



CHARLES UNIVERSITY
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Patterns and processes in desmid communities:
Insights from functional and phylogenetic approaches

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Doctoral thesis

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Author's declaration

I hereby declare that I have written thesis independently using listed references. I have submitted neither this thesis nor its parts to acquire any other academic degree.

In Prague 25. 03. 2019

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I would like to thank everybody who has been with me through those exciting years. I know that it has not been always easy, but you encouraged me anyway.

Thanks to my family for all the support you provided to me, not just during my Ph.D. Moreover, I admire the patience you had to repeatedly ask all those years: “So when you will write it?” I can finally provide you the answers: “Now”. I would like to remind my brother that we had a bet who will finish first, and surprisingly I won...

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Papers included in the thesis

Paper 1: **Helena Bestová**, François Munoz, Pavel Svoboda, Pavel Škaloud and Cyrille Violle (2018) Ecological and biogeographical drivers of freshwater green algae biodiversity: from local communities to large-scale species pools of desmids; *Oecologia* 186 (4): 1017–30

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Authors' contributions:

Paper 1 HB, CV and FM designed the study, HB and PSv collected data, HB, CV and FM analysed data and wrote the manuscript, PŠ revised the manuscript

Paper 2 HB designed the study, collected data, carried out analysis and wrote the manuscript, PŠ revised the manuscript

Paper 3 HB, CV and TL conceived the idea, designed the study. KvS cultivated algal strains. HB carried out the experiment. HB and JS analysed the data and carried out the statistical analysis. HB and CV wrote the manuscript. TL, JS, PŠ, KvS revised the manuscript

On behalf of all the co-authors, I declare the keynote participation of Helena Bestová in completing research and writing papers, as described above

Pavel Škaloud

Abstract

For long, microorganisms have been thought to have unlimited dispersal and therefore to follow fundamental different assembly rules than macroorganisms. This premise is slowly changing, and ecological structure of microbial communities could gain insights from the theoretical and methodological developments applied on macroorganisms. While scarcely used, functional and phylogenetic metrics represent complement facets of diversity that should add valuable information to understand microorganisms' diversity structuration.

We used freshwater phyto-benthic algae desmids (Desmidiaceae, Streptophyta) as a model group. The main aim of this thesis was to identify the processes creating distinct patterns of desmid diversity using functional and phylogenetical information. We sampled over a hundred communities within Europe, used a well-resolved phylogeny, and size-related functional traits. Moreover, we experimentally measured the population growth rate of desmids, to determine the link between functional traits and organism performance.

Desmid communities showed non-random patterns of size and phylogenetic distributions on both large and local scales, advocating for a strong influence of niche-based processes. At large scale, desmids were distributed into environmentally and geographically separated species pools. At the local scale, phylogenetic diversity was linked with a gradient of pH and functional diversity with climatic variation. The population growth rates were strongly related to the size of desmids while no signal was found with other morphological traits.

We show that large scale diversity of European desmids is driven by niche conservatism, large scale environmental filtering, dispersal limitations and historical biogeography. Locally, functional and phylogenetic structuring in communities revealed the influence of environmental filtering and biotic interactions. Phylogeny and size-related traits apparently represented different ecological axes of desmids, as they responded to different environmental drivers. Size is strongly linked with organism performance and thus is a pivotal functional trait to infer desmids' community dynamics. The diversity of desmids communities is shaped by similar processes affecting macroorganismal communities.

Abstrakt

Dlouho se předpokládalo, že společenstva mikroorganismů jsou formována rozdílnými procesy než ta makroorganismální, především díky možnosti neomezeného šíření. Tento pohled se sice začíná měnit, ale k objasnění ekologické struktury mikrobiálních společenstev se nadále využívá převážně taxonomická diverzita a často se opomíjí teoretický a metodologický posun v ekologii společenstev makroorganismů. Například, funkční a fylogenetická diverzita odrážejí procesy formující společenstva lépe než pouhá druhová bohatost, přesto se jen zřídka používají v ekologii mikroorganismů.

V této práci jsme se zaměřili na sladkovodní zelené řasy – krásivky (Desmidiaceae, Streptophyta). Hlavním cílem bylo porozumět procesům utvářejícím diversitu společenstev krásivek za použití jejich fylogenetických a funkčních vztahů. Analyzovali jsme přes sto společenstev z Evropy, zkonstruovali fylogenezi skupiny a použili funkční vlastnosti spojené s velikostí a tvarem buněk. Dále jsme experimentálně testovali vztah mezi funkčními vlastnostmi a populační růstovou rychlostí krásivek.

Společenstva krásivek byla funkčně i fylogeneticky nenáhodně strukturovaná, a to jak v regionálním, tak i lokálním měřítku, tudíž pod dominantním vlivem nikových procesů. V regionálním měřítku, byly metaspolečenstva rozdělená geograficky i environmentálně s rozdílnými funkčními vlastnostmi. Takovéto rozdělení svědčí o limitovaném šíření, vlivu regionální environmentální variability a historických faktorů. Na lokálním měřítku jsou společenstva ovlivněna kompeticí a environmentálním filtrováním, které se odrážejí v nenáhodném fylogenetickém a funkčním složení společenstev. Fylogenetická diverzita se měnila s gradientem pH a funkční diverzita s klimatickým gradientem. Tento rozdíl v dominantním faktoru prostředí svědčí o tom, že funkční a fylogenetická diverzita reprezentují rozdílnou ekologickou niku krásivek. Experimenty ukázaly, že velikost je hlavní vlastností definující populační růstové rychlosti, je tudíž zásadní funkční vlastností pro predikci dynamiky krásivkových společenstev. Celkově jsme ukázali, že diverzita evropských krásivek je strukturována interakcí evolučních a ekologických procesů i historickou biogeografií a pro objasnění jejich dynamiky lze využít jednoduché funkční vlastnosti a evoluční vztahy mezi druhy ve společenstvech.

