (*F.* spN and *F.* spV). No such pattern has been observed for the other morphotypes identified, which had rather lower abundances in the assemblage but were distributed quite evenly.

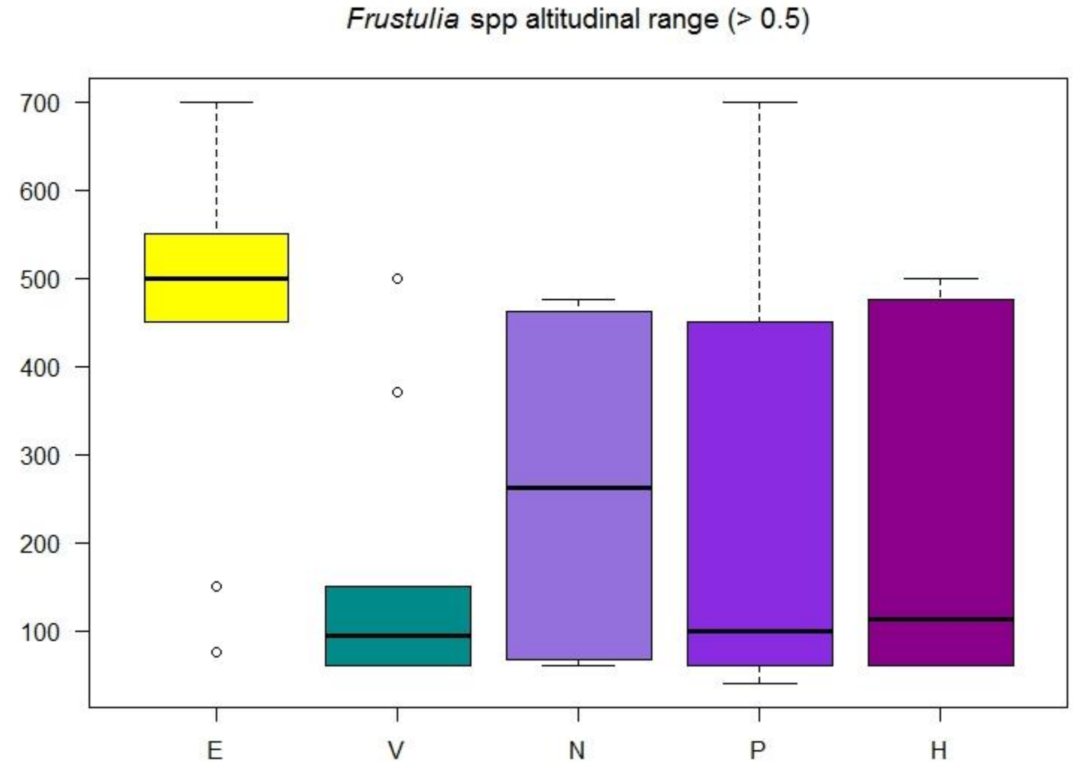

Figure 4.21. *Altitudinal range of* Frustulia *spp. distributed along the altitudinal gradient on Gough Island. Only samples with relative abundances of* Frustulia *valves in sample of more than 0.5 % were taken into account.*

Elliptic Fourier analysis seemed to be a strong tool in delimitation of morphotypes that were distinguished visually. Morphotypes were separated according to their valve outlines, with the only exception of morphotype H which was overlapping with morphotype V. Here, it might suggest that the previous delimitation based on very parallel side margins of *Frustulia* spH, in comparison with slightly undulate margins of *F.* spV, was erroneous and that these actually represents the same, or as an alternative hypothesis, that elliptic Fourier analysis is not sensitive enough to encounter the undulate margins. *F.* spH was found only rarely and always accompanied by *F*. spV which further supports this similarity. When morphotype groups were disregarded, LDA identified groups similarly as when taken into account. The most important discriminants of the variation for the separation of morphotypes were ‘size’/‘valve width’ and ‘necking’.

DISCUSSION AND CONCLUSION

The morphotypes identified in this study all belonged to the *Frustulia crassinervia-saxonica* complex, especially based on valve dimensions and their ultrastructure. However, according to the unique valve outline, several unknown morphotypes could be separated and are described in the presented study as different morphotypes or ‘sp.’s. Although they could only be considered as ‘morphospecies’ since their genetic structure is unknown, it was shown that morphospecies delimited after fine morphometric characters usually have also distinctly unique genotypes (Mann et al. 2004; Poulíčková et al. 2010). Lange-Bertalot (2001) listed *F. saxonica* with a very large length range (28–105 µm), but separated ‘morphotype I’ and ‘morphotype II’, pointing to the high morphological variability of this taxon. This separation was however ‘*without taxonomic significance*’ as it was interpreted by Siver and Baskette (2004). In the case study of an emended diagnosis of *F. disjuncta* Lange-Bertalot, Lange-Bertalot and Sterrenburg (2004) suggested, that morphotypes of *F. saxonica* of two size series represent the same entity, probably as a result of periodic sexuality in a population which results in bimodal size distribution. The difficult morphological variation of *F. saxonica* was, however, not stopping scientists from describing new species highly resembling this taxon in features such as rhombic-lanceolate outlines and less protracted apices [*e.g. Frustulia floridana* (in Lange-Bertalot and Sterrenburg 2004), *F. lebouvieri* (Van de Vijver and Gremmen 2006) or *F. lacus-templi* (Blanco et al. 2012)].That *F. saxonica* actually represent a polyphyletic species complex together with *F. crassinervia,* was only later shown in Urbánková and Veselá (2013). In comparison to *F. floridana,* which has a length range of 86–148 µm (Lange-Bertalot and Sterrenburg 2004), morphotypes identified on Gough Island with length between 38 and 100 µm (main populations) all fits into the *F. saxonica* length range (28–105 µm sensu Lange-Bertalot 2001) and they also overlap in width and striation values. They, however and apart from some, possess more protracted apices or less, but with pronounced widening (identified as 'negative necking'), more importantly, have distinct valve outline shapes which are unfortunately hardly to analyse without geometric morphometry data for *F. saxonica* sensu Lange-Bertalot (2001).

These could also be used in order to delimit different morphotypes present in moss habitats of Gough Island. They were, however, quite similar in ultramorphology, making the identification of different morphotypes under SEM challenging, but might suggests that these could represent closely related species. The characteristic ultrastructural features (such as raphe endings, shape of the areolae, circumpolar areolae, central nodulus, hymenes and helictoglossae or porte-crayon structure) appears to be more or less similar within morphotypes identified (or differed irregularly throughout the assemblage). Although ultramorphological features connect the *Frustulia* spp. of Gough Island with the *Frustulia crassinervia-saxonica* species complex on the first sight, recent molecular findings suggests that ultrastructural features of *Frustulia* spp. are highly similar and might not be powerful enough in order to separate distinct species (Scharfen 2014). The ultrastructural resemblance of distinct morphotypes present on Gough Island could possibly present a case of islandic allopatric speciation, similarly as was suggested for *Eunotia praerupta* Ehrenberg species complex in the Antarctic region (Van de Vijver et al. 2014).

On the other hand, *Frustulia* spp. on Gough Island were morphologically quite variable among different sites, sometimes overlapping in outline variables identified for distinct morphotypes, which could not be encompassed thoroughly in the presented study as it would significantly widen the set of images that would have to be identified for the outline. However, the observed morpho-variability of *Frustulia* representatives could itself reflect an ecologically conditioned plasticity. Phenotypic plasticity has previously been observed in diatoms [*e.g. Diadesmis gallica* (Cox 2006)]. The variation in valve outline of natural populations of the *F. crassinervia-saxonica* complex can be promoted by environmental factors (Kulichová and Fialová 2016) and therefore, also the Antarctic morphotypes could actually represent morphologically distinct ecotypes rather than distinct biological species or phylogenetic entities. Smaller valves of *Frustulia* spP highly resemble those of the larger valves of *F. lebouvieri* which was described from Ile Amsterdam (South Indian Ocean) in Van de Vijver and Gremmen (2006). Although *F.* spP has more acutely rounded apices and helictoglossae, the morphotype seemed to be less round than valves of *F. lebouvieri,* the striae being slightly convergent in both, while the central nodulus and the T-shaped raphe ends look similar. This further supports the close relativeness of these two. *F. lebouvieri* was (similarly) found in 'submerged mosses and plants in bog ponds and lakes, with no altitudinal preference' (Van de Vijver and Gremmen 2006) and from the accompanying species, *Distrionella germainii* was accompanying both, *F. lebouvieri* of Ile Amsterdan and *F.* spP from Gough Island. *F.* sp was the only one from Gough accompanied by the genus *Distrionella* in all samples where it was counted and in high relative abundances, suggesting a similar niche of both *Frustulia* species. It was, however, the morphotype of generally lower abundances on Gough found in only four samples during the counting. The suggested relation of these two should be further studied, in order to understand better the distribution and possible dispersion of diatom species in and around Antarctica, and also the phenotypic plasticity of *Frustulia* representatives. Kulichová and Fialová (2016), regardless of species or genetic identity, tested whether the *F. crassinervia-saxonica* complex morphologically reflects environmental conditions and identified significant correspondence between them. They found that conductivity and pH significantly determined the size variation pattern of *Frustulia* cells, as larger cells were found on habitats with higher values (minerotrophic lowland mires) and smaller cells on habitats with generally lower values (ombrotrophic peat bogs). The same conclusion cannot be applied to the *Frustulia* cells found on Gough as those only reflected significantly the altitudinal gradient and, unfortunately, we have no measured conductivity or pH values for the sites sampled so the elevation might be correlated with some unknown environmental variables. However, one sample of low altitudes was described as 'within seepage water reach' and taking the vicinity of sea into account, this could indicate higher conductivity and pH values caused by the sea influence. The overall larger size of *Frustulia* cells was characteristic for this sample, which could be explained by the association with adaptation to ecophysiological parameters stated. Nevertheless, the same is hardly to interpret after the distribution of different morphotypes along altitudinal gradient.

Although *Frustulia* spE was typically found at higher altitudes whereas *Frustulia* spV at the lower ones, by the size these two were highly similar. They were, however, highly distinct in mean roundness, rectangularity and triangularity. When applied also with other morphotypes, these three heuristic shape descriptors reflected the distribution of morphotypes along the altitudinal gradient. If applied, it seems that being rounder and more rectangular is better for species of lower altitudes on Gough whereas higher triangularity is characteristic for the species of rougher, more exposed, higher altitudes. Survey on dead material only unfortunately prohibits any phylogenetic testing or biological crossing experiments, which would certainly help to unravel this case.

In conclusion, several morphologically distinct species belonging to the *Frustulia crassinervia-saxonica* complex were identified from moss material of Gough Island. These were characterized by different size, valve width, the extent to which apices were protracted and constricted, and the type of marginal undulation, all being characteristic for the variation in valve outlines. At least three of them, *Frustulia* spE, *Frustulia* spN and *Frustulia* spV, had a sufficient number of valves analyzed using EFA and heuristic shape descriptors, and could be proposed as new species ('morphospecies') to science. However, it is questionable whether these morphotypes should be formally described as new species, because it is not entirely clear to what extent their morphological variation reflects environmental conditions (lack of ecological data needed) and if these aren't phenotypically distinct ecotypes rather than distinct phylogenetic entities. Nevertheless, the results of presented study could also serve as a solid ground material and morphological link for possible further study on living diatom material from Gough Island.

CHAPTER 5

GENERAL DISCUSSION

A synthesis is made based on the diatom flora of Gough Island. The diversity, composition, ecology, and general biogeography of the flora are presented in the frame of studies from southern hemisphere. Topics concerning dispersion and distribution of microorganisms, dispersal hypotheses and events are discussed.

GENERAL DISCUSSION

During the present survey of moss-inhabiting diatoms of Gough Island (and partly also of Inaccessible and Tristan da Cunha Island), a unique flora was observed. A total of 141 diatom taxa (including species, varieties, subspecies and formas) has been identified from Gough Island, and additional nine taxa from Tristan da Cunha or Inaccessible Islands. During the counts, 124 taxa of these have been observed. The flattening toward the end of the species accumulation curve indicates that a high portion of the (theoretically possible) flora was recorded. Also the traditionally used species richness estimators (ICE and Chao2) indicated that about 94 % of all taxa on Gough Island have been found in our counts. About half of the species described by Carter in 1966 from the Tristan da Cunha archipelago has been observed and identified during this survey. Most of these observed in our samples on Gough Island moss habitats were originally only found by Carter on Tristan da Cunha (*see* **Appendix 1**). We already clarified the taxonomical position of one intriguing *Eunotia* species identified based on the description of Carter (1966) which was found during this survey (Vinšová et al. 2016 – **Chapter 3**). The observed flora was represented by only a few diatom genera, which is typical for oceanic islands. Also only 11 taxa account for about 67 % of the total number of valves counted during the study. Although the fact that only a few diatom taxa entirely dominated the flora of the moss habitats on Gough Island, most of them were unique and possibly endemic to the Tristan da Cunha archipelago in general, or even Gough Island in particular. Otherwise, diatom taxa already known to the science and observed on the Gough Island were rather cosmopolitan in distribution, than truly sub-Antarctic. The specificity of the Gough Island diatom flora is mainly reflected in the species composition of several highly diversified genera such as *Eunotia, Frustulia,* and *Pinnularia*, typical genera for acidic, oligotrophic habitats. Some species of these show a high morphological variability, and might therefore likely represent species complexes containing taxa that are hard to separate. Several morphotypes of the frequently found *Frustulia crassinervia-saxonica* species complex have been separated during this survey based on geometric morphometry techniques, in order to access the flora diversity in the most detailed way. Unfortunately, it is not easy to establish the correct taxonomical position of these morphotypes without molecular sequencing, as the distinct morphology might only be reaction to different environmental conditions (*see* **Chapter 4**).

Species diversity, composition and general biogeography

The observed species richness was rather low and about one third lower than observed on the moss-inhabiting diatom communities on sub-Antarctic islands (Van de Vijver and Beyens 1997; Van de Vijver et al. 2004, 2008) in the southern Indian Ocean. The diversity is however comparable to Livingston Island in Maritime Antarctica (Kopalová et al. 2014) and Ile Amsterdam in the cool-temperate zone of the Indian Ocean (Chattová et al. – *unpublished results*). Gough had more than double the species number recorded on James Ross Island (Kopalová et al. 2014), mainly due to the absence of a large number of environmentally diverse habitats on the latter. It is generally accepted that diversity decreases moving southward in the Antarctic region (Jones 1996; Van de Vijver & Beyens 1999b), which is clearly demonstrated by a decreasing diatom diversity moving from the sub-Antarctic islands to the (more southerly positioned) islands of Maritime Antarctica. Gough Island is located in the cool-temperate zone of South Atlantic, with an annual mean temperature about two to three times higher than more southward located islands (Greve et al. 2005). At the same time, the island has a lower diatom species diversity than found in the moss-inhabiting diatom assemblages of sub-Antarctic islands. One of the main reasons is the fact that Gough Island is one of the most remote islands in the world, and the more isolated a locality gets, the lower the species number might be (Cox & Moore 2010). So, even when the evolution rates get promoted on isolated islands, the species equilibrium cannot be achieved, unless the island is located near a major species source = larger landmasses such continents, island-continents (Schoener 2009). This is well-reflected by the disharmonic fauna and flora composition of such locality (Cowie and Holland 2006; Gillespie 2007), resulting in only a limited number and variation of diatom taxa (Preece et al. 1986).

Typically for Gough Island, some diatom genera are overrepresented whereas others are lacking. For example, the diatom genera *Eunotia* accounted for 42 % of all recorded taxa, whereas other generally species-rich genera such as *Navicula* or *Nitzschia* were far less abundant. The two most abundant genera on the island, *Eunotia* and *Pinnularia* (14 %), were also the most species-rich, with 16 and 17 taxa respectively. Similarly to the generic richness, only few taxa dominate the diatom moss-inhabiting flora (**Figure** 5.1). The group of *Eunotia paludosa* var. *paludosa* (16 %) was followed by *Eunotia johncarteri* (14 %) and *Eunotia* cf. *fallax, Psammothidium atalanta,* and *Pinnularia restituta,* all of which accounted for more than 5 % of all taxa.

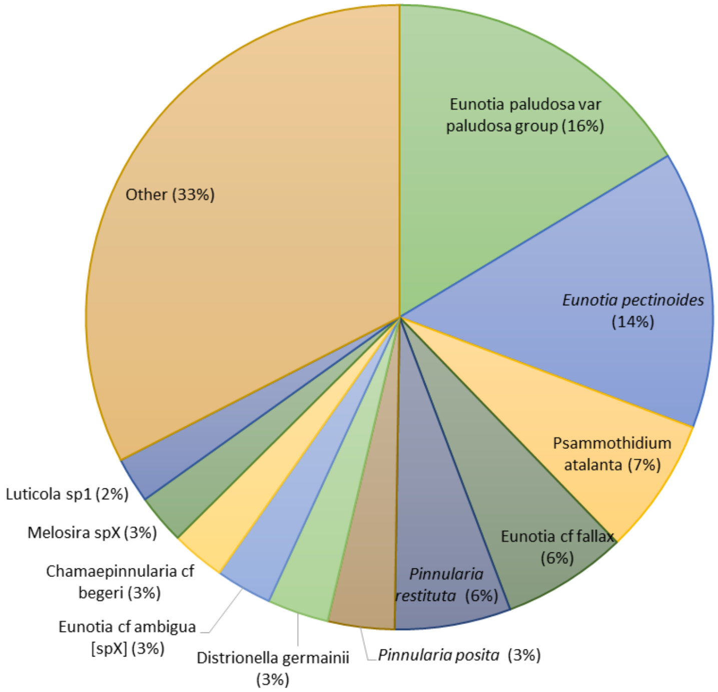

Figure 5.13. Proportional representation of diatom taxa as counted in samples. Taxa of relative abundances less than 2.0 % are included within 'Other'.

The generally low number of abundant species and genera is a typical feature of remote islands (Chattová et al. 2014). Even though a number of species restricted to the island (or locally restricted to the archipelago) have been observed, the most dominant taxa, *Eunotia paludosa* var. *paludosa*, is a rather cosmopolitan in distribution (Van de Vijver et al. 2008b; Van de Vijver et al. 2014a) and the species has also been observed to be a dominant taxon in the moss habitats of other southern oceans islands – *e.g.,* Heard Island (Van de Vijver 2004), Ile Amsterdam (Van de Vijver et al. 2008b; Chattová et al. – *unpublished results*), or soils of Ile de la Possession (Van de Vijver and Beyens 1999a; Moravcová et al. 2009). *Eunotia johncarteri* was a comparably abundant species present on Gough Island moss habitats. This species was identified after Carter's description [as *E. pectinoides* in Carter (1966)] although separated into two, morphologically distinct forms, *E. johncarteri* forma *thin* and *E. johncarteri* forma *thick,* during the community analysis. However, in sample 2000-195, both forms of *Eunotia johncarteri* were found and could be hardly separated as a large proportion of intermediate forms were observed. Both forms were found in equal abundances mostly in similar samples, suggesting that the species is a morphologically highly variable taxon. Apart from *E.* cf. *fallax* and *Distrionella germainii*, the remaining abundant species (> 3.0 %) consisted of those identified after Carter (1966) which have never been reported from outside the Tristan da Cunha archipelago. Here, *Psammothidium atalanta, Pinnularia restituta* and *P. posita* were both abundant and frequently found in the sample set. Although species described as new from the oceanic islands are not necessarily endemic (Flower et al. 2012), it is highly probable that these, described 50 years ago, are restricted in their distribution to the archipelago, as remote islands tends to have higher number of unique diatom endemic flora (Vyverman et al. 2007; Van de Vijver et al. 2012).

Moss-inhabiting diatom communities

The composition of non-marine diatoms observed from moss-dominated microhabitats of Gough Island is truly bryophytic. Abundant dominant diatom genera such as *Chamaepinnularia, Distrionella, Eunotia* or *Frustulia,* are typical aerophilic moss inhabitants reported from sub-Antarctica (Van de Vijver et al. 2004; Van de Vijver and Gremmen 2006; Kopalová et al. 2015) and Europe (Lange-Bertalot and Metzeltin 1996; Lange-Bertalot 2001; Wetzel et al. 2013). Aerophilic genera such *Orthoseira, Pinnularia* or *Hantzschia* were also frequently present in the samples, however they were significantly less abundant. *Pinnularia* and *Humidophila* are rather typical for semi-wet to dry terrestrial mosses (Van de Vijver et al. 2004). The *Pinnularia borealis* species complex of Gough Island might be composed of several, probably new, species. This complex is typically terrestrial, similar to the *Hantzschia amphioxys* complex or *Humidophila arcuata* reported from rather drier environments (Van de Vijver & Beyens 1997; Kopalová et al. 2014).

On Gough Island, the habitat type had a generally lower influence in structuring diatom communities than individual environmental variables. Groups identified based on the cluster analysis had more significant power in shaping diatom composition in mosses. Dominant diatom taxa of these can be found in **Figure 5.2–5.22)**. The first identified group was dominated by *Luticola* sp1 and *Humidophila contenta.* These taxa belong to typically aerophilic genera and are frequently found as dominant taxa in terrestrial habitats of the Antarctic Region (Van de Vijver and Mataloni 2008; Kopalová et al. 2009, 2015). The second group was dominated by *Achnanthes okamurae* (sensu Carter 1966) and *Planothidium pericavum*, both reported from other localities worldwide although their taxonomical status is unclear (Van de Vijver, pers. comm.). The third group is dominated by *Melosira* spX and *Eunotia* cf. *lecohui*. These three groups, although identified as significantly different based on their species composition, can be together characterized as 'areas close to the sea' found on very low altitude (< 35 m a.s.l.) and positive values of sea spray. They also lack diatom taxa characteristic for higher altitudes. The PCA clustered these groups together, suggesting their similar nature, and the dbRDA analysis identified sea spray influences to be the most significant environmental variable separating these from the others. Samples of these were collected on beaches, in rockpools or on rocky cliffs, and two samples of the third group came out from tuffaceous highly sheltered deposits of steep slopes – the only habitat in which *Melosira* spX clearly dominated. It was also the characteristic habitat for *Eunotia linearis* described from the island, which otherwise occurred in less than 1 % of relative abundances, suggesting the very narrow ecological preferences of *E. linearis*. Those can be summarized as typically wet and sheltered moss microhabitats, within the limited reach of sea spray, probably preferring higher conductivity values or directly brackish semi-terrestrial habitats. Importantly, the connectivity of first three groups would be lost on the generic level only, where *Eunotia* and *Melosira* would clearly separate the third group from the previous two based on its preferences for highly sheltered habitats.

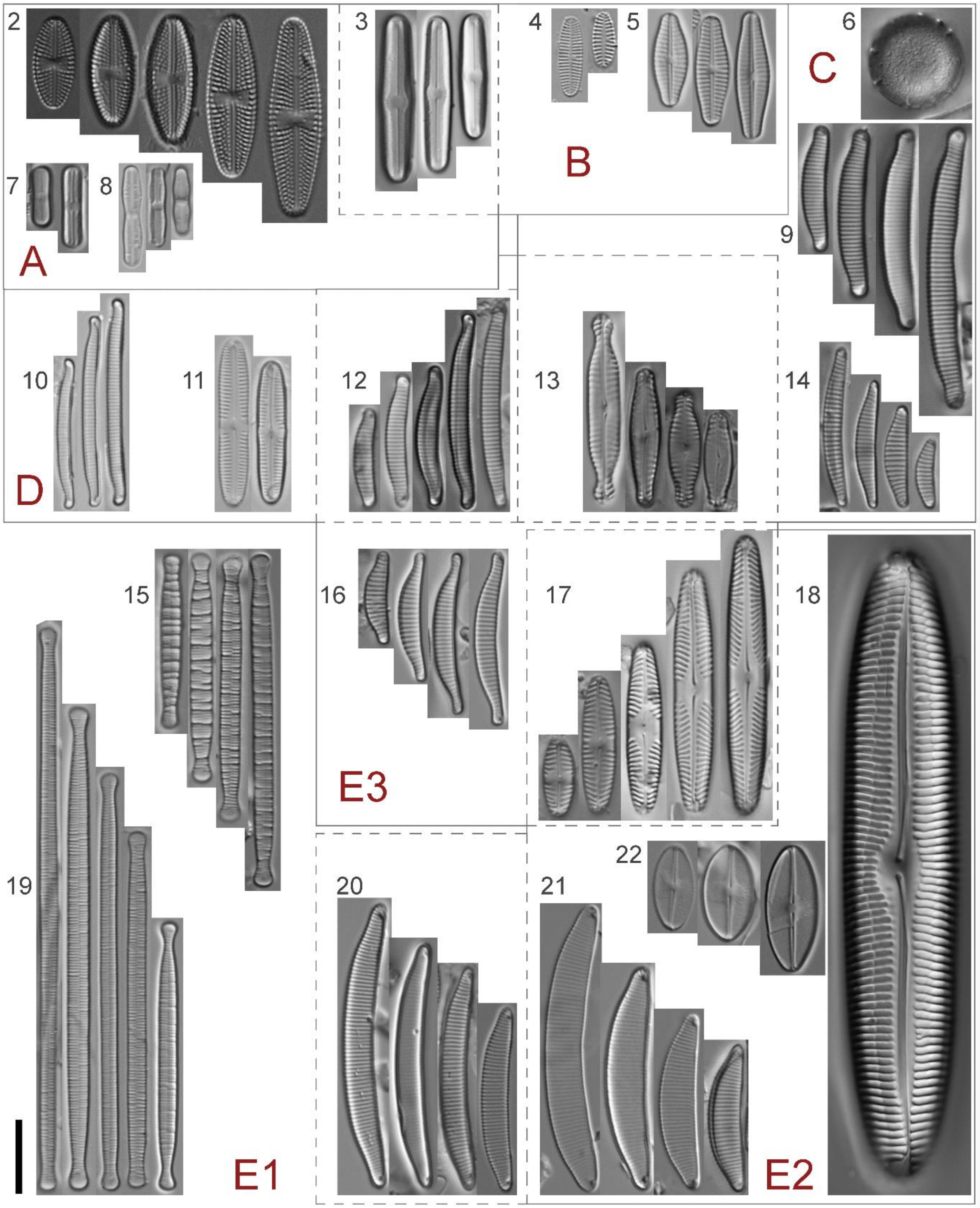

Figure 5.2–5.22. *Dominant diatom taxa of groups and sub-groups*. *(2)* Luticola *sp1, (3)* Humidophila *sp177, (4)* Achnanthes okamurai*, (5)* Planothidium pericavum, *(6)* Melosira spX, *(7)* H. contenta*, (8)* H. nienta*, (9)* Eunotia *cf.* lecohui, *(10)* E. paludosa *var.* paludosa, *(11)* Chamaepinnularia *cf.* begeri*, (12)* E. *cf.* fallax*, (13)* Pinnularia restituta*, (14)* E. ambigua*, (15)* Distrionella germainii*, (16)* E. *spX* [*cf.* ambigua]*, (17)* P. posita*, (18)* P. vapilla*, (19)* D. germainii *f.* acostata*, (20)* E. johncarteri *f.* thin*, (21)* E. johncarteri *f.* thick*, (22)* Psammothidium atalanta. *Scale bar represent 10 µm.*

The fourth group in the moss assemblages is characterized by the clear dominance of *E. paludosa* var. *paludosa,* a cosmopolitan species, frequently reported from sub-Antarctica and typically found in semi-wet terrestrial mosses or small acid pools of lower conductivity (Van de Vijver et al. 2014a). This *E. paludosa* var. *paludosa* group is typical for localities found at higher altitudes (mean 500 m a.s.l.) and characterized by the lowest Evenness diversity index (0.11) found in this study, higher moisture levels, but being intermediately exposed. Samples of this group were collected from aquatic habitats, peat bogs and slopes, or from drainage channels and are characteristic by a sometimes almost monospecific community of *E. paludosa* var. *paludosa*, similarly as was noted from Ile Amsterdam (Van de Vijver et al. 2008; Chattová 2016 – *unpublished results*) and Ile de la Possession (Van de Vijver & Beyens 1999a). When other taxa were present, they mostly belonged to *E.* cf. *fallax* or *Chamaepinnularia* cf. *begeri*. Only in two samples, *Ch.* cf. *begeri* was more abundant than *E. paludosa* var. *paludosa,* in samples taken from upland mires (2000-137, 2000-711).

Contrary to the first three groups, the fifth group was composed of 28 samples in total, and of diatom taxa with different influences shaping community structure. The 'inkspot' and PCA ordination helped to subdivide this group into three sub-groups, which can help in better understanding of environmental influences. In general, the diatom genera *Eunotia, Pinnularia* and *Psammothidium* dominated this group, although the first, and smallest, sub-group was dominated by *Distrionella germainii.* This sub-group can be characterized by the highly moist habitats (very wet peat or seepages) of higher altitudes. *Distrionella germainii* was found to be typical for medium altitudes at sub-Antarctic Iles Kerguelen (Gremmen et al. 2006), typically in some form of flowing water (Van de Vijver, pers. comm.). On Gough, this species highest relative abundance was measured in sample taken from a moss cushion on rock on the river edge, but was also found to be abundant in a sample of a wet sloping mire, optimally at ca. 400 m above sea level. This finding might suggest that even more southward, the ecological optimum is similar at least for some species in the moss microhabitats.

The ordinations nicely separated the two other subgroups. In the second sub-group, the most dominant species are *E. johncarteri* and *Psammothidium atalanta* whereas in the third group it is mostly *Pinnularia restituta* and *E.* cf. *fallax,* lacking the *Psammothidium* taxa. These two sub-groups are, however, highly similar in environmental conditions. The second sub-group is found on slightly more exposed and slightly wetter localities, with more samples taken from rocks and from running water. This might favour *Psammothidium atalanta*, originally described from Crater Lake of Tristan da Cunha (Carter 1966) in association with *Eunotia* taxa. *Eunotia johncarteri* was, on the other hand, first observed on the sea level of Gough Island by Carter, but in our recent observations, the species seems to be widespread on the entire island, mostly found in running waters or very exposed places. *Pinnularia restituta*, on the other hand, seems to have a very broad range of distribution, inhabiting mosses on peat slopes, rocks in or above water level of small streams, beaches or inland habitats. Similarly as the fourth group (*E. paludosa* group) the last sub-group had typically lower species number per sample.

Altitude appeared to be the most important environmental variable explaining the diatom species community structure. This was also observed on the sub-Antarctic island Kerguelen (Gremmen et al. 2006). Altitude was negatively correlated with salinity, caused by the habitat distribution along the altitudinal gradient. Localities influenced by sea spray were found on beaches and cliffs of lower altitudes and this sea influence disappears with the increasing altitude. This results in a highly different floral composition for (i) areas close to the sea, and (ii) inland areas. This further suggests that salinity has a high selective influence on the diatom composition. Gremmen et al. (2006) found that cosmopolitan taxa occur at lower altitudes and local, specific diatoms inhabited higher altitudes. On the seepage areas of Gough Island the occurrence of cosmopolitan *H. contenta* and *Achnanthidium* taxa were noted in low relative abundances. However, apart from *E. paludosa* var. *paludosa* being rather cosmopolitan and typically found at higher altitudes of Gough, the dominating diatom taxa are usually not influenced by altitude. However, the lower diversity in samples taken at higher altitudes on Gough Island is clear and might even be similar to the latitudinal diversity gradient of the southern hemisphere (Jones 1996; Van de Vijver & Beyens 1999b).

Long-distance and local dispersion, dispersal events and hypotheses

On oceanic islands, dispersion is highly pronounced for microbial life as well as for plants in general. Since a large number of those islands have a volcanic origin, they have never been connected to the larger landmasses such as continents. Any species present on these island, needed to actively colonize these islands following a successful dispersion. In the Southern Hemisphere, strong west-wind drift and circumpolar marine currents cause high rates of aeolian mediated dispersion sometimes across thousands of kilometers (Moore 1979), but this is not the restricted way, and plant propagules are also being transported in different directions (reviewed in Winkworth et al. 2002). Nevertheless, the west-wind drift might cause a higher floristic similarity of localities that are 'on wind' rather than in close proximity. Gough Island is a good example, sharing no unique non-vascular species with Bouvet Island, and having generally lower floristic (mosses, lichens, liverworts) similarities (positioned southward, distance ~ 1860 km), but shares at least 18 unique ones with Ile Amsterdam (Indian Ocean, distance ~ 8000 km) (Muñoz et al. 2004; Aptroot et al. 2011; Vinšová 2014). Fragments of mosses, lichens or liverworts can be possibly used by moss-inhabiting diatoms as a passive aerial transport mean similar to Antarctic soil invertebrates that can disperse while being stuck in microbial mats (Nkem et al. 2006). Similarly, a long-distance dispersal from South America was suggested on pollen of *Ephedra* L. or *Pinus* L. observed from a peat core analysed on Inaccessible Island (Preece et al. 1986). But recently a similar process has been observed on an 'Island tree', *Phylica arborea.* Based on genotypic distinctness, Richardson et al. (2003) identified that the representatives of *P. arborea* on Gough Island are similar to those of Ile Amsterdam, to where they dispersed in a single colonization event from Gough. Above that, the *P. arborea* population on Gough is very distinct from those on Tristan da Cunha, indicating two separate dispersal events from different lines of the mainland progenitor (southern Africa). With an observed hybrid phenotype on Tristan da Cunha, Richardson et al. (2003) suggest possible recolonization of *P. arborea* from Gough to Tristan da Cunha, but none in the opposite direction.

Another proposed means of dispersion is bird-mediated transport. This can be especially pronounced in fruity plants, *e.g.* on crowberries (*Empetrum* L.) which exhibit an extreme bipolar disjunction, with *E. rubrum* Vahl ex. Wild occurring on the South. Crowberries were most likely transported by a single dispersal bird-mediated event first from northwestern North America to southernmost South America in Mid-Pleistocene, then subsequently transported to the Falklands, Tristan da Cunha and Gough Island (Popp, Mirré and Brochmann 2011). This biogeographic dispersion patterns following west wind-drift was recently supported by the observed dispersion of a northern rockhopper penguin *Eudyptes moseleyi* to the Kerguelen Islands (Southern Indian Ocean). The species is different from the more southerly breeding *E. filholi* and is restricted to Tristan da Cunha, Ile Amsterdam and Ile St Paul, but an individual bird has been recently found on Kerguelen Islands, and molecular data showed that this specimen may have come from the 6.000 km away Gough Island, and not from Ile Amsterdam.

Dispersion for diatoms is not well understood. Since most species have well-defined ecological preferences, they are not able to survive in every habitat whereto they got dispersed. Stream diatoms have been reported to possess a stronger level of environmental control than benthic macroinvertebrates or bryophytes, and that this control affects them more than spatially-limited dispersal (Astorga et al. 2012). Physiological research dealing with the ability of diatoms to survive desiccation stress and freezing (- 20 °C) has been performed in the past. These forms of stress reflect the conditions that a passively travelling diatom cell would have to undergo in the cold, higher atmosphere. According to these studies, freezing and desiccation can be lethal for vegetative diatom cells that are generally highly sensitive, with only some strains of terrestrial representatives being able to survive (Souffreau et al. 2010). Marine planktonic and some semi-terrestrial diatom taxa are able to condense their cytoplasm, lower respiration and photosynthetic rates, therefore creating a resting stage which is morphologically identical to its vegetative cell (Anderson 1975, 1976; Sicko-Goad et al. 1986; Kuwata et al. 1993) with increased survival ability. Sometimes, it is possible to observe heavily silicified 'ghost valves' under scanning electron microscope, which could potentially be some kind of a resting stage

(Van de Vijver et al. 2014b). Higher desiccation and freezing survival rates for terrestrial diatoms have been observed, mainly for their resting stages, suggesting their higher ability to survive long-distance dispersal events (Souffreau et al. 2010, 2013). Yallop and Anesio (2010) where able to retrieve a large number of living diatoms from cryoconite debris after 1-2 years being frozen. Indeed, in the case of the Arctic environment, there might already be some diatoms selected to successfully survive the frozen stage and get back into their vital stage soon after the melt, especially when they are provided with an ideal substrate. Otherwise, diatoms from cryoconite holes of the Arctic can also inform investigators about the regional diversity and dispersion trends (Yallop and Anesio 2010; Vinšová et al. 2015) as they serve well as catchment traps. Similarly, Antarctic diatoms from streams of Dry Valley are being frozen for months, and some only get back to aquatic conditions a few times in a decade, thus they need to be able to survive extended periods of desiccation stress (Stanish et al. 2012). Mostly aerophilic diatoms have been observed by Stanish et al. (2012) on such localities, and their highest autotrophic index observed indicates short periods of rapid growths implying that diatoms present are able to reactivate themselves immediately when water becomes available.

Biogeographical position of Gough Island based on the diatom flora

In the discussed theories on the distribution of micro-organisms, Gough Island represents a locality with a considerable barrier to successful natural colonization caused by its geographically remote position (Jones et al. 2003), and can be used well to test distribution theories in diatoms. Carter (1966) described a significant number of new diatom taxa from Tristan da Cunha, which were recorded during this survey on Gough Island's moss material (*e.g., Pinnularia sistassa,* more in **Appendix 1**). Another connection exists between diatom communities of Gough and Inaccessible island, as here, unique and probably new taxa of *Luticola* and *Melosira* have been recorded to be similar (this survey). The similarity analysis based on Dice-Sorensen index and the cluster analysis, both placed Gough, Inaccessible and Tristan da Cunha Island together. These results, although illustrational, implying the highly pronounced connection between islands of this archipelago, which now could be caused by human migration, but previously was probably mainly bird-mediated (*e.g., Eudyptes moseleyi*).