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1. General introduction



Community assembly of protists

While much attention has been devoted to unravelling the mechanisms beyond the community assembly of macro-organisms (Keddy 1992; Götzenberger et al. 2012; Lortie et al. 2004), the understanding of microbial community assembly is still in its infancy. Biodiversity organization is the outcome of many processes acting at different spatial scales (Ricklefs 1987). Each level of organization, from global to local diversity, is affected by both ecological and evolutionary processes (Hanson et al. 2012) which can be summed up into four categories: dispersal, selection, drift and speciation (Figure 1.1) (Vellend 2010). Although the relative importance of each of these four processes has been acknowledged for several decades in macro-organisms (Hubbell 2001; Ricklefs 1987; MacArthur and Wilson 1967), microbial community was for long considered as being fundamentally different.

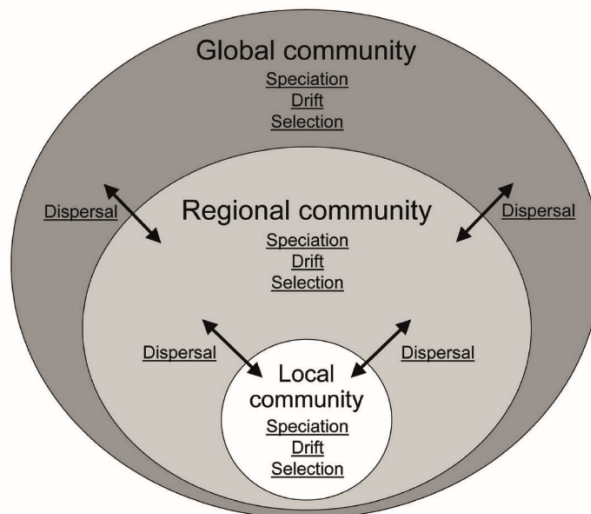


Figure 1.1 Schematic representation of multiscale influence of community assembly processes. From Vellend (2010)

The famous quotation of Baas Becking (1934), “Everything is everywhere, but the environment selects”, illustrates the fact that the influence of **dispersal** in microorganisms community assembly should be distinct from its importance in other biological groups. Indeed, their miniscule size makes them extremely sensitive to highly dispersive forces such as wind (Wilkinson 2001). Moreover, small sizes were early

associated with huge population densities (Fenchel and Finlay 2004). Both of these consequences based on tiny size should enable microorganisms to disperse through space in an unlimited manner. The arrival to local communities should then not be limited by dispersal, also, microbes should not have distinct biogeographical patterns and have only weak spatial structuring (Van der Gucht et al. 2007). However, this assertion has been challenged (Martiny et al. 2006). In this line, many microbes have been shown to have distinct biogeographical (Telford, Vandvik, and Birks 2006; Vyverman et al. 2007) and spatial diversity patterns (Green et al. 2004; Heino et al. 2010). Additionally, air dispersal is strongly linked to size and never unlimited. Computer simulation of atmospheric circulation have shown that the smallest particles never dispersed across hemispheres, whilst larger particles (over 40 μm) have very limited long-distance dispersal (Wilkinson et al. 2012). These findings challenge the idea of unlimited dispersal even for great disperser groups such as soil bacteria. Larger organisms in restricted and patchy habitats such as freshwater protists should consequently be even more dispersal-limited. Moreover, a lot of freshwater protists cannot form resting stages able to survive desiccation. Therefore, getting a long distance hitch on macroorganisms (Nemergut et al. 2013) would be limited by desiccation. Recent evidence shows that microbial communities are often dispersal limited and previously observed weaker spatial patterns could be the result of sampling artifacts (Meyer et al. 2018).

Selection acts via fitness differences between taxa and is analogous to niche-based processes (Kraft, Valencia, and Ackerly 2008) or species sorting (Leibold et al. 2004). It results from environmental filtering and biotic interactions (Hanson et al. 2012). The selection was shown to be the dominant driver for most of the passive dispersing organisms, including plants and freshwater plankton (Cottenie 2005). The environmental selection has been long acknowledged in microbial communities, as highlighted in Baas Becking's quote (1934). A large number of studies illustrated environmentally related diversity patterns for various microbes (e.g. (Fierer, Bradford, and Jackson 2007; Soininen and Weckström 2009; Mataloni 1999; Singer et al. 2018)). However, the role of biotic interactions in microbial communities is still being overlooked (Nemergut et al. 2013). In spite of the fact that early models of competition were based and tested on microbes (Gause, Nastukova, and Alpatov 1934; Tilman 1977). Evidencing the biotic interaction in microbial communities is difficult (Nemergut et al. 2013) and most of the research in this

area remains experimental (Violle et al. 2011; Violle, Pu, and Jiang 2010; Müller, Hauzy, and Hulot 2012). However, some studies have related microbial diversity patterns to apparent competition (e.g. Horner-Devine and Bohannan 2006; Klais et al. 2017).

Drift corresponds to neutral, or stochastic, dynamics generated by the frequencies of species at a regional scale and an associated low dispersal rate (Hubbell 2001). In this theory, species are thought to be functionally equivalent and their local abundances are the result of large-scale demographic fluctuations (Hubbell 2001). The influence of drift is supposed to be more important when abundances are low (Hubbell 2001). Therefore, if effective sizes of microbial populations are large (Finlay 2002), drift dynamics should be less important for microorganisms. However, most of the microorganisms are locally found in low abundances (Nemergut et al. 2013). Indeed, several microbial studies observed diversity patterns that could be attributed to drift (Kruk et al. 2016; Segura et al. 2010; Ren et al. 2015; Soininen et al. 2007).

Diversification generates new genetic variation potentially leading to speciation (Nemergut et al. 2013). Initially it was expected that efficient dispersal of microorganisms would produce unlimited gene flows, and together with large population numbers would lead to low speciation rates (Fenchel and Finlay 2004). Moreover, the predominance of asexual reproduction, dormant cells in “microbial seed bank”, and common horizontal gene transfer are expected to change the dynamics of diversification, either as homogenizing or diversification factors, or separation from the recent environment (Nemergut et al. 2013). However, populations of microbes were shown to be differentiated even on relatively small distances (Rengefors, Logares, and Laybourn-Parry 2012) and several studies brought evidence for very recent ecological speciation (Škaloud et al., n.d.; Logares et al. 2007). Therefore diversification among microbes can be more similar to macroorganism than previously expected (Škaloud et al., n.d.).

These four major assembly processes act on various temporal scales with current diversity patterns affected by the historical legacies of past colonization, speciation, and extinction events (Ricklefs 2007). Imprints of historical processes on communities of microorganisms are usually considered only in form of priority effects – first comes dominates (Fukami 2015; Urban and De Meester 2009; Pu and Jiang 2015). Historical biogeography is rarely investigated whilst biogeographical barriers are known to

influence diversity patterns (Martiny et al. 2006). The unlimited dispersal paradigm is slowly changing with microbial and protists communities being more and more believed to follow similar community assembly rules as macroorganisms (Horner-Devine et al. 2015). The pitfall of driving a general conclusion about microbial community assembly is that microbes consist of very distinct organisms, defined only by their small size (Caron et al. 2009). Microorganisms are a phylogenetically diverse group (bacteria, fungi, and protists from various supergroups of the tree of life), with all imaginable life forms, strategies and occupying a vast range of environments. Within microbes, most of the research has been focused on bacteria, whereby protists (eukaryotic microbes) have been frequently overlooked (Caron et al. 2009). Regarding photoautotrophic microbes, it is phytoplankton which has captured all the attention. Much work remains to be done in the face of large morphological, ecological, phylogenetical and functional variability of protists. In my thesis, I focus on the phylogenetical and functional diversity of an underrepresented group of phototrophic freshwater benthic protists.

Beyond taxonomic identities: phylogenetic and functional community ecology

Taxonomic diversity treats species as independent units, neglecting the fact that they share a common evolutionary history and functional characteristics (Cadotte, Cardinale, and Oakley 2008). Therefore, phylogenetic and functional aspects represent complementary facets of biodiversity which are informative about community assembly processes (Webb et al. 2002; Kraft and Ackerly 2010). Despite this additional component of information, they both have been overlooked in ecology of protists.

Phylogeny reflects the shared ancestry of species. Large scale phylogenetic patterns are influenced by evolutionary history made up of speciation and radiation events and also the results of historical boundaries (Pavoine and Bonsall 2011). Moreover, phylogenetic information has been advocated to be a good proxy for functional similarities of species (Cadotte et al. 2009; Srivastava et al. 2012), since phylogenetically close species have been diverging more recently and share a longer common history. At a local scale, non-random community phylogenetic patterns can result from selection processes (Webb et al. 2002) (Figure 1.2). Co-occurrence of closely related species,

phylogenetic underdispersion, in comparison with the phylogenetic structure at a regional scale, indicates the dominant influence of selection toward species sharing favorable characteristics. Environmental filtering that selects species with physiological tolerance for a given environment can lead to such patterns (Webb et al. 2002). The opposite pattern, co-occurrence of distant relatives, phylogenetic overdispersion, results from a process that limits the degree of species similarity such as interspecific competition (Webb et al. 2002).

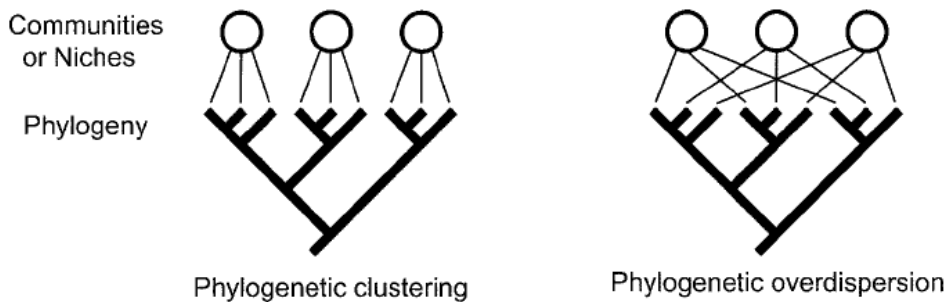


Figure 1.2 Phylogenetic structure of communities. On the left communities are phylogenetically underdispersed, composed of closely related species. On the right they are overdispersed, composed of species from different clades. From Cavender-Bares et al. (2004)

This “community phylogenetics” approach is based on the premise that important niches are phylogenetically conserved and competition is stronger between closely related, and therefore more similar taxa (Cahill et al. 2008; Violle et al. 2011). Nevertheless, community phylogenetics has been recently criticized because contrasting processes such as competition and environmental filtering can lead to similar phylogenetic patterns (Mayfield and Levine 2010). Moreover, phylogenetic niche conservatism may not be implicit (Losos 2008) with a limited amount of experimental evidence supporting the competitive relatedness hypothesis (Gerhold et al. 2015). However, we believe that non-random phylogenetic patterns in communities, and especially changes in those patterns along environmental gradients, can help to build the hypothesis about the origins of such distribution (Cadotte and Tucker 2017; Helmus et al. 2007; Graham and Fine 2008).