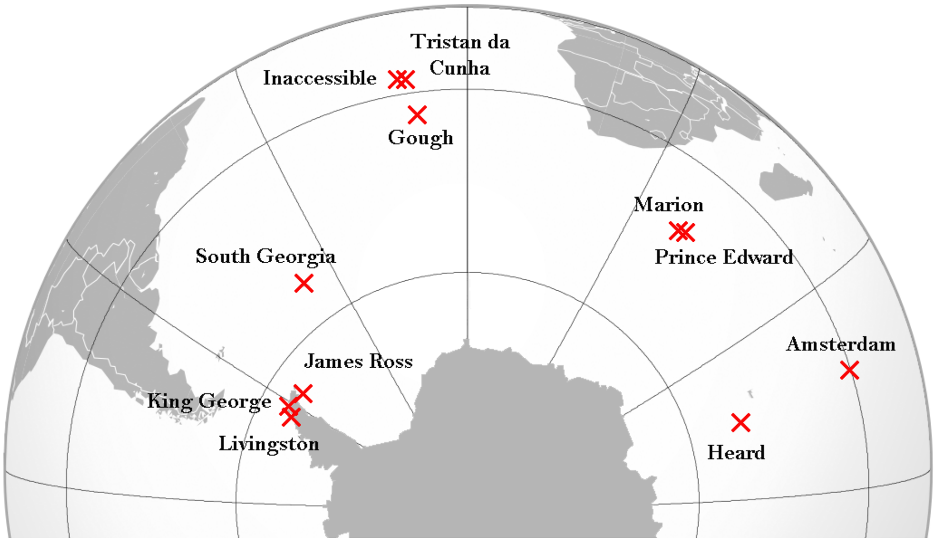

Figure 5.14. *Map of the region. Islands of southern oceans (Altantic and Indian Oceans) analyzed for the similarity of diatom floras from moss habitats.*

The extremely low similarity values between Gough Island and the other islands in the southern oceans outside the archipelago (< 0.22), indicate a highly unique floral structure. Based on the cluster analysis, roughly three biogeographical groups of islands could be identified (**Figure 5.3**): (i) islands of Maritime Antarctica and sub-Antarctica in the South Atlantic Ocean, (ii) islands in the Southern Indian Ocean, except of (iii) Ile Amsterdam, which was more clustered together with Gough Island. The observed flora of Gough generally lacks the species typical for sub-Antarctica (Van de Vijver et al. 2004; Van de Vijver and Beyens 1997) and the Maritime Antarctic region (Kopalová et al. 2014; Sterken et al. 2015), which would otherwise increase the similarity values. Some species typical for sub-Antarctica were observed in the samples, but typically in very low relative abundances, such as *Distrionella husvikensis* or *Pinnularia subantarctica.* The quite abundant *P. lindanedbalovae* is shared with the islands of the Southern Indian Ocean (Van de Vijver et al. 2002, 2004, 2008). Much of the shared diatom taxa were, however, presumably cosmopolitan in terms of distribution, such are *Eunotia paludosa* var. *paludosa, Humidophila contenta, Psammothidium investians* or *Navicula gregaria. Eunotia* was the most abundant genus in the moss samples of Gough Island.

Eunotia paludosa is a species having a broad distribution range frequently found in the sub-Antarctic islands but absent from the islands of Maritime Antarctica or the continent where it is replaced by *E. australopaludosa* (Van de Vijver et al. 2014a).

However, there might be several other issues contributing to the extremely low similarity values, which should also be noted here. Firstly, dissimilar sampling effort could pronounce the divergences of studied localities. Gough Island has high values (94 % of traditionally used ICE and Chao2 estimates), indicating that most of the flora has been observed, whereas *e.g.* James Ross Island's counting score achieved between 62 and 69 % only, and for most of the others the sampling efforts are unknown. Even though the analysis was restricted to the diatom flora from mosses, it is not possible to discern the characteristic environmental nature of distinct islands analyzed. sub-Antarctic islands of are dominated by mosses (Van de Vijver et al. 2002), whereas in the Maritime Antarctic mosses are restricted to wet areas around ponds, streams and to seepages, and the generally more dry nature of the region promotes the absence of a typical moss-inhabiting diatom flora (Kopalová et al. 2014). Finally, identification errors could also lead to differences. Nevertheless, remote localities are generally more distinct in their diatom flora present (Chattová et al. 2014), especially when there was sufficient time to evolve (*see* also Jones et al. 2003). Therefore, the actual low similarity value observed for the moss-inhabiting diatom community of Gough Island can be an appropriate one corresponding to the island's history and geographical position.

Insular context observed in diatom moss-inhabiting species

Some of the observed diatom species, and not only in the genus *Frustulia*, showed a high morphological variability in the assemblages, *i.e. Eunotia paludosa* var. *paludosa, E. johncarteri, Pinnularia restituta, P. posita* and *P. sistassa.* There are two possible hypotheses to explain this pattern in morphological variation. First, the change in morphology could be invoked by differences in the environmental parameters of the sampled localities, and the resulting different outline of a species could therefore represent only a different ecotype, but not truly different biological species. An alternative hypothesis would suggest that rather than ecologically invoked morphological response, these represent species complexes of phenotypically distinct species which are on their diversification onset, as a result of pronounced radiation rates of an isolated island. In the Antarctic region, allopatric speciation was suggested for some species [*Eunotia subantarctica* Van de Vijver, de Haan & Lange-Bertalot (present on several islands in the southern Indian Ocean) and *E. ralitsae* Van de Vijver, de Haan & Lange-Bertalot (South Georgia) belonging to *E. praerupta* Ehrenb. complex (Van de Vijver et al. 2014a)]. Also a clear bipolar and Antarctic intra-regional distinctness was reported for species of *Stauroneis* Ehrenb. (Van de Vijver et al. 2005). Adaptive radiation and allopatric speciation promoted on Galápagos Islands due to a geographic isolation is one of the hypotheses possibly explaining the higher diversity in the *Navicula* genus (Seddon et al. 2011).

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APPENDICES

The following appendix contains (1) a short presentation of species (using lists and figures) identified after the new species described in Carter (1966) which were found in moss-habitats of Gough Island, (2) a list of all diatom taxa and their authorities identified from the moss material during this survey, (3) abstracts of two posters that were presented on international conferences during my master studies dealing with the topic, and finally (4) a paper published in the second year of my master studies about diatom communities from Svalbard (High Arctic), therefore not concerning the topic of this thesis, but answering a similar ecological dispersal-related question, as to what extent adjacent habitats are responsible for structuring diatom assemblages.

APPENDIX 1

Diatom species identified after Carter (1966)

***List of species and formas described in Carter (1966) from Tristan da Cunha and Gough Islands**. Current names (if changed) or corrected names (if wrongly identified). Species in bold are in following figures. Distribution:* T – Tristan da Cunha Island, G – Gough Island, N – Nightingale Island, I – Inaccessible Island, C – reported also outside the archipelago. × *- sp. was found during counting,* (×) *– species has been observed only outside the counts.*

TAXON NAME IN CARTER (1966)	Current name	Distribution	If found
Achnanthes atalanta	***Psammothidium atalanta*** (J.R.Carter) L.Bukhtiyarova	T, G	×
Achnanthes investians	***Psammothidium investiens*** (J.R.Carter) L.Bukhtiyarova	T, G	×
Achnanthes lanceolata f. *rhombica*		T	
Achnanthes natrata		T	
Achnanthes pericava	***Planothidium pericavum*** (J.R.Carter) Lange-Bertalot	T, G, C	×
Achnanthes sumara	(As *Achnanthes petersenii* Hust. in Lange-Bertalot & Krammer (1989: 117))	T	
Amphora incurvata		T	
Caloneis liophilla		G	
Caloneis toxa		T	
Caloneis transepta		T	
Cyclotella stelligeroides f. *irregularis*		G	
Eunotia ambigua		T, G	×
Eunotia diverta		G	×
Eunotia incurva		T	
Eunotia morbida		G	×
Eunotia pectinoides	***Eunotia johncarteri*** (Carter) D.M.Williams	G	×
Fragilaria brevistriata f. *australis*		T	
Gomphonema asymmetricum	*Gomphonema johncarteri ('johncarterii')* (Carter) D.M.Williams	T	
Gomphonema spatulum		T, G	×
Melosira dispersa			
Melosira incorrupta			
Melosira setosa	***Melosira johncarteri*** (Carter) D.M.Williams	G	×
Navicula abica		G	
Navicula amerinda		T	
Navicula brockmanii f. *concave*		T	
Navicula decissa	***Humidophila decissa***	T, G	(×)
Navicula frisca	[as Chamaepinnularia sp2, this thesis]	T, G	×
Navicula nienta	***Humidophila nienta*** (Carter) Lowe, Kociolek, Johansen, Van de Vijver, Lange-Bertalot & Kopalová	T, G, C	×
Navicula orbita		T	
Navicula riparta		G	
Navicula scalpia		G	
Nitzschia eppressa		T	
Nitzschia navia		T, G	×
Nitzschia oscilla		T	(×)
Nitzschia palmida		G	
Nitzschia petulla		T, G	×
Nitzschia pistilla		T	
Nitzschia rotula	***Nitzschia terrestris*** (J.B.Petersen) Hustedt	T, G, C	×
Nitzschia salta		T	
Nitzschia serpentiformis		G	
Nitzschia sonora		G	×
Pinnularia dispersa	***Pinnularia johncarteri*** (Carter) D.M.Williams	T, G	×
Pinnularia extenda		T	
Pinnularia impassa		T	

TAXON NAME IN CARTER (1966)	Current name	Distribution	If found
Pinnularia leptosomiformis		G	
Pinnularia posita		G	×
Pinnularia redunda		G	
Pinnularia restituta		G	×
Pinnularia ritarda		T	
Pinnularia seriata		G	(×)
Pinnularia sistassa		T, G	×
Pinnularia subcapitata f. *plana*		T	
Pinnularia vapilla		T, G	×
Pseudoeunotia linearis	***Eunotia linearis*** (Carter) Vinsová, Kopalová & Van de Vijver	G	×
Pseudoeunotia duplex		G	×

In the following part, each species identified during this study after what Carter (1966) described as new to the science, is shortly presented following the order of original taxon names as listed in the previous list. Nomenclature changes or corrected names are specified together with the island where the species was originally identified from. If not specified otherwise, the species has been observed and identified from Gough material during this study. Most of the species are also accompanied in respective figures of paired Carter's original drawings and pictures of light microscope (LM). Some of them are represented also by scanning electron microscope (SEM) pictures. Notes regarding morphological features are added when appropriate. One species, *Eunotia (Pseudoeunotia) linearis* is presented as a separate (published) paper in **Chapter 2**.

Psammothidium atalanta (Carter) Bukhtiyarova (Bukhtiyarova et al. 2013)
Basionym*:* ***Achnanthes atalanta*** Carter, 1966: 444, pl. 1, figs. 15, 16, 19, 20.
Locality. Tristan da Cunha (Carter 1966).
Figures A1.19, **A1.38**.

Psammothidium investians (J.R.Carter) L.Bukhtiyarova in Bukhtiyarova & Round 1996: 26 (as *'P. investians'*). Basionym: ***Achnanthes investians*** J.R. Carter 1966: 445, pl. 1, figs. 23-28.
Locality. Tristan da Cunha (Carter 1966).
Figure A1.22.

Planothidium pericavum (J.R.Carter) Lange-Bertalot 1999: 278.
Basionym: ***Achnanthes pericava*** J.R. Carter 1966: 447, pl. 1, figs. 5-8.
Locality. Tristan da Cunha (Carter 1966).
Figures A1.21.

Amphora incurvata J.R. Carter 1966: 448, pl. 3, figs. 29-31.

Locality. Tristan da Cunha (Carter 1966).

Figure A1.30. SEM photo from Carter's original material.

Eunotia ambigua J.R. Carter 1966: 451, pl. 2, figs. 1 1-15, 20.

Locality. Tristan da Cunha (Carter 1966).

Figure A1.1. Upper LM pictures present what has been identified as this species and below, the three valves which were identified as *E.* cf. ambigua.

Eunotia diverta J.R. Carter 1966: 452, pl. 9, figs. 6-7.

Locality. Gough Island (Carter 1966).

Figures A1.6, A1.25–A1.26.

Eunotia morbida J.R. Carter 1966: 453, pl. 9, figs. 23-24.

Locality. Gough Island (Carter 1966).

Figure A1.3, A1.23–A1.24.

Eunotia johncarteri D.M.Williams in D.M.Williams & G.Reid 2002: 142 (as *'E. johncarterii'*)
Heterotypic synonym: ***Eunotia pectinoides*** J.R. Carter 1966: 454, pl. 9, figs. 4-5, 22.

Locality. Gough Island (Carter 1966).

Figure A1.4.

Note. Two forms of *E. johncarteri* have been identified and separated during the analysis of samples from Gough. However, in sample 2000-195 both of them, *E. johncarteri* forma thin and *E. johncarteri* forma thick, have been found together quite inseparably, which implies that it might be one species with a wider morphological variability (phenotypic plasticity).

Gomphonema spatulum J.R. Carter 1966: 456, pl. 6, fig. 12, pl. 7, fig. 23.

Locality. Tristan da Cunha (Carter 1966).

Figures A1.2, A1.27–A1.28.

Melosira johncarteri D.M.Williams 2001: 143.
Heterotypic synonym: ***Melosira setosa*** J.R. Carter 1966: 458, pl. 9, figs. 12-14.

Locality. Gough Island (Carter 1966).

Figure A1.5. Very rarely seen.

Navicula decissa J.R. Carter 1966: 461, pl. 3, fig 11.

Locality. Tristan da Cunha (Carter 1966).

Figure A1.14.

Navicula friska J.R. Carter 1966: 462, pl. 3, figs. 7-10.

Locality. Tristan da Cunha (Carter 1966).

Figure A1.7, A1.29.

Note. *Navicula frisca* is most probably the same as species identified under *Chamaepinnularia* sp2. Absence of gaps in the middle valve face area firstly refused pairing of these two, but it seems that the species might possess both features. Also the identity of *Ch.* sp1 in comparison to *Ch.* sp2 and the original species of Carter should be made prior the formal change.

Humidophila nienta (Carter) Lowe, Kociolek, Johansen, Van de Vijver, Lange-Bertalot & Kopalová 2014: 353. Basionym: ***Navicula nienta*** J.R. Carter 1966: 464, pl. 3, fig. 17.
Heterotypic Synonyms: *Diadesmis langebertalotii* Le Cohu & Van de Vijver 2002; *Diadesmis pseudolangebertalotii* Metzeltin, Lange-Bertalot & García-Rodríguez 2005

Locality. Tristan da Cunha (Carter 1966).

Figure A1.8, A1.31.

Nitzschia navia J.R. Carter 1966: 468, pl. 7, figs 1, 7.

Locality. Tristan da Cunha (Carter 1966).

Figure A1.11.

Note. Identified as 'cf. *navia'* due to only solely observed valves.

Nitzschia oscilla J.R. Carter 1966: 468, pl. 7, figs. 21-22.

Locality. Tristan da Cunha (Carter 1966).

Figure A1.10.

Note. Identified as 'cf. *oscilla'* due to only solely observed valves.

Nitzschia petulla J.R. Carter 1966: 469, pl. 6, figs. 18-23.

Locality. Tristan da Cunha (Carter 1966).

Nitzschia sonora J.R. Carter 1966: 470, pl. 8, figs. 21-22.

Locality. Gough Island (Carter 1966).

Note. Identified as 'cf. *sonora'* due to only solely observed valves.

Nitzschia terrestris (J.B.Petersen) Hustedt 1934: 396**.**
Erroneously described as new, ***Nitzschia rotula*** J.R. Carter 1966: 470, pl. 7, figs. 19, 26.

Locality. Tristan da Cunha (Carter 1966).

Figure A1.12.

Pinnularia johncarteri (Carter) D.M.Williams 2001: 149.
Heterotypic synonym: ***Pinnularia dispersa*** J.R. Carter 1966: 472, pl. 5, figs. 7-9.

Locality. Tristan da Cunha and Gough Island rarely (Carter 1966).

Figures A1.13, A1.32–A1.33.

Pinnularia posita J.R. Carter 1966: 475, pl. 8, figs. 7-8.

Locality. Gough Island (Carter 1966).

Figures A1.15, A1.35.

Note. Abundant species, the size range varies in assemblage (grouped photos in **Figure A1.15**).

Pinnularia restituta J.R. Carter 1966: 475, pl. 8, figs 3-5.

Locality. Gough Island (Carter 1966).

Figures A1.18, A1.34.

Note. Very abundant and apparently quite morphologically variable species.

Pinnularia seriata J.R. Carter 1966: 476, pl. 8, fig. 12.

Locality. Gough Island (Carter 1966).

Pinnularia sistassa J.R. Carter 1966: 477, pl. 5, figs. 3-6.

Locality. Tristan da Cunha (Carter 1966).

Figures A1.17, A1.36–A1.37.

Note. Abundant species, costae may or may not be present along the entire valve (**Figure A1.17**).

Pinnularia vapilla J.R. Carter 1966: 478, pl. 4, figs. 1-2.

Locality. Tristan da Cunha (Carter 1966).

Figure A1.20.

Eunotia linearis (Carter) Vinsová, Kopalová & Van de Vijver (2016: 122).

Basionym/Homotypic synonym: ***Pseudoeunotia linearis*** J.R. Carter 1966: 479, pl. 9, figs. 15-19.

Locality. Gough Island (Carter 1966).

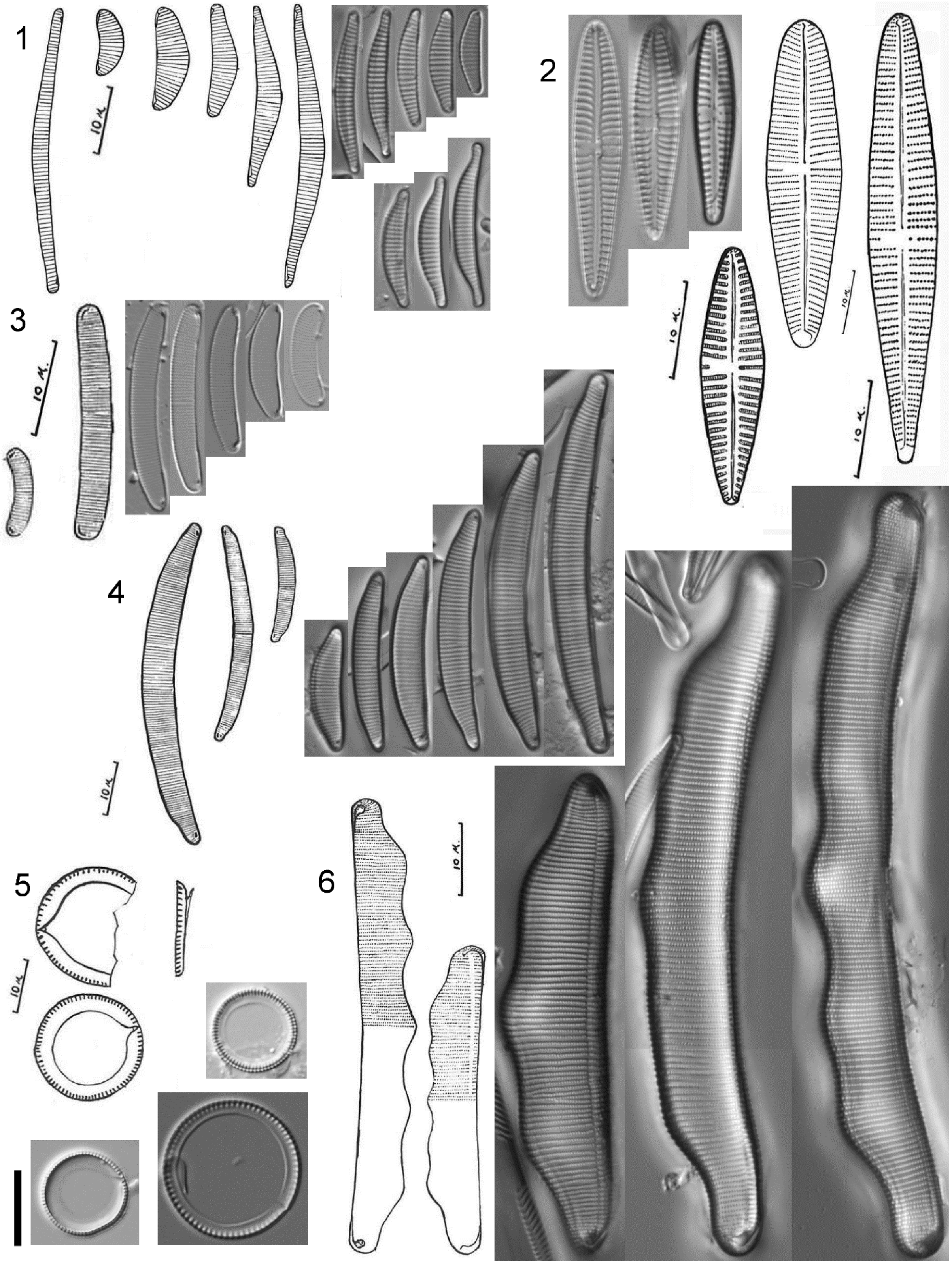
Figures can be found in **Chapter 2** of the thesis.