Functional diversity considers the characteristics that organisms possess, how they relate to the environmental fitting and how they affect their ecosystem (Westoby and Wright 2006). These characteristics are called functional traits when they constitute a

surrogate for organism performance (Violle et al. 2007; McGill et al. 2006). Traits reflect key properties, such as the ability to disperse, establish, survive and reproduce in a given environment (Violle et al. 2007). Therefore, at the species level, trait variations represent the ecological niche of the organism while functional patterns at the community level might mirror the processes structuring it (McGill et al. 2006). The selection at each spatial scale would influence trait distribution. At larger scales, macroecological filtering shapes covariation of traits with the environment (Violle et al. 2014) and functional variation within communities reflects niche-based assembly process (Kraft and Ackerly 2010). The environmental filtering is expected to reduce the range of viable functional strategies at the community scale, with trait values potentially influencing the performance of species and therefore the relative abundances of coexisting species (Figure 1.3) (Kraft and Ackerly 2010; Bernard-Verdier et al. 2012).

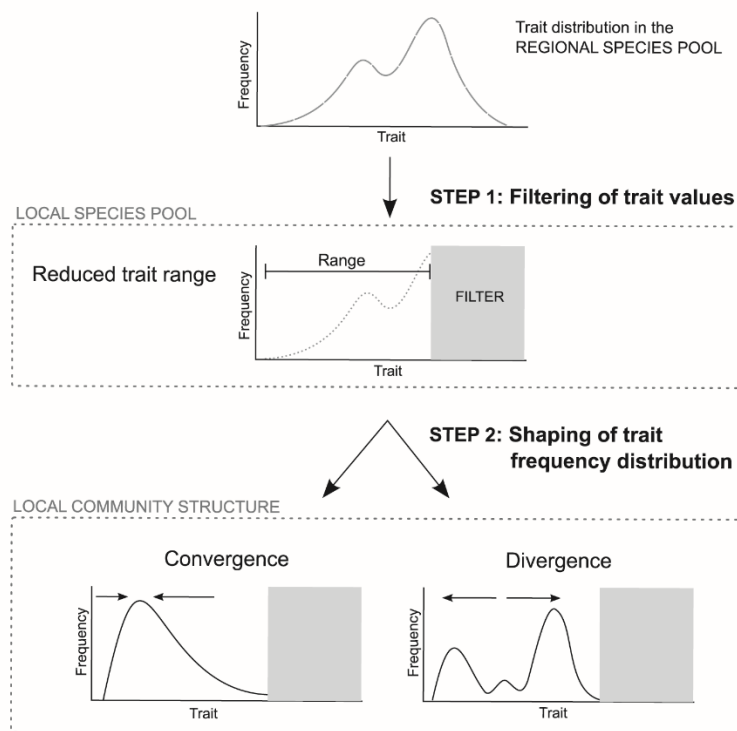


Figure 1.3 Schematic representation of trait-based community assembly. Environmental filtering reduces the range of trait values and subsequently trait determines success of species in community. This may lead to functional convergence, dominance of one preferential strategy or divergence, functional overdispersion, when several successful strategies coexist. Modified according Bernard-Verdier et al. (2012).

Moreover the community mean trait value should reflect a collective physiological response to abiotic drivers (Westoby and Wright 2006). Unlike phylogeny which should mirror the multidimensional niche (Mouquet et al. 2012), traits represent specific functions of the organism (Violle et al. 2007). Therefore, a distinct set of traits are related to contrasting functions and respond differently to the environment (Bernard-Verdier et al. 2012).

Traits

In recent years functional (trait-based) ecology is being increasingly used in studies of protists (e.g. Weisse 2017; Weisse et al. 2016; Tapolczai et al. 2016), notably with phytoplankton (Reynolds et al. 2002; Kruk et al. 2011; Kruk et al. 2016; Klais et al. 2017). Unfortunately, the selection of traits for microorganisms is not straightforward (Nemergut et al. 2013). Litchman and Klausmeier (2008) summarized the main axes of ecological variation in phytoplankton and the related traits (Figure 1.4).

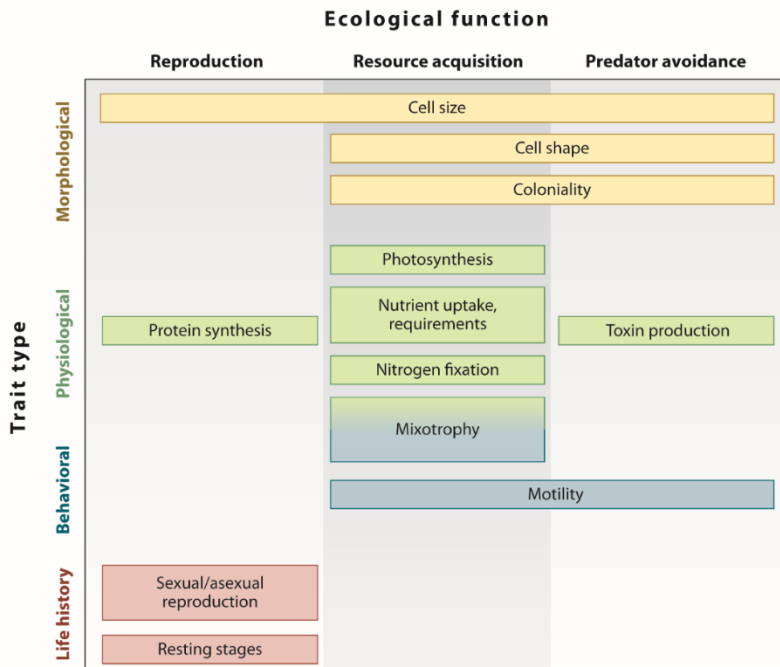


Figure 1.4 Summary of ecological functions of phytoplankton and related traits related to them. From Litchman and Klausmeier (2008)

This classification is focused on phytoplankton, but most of these traits are applicable to algae in general. However, a lot of these traits can only be determined through experimental measurements. This implies the necessity to collect species, cultivate them in controlled conditions and measure the variable of interests, which is impossible for a larger number of species. Having morphology-based traits covering up functional variations across species and simple to estimate would allow to bypass cultivation steps (Reynolds et al. 2002). For example, Kruk et al. (2010) developed morphological-based categories of phytoplankton corresponding to distinct functional guilds. These categories are based on the presence or absence of flagella, mucilage, aerotopes, siliceous structures, and size. However, this type of classification that reflects the ecological niches of phytoplankton (Kruk et al. 2010; Kruk et al. 2011) is not directly transferable to other groups of algae. For example, phytobenthic organisms fit into very few groups within this framework, and with this classification therefore fails to reflect a large spectrum of strategies and functions within phytobenthos. Additional more detailed morphological classifications that have been proposed (reviewed in Salmaso, Naselli-Flores, and Padisák 2015) are similarly barely applicable outside the context of phytoplankton. Moreover, such functional categories cannot be directly linked to an organism's performance. A big step forward in community ecology of protists would thus be the creation of trait database with standardized measurements, clear guidelines and identified links between traits and local performance. The first attempts of this kind with algae are still centered around phytoplankton (Edwards, Klausmeier, and Litchman 2015; Klais et al. 2017; Rimet and Druart 2018). Meanwhile, there is one easily obtainable trait that influences all the ecological functions of algae – size (Litchman and Klausmeier 2008).

Size

Size is sometimes called “master trait” (Passy 2007) as it associates with all the important ecological niches of unicellular organisms (Figure 1.4) (Litchman and Klausmeier 2008). Violle et al. (2007) classified traits into performance and functional traits. Performance traits directly influence the fitness of organism and functional traits have impacts on performance traits and hence through them on fitness. Size represents both a performance and functional trait. Indirect effects of size on fitness of unicellular

algae include nutrient uptake (Nielsen 2006; Passy 2007; Yoshiyama and Klausmeier 2008; Hein, Pedersen, and Sand-Jensen 1995), storage capacity (Verdy, Follows, and Flierl 2009), minimal nutrient quotas (Marañón et al. 2013), and light acquisition (Mei, Finkel, and Irwin 2009; Wirtz 2011). Moreover, size directly influences organismal performance and fitness. It was shown to be connected to predation rates (Thingstad et al. 2005) and mortality linked with sinking in phytoplankton (Padisák, Soróczki-Pintér, and Rezner 2003). Last but not least, size is related to metabolic and growth rates (Brown et al. 2004).

Indeed size, alongside with temperature, has been shown to be the main determinant of metabolism and of all the reliant processes including population growth rate (μ_{max}) (Brown et al. 2004). Metabolic rate and other biological processes were shown to vary predictably with size. This relationship can be summarized by the allometric equation:

$$\text{Eqn. 1} \quad Y = Y_0 M^b$$

where Y is the characteristic of interest, for example, μ_{max} , M organism's body mass, Y_0 a normalization constant and b the scaling exponent. According to the Metabolic Scaling Theory (MST), the scaling exponent b is predicted to be proportional to $1/4$ across all organisms, the so-called "quarter-power law" (West, Brown, and Enquist 1997; West, Enquist, and Brown 1999; Savage, Deeds, and Fontana 2008). However, it is not clear whether quarter-power law is valid for protists. On one hand, it was shown that the scaling of protists' μ_{max} takes the value of $-1/4$ (Fenchel 1974). On the other hand, the universality of quarter-power law has been challenged (DeLong et al. 2010; Marañón et al. 2013). DeLong et al. (2010) argued that scaling changes along evolutionary transitions prokaryote-protist-metazoans, because of changing biophysical constraints underlying it. The main constraint of metabolism in bacteria should be the genome size, in protist external nutrient uptake and internal nutrient transport, and in metazoans resource distribution. Therefore, MST is potentially valid only for multicellular organisms, with bacteria and protists having different scaling exponents (DeLong et al. 2010). Moreover, biophysical constraints limiting unicellulars intensify with increasing size (DeLong et al. 2010; Marañón 2014), which should result in reduced metabolic efficiency at the upper limit of protist size spectra (DeLong et al. 2010). Additionally, Marañón et al. (2013) experimentally showed unimodal size- μ_{max} relationship for phytoplankton that was

directly related to cell size, not to prokaryote-protist transition. For pico- and nano-plankton μ_{max} increased with size, reaching the maximum at intermediate sizes and decreased for large phytoplankton (Marañón et al. 2013). Organisms with a size smaller than 6 μm were shown to deviate because of an unbalance between nutrient uptake and requirement (Marañón et al. 2013). However, the situation on the other end of the protist size spectra is unclear. A predictable relationship between processes and size would make the size-distribution the most informative characteristics of a community (Kerkhoff and Enquist 2006). This knowledge gap is limiting the ability to scale organismal processes to ecosystem levels (Enquist, Michaletz, and Kerkhoff 2016) and prediction of ecosystem functions (e.g. primary productivity, nutrient cycling) from known size-structure of the community (Kerkhoff and Enquist 2006; Enquist et al. 2015).