Pseudoeunotia duplex J.R. Carter 1966: 479, pl. 9, fig. 29.

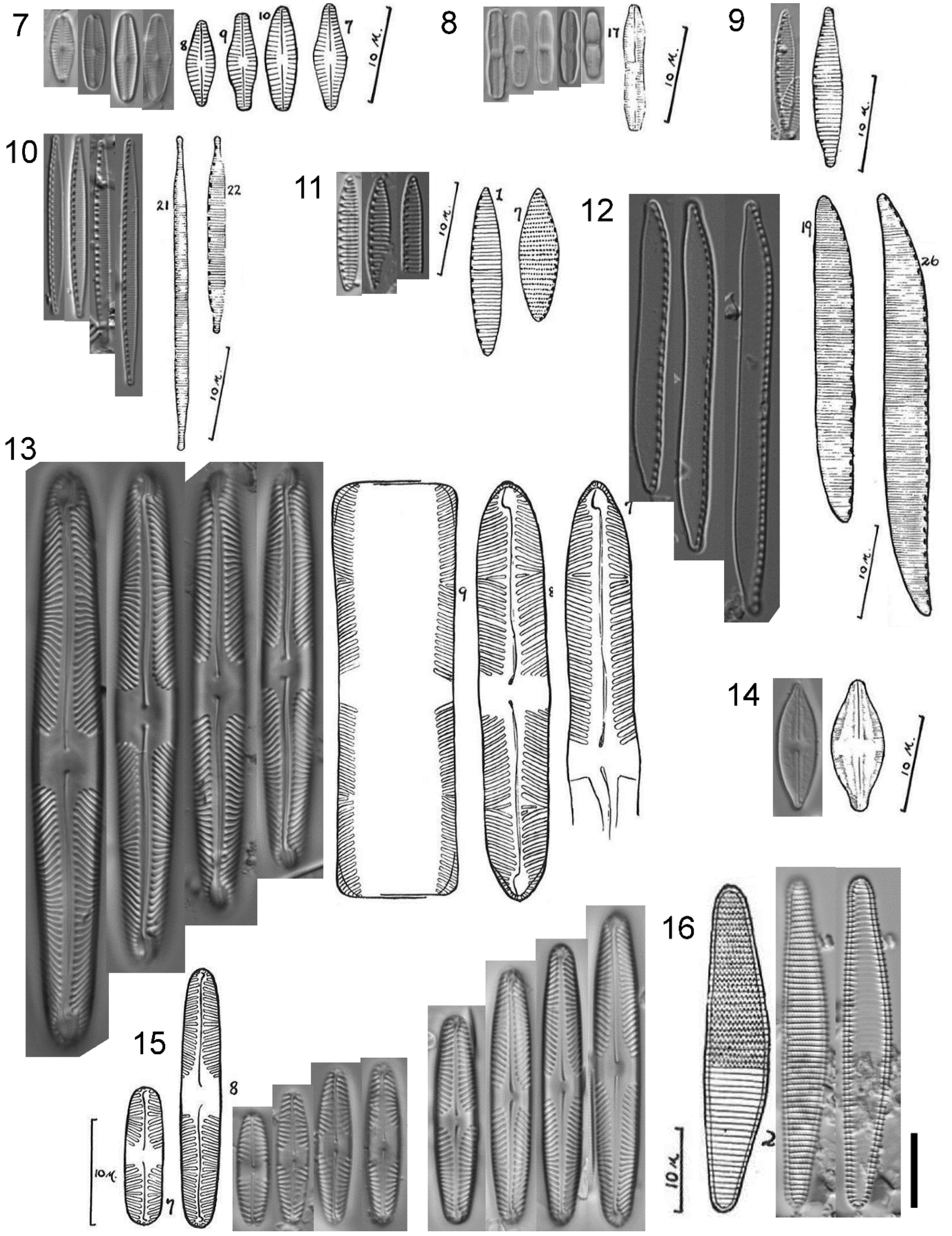
Locality. Gough Island (Carter 1966).

Figure A1.16. LM photos of one valve under different focus.

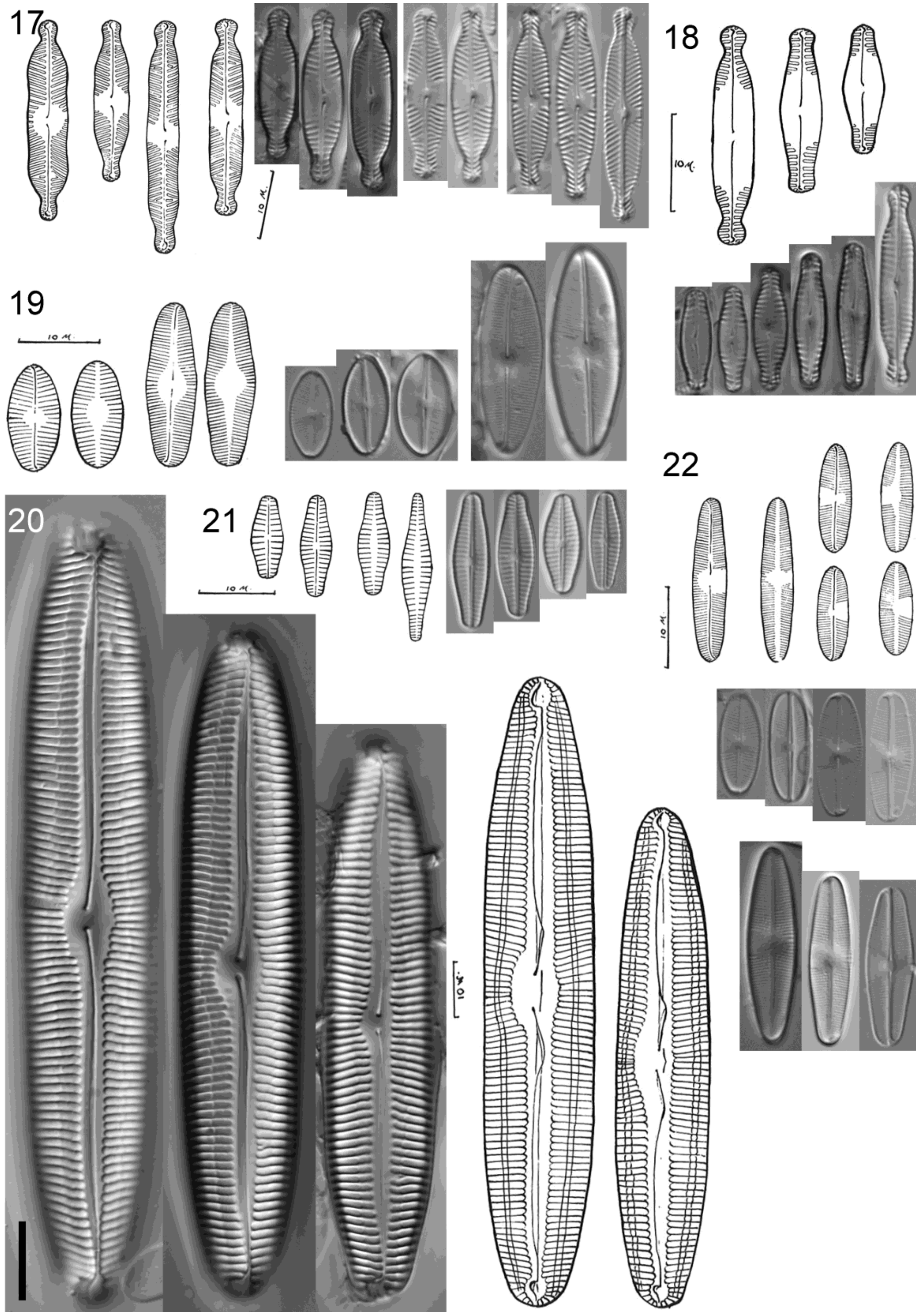
Note. Marine species. Only one entire valve was found, and numerous fragments which suggests that the species is not native to moss habitats.



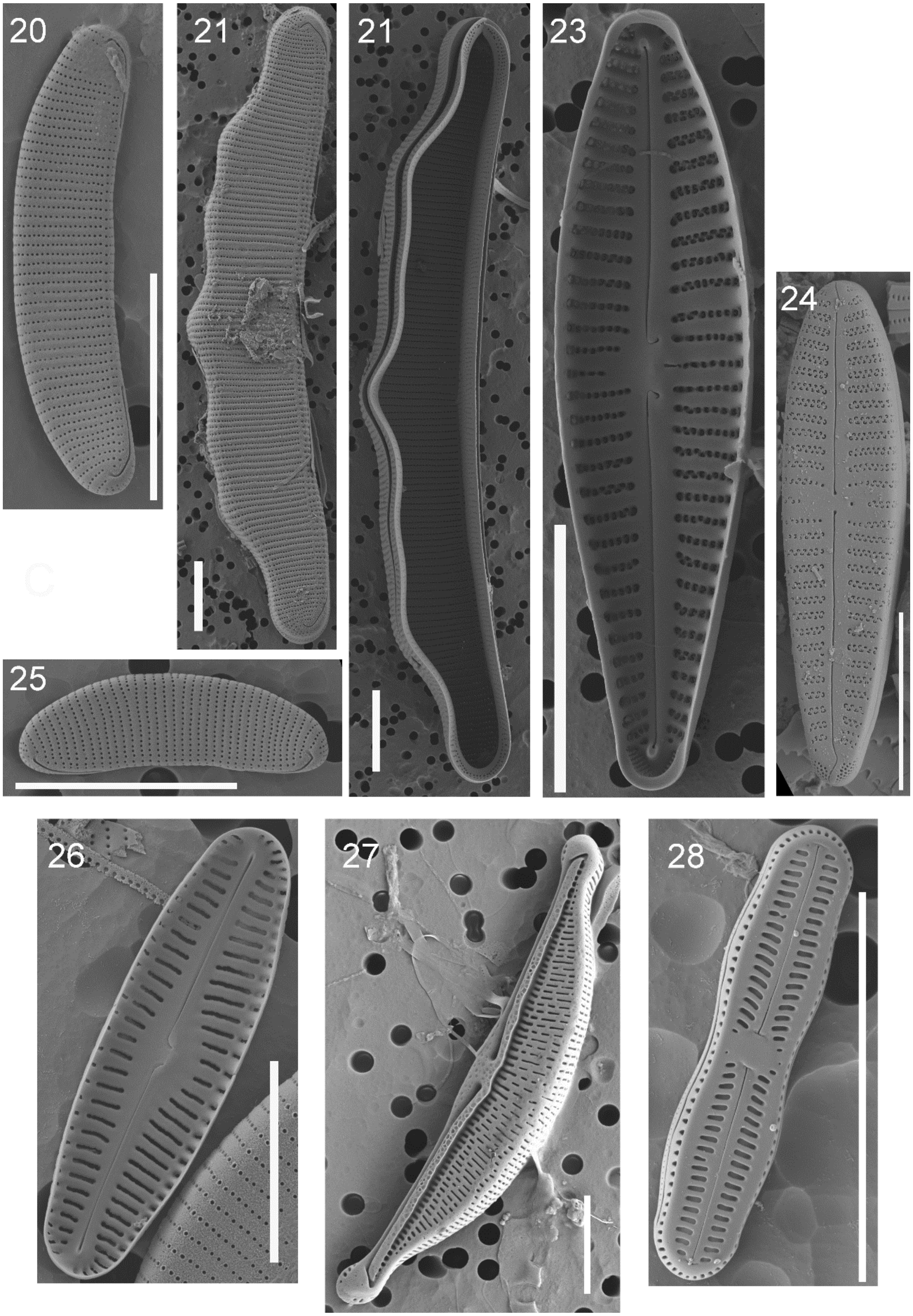
Figures A1.1–A1.6. *(1)* Eunotia ambigua, *(2)* Gomphonema spatulum, *(3)* Eunotia morbida, *(4)* Eunotia johncarteri, *(5)* Melosira johncarteri, *(6)* Eunotia diverta. *Scale bars represent 10 µm, drawn ones correspond to each drawing.*



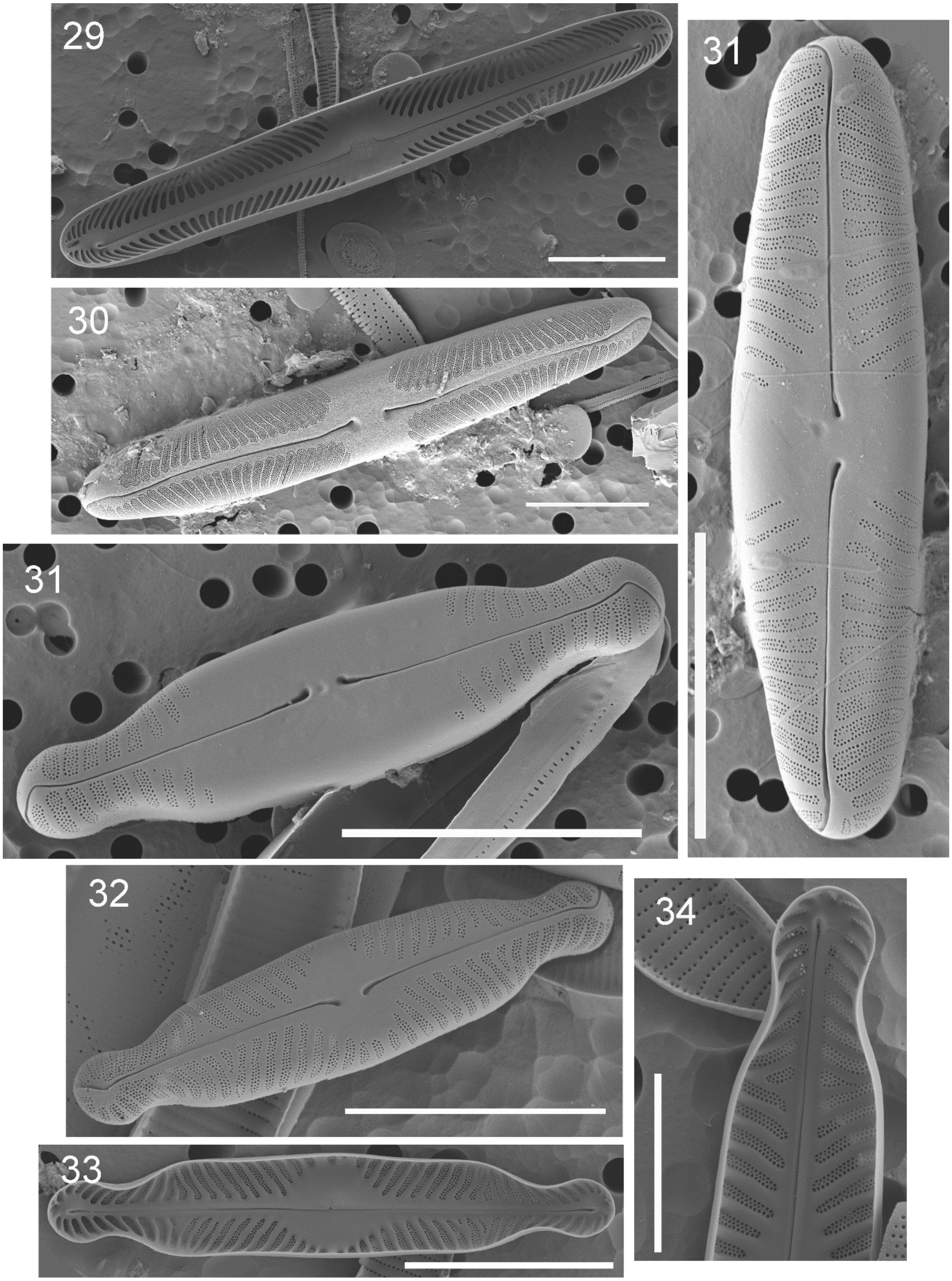
Figures A1.7–A1.16. (7) Chamaepinnularia frisca, *(8)* Humidophila nienta, *(9)* Nitzschia pettula, *(10)* Nitzschia oscilla, *(11)* Nitzschia navia, *(12)* Nitzschia terrestris, *(13)* Pinnularia johncarteri, *(14)* Navicula decissa, *(15)* Pinnularia posita, *(16)* Pseudoeunotia duplex. *Scale bars represent 10 µm, drawn ones correspond to each drawing.*



Figures A1.17–A1.22. (17) Pinnularia sistassa*, (18)* Pinnularia restituta, *(19)* Psammothidium atalanta, *(20)* Pinnularia vapilla, *(21)* Planothidium pericavum, *(22)* Psammothidium investians*. Scale bars represent 10 µm, drawn ones correspond to each drawing.*



Figures A1.23–A1.31. *(23-24)* Eunotia morbida, *(25-26)* Eunotia diverta, *(27-28)* Gomphonema spatulum, *(29)* Chamaepinnularia frisca, *(**30**)* Halamphora incurvata,*(**31**)* Humidophila nienta. *Scale bars represent 10 µm.*



***Figures A1.33–A1.38**. (32-33)* Pinnularia johncarteri*, (34)* Pinnularia restituta, *(35)* Pinnularia posita, *(36-37)* Pinnularia sistassa*,*
(38) Psammothidium atalanta*. Scale bars represent 10 µm.*

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APPENDIX 2

List of species identified from the moss material of Gough Island

APPENDIX 2. Taxonomic list of all diatom taxa with authorities, observed in the present study. Taxa unidentified to the species level are given a proper provisional code.