Shape

The shape is another important characteristic of organisms (Figure 1.4) (Litchman and Klausmeier 2008). In multicellulars, the role of morphological optimization on biological scaling is largely acknowledged (G. B. West, Enquist, and Brown 1999). In protists, the shape is commonly related to variation in the surface-to-volume ratio (Niklas 2000; Okie 2013), predator avoidance and sinking prevention (Litchman and Klausmeier 2008; Padiśák, Soróczki-Pintér, and Reznér 2003). For example, phytoplankton was shown to have distinct size-related shape distribution, which apparently modifies surface-to-volume scaling (Niklas 1994b). Such changes in the external surface area must have an influence on biophysical constraints which limit nutrient uptake and consequently influence the pace of metabolism. However, the role of morphology on the scaling of processes in protists has been neglected. Protists might optimize phenotype to maximize metabolism similar to multicellular organisms. Therefore, the shape can be a very important trait, possibly directly linked to metabolism and growth rate.

Desmids

In my thesis, I focused on a group of freshwater phyto-benthic green algae desmids (Streptophyta, Desmidiatales). Desmids are mostly unicellular with few colonial species. Desmids have striking morphological variability with a rigid ornamented cellulose cell wall and a high degree of symmetry (Coesel and Meesters 2007a). Their morphology is reflected in their Czech name “krásivky” – the beautiful ones (Figure 1.5). Desmids are a monophyletic group within the class Zygnematophyceae, which is a sister group to land plants (Ruhfel et al. 2014). They are dominantly found in oligo- to mesotrophic wetlands such a peat bogs and lake littorals (Brook 1981). The majority of desmids are benthic with few planktonic or tychoplanktonic species (Brook 1981). Desmids were one of the first algal groups with described biogeographical distribution (West 1909). Within Europe, they display pronounced arcto-atlantic vs continental aspect with higher species richness and endemism on the Atlantic coast (Coesel and Krienitz 2008). Desmid diversity is linked with gradients of pH (Neustupa, Černá, and Šťastný 2009; Coesel 2001; Neustupa, Veselá, and Šťastný 2013) and conductivity (Mataloni 1999). However, it is still a puzzle to identify what are the factors determining those diversity patterns.



Figure 1.5 Microphotograph of desmid community, photo by Jan Šťastný

Research objectives and methods

I refer to the following Chapters 2-4 as Ch2-4.

The main objective of this thesis was to provide a better understanding of community assembly of freshwater algae. I aimed at linking diversity patterns with multiscale processes structuring them using phylogenetic and functional characteristics of species. In addition, I aimed at linking functional traits with organism performance. I chose desmids as a model group for algal community assembly for several reasons i) Phytobenthic communities are understudied compared to phytoplankton. Therefore, processes behind their diversity patterns are largely unexplained. ii) Desmids live in patchy habitats of wetlands with distinct spatial structure. iii) They are known to have relatively temporally stable communities without important yearly fluctuations (Svoboda, Kulichová, and Šťastný 2013). iv) Desmids have reasonably good species-concept based on morphological identification, which is rare among protists. Several recent phylogenies of desmids were published along with many sequences in the GeneBank database (e.g. Gontcharov 2008; Gontcharov, Marin, and Melkonian 2003; Gontcharov and Melkonian 2011). v) Desmids have a large span of sizes and their rigid cell wall limits size-environment accommodation. vi) Easy cultivation of desmids enables to carry out experiments readily. Indeed, several studies have tried to move beyond taxonomical diversity in desmids describing phylogenetic patterns of species:genus ratio (Coesel 1982) and also size distribution and its relation to the environment (Neustupa, Veselá, and Šťastný 2013). However, the understanding of ecological and evolutionary processes behind those patterns is lacking.

Specifically, I sought to answer the following questions:

What are the large-scale drivers of the diversity of desmids? (Ch2 and 3) Can we find imprints of dispersal limitations and historical legacies? What are the most influential macroecological drivers?

What are local-scale drivers of the diversity of desmids? (Ch2 and 3) Do local scale sorting processes such as environmental filtering and biotic interaction result in the non-random functional and phylogenetic composition of communities?

What is the role of phylogenetic and functional characteristics of species? (Ch 2, 3, and 4) Does phylogeny reflect an ecological niche of desmids? What is the relationship between size, shape, and performance of desmids?