TAXON NAME	Abbreviations	If only outside of the counts
Achnanthes coarctata (Brébisson ex W.Smith) Grunow in Cleve & Grunow		×
Achnanthes muelleri G.W.F.Carlson		×
Achnanthes okamurae Skvortzov	Ach	
Achnanthes sp		×
Achnanthidium minutissimum (Kütz.) Czarnecki	Achn	
Achnanthidium cf. *modestiforme* (Lange-Bertalot) Van de Vijver	Achn1	
Adlafia bryophila (J.B.Petersen) Lange-Bertalot	Adl	
Amphora incurvata J.R.Carter		×
Aulacoseira ambigua (Grunow) Simonsen	Aul	
Brachysira sp	Bra	
Caloneis cf. *bacillum* (Grunow) Cleve	Cal	
Caloneis sp1	Cal1	
Caloneis sp2	Cal2	
Cocconeis placentula Ehrenberg	Coc	
Chamaepinnularia cf. *begeri* (Krasske) Lange-Bertalot	Cha	
Chamaepinnularia evanida (Hustedt) Lange-Bertalot	Cha1	
Chamaepinnularia sp1	Cha2	
Chamaepinnularia sp2 [*Navicula frisca* Carter]	Cha3	
Craticula vixneglidenda Lange-Bertalot	Cra	
Cymbella gracilis (Rabenhorst) Cleve	Cym	
Denticula sp	Den	
Distrionella germainii (Reichardt & Lange-Bertalot) Morales, Bahls & Cody	Dis	
Distrionella germainii f. *acostata* Lange-Bertalot & Rumrich	Dis1	
Distrionella husvikensis (Van de Vijver, Denys & Beyens) Morales, Bahls & Cody	Dis2	
Encyonema sp	Enc	
Encyonopsis sp	Encs	
Eunotia ambigua J.R.Carter	Eun	
Eunotia cf. *ambigua* J.R.Carter [spX]	Eun1	
Eunotia diverta J.R.Carter	Eun2	
Eunotia elegans Østrup	Eun3	
Eunotia cf. *fallax* A.Cleve	Eun4	
Eunotia cf. *lecohui* Van de Vijver, Beyens & Lebouvier	Eun5	
Eunotia linearis (Carter) Vinsová, Kopalová & Van de Vijver	Eun6	
Eunotia morbida J.R.Carter	Eun7	
Eunotia cf. *naegelii* Migula	Eun8	
Eunotia paludosa var. *paludosa* Grunow group	Eun9	
Eunotia aff. *papilio* (Ehrenberg) Grunow		×
Eunotia johncarteri (Carter) D.M.Williams [forma *thick*]	Eun10	
Eunotia johncarteri (Carter) D.M.Williams [forma *thin*]	Eun11	
Eunotia pseudoparalleloides (Grunow) Nörpel-Schempp & Lange-Bertalot	Eun12	
Eunotia sp2	Eun13	
Eunotia sp4	Eun14	
Eunotia sp5	Eun15	
Eunotia cf. *superbidens* Lange-Bertalot		×
Fragilaria construens (Ehrenberg) Grunow forma s.l. Carter	Fra	
Fragilaria cf. *parva* (Grunow) A.Tuji & D.M.Williams	Fra1	
Frustulia crassinervia (Brébisson ex W.Smith) Lange-Bertalot & Krammer	Fru	
Frustulia saxonica Rabenhorst	Fru1	
Frustulia spE	Fru2	

Frustulia spH	Fru3	
Frustulia spL	Fru4	
Frustulia spN	Fru5	
Frustulia spP	Fru6	
Frustulia spV	Fru7	
Gomphonema coronatum Ehrenberg	Gom	
Gomphonema cf. *longiceps* Ehrenberg	Gom1	
Gomphonema longiceps var. *subclavata* Grunow s.l. Carter	Gom2	
Gomphonema parvulum (Kützing) Kützing group	Gom3	
Gomphonema parvulum var. *lagenula* (Kützing) Frenguelli	Gom4	
Gomphonema cf. *spatulum* J.R.Carter	Gom5	
Gomphonema sp3		×
Halamphora sp	Hal	
Hantzschia abundans Lange-Bertalot		×
Hantzschia amphioxys (Ehrenberg) Grunow	Han	
Humidophila arcuata (Lange-Bertalot) Lowe, Kociolek, Johansen, Van de Vijver, Lange-Bertalot & Kopalová	Hum	
Humidophila cf. *australis* (Van de Vijver & Sabbe) Lowe, Kociolek, Johansen, Van de Vijver, Lange-Bertalot & Kopalová		×
Humidophila contenta (Grunow) Lowe, Kociolek, J.R.Johansen, Van de Vijver, Lange-Bertalot & Kopalová	Hum1	
Humidophila cf. *ingeae* (Van de Vijver) Lowe, Kociolek, Johansen, Van de Vijver, Lange-Bertalot & Kopalová	Hum2	
Humidophila nienta (Carter) Lowe, Kociolek, Johansen, Van de Vijver, Lange-Bertalot & Kopalová	Hum3	
Humidophila spE	Hum4	
Humidophila sp177	Hum5	
Humidophila sp2	Hum6	
Karayevia oblongella (Østrup) Aboal	Kar	
Luticola cf. *beyensii* Van de Vijver, Ledeganck & Lebouvier	Lut	
Luticola sp1	Lut1	
Luticola sp2	Lut2	
Luticola sp3	Lut3	
Mayamaea atomus (Kützing) Lange-Bertalot	May	
Mayamaea atomus var. *permitis* (Hustedt) Lange-Bertalot	May1	
Melosira cf. *echinata* Manguin	Mel	
Melosira jonhcarteri (Carter) D.M.Williams	Mel1	
Melosira varians C.Agardh	Mel2	
Melosira spX	Mel3	
Melosira spY	Mel4	
Meridion circulare (Greville) C.Agardh	Mer	
Navicula arvensis Hustedt	Nav	
Navicula bicephala Hustedt	Nav1	
Navicula cryptotenelloides Lange-Bertalot	Nav2	
Navicula decissa J.R.Carter		×
Navicula exilis Kützing	Nav3	
Navicula gregaria Donkin	Nav4	
Navicula lapidosa Krasske	Nav5	
Navicula aff. *lapidosa* Krasske	Nav9	
Navicula pseudoventralis Hustedt	Nav6	
Navicula cf. *radiosa* Kützing	Nav7	
Navicula veneta Kützing	Nav8	
Navicula vilaplanii (Lange-Bertalot & Sabater) Lange-Bertalot & Sabater		×
Neidium sp	Nei	

Nitzschia debilis (Arnott ex O'Meara) Grunow in Cleve & Grunow	Nit	
Nitzschia cf. *navia* J.R.Carter	Nit1	
Nitzschia cf. *oscilla* J.R.Carter	Nit2	
Nitzschia palea (Kützing) W.Smith	Nit3	
Nitzschia perspicua Cholnoky	Nit4	
Nitzschia petulla J.R.Carter	Nit5	
Nitzschia cf. *sonora* J.R.Carter	Nit6	
Nitzschia terrestris (J.B.Petersen) Hustedt	Nit8	
Nitzschia sp675	Nit9	
Nupela cf. *acaciensis* Vouilloud & Sala		×
Orthoseira gremenii Van de Vijver & Kopalová	Ort	
Orthoseira roseana (Rabenhorst) O'Meara	Ort1	
Pinnularia austroshetlandica (Carlson) A.Cleve	Pin	
Pinnularia borealis Ehrenberg	Pin1	
Pinnularia borealis var. 65A	Pin2	
Pinnularia borealis var. *scalaris* (Ehrenberg) Rabenhorst	Pin3	
Pinnularia cf. *johncarteri* (Carter) D.M.Williams	Pin4	
Pinnularia cf. *laucensis* Lange-Bertalot	Pin5	
Pinnularia divergentissima (Grunow) Cleve forma *elongata* A.Cl.		×
*Pinnularia lindanedbalovae V*an de Vijver & Moravcová	Pin6	
Pinnularia cf. *microstauron* (Ehrenberg) Cleve s.l. Carter	Pin7	
Pinnularia cf. neomajor Krammer		×
Pinnularia perirrorata Krammer	Pin8	
Pinnularia posita J.R.Carter	Pin9	
Pinnularia restituta J.R.Carter	Pin10	
Pinnularia seriata J.R.Carter		×
Pinnularia sistassa J.R.Carter	Pin11	
Pinnularia cf. *shoenfelderi* Krammer	Pin12	
Pinnularia sp11	Pin13	
Pinnularia spO	Pin14	
Pinnularia subacoricola Metzeltin, Lange-Bertalot & García-Rodríguez		×
Pinnularia subantarctica var. *elongata* (Manguin) Van de Vijver & Le Cohu		×
Pinnularia vapilla J.R.Carter	Pin15	
Pinnularia viridis (Nitzsch) Ehrenberg	Pin16	
Planothidium cyclophorum (Heiden) Van de Vijver	Pla	
Planothidium cf. *densistriatum* Van de Vijver & Beyens	Pla1	
Planothidium frequentissimum (Lange-Bertalot) Lange-Bertalot	Pla2	
Planothidium pericavum (J.R.Carter) Lange-Bertalot	Ach1	
Psammothidium abundans (Manguin) Bukhtiyarova & Round	Psa	
Psammothidium atalanta J.R.Carter	Psa1	
Psammothidium investians J.R.Carter	Psa2	
Pseudoeunotia duplex J.R.Carter	Pse	
Rhopalodia rupestris (W.Smith) Krammer	Rho	
Sellaphora atomoides (Grunow) C.E. Wetzel & Van de Vijver	Sel	
Sellaphora saugerresii (Desm.) C.E. Wetzel & D.G. Mann	Sel1	
Stauroneis sp	Sta	
Staurosira venter (Ehrenberg) Cleve & J.D.Möller	Sta1	
Staurosira sp1	Sta2	
Surirella sp1	Sur	
Tabellaria flocculosa (Roth) Kützing	Tab	
Thalassiosira sp	Tha	

APPENDIX 3

Abstracts

Poster presented at *9th Central European Diatom Meeting,*
Bremerhaven, Germany 03/2015.

THE GENUS *FRUSTULIA* ON GOUGH ISLAND (TRISTAN DA CUNHA ARCHIPELAGO)

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Gough Island (40°21′ S, 9°53′ W), part of the Tristan da Cunha archipelago, is a small, uninhabited island, situated in the cool-temperate zone of the southern Atlantic Ocean. Large parts of the island are quite mountainous with most of the island above 400 m. This volcanic island is typically oceanic and has never been part of a continental landmass. The climate is cool and wet, with a mean annual temperature (at sea level) of 11° C, a mean annual precipitation in excess of 3000 mm and with frequent gale-force winds.

Despite its very interesting geographical position, its oceanic origin and the number of interesting habitats, the non-marine diatom flora of Gough Island is only poorly known. Carter published in 1966 on the diatom flora from the Tristan da Cunha Island group, reporting the presence of a very diverse diatom flora with a large number of new taxa that so far have been rarely observed elsewhere. Recently, a new survey of the moss-inhabiting diatoms on Gough Island has started. During this survey, several taxa were discovered belonging to the genera *Pinnularia*, *Eunotia* and *Frustulia*, that could not be identified using the currently available literature.

The poster illustrates and discusses the diversity within the genus *Frustulia*. Apart from the presumably cosmopolitan species *F. vulgaris* (Thwaites) De Toni, six unknown *Frustulia* taxa were observed. Based on detailed LM and SEM observations, it is highly likely that these six taxa need to be described as new species. They differ from each other and from already described taxa by a series of morphological features such as the overall valve outline, the shape and structure of the helictoglossa, the structure of the central area and the external proximal and distal raphe endings. All differentiating characters are highlighted on the poster. Brief notes on the ecology of the six taxa are added.

References: Carter, J. (1966) Some freshwater diatoms of Tristan da Cunha and Gough Island. *Nova Hedwigia*, 9: 443-492.

Poster presented at *6th International Conference on Polar and Alpine Microbiology*,
České Budějovice, Czech Republic 09/2015.

A NEW INTERESTING SPECIES OF *EUNOTIA* (BACILLARIOPHYTA) FROM GOUGH ISLAND (TRISTAN DA CUNHA ARCHIPELAGO)

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Gough Island (40°21’ S, 9°53’ W), is a small remote uninhabited island of a volcanic origin, positioned in southern Atlantic Ocean and some 350 km south of the rest islands from Tristan da Cunha archipelago. Despite its very interesting geographical position, its oceanic origin, an isolation and the number of interesting habitats, the non-marine diatom flora of Gough Island is only poorly known. Carter described in 1966 numerous of new diatom species from Tristan da Cunha Island group, reporting the presence of a very diverse diatom flora with a large number of new taxa that so far have been only rarely observed elsewhere. Recently, a new survey of moss-inhabiting diatom flora on Gough Island has started and so far, several taxa were discovered belonging to the genera *Eunotia, Frustulia* and *Pinnularia*, that could not be identified using the currently available literature.

One species belonging to the genera *Eunotia* is of our particular interest. This species, which Carter (1966) newly described as *Pseudoeunotia linearis*, has some morphological features that clearly differs it from the other species of the same genera known so far. Based on a detailed LM and SEM observation, we can now present a complex description of this highly interesting species that differs morphologically (i.e., the overall valve shape, the presence of spines on sides, areolae and striae density and their structure based on the position, as well as the absence of a terminal nodules). All differentiating characteristics are highlighted on the poster. The comparison and the unification with the same species found in original material of Carter (1966) is added and also the correction of the species name is suggested. Notes on the ecology are also added.

KEYWORDS: GOUGH ISLAND, DIATOMS, EUNOTIA

REFERENCES: Carter, J. (1966): “Some freshwater diatoms of Tristan da Cunha and Gough Island”. Nova Hedwigia 9: 443-492.

APPENDIX 4

Diatoms in cryoconite holes and adjacent proglacial freshwater sediments, Nordenskiöld glacier (Spitsbergen, High Arctic)

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DIATOMS IN CRYOCONITE HOLES AND ADJACENT PROGLACIAL FRESHWATER SEDIMENTS, NORDENSKIÖLD GLACIER (SPITSBERGEN, HIGH ARCTIC).

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ABSTRACT

Cryoconite holes are small, extreme habitats, widespread in the ablation zones of glaciers worldwide. They can provide a suitable environment for microorganisms including bacteria, cyanobacteria, algae, fungi, and invertebrates. Diatoms have been previously recovered from cryoconite holes of Greenland and of Svalbard, and recent findings from Antarctica suggest that cryoconite holes may harbor a unique diatom flora distinct from other aquatic habitats nearby. In the present study, we characterize the diatom communities of Nordenskiöld glacier cryoconite holes in Billefjorden (Svalbard, Spitsbergen), and multivariate approaches were used to compare them with three freshwater localities in the immediate vicinity to investigate possible sources of the species pool. We found cryoconite holes to have similar or greater average genus-richness than adjacent lake/ponds habitats, even though lower numbers of valves were recovered. Overall, cryoconite hole diatom communities differed significantly from those observed in lakes, suggesting that other sources actively contribute to these communities than nearby lakes alone. This further suggests that (i) diatoms present in cryoconite might not exclusively originate from aquatic habitats, but also from (semi-)

terrestrial ones; and (ii) that a much wider area than the immediate surroundings should be considered as a possible source for cryoconite diatom flora.

KEYWORDS
Ecology, limnology, lakes, cryosphere, Svalbard, polar region

INTRODUCTION

Glaciers and ice sheets cover almost 10% of the Earth's landmass (Clark 2009), and as a result, their suitability for life is of great importance. Cryoconite holes represent icrohabitats formed by dust transported into the ablation zones of the glacial surface, which leaves behind small water-filled depressions during thawing periods. These supraglacial habitats are found worldwide and can persist for days to decades (Hodson et al. 2008). Since the 1930's, cryoconite holes have been recognized as a micro-environmnent harboring life (Steinbock 1936), and modern investigations have shown that they support a range of micro-organisms including archaea, heterotrophic bacteria, fungi, microalgae, filamentous cyanobacteria, nematodes, rotifers and tardigrades (*e.g*. Wharton et al. 1981 and 1985, Mueller et al. 2001, Edwards et al. 2013a). In contrast to the surrounding glacier surface, cryoconite holes supply liquid water to organisms, can shelter microorganisms from UV light, and are presumably also less susceptible to temperature extremes. Biovectors and wind are thought to serve as dispersal agents for microbial propagules to the glacier surface. Both local and long range aeolian input (Šabacká et al. 2012, Budgeon et al. 2012), as well as debris from avalanches and erosion, can supply the surface with sediment (Hodson et al. 2008) and organic carbon (Stibal et al. 2008).

Antarctic cryoconite holes may be isolated from the atmosphere for multiple melt seasons by a thick ice lid (Fountain et al. 2004), allowing the development of unique habitats that harbor diverse communities (Mueller et al. 2001). In contrast, only a thin ice lid may develop during summer months in the Arctic, resulting in hydrologically connected environments that are frequently flushed with meltwater, promoting a more homogenous resident community (Edwards et al. 2011). Nevertheless, biological activity of inhabitants (*e.g.* granule formation, darkening processes and photosynthetic activity) remains surprisingly high for such an extreme habitat (Vonnahme 2014), and as known from the Antarctic, cryoconites can serve as refuges for aquatic and terrestrial microorganisms (Foreman et al. 2007, Stanish et al. 2013). Cryoconite communities may also (re-)seed downstream microbial communities residing in proglacial lakes and streams with cells and propagules (Yallop et Anesio 2010, Stanish et al. 2013) that can stand at the very onset of microbial colonization (Stibal et al. 2006).

Most studies of glacial microbial communities have focused on bacteria or cyanobacteria (Mueller et al. 2001, Christner et al. 2003, Cameron et al. 2012, Edwards et al. 2013b), which are the most abundant primary producers in these habitats (Mueller et al. 2001, Porazinska et al. 2004, Stibal et al. 2006, Stibal et Tranter 2007). Our recent knowledge of other cryoconite hole phototrophs such as diatoms (Bacillariophyceae) is, however, rather poor. It is in spite of the fact that diatoms are one of the most successful groups of unicellular algae worldwide, inhabiting a wide range of aquatic and terrestrial environments including polar regions (Jones 1996, Van de Vijver et Beyens 1999, Sabbe et al. 2003, Van de Vijver et al. 2005, Antoniades et al. 2008, 2009). Furthermore, the speciesspecific characteristics of their outer silica cell-wall, as well as individual responses to the physico-chemical environment make diatoms excellent bio-indicators in applied sciences such as paleo-ecology and biogeography (Spaulding et al. 2010).

While diatoms have been previously recorded from supraglacial habitats (Mueller et al. 2001, Van de Vijver et al. 2010b, Cameron et al. 2012), speculation persists as to how these communities are assembled. It is possible that diatoms, together with other micro-organisms present in microbial mats, can be delivered to the supraglacial habitats from nearby lakes and streams by winds (Nkem et al. 2006) and solely reflect those in transport. On the other hand, they may constitute independently functioning communities with a composition similar, yet distinct from their surroundings. To our best knowledge, only two diatom-focused studies of cryoconite holes exist at this time. One relates to the Arctic (Yallop et Anesio 2010), the other one to the Antarctic (Stanish et al. 2013). The former one reports cultured diatoms from cryoconite holes and compares these with database samples. The latter one compares cryoconite hole communities with microbial mats from adjacent streams. Therefore, much work remains for the diatom-related scientific community in order to gain a better understanding of the structure and function of these extreme habitats.

To address such gap in our knowledge, we characterize diatom communities from cryoconite hole sediments of the Nordenskiöld glacier (Adolfbukta, Billefjorden, Svalbard) and compare these results with adjacent aquatic habitats that could potentially serve as a source. We hypothesize that if cryoconite holes are seeded by these adjacent habitats, supraglacial diatom communities should reflect the diatom communities in surrounding lakes and ponds. An alternative to this hypothesis is the potential importance of other nearby habitats such as terrestrial areas and streams, and/ or more long range transport from other sources. To answer this question, a simple genus-based approach was applied to reduce the effects of taxonomic uncertainty, and the results were analyzed using multivariate approaches to determine the similarity of communities between habitats.

MATERIAL AND METHODS

Sample collection

Since 2007, summer research campaigns organized by the Centre for Polar Ecology (University of South Bohemia in České Budějovice, Czech Republic) have annually taken place in Petuniabukta (*see* reports of the research activities at website CPE: http://polar.prf.jcu.cz/docs.htm or http://polar.prf.jcu.cz/index.htm) located in the central part of Spitsbergen (Svalbard Archipelago) (*see* Fig. A4.1). The fjord is surrounded by a lowland area of marine terraces, and steep slopes up to 937 m. Its climate is characterized by low precipitation rates (about 200 mm y^{-1}), relatively warm winters (-6.7 to -17°C), and wind speed ranging from 2.8 to 23.6 m s^{-1} (at 78° 42’ N and 16° 27’ E) (Rachlewicz et al. 2007, Láska et al. 2012).

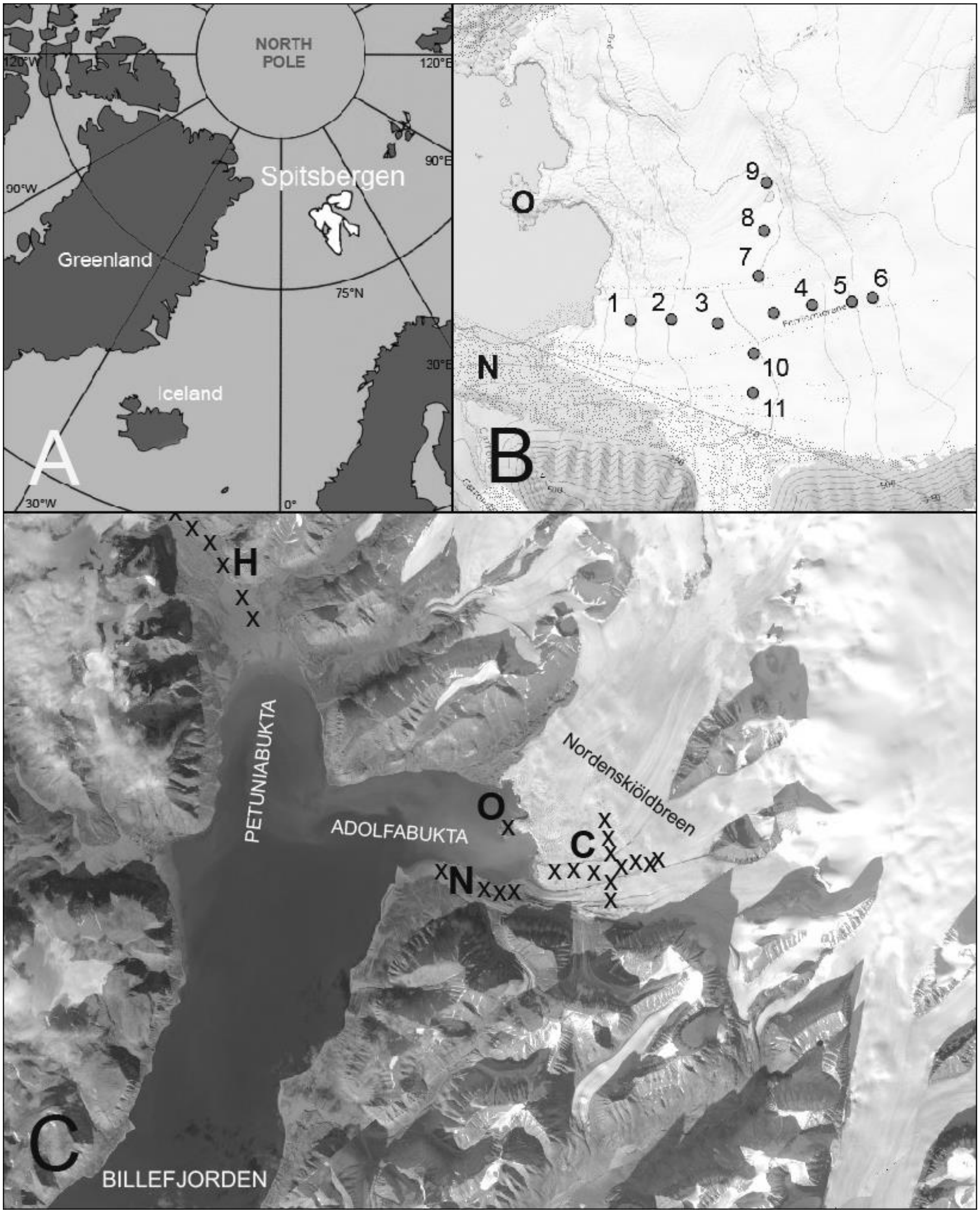

Figure A4.1. Map of the study sites. Sampling points are indicated by crosses. On the last image, C refers to cryoconite holes, H to Hørbye lakes, N to Nordenskiöld lakes, and O to Retrettøya ponds site.

During the 2014 boreal summer season, 12 samples from cryoconite holes were collected (Fig. A4.2) along two sampling lines on the Nordenskiöld tidewater glacier (glacier description in Rachlewicz et al. 2007). The first line was situated on an altitudinal gradient from the glacier front upwards, and the second one from the side marginal moraine to the glacier center (Fig. A4.1B and Fig. A4.2). These sites were chosen to study possible patterns in aeolian transport of diatoms from the surrounding environments, which should occur from the sea and/or from the marginal zones towards the glacier surface. Sediment samples from small cryoconite holes (3 – 5 cm in diameter, 10 – 15 cm in depth) were collected with pipettes with enlarged openings, stored in 25 mL tubes, and preserved with 96% ethanol. A hand-held GPS was used to determine altitude and location, and pH, temperature and conductivity were measured in the field using a HANNA Instrument HI 98129 Waterproof pH/Conductivity/ TDS Tester (Hanna Instruments Czech s.r.o., Czech Republic). Additionally, the water temperature of one stable cryoconite hole on Nordenskiöld glacier surface was measured every hour for 28 days between the 22nd of July and 25th of August using a temperature datalogger 'minikin T' (EMS Brno, Czech Republic), revealing stable values between – 1°C and 1°C.

During the 2011 and 2013 summer campaigns, benthic epilithon/epipelon samples from the littoral zones of freshwater lakes and ponds were collected for diatom analysis as described above for cryoconite holes. For a full species report of freshwater diatoms from Petuniabukta, *see* Pinseel (2014). Three of these localities from Pinseel (2014) (Fig. A4.1C) were selected for comparison with the cryoconite samples. The first locality, Retrettøya (O) (known also as 'roche moutonnée' or 'Oblik'), is a peninsula located in front of the tide-water glacier Nordenskiöld. The area is rather freshly deglaciated, being covered by the Nordenskiöld glacier until about 30 years ago, and therefore open to organismal colonization. Several ponds on this peninsula, situated in eroded tectonic faults later remodeled by glacial erosion (Pinseel 2014), are located in close vicinity of the sea, surrounded by a large colony of Arctic terns, Sterna paradisaea Pontoppidan. Altogether, glacial influence, the presence of the Arctic terns colony, and possible sea spray influence make this peninsula a unique locality in terms of nutrient sources. The other two localities are comprised of kettle lakes located in the frontal moraines of the Nordenskiöld (N) and Hørbye (H) glaciers, the latter being located in the northern part of Petuniabukta. All samples used in this study (both lakes and cryoconite holes), together with their parameters, are listed in Table 1.

Slide preparation and enumeration

For light microscopy analysis, subsamples were cleaned by a modified method described in Van der Werff (1955). The sub-samples were added 37% H2O2 and then heated to 80°C for about 1h. The reaction was completed by addition of saturated KMnO4. Following digestion and oxidation, samples were rinsed three times with distilled water alternated with centrifugation (10 minutes at 3500 x g). The cleaned diatom material was diluted with distilled water on microscope cover slides, dried, and mounted in Naphrax®.

In each sub-sample, diatom valves were identified to the lowest taxonomic level possible (taxa with uncertain taxonomic status were indicated with ‘cf.‘ (confer: species identification is uncertain) or ‘sp.‘, and sometimes only genus level was possible) and enumerated at 1500 x magnification under immersion oil using an Olympus® BX51 microscope equipped with Differential Interference Contrast (Nomarski) optics. Diatoms were very rare in the cryoconite hole samples, and therefore entire microscope slides were counted for diatom valves. In total, 9 samples were used for the community analysis, with 2 additional samples (C10 and C11, with only a few recovered valves) used in the “inkspot” diagram (for explanation, *see* below). Of these, total counts ranged between 40 and 216 valves per sample. One sample (C12) did not contain any frustules and was therefore removed from further analysis.

For the identification of diatom species from cryoconite hole material, we primarily consulted Pinseel (2014). Along with diatoms, the presence of Chrysophyte stomatocysts (golden brown algae) was also noted, although abundances were generally very low. Following Yallop et Anesio (2010), count data were combined at the genus level. Due to the widespread practice of “force-fitting” Arctic taxa into their Europeanand North-American relatives (Tyler 1996), or the use of a too broad morphospecies concept (Mann 1999), a substantial number of taxa has been incorrectly identified in the past. As a consequence, many studies which identified Arctic diatoms to the species level might not be reliable (Pinseel 2014). On the contrary, the genus level is taxonomically more robust and ensures consistency between datasets. Moreover, the

Table A4.1. List of all samples used in this study and physico-chemical parameters of the study sites.

Study code (sample code)	Location/ ake type	Temper-ature (°C)	Conduc-tivity (µS/cm)	pH	Altitude a.s.l. (m)	GPS N	GPS E
Cryoconite holes							
C1 (N002)	Glacier surface	0.5	1	8.30	130	78° 38' 24.7"	16° 58' 37.1"
C2 (N003)	Glacier surface	0.7	1	8.60	201	78° 38' 24.6"	17° 00' 05.1"
C3 (N004)	Glacier surface	0.6	1	8.30	244	78° 38' 22.2"	17° 01' 44.6"
C4 (N006)	Glacier surface	0.5	1	9.10	278	78° 38' 28.3"	17° 05' 11.0"
C5 (N007)	Glacier surface	0.4	7	9.30	361	78° 38' 29.2"	17° 06' 37.6"
C6 (N008)	Glacier surface	0.4	4	9.80	393	78° 38' 30.5"	17° 07' 22.7"
C7 (N009)	Glacier surface	0.3	1	8.50	262	78° 39' 21.4"	17° 03' 40.9"
C8 (N010)	Glacier surface	0.4	2	8.40	263	78° 39' 01.0"	17° 03' 31.9"
C9 (N011)	Glacier surface	0.5	12	10.10	267	78° 38' 41.5"	17° 03' 16.2"
C10 (N012)	Glacier surface	0.5	2	9.20	237	78° 38' 08.6"	17° 03' 01.0"
C11 (N013)	Glacier surface	0.4	11	8.80	233	78° 37' 51.9"	17° 02' 55.3"
C12 (N005)	Glacier surface	0.5	2	9.00	271	78° 38' 25.6"	17° 03' 46.9"
Nordenskiöld lakes (Pinseel 2014)							
N1 (SP20)	Kettle lakes	8.3	443	8.52	28	78° 38' 19.2''	16° 49' 35.8''
N2 (SP21)	Kettle lakes	8.6	329	8.55	24	78° 38' 19.4''	16° 49' 41.1''
N3 (SP22)	Kettle lakes	8.4	658	8.33	29	78° 38' 17.6''	16° 50' 1.5''
N4 (SP23)	Kettle lakes	8.7	566	8.60	26	78° 38' 17.6''	16° 50' 5.1''
Hørbye lakes (Pinseel 2014)							
H1 (SPH1B)	Kettle lakes	ND	ND	ND	37	78° 44' 05.7"	16° 26' 52.5"
H2 (SPH1C)	Kettle lakes	10.9	861	8.18	23	78° 44' 08.1"	16° 26' 51.9"
H3 (SPH2A)	Kettle lakes	11.7	461	8.39	41	78° 44' 13.4"	16° 26' 27.7"
H4 (SPH2B)	Kettle lakes	12.0	760	8.13	43	78° 44' 11.2"	16° 26' 15.2"
H5 (SPH2C)	Kettle lakes	11.6	756	8.20	44	78° 44' 11.8"	16° 26' 10.1"

H6 (SPH3A)	Kettle lakes	13.3	338	8.41	49	78° 44' 22.0"	16° 25' 04.8"
H7 (SPH3B)	Kettle lakes	12.7	306	8.42	50	78° 44' 23.9"	16° 24' 56.3"
H8 (SPH3C)	Kettle lakes	14.2	339	8.54	46	78° 44' 21.4"	16° 25' 08.2"
H9 (SPH4B)	Kettle lakes	11.3	423	8.28	52	78° 44' 34.2"	16° 24' 42.4"
H10 (SPH4C)	Kettle lakes	14.5	655	8.32	50	78° 44' 34.2"	16° 24' 48.7"
H11 (SPH5A)	Kettle lakes	13.9	1428	8.12	54	78° 44' 43.9"	16° 24' 27.4"
H12 (SPH5B)	Kettle lakes	12.5	1805	8.18	57	78° 44' 45.4"	16° 24' 37.0"
H13 (SPH5C)	Kettle lakes	13.3	1208	8.32	57	78° 44' 47.2"	16° 24' 39.5"
H14-15 (SP41-42)	Kettle lakes	11.6	1325	8.18	35	78° 44' 05.0"	16° 26' 34.1"
H16 (SP44)	Kettle lakes	12.7	180	8.45	40	78° 44' 13.0"	16° 26' 14.1"
H17 (SP45)	Kettle lakes	12.2	218	8.54	50	78° 44' 35.4"	16° 24' 44.9"
H18 (SP46)	Kettle lakes	9.2	198	9.20	106	78° 45' 14.0"	16° 21' 46.2"
H19-20 (SP47-48)	Kettle lakes	8.7	182	8.39	107	78° 45' 24.5"	16° 22' 00.8"
Retrettøya ponds (as ‘roche moutonnée/Oblík’ in Pinseel 2014)							
O1 (SP62)	Tectonic related	8.2	592	8.80	20	78° 39' 24.9''	16° 54' 46.5''
O2 (SP63)	Tectonic related	8.1	256	9.60	7	78° 39' 27.8''	16° 54' 34.3''
O3 (SP64)	Tectonic related	8.5	377	8.90	24	78° 39' 23.0''	16° 54' 44.4''
O4 (SP65)	Tectonic related	8.1	281	9.30	18	78° 39' 19.3''	16° 54' 37.8''
O5 (SP66)	Tectonic related	7.4	510	8.70	20	78° 39' 21.4''	16° 54' 39.9''
O6 (SP67)	Tectonic related	8.0	319	8.70	28	78° 39' 18.6''	16° 54' 38.5''
O7 (SP68)	Tectonic related	8.4	174	9.80	24	78° 39' 16.3''	16° 54' 21.9''
O8 (SP69)	Tectonic related	9.0	347	9.20	7	78° 39' 8.5''	16° 54' 51.4''
O9 (SP70)	Tectonic related	9.0	450	9.40	11	78° 39' 14.6''	16° 55' 28.5''
O10 (SP71-72)	Tectonic related	9.1	147	9.60	26	78° 39' 14.1''	16° 55' 16.4''
O11 (SP73)	Tectonic related	9.7	133	10.90	17	78° 39' 9.0''	16° 55' 8.7''

large amount of debris, together with an association of cells with mineral particles, makes the observation of single small valves rather difficult (Stibal et al. 2015), and sometimes even unfeasible when looking for diatoms in living samples (Vonnahme 2014), impeding identification of diatom valves up to the species level.

Statistical analyses

To investigate the similarity of the cryoconite diatom flora with those of nearby freshwater habitats, we compared our cryoconite hole dataset with diatom counts from Pinseel (2014), who counted and identified 400 diatom valves in littoral samples from freshwater ponds and lakes in Petuniabukta, using the methods described above. Relative abundances were first calculated from diatom counts from both datasets, and an "inkspot" plot was created using the *rioja* R package (Juggins 2012) to manageably view diatom community structure among samples.

Diatom communities were statistically analyzed using the approaches applied by Stanish et al. (2012, 2013). Briefly, nonmetric multidimensional scaling (NMDS) analyses were performed to visualize relationships between communities from different samples, sites, and habitats. Rare species (< 1.0%) were removed, all data square-root transformed, and a distance matrix was calculated based on Bray–Curtis dissimilarity using the *vegan* R package (Oksanen et al. 2011). From this, a three dimensional model was created, which produced a Kruskal's "stress" value of 9.88%, and strong nonmetric (R2 = 0.99) and linear fits (R2 = 0.94). To discern which genera drive patterns between samples, a corresponding NMDS figure was produced with genera > 5.0% in relative abundance superimposed.

Lastly, a hierarchical dendrogram was produced by creating a distance matrix as described above, utilizing the "average" clustering method (coeff = 0.81). To test for significant differences between the lake and cryoconite communities in general, the cryoconite hole diatoms communities were tested against the pooled lake diatom community data using permutational multivariate analysis of variance (PERMANOVA), with $\alpha = 0.05$. All analyses were performed using the R statistical environment (R Core Team 2014, *see* References – Other sources).