To answer the above-outlined questions, I combined observational with experimental work. I collected over 100 communities of desmids from several regions in Europe and diverse environments spanning a large gradient of pH and conductivities. This sampling was the basis for analysis of community structure with a focus on functional biogeography (Ch2) and community phylogenetics (Ch3). I used metacommunity framework analysis to define large-scale species pools of desmids and to determine the origin of those pools (Ch2). I used size (biovolume, surface area, and surface-to-volume ratio) as traits. I constructed a phylogeny based on chloroplast-encoded *rbcL* gene, as well as using sequences available in GeneBank database, supplemented with my own sequences from cultivated strains. I investigated patterns of phylogenetic β -diversity to identify evolutionary processes structuring those communities (Ch3) and large trait distribution to identify macroecological processes. I used null models to search for non-random patterns in the functional and phylogenetic compositions to disentangle the processes acting on local communities (Ch2 and 3). Further, I carried out an experiment with genus *Micrasterias* to quantify size dependence of population growth rate and investigate the role of morphology (Ch4). In this experiment, we directly measured population growth rate and dry mass of 24 species of *Micrasterias* and estimated their size and morphology-related traits.

Key results and conclusions

Results of presented studies show non-random patterns in the functional and phylogenetic distribution of desmids on both regional and local scales. We argue that assembly mechanisms of desmids are not different from macroorganisms including historical legacies, dispersal limitation, environmental filtering, and competition.

Large scale diversity patterns

We showed that selection and dispersal influence large scale diversity patterns in desmid communities. We found geographical and environmental separation of regional species pools which were also reflected in the distinct distribution of traits (Ch2). Geographical structuring of regional pools and spatial taxonomical turnover within them supported the dispersal limitations of desmids communities (Ch2 and 3). However, the percentage of total taxonomical turnover explained purely by distance was small (Ch3), stressing the importance of large-scale environmental filtering. Moreover, we did not find spatial structuring of phylogenetic β -diversity. This is congruent with the lack of allopatric speciation and single evolutionary origin of desmid diversity within Europe. Therefore, the higher species richness of communities on Atlantic sites, without higher phylogenetic diversity, may not be caused by speciation. We also did not find effects of higher habitat availability on this richness pattern. The geographic arrangement of species pools evoked the hypothesis that this distribution could be an imprint of glacial refugia, whereby refugia of plants are linked with higher contemporary species richness (Médail and Diadema 2009). Actually, such refugia on the Atlantic coast have been shown for macroalgae (Provan, Wattier, and Maggs 2005) and mosses (Szövényi et al. 2007). Historical legacies are rarely considered in freshwater algae or protists in general (but see e.g. Pu and Jiang (2015)), even if there is no doubt that current diversity patterns are the outcome of recent and past processes (Fukami 2015; Ricklefs 2007). Environmental gradients determined the taxonomical, functional, and phylogenetic turnovers in desmid communities. However, the most important macroecological driver was climate. Climate together with pH separated different species pools (Ch2). Climatic gradient, from milder oceanic to continental climate, was also linked with changes in mean community volume and surface-to-volume ratios, with cells being larger in a continental climate.

Phylogenetic turnover was also associated with climate and pH (Ch3), testifying for niche conservatism in desmids where lineages retain environmental preferences (Jin, Cadotte, and Fortin 2015).

Local diversity patterns

Selection processes namely environmental filtering and competition are driving forces for local diversity patterns. We found imprints of those processes on both the functional and phylogenetic compositions of communities (Ch2 and 3). However, dominant environmental drivers differed for each biodiversity facet. The functional composition was linked with the gradient of climate (Ch2). In a continental climate, the size range was reduced toward larger species, which can be better for survival (Reynolds 1987). In contrast, a milder oceanic climate, with diminished winter freezing stress promoted functional convergence towards more competitive phenotypes (Mayfield and Levine 2010), with smaller species and higher populations growth rates (Ch4). Contrastingly to climate-associated functional variation, local phylogenetic structure covaried with pH (Ch3). Communities were composed of closely related species in highly acidic sites. In less acidic habitats with $\text{pH} > 5$, distantly related species were co-occurring. Moreover, we found a phylogenetic signal of pH preferences, where closely related species had similar pH optima. We concluded that the dominant assembly process changed along the gradient of pH. Low pH represented a strong environmental filter, selecting for closely related species sharing some characteristics favoring them in these harsh conditions (Webb et al. 2002). When the influence of this filter was relaxed, communities were composed of distantly related species, most likely because of competitive exclusion limited the degree of relatedness (functional similarity) of species (Kembel 2009). The discrepancy between environmental drivers of local functional and phylogenetic diversity highlights the complexity of processes structuring desmid communities. Even though there is phylogenetic signal in sizes (volume: Pagel's $\lambda = 0.89$ P -value = 0.001, surface area: $\lambda = 0.90$ P -value = 0.001, surface-to-volume: $\lambda = 0.88$ P -value = 0.001), phylogeny apparently reflects a different niche axis of desmids. Therefore, it responded to different environmental gradients (Violle et al. 2007). This underlines the importance of selecting meaningful traits for answering the question of interest. It would be difficult to pinpoint

a specific trait linked to low pH resistance, but we showed that phylogenetical relatedness between species is a good representation of their similarities in response to pH.

Size and shape

We show that the size scaling of population growth rate follows predictions made by the Metabolic Scaling Theory ($^{-1/4}$) (Brown et al. 2004) (Ch4). We support the universality of quarter-power scaling, even for large protists since we did not find a reduction of metabolic efficiency. Marañón et al. (2013) showed that for pico and nano-plankton growth rate increases with size. However, desmids have very few species smaller than 10 μm . Based on our experiment we show that μ_{max} , and therefore fitness of desmids, can be predictably estimated from size following MST. We also showed that μ_{max} was independent of morphology. Size-related shape change elevated surface area scaling (Niklas 2000; Okie 2013). Along with size-dependent density decrease, it led to an unexpected linear increase of surface area with body mass. We hypothesized that even though allometric optimizations do not directly influence growth rate, they are crucial for overcoming external constraints imposed on large cells. Linear surface area increase provides adequate nutrient and CO_2 uptake whilst enabling to reach optimal quarter-power scaling. However, size, was the only predictor of organismal performance (population growth rate). This supports that size is the most influential trait (Enquist et al. 2015).