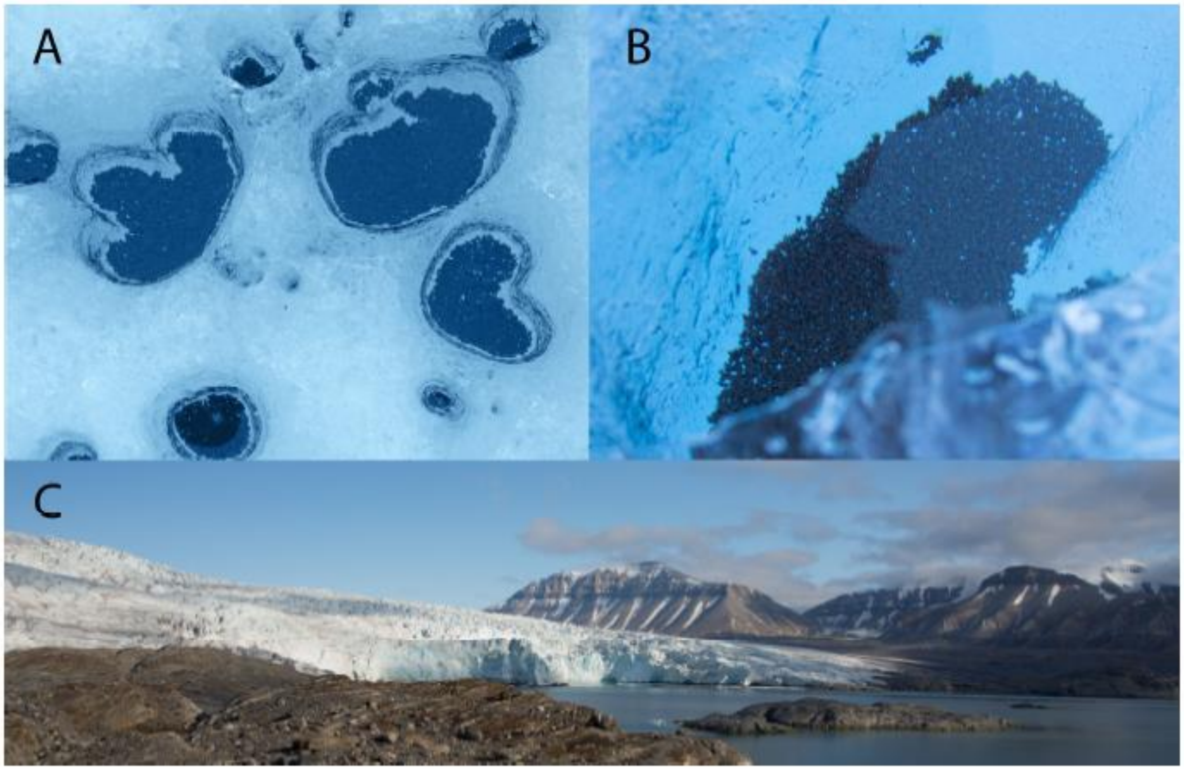

*Figure A4.2. Examples of cryoconite holes (**A–B**), and an overview of the Nordenskiöld tide-water glacier (**C**).*

RESULTS

Species composition of the cryoconite holes

A total of 58 diatom taxa (including species, subspecies, varieties and formas) belonging to 46 genera were identified in the cryoconite material (Fig. A4.3). An additional 26 diatom frustules could not be identified below genus level. Genus richness of the cryoconite samples ranged from 9 to 24 with a median of 18. Some genera were common throughout the sampling sites, such as *Pinnularia* Ehrenberg (in all samples), *Nitzschia* Hassall (in 8 out of 9 samples), *Staurosirella* D.M.Williams (8/9), *Gomphonema* Ehrenberg (8/9) and *Luticola* D.G.Mann (8/9). The dominance of these genera are also reflected in the number of counted valves: *Nitzschia* (17% of all counted valves), *Psammothidium* L. Bukhtiyarova (14%), *Pinnularia* (12%), *Staurosirella* (6%), *Gomphonema* (5%) and *Luticola* (5%).

The most species-rich genera were *Pinnularia, Nitzschia* and *Eunotia* Ehrenberg. Six *Pinnularia* taxa could be identified – *P.* cf. *brebisonii* (Kützing) Rabenhorst*, P. obscura* Krasske*, P. intermedia* (Lagestedt) Cleve*, P. schimanskii* Krammer*, P. rabenhorstii* (Grunow) Krammer*,* and *P. borealis* Ehrenberg (Fig. A4.3). The lattermost actually represents one of the most common species complexes (in 8 out of 9 samples) on the site, together with *P. intermedia* (7/9)*, Nitzschia perminuta* complex form 2 (Pinseel 2014; 7/9), *Gomphonema* aff. *nathorstii* (Pinseel 2014; 8/9), and *Psammothidium* sp1 (Pinseel 2014; 7/9) - *see* Fig. 3.

Several other *Nitzschia* taxa*,* such as *N. amphibia* Grunow*, N. communata* Grunow*, N. flexoides* Geitler*, N. palea* (Kützing) W.Smith and *N.* sp8 (Pinseel 2014) have been observed in low numbers (containing less than 2.5 % out of the total species counts). Four *Eunotia* taxa have been identified: *E.* cf. *ambivalens* Lange-Bertalot & Tagliaventi*, E. curtagrunowii* Nörpel-Schempp & Lange-Bertalot (Fig. A4.3)*, E. islandica* Østrup and *E. nymanniana* Grunow. Three species out of the most common taxa also dominated the cryoconite sites in terms of relative abundances: *Nitzschia perminuta* form 2, *Psammothidium* sp1 and *Pinnularia borealis* complex, represented 12%, 10% and 5% respectively.

Local spatial comparison

To examine diatom community similarity between localities, the genus richness of the cryoconite holes and nearby lake habitats was compared. All observed genera, their abbreviations used for analyses and their distribution in between compared localities, can be found in Table 2. Even though only a low number of diatom valves could be enumerated from the cryoconite samples, overall genus richness was comparable, and sometimes even greater, than some of the lake sites (Fig. A4.4).

Nonmetric multidimensional scaling (NMDS) of lake and cryoconite hole communities revealed a strong separation between the two habitat types, both on NMDS axis 1 and 2 (*see* Fig. A4.5). Cryoconite hole diatom communities had greater proportions of the genera *Eunotia* (not visible on figure), *Aulacoseira* Thwaites, and *Gomphonema*, all of which strongly influenced sample orientation on both NMDS axes. Lake habitats were strongly influenced by *Adlafia* Moser, Lange-Bertalot and Metzeltin, *Encyonema* Kützing, and *Mayamaea* Lange-Bertalot on NMDS axis 2. Habitat differences were further influenced by *Staurosira* Ehrenberg, *Staurosirella*, *Luticola*, *Pinnularia* and *Hantzschia* Grunow on axis 1 for the cryoconite holes, and *Encyonema*, *Cymbella* Agardh, *Diatoma* Bory de Saint-Vincent, and *Denticula* Kützing for the lakes. The Bray-Curtis cluster analysis separated communities into several distinct groups, of which, one exclusively consisted of all samples from the cryoconite holes (*see* Fig. A4.6). When the community data were compared with PERMANOVA, cryoconite hole diatom

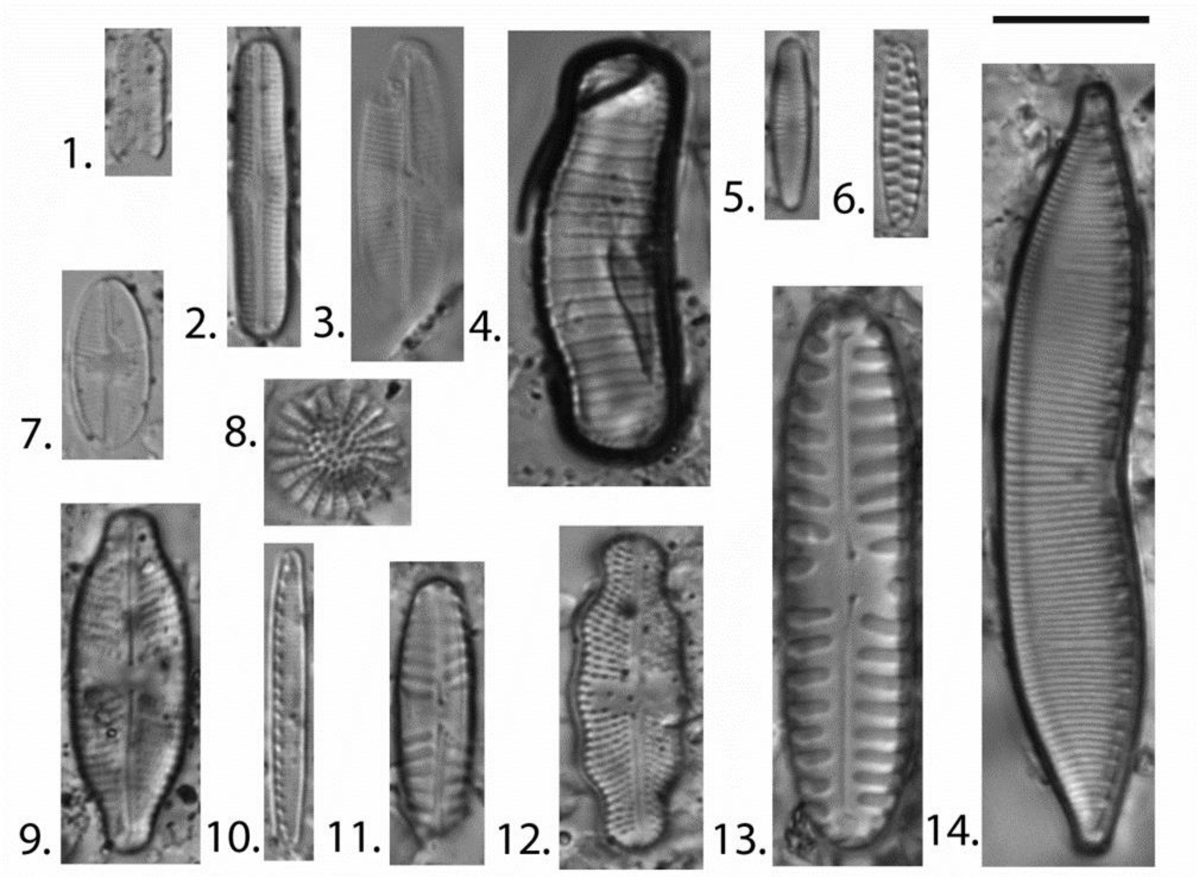

Figure A4.3. *Selected diatom species observed in cryoconite holes of Nordenskiöld glacier. Scale bar = 10 µm.* ***1***. Humidophila *cf.* contenta. ***2***. Chamaepinnularia *sp. 3.* Rossithidium petersenii. ***4***. Eunotia curtagrunowii. ***5***. Achnanthidium minutissimum *complex.* ***6***. Staurosirella *sp.* ***7***. Psammothidium *sp1.* ***8***. Stephanodiscus *cf.* minutulus. ***9***. Gomphonema *aff.* nathorstii. ***10***. Nitzschia perminuta *forma 1****. 11***. Pinnularia intermedia. ***12***. Luticola nivalis. ***13.*** Pinnularia borealis *complex****. 14***. Hantzschia amphioxys *complex.*

communities were significantly different from the pooled lake samples (df = 43, F = 15.64, R2 = 0.27, p = 0.001).

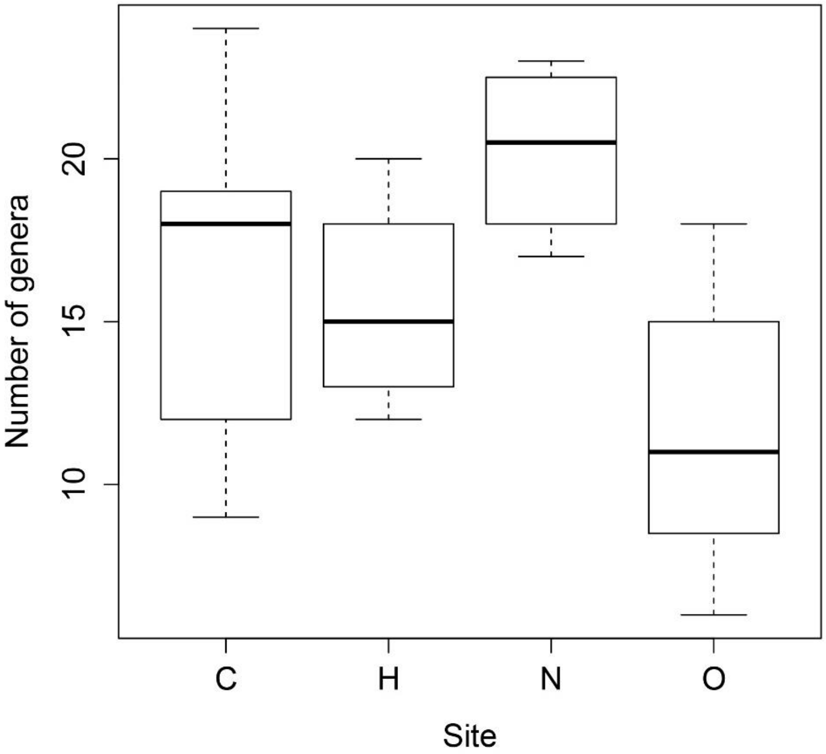

Figure A4.4: *Genus richness of cryoconite holes (**C**), Hørbye lakes (**H**), Nordenskiöld lakes (**N**), and Retrettøya (**O**) sites.*

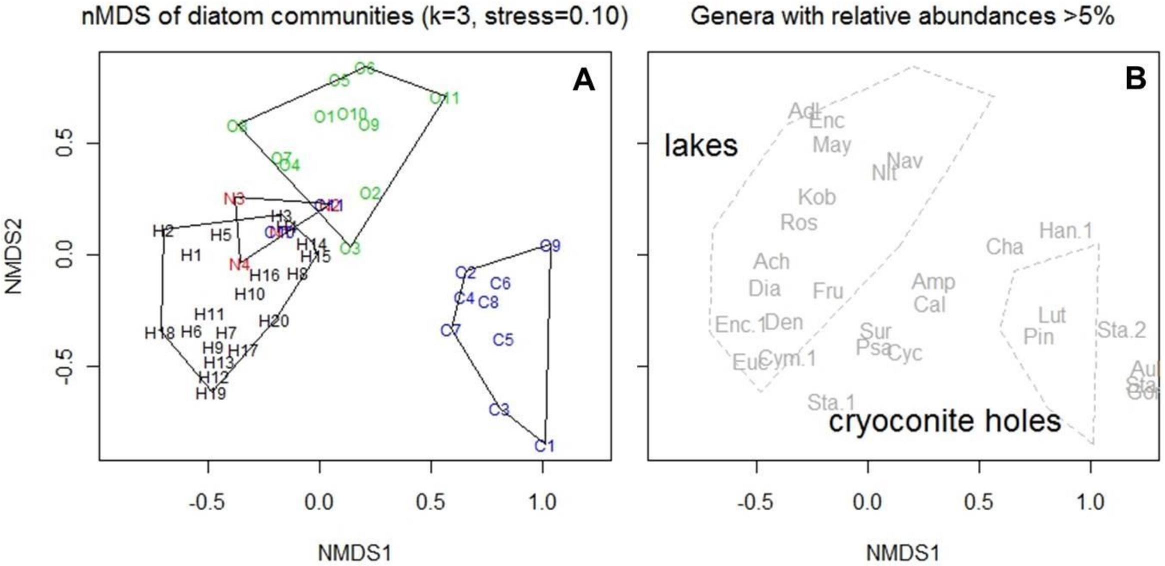

Figure A4.5. *Non-metric multidimensional scaling (NMDS) of diatom communities from cryoconite holes and nearby lakes, indicating separation of the two habitats. **C1–C9** for cryoconite holes, **H1–H20** for Hørbye lakes**, N1 – N4** for Nordenskiöld lakes, and **O1–O11** for Retrettøya. Diatom genus abbreviations are given in **Table 2**.*

Table A4.2. *List of diatom genera (with abbreviations) from cryoconite holes (**C**), Hørbye lakes (**H**), Nordenskiöld lakes (**N**) and Retrettøya ponds (**O**).*

Genus	C	H	N	O
Achnanthidium (Ach)	x	x	x	x
Adlafia (Adl)		x	x	x
Alveovallum (Alv)	x			
Amphora (Amp)	x	x		
Aulacoseira (Aul)	x			
Brachysira			x	
Caloneis (Cal)	x	x	x	x
Cavinula	x			
Chamaepinnularia (Cha)	x	x		x
Cocconeis (Coc)	x			
Craticula (Cra)		x		x
Cyclotella (Cyc)	x		x	
Cymbella (Cym)		x	x	x
Cymbopleura (Cym.1)	x	x	x	x
Denticula (Den)	x	x	x	
Diatoma (Dia)	x	x	x	x
Diploneis (Dip)	x	x	x	
Encyonema (Enc)	x	x	x	x
Encyonopsis (Enc.1)	x	x	x	
Epithemia (Epi)	x			
Eucocconeis (Euc)	x	x	x	x
Eunotia (Eun)	x			
Fallacia				x
Fistulifera				x
Fragilaria		x	x	
Frustulia (Fru)		x		
Geissleria				
Gomphonema (Gom)	x			
Gyrosigma		x		
Halamphora (Hal)		x	x	x
Hannaea (Han)	x	x		
Hantzschia (Han.1)	x	x	x	x
Humidophila (Hum)	x	x	x	
Hygropetra (Hyg)	x			
Kobayasiella (Kob)		x	x	x
Luticola (Lut)	x	x	x	x
Mayamaea (May)		x	x	x
Melosira	x			
Microcostatus			x	
Muelleria (Mue)	x	x		
Navicula (Nav)	x	x	x	x
Neidium (Nei)	x	x		x
Nitzschia (Nit)	x	x	x	x
Orthoseira (Ort)	x			
Pinnularia (Pin)	x	x	x	x
Placoneis (Pla)	x			
Planothidium (Pla.1)	x			
Psammothidium (Psa)	x	x	x	x
Pseudostaurosira (Pse)	x			
Rossithidium (Ros)	x	x	x	x
Sellaphora (Sel)		x		
Simonsenia			x	
Stauroforma (Sta)	x			
Stauroneis (Sta.1)	x	x	x	
Staurosira (Sta.2)	x			
Staurosirella (Sta.3)	x			
Stephanodiscus (Ste)	x			
Surirella (Sur)	x	x		x
Tabellaria (Tab)	x			

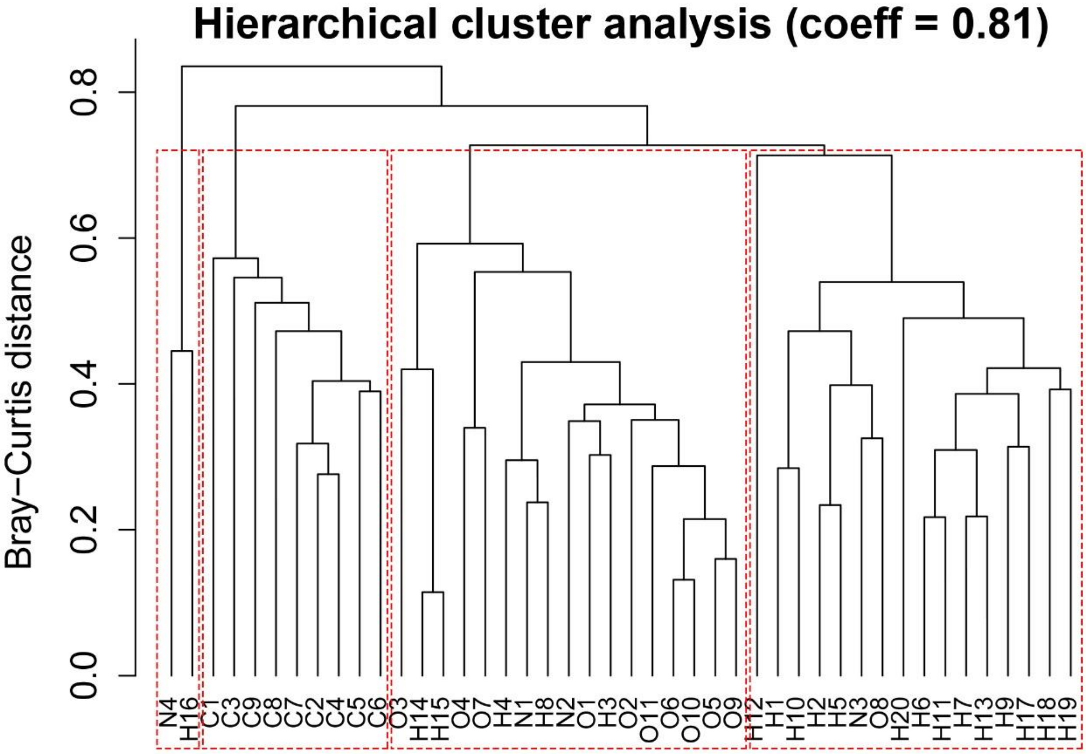

Figure A4.6. Bray-Curtis cluster analysis. ***C1–C9*** *for cryoconite holes,* ***H1–H20*** *for Hørbye lakes,* ***N1 – N4*** *for Nordenskiöld lakes, and* ***O1–O11*** *for Retrettøya.*

Despite such clear split of both habitat types, some genera were present between both localities studied as seen in the 'inkspot' plot. This diagram, which can be used to visualize the community structure among samples using the raw relative abundance data (Fig. A4.7), resulted in a clear separation of three groups. The first consisted entirely of samples from cryoconite holes. The second group linked both localities of the Hørbye and Nordenskiöld moraine kettle lakes. The third group consisted entirely of samples of Retrettøya. Some diatom genera showed clear differences between these three groups: *e.g., Encyonema* and *Adlafia* were both abundant in the third group. *Nitzschia*, although prevailing in the entire dataset, was clearly less abundant in the samples of the second group. *Psammothidium* was almost equally abundant in the first two groups, and, finally, *Pinnularia, Luticola, Staurosirella*, together with other genera (lower part of Fig. A4.7), clearly separated the first group from the remaining two.

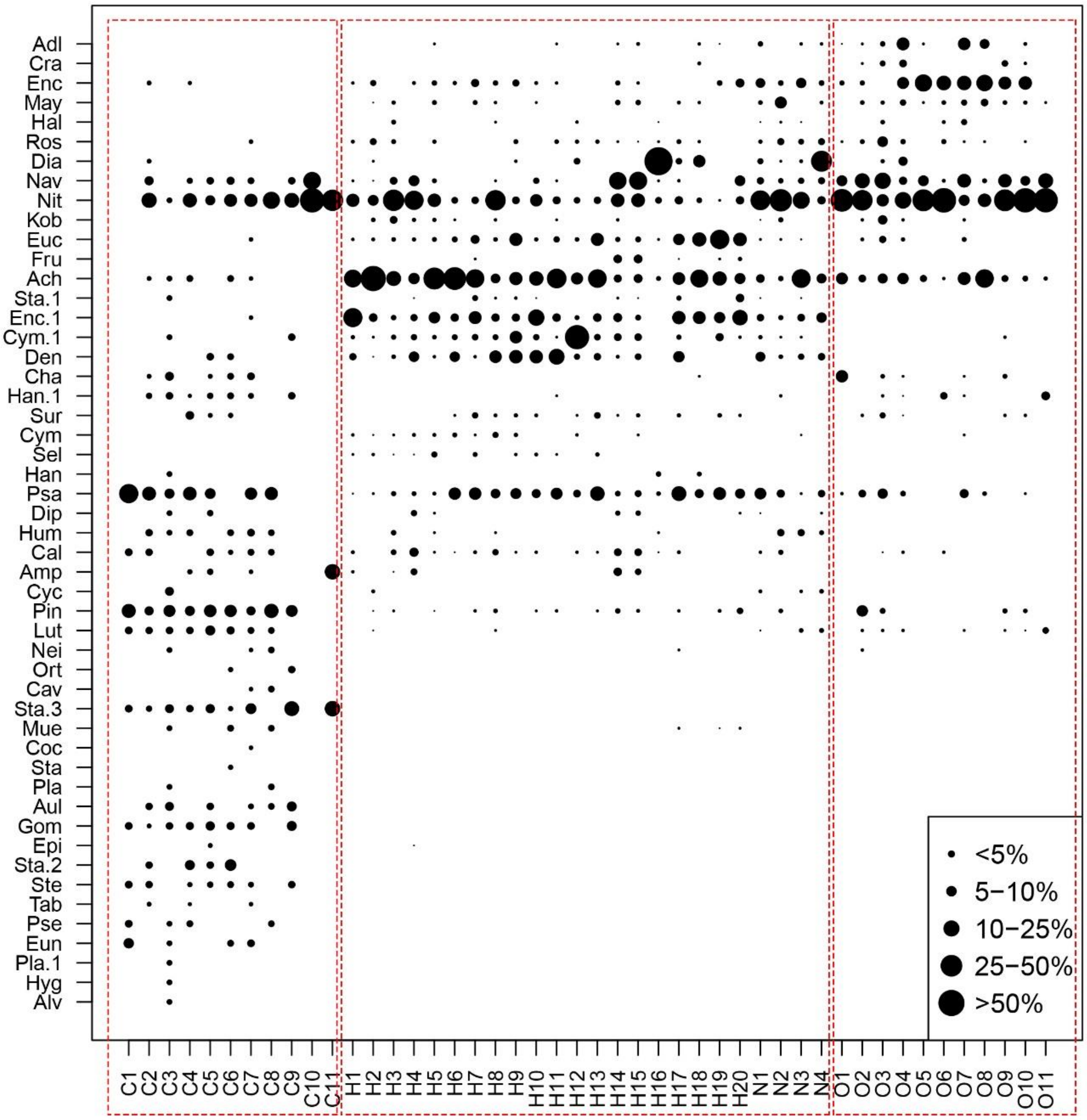

Figure A4.7. An 'inkspot' plot visualizing the diatom community structure among samples using relative abundance data. Separation of three groups is shown. ***C1–C11*** *for cryoconite holes,* ***H1–H20*** *for Hørbye lakes,* ***N1 – N4*** *for Nordenskiöld lakes, and* ***O1–O11*** *for Retrettøya ponds.*

DISCUSSION

Although numerous studies have reported the presence of diatoms in cryoconite holes, many questions remain as to their origin, viability, community structure, and assembly. Here, our aim was to describe and compare the diatom assemblages from cryoconite holes of Nordenskiöld glacier to the communities from lake habitats in the immediate vicinity which might serve as a potential source. We hypothesized that if diatoms are being transported from surrounding aquatic habitats to cryoconite systems, then cryoconite communities should be highly similar to the communities from the habitat of origin.

We found that the diatom communities of the cryoconite hole sediments were distinctly different from those from the adjacent lake habitats. Not only did cryoconite sediment contain different genera not observed in lake habitats, but often a higher number of genera was observed in the cryoconite communities compared to the lake sediment samples, even when only a fraction of the amount of valves was counted. This may suggest that (i) cryoconite hole diatom communities have a unique structure, albeit at low cellular densities, or that (ii) these cryoconite communities are not self-sustaining and are dependent on dispersed cells, but are derived from more varied sources than our surveyed lakes.

If these communities were actively reproducing, it could be argued that this should be reflected in a community dominated by only a few species or genera. On the contrary, our results show the presence of a lot of different genera with a low number of species and valves within the cryoconite holes, which suggest that these assemblages are more likely to be formed as a result of aeolian dispersion and deposition. While there is much left to learn about these extreme environments, our study adds further evidence that the diatom flora from cryoconite holes is unique and distinct from adjacent freshwater habitats. Recently, there is a need for further study that could help in developing a more complete understanding of diatom biogeography, dispersal, and evolution.

Ecology of individual diatoms

Our results showed that the cryoconite diatom community composition exhibited only little similarity to any of the lake diatom communities in the vicinity, despite the fact that 25 genera occur in both habitat types. These shared genera may indicate that these lakes contribute to the diatom community in the cryoconite holes (though investigation at a finer resolution would be necessary to support this claim). On the other hand, it is clear that at least one other source is necessary to explain the higher number of genera in the Nordenskiöld glacier cryoconite holes, a number of which are rare or uncommon to the surrounding lakes. Similar results have been reported by Stanish et al. (2013), who found that cryoconite hole communities were distinctly different from adjacent stream habitats in the McMurdo Dry Valleys (though they certainly shared more taxa than in our study). Another supporting data come from Edwards et al. (2013c), who found that cryoconite bacterial communities significantly differed from those from the glacier margins in Svalbard.

A number of diatom genera found in cryoconite holes, including *Hantzschia, Humidophila* Lowe et al., *Orthoseira* Thwaites, *Pinnularia* and *Luticola,* are typically aerophilic genera, known to thrive mainly in moist terrestrial soil or moss habitats. Diatom cells from soils can be easily transported by wind together A number of diatom genera found in cryoconite holes, including *Hantzschia, Humidophila* Lowe et al., *Orthoseira* Thwaites, *Pinnularia* and *Luticola,* are typically aerophilic genera, known to thrive mainly in moist terrestrial soil or moss habitats. Diatom cells from soils can be easily transported by wind togetherwith sediment or volcanic dust (Dagson-Waldhauserová et al. 2015), or be attached to small moss patches able to overgrow small cryoconite holes and thus becoming a 'glacial mouse' (Vonnahme 2014). *Pinnularia borealis* and *Hantzschia amphioxys* (Ehrenberg) Grunow, two species highly abundant in cryoconite samples, are frequently reported from polar soils and also commonly recorded from mosses (Beyens 1989, Van de Vijver et al. 2003, Vinocur et Maidana 2010). Terrestrial diatoms make good candidates for cryoconite colonization because they are more able to resist freezing and desiccation than freshwater species (Souffreau et al. 2010, Yallop et Anesio 2010). Abrupt freezing can be lethal for diatom vegetative cells, especially for non-terrestrial species (Souffreau et al. 2010, 2013). Furthermore, some of these diatoms could be present in resting stages, increasing thus the ability to survive freezing stress (Souffreau et al. 2013).

In our study, rather than more, few genera such as *Adlafia*, *Encyonema*, *Navicula* and *Nitzschia*, were present in greater relative abundances from the Retrettøya proglacial site, in contrast to the other two lake localities. According to our field observations, no direct hydrological connection exists between ponds on Retrettøya and the glacier. However, plenty of fresh glacial sediment, including cryoconite material, was present drying on the margin and mobilized by wind, which blows from the glacier towards the fjord.

Consequently, the Retrettøya ponds might be supplied by cryoconite communities, similar as suggested by Vonnahme (2014) for Hørbye glacier and proglacial ponds in its frontal moraine.

Species of the genera *Achnanthidium* Kütz.*, Denticula* Kütz.*, Encyonema,* and *Eucocconeis* Cleve had relatively high abundances in moraine kettle lakes. These genera were uncommon or rare in the cryoconite samples, most likely suggesting their preference for more established (aquatic) habitats. The *A. minutissimum* (Kütz.) Czarnecki complex reaches high abundances in various freshwater habitats across Petuniabukta and actually presents the most common freshwater diatom complex in the area (Pinseel 2014). Although this genus was rarely found in the cryoconite holes sampled for diatoms (this study), fresh cryoconite material from the same area observed by Vonnahme (2014) in the field laboratory revealed a few viable individuals of *Achnanthidium*. *Psammothidium* is another very interesting genus that connects the cryoconite holes to the moraine kettle lakes of Nordenskiöld and Horbye. Moreover, species of this genus prefer habitats with sandy bottoms (Round et Bukhtiyarova 1996). This is in contrast with another highly abundant genus, *Nitzschia,* which dominates both the cryoconite habitats and the Retrettøya ponds, but usually occurs in lower abundances in the kettle lakes.

Luticola was a rather common genus in cryoconite holes involved into our study. Previously, *L. ventricosa* (Kütz.) Mann and *L. nivalis* (Ehrenberg) Mann have been retrieved alive from frozen cryoconite material (Yallop et Anesio 2010). *Luticola nivalis* was also observed in the present study, together with *L. frequentissima* (*see* Fig. 3). Species of the genus *Muelleria* (Frenguelli) Frenguelli are not abundant in the Arctic, and only one species resembling the cosmopolitan *M. terrestris* (Petersen) Spaulding & Stoermer was observed in this study. The species was previously reported from cryoconite holes by Yallop et Anesio (2010). *Muelleria* is, however, one of the most dominant genera of Antarctic cryoconite diatom communities, accompanied by species from genera *Humidophila* and *Luticola* (Mueller et al. 2001, Van de Vijver et al. 2010, Stanish et al. 2013, Kohler et al. 2015).

Insights into microbial dispersal

While we did not check for cell viability in our samples due to the limited amount of material, we can postulate that viability may have been low, as recovered valves were often broken in addition to being sparse. However, earlier studies have suggested that at least some cryoconite diatom cells are viable. Stanish et al. (2013) and Vonnahme (2014) have both reported viable valves by microscopic analysis, and Yallop et Anesio (2010) were able to culture 27 diatom genera from cryoconite material, even after being frozen for 1-2 years. Vonnahme (2014) analyzed only a few samples (*n* = 3) of fresh material from cryoconite holes of Nordenskiöld and Hørbye glaciers for diatoms content, and reported following genera and species as living, although in low numbers: *Achnanthidium* sp*., Encyonopsis laevis* Nägeli and *E. subminuta* Krammer & E. Reichardt*, Humidophila* sp*., Pinnularia* cf. *obscura,* and *Psammothidium* cf. *marginulatum* (Grunow) Bukhtiyarova and Round. All of these were also identified in our study of fixed material, albeit present in various abundances.

In Antarctica, some diatom species, such as *Muelleria cryoconicola* Stanish & Spaulding (Van de Vijver et al. 2010), *Luticola bradyi* Kohler, and *L. spainiae* Kohler & Kopalová are thus far found almost exclusively in cryoconite holes, and have been suggested to be endemic to these habitats. According to Kohler et al. (2015), transportation of diatom cells from cryoconite holes to surrounding aquatic habitats might even take place, as suggested for *L. bradyi*, a species found in a large population in cryoconite material, but only very rarely observed in glacial meltwater streams. These above observations, together with the differences in cryoconite diatom communities from marginal habitats, provide evidence that while diatoms may be seeded from adjacent habitats, at least some survive, live, and possibly reproduce while in cryoconite holes. While unique cryoconite diatom communities are thought to develop in stable cryoconite systems found in Antarctica (Stanish et al. 2013), Arctic systems are much more exposed to the outside world and do not persist as long due to glacier hydrology and melting. Despite this, they can still be colonized by living diatoms (Yallop et Anesio 2010), and these habitats might subsequently select the most adapted species to live in.

In our study, many genera observed in the cryoconite material were rare in the lakes and ponds of Petuniabukta. For example, the genera *Aulacoseira, Tabellaria* Ehrenberg, *Melosira* Agardh, *Orthoseira*, and *Stephanodiscus* Ehrenberg were present in the cryoconite holes, but have never been observed in the lakes and ponds of Petuniabukta (> 50 lakes studied, in Pinseel 2014). One *Gomphonema* taxon (*i.e. Gomphonema* aff. *nathorstii,* Fig. 3) was recorded as common in almost all (eight out of nine) cryoconite samples but was visually absent from the surrounding lakes. However, the same species has been observed previously in a temporal pond on a mountain top in Mimerdalen (Pinseel, *unpublished data*), suggesting that this taxon prefers emphemeral habitats. Several other species of *Gomphonema* were present within the cryoconite samples, although absent from nearby lakes, and the same is true for species of *Staurosira, Staurosirella* and *Eunotia.* Some genera (*e.g. Achnanthidium, Gomphonema, Psammothidium, Staurosirella*) may produce (mucilaginous) stalks (Potapova 2009, Gesierich et Rott 2012) so they can be attached to solid objects, hypothetically favoring them in aeolian dispersion. It was striking that no marine species have been identified from the cryoconite material, despite the immediate vicinity of the sea, even though direct evidence of marine diatom deposition on ice sheets was previ ously reported before from the Antarctic (Budgeon et al. 2012). This is probably because the prevalent wind direction is in the opposite way, which reduces the extent of marine deposition of diatoms compared to those that are limnoterrestrial in origin.

As light microscope studies of cryoconite material are challenging, it is possible that the lack of records of diatom species inhabiting cryoconite holes is a result of under-sampling, as previously hypothesized by Yallop et Anesio (2010). In total, some 84 taxa were identified in our study, and broader sampling efforts would certainly reveal more taxa. Our gradient sampling also revealed that sites closer to the side of the glacier contained less diatom valves than those situated more inside. This could further support the suggestion of aeolian input of cells rather than the cell input from avalanches of eroded material that occurs on glaciers sides (Landford et al. 2011). On the contrary, sites that were situated the most to the center of the Nordenskiöld glacier (C8 and C9) contained less (roughly half) of the average counted valves. It might be interesting to sample across the whole ablation zone of the glacier by adding further sampling sites, as it could reveal wind patterns on a local scale (*i.e.* considering katabatic winds).

Future directions and conclusions

Contrary to the Antarctic diatom flora, which has been recently revised based on a more narrow morphology-based species concept (ex. Van de Vijver et al. 2010a, 2011, 2013, Kopalová et al. 2012, 2013, Taylor et al. 2014, among others), the Arctic diatom morphological taxonomy is insufficient at this time to make fine-scale comparisons between habitats. Moreover, Pinseel (2014) have identified a high number of new diatom taxa ready to be described. However, in this study, we found the genus-level to be of great use to compare cryoconite holes with adjacent freshwater habitats, and it became clear that our comparison set of localities in the immediate vicinity was not broad enough to fully assess 'the source' of the cryoconite diatom flora. It is possible that a more broad sampling effort to include non-aquatic habitats nearby, as well as more distant localities such as Iceland (Dagsson-Waldhauserova et al. 2015) are necessary to discern the cryoconite diatom flora 'source'. In any event, the diatom communities of cryoconite holes have the potential to inform researchers about microbial dispersal patterns through comparing the regional distribution of diatom taxa in between distinct polar habitats, and deserve further study in our investigation of the cryosphere.

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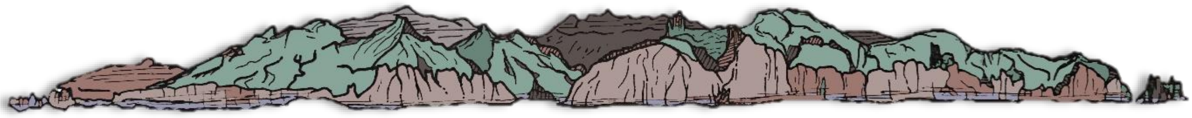

Gough Island

Mountains in the Sea