Results presented in this thesis bring new insights into community assembly of desmids and the role of their functional characteristics. I have showed that phylogeny and size represent a meaningful niche axis of unicellular green algae, which can be used to drive some general conclusions about processes driving their diversity on large and local scales. Moreover, I showed that size is a solid performance trait, which can be used to predict population growth rate and therefore link size distribution of communities to their functioning. The conclusions and outcomes from this thesis have the potential to serve for future development of predictive modelling of phytobenthos functioning.



2. Ecological and biogeographical drivers of freshwater green algae biodiversity: from local communities to large-scale species pools of desmids

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ABSTRACT

Dispersal limitation, niche-based processes as well as historical legacies shape microbial biodiversity, but their respective influences remain unknown for many groups of microbes. We analysed metacommunity structure and functional trait variation in 148 communities of desmids, freshwater green algae, distributed throughout Europe. We delineated biogeographic modules for both taxa and sites using bipartite network analysis given that the taxa of a module co-occurred more often than expected by chance in sites of the same module. The network analysis distinguished two main acidic and neutral habitats, reflecting environmental filtering, and within each habitat separated species pools with distinct geographic locations, representing a plausible influence of historical biogeography. The geographic differentiation was consistent with a hypothesis of glacial refugia on Atlantic coast. Distance decay in community composition in addition to environmental influence further suggested a role of dispersal limitation. Next, we quantified the variation in cell volume and surface-to-volume of taxa within and among communities, to examine morphological and physiological adaptations of desmids in varying environments. Communities from continental climate contained larger desmids. Conversely, we found a functional convergence of smaller, fast-growing, desmids in oceanic regions. Overall, our findings suggest that niche-based processes, dispersal limitation, and historical legacy together drive the distribution and structure of desmid communities. Combining trait- and network-based analyses can resolve long-lasting questions in microbial ecology and biogeography and could be successfully used in macrobial ecology too.

Key words: Community assembly, functional trait, bipartite network, biogeography, green algae

INTRODUCTION

What drives the local and regional biodiversity of microorganisms is a much-debated issue. Do neutral or niche-based processes predominate (Nemergut et al. 2013)? What is the extent of dispersal limitation (Fenchel and Finlay 2004; Foissner 2006; Martiny et al. 2006; Cermeño and Falkowski 2009; Hanson et al. 2012)? Recent evidence supports the early hypothesis of Baas Becking (1934) that environmental filtering selects local assemblages depending on the niche of microbes (Stegen et al. 2012; Wang et al. 2013), but also tend to disprove its hypothesis of unlimited dispersal (Telford, Vandvik, and Birks 2006; Chytrý et al. 2012; Bates et al. 2013; Zinger, Boetius, and Ramette 2014). Conversely, several microbial communities show apparent neutrality (Segura et al. 2010) and lack of dispersal limitation. Therefore it is unclear whether general rules can be derived for microbial communities since microbes encompass various organisms and life strategies and occupy very diverse environments (e.g. marine plankton vs. freshwater algae in patchy habitats). Consequently, macroecology of many microbes remains poorly known, which prevents getting a comprehensive picture of their dynamics (Prosser et al. 2007). One of those scarcely explored microbial groups are desmids, Streptophyte green algae (but see Coesel 1996; Neustupa, Černá, and Šťastný 2009; Neustupa, Veselá, and Šťastný 2013). They mostly inhabit benthos of non-marine and patchy aquatic ecosystems. Here we investigated the role of ecological and biogeographical processes in shaping biodiversity of desmids across Europe.

The influence of niche-based processes can be grasped by analysing the diversity of key functional traits reflecting the ability of organisms to disperse, establish, survive and reproduce in their local environment (Violle et al. 2007). Addressing the nature and role of functional traits in microbial community dynamics have recently gained momentum (Fierer, Barberán, and Laughlin 2014; Krause et al. 2014; Nemergut, Shade, and Violle 2014), notably in phytoplankton systems (Reynolds et al. 2002; Litchman and Klausmeier 2008; Kruk et al. 2011). Desmids' cell sizes range from under 10µm up to 1mm (Brook 1981) and represent a potentially broad spectrum of ecological niches (Litchman and Klausmeier 2008), given that the size of photoautotrophs is tightly linked to ecophysiological abilities for nutrient and light acquisition (Passy 2007). Size appears to

be the key functional trait for unicellular organisms (Margalef 1978; Reynolds et al. 2002). Generally, small cell size is associated with higher population growth rate (Nielsen et al. 1996). High cell surface-to-volume ratio is expected to increase nutrient uptake in oligotrophic environments (Passy 2007), while a lower ratio limits osmotic stress in highly acidic environments (Černá and Neustupa 2009). Therefore, we hypothesized that variation in cell volume and surface-to-volume ratio within and among desmid communities should reflect the influence of niche-based assembly processes in varying environmental contexts (Cornwell, Schwilk, and Ackerly 2006; McGill et al. 2006; Escudero and Valladares 2016).

We addressed the role of assembly processes by deciphering their signature in functional trait distribution (Enquist et al. 2015) within desmid communities. First, the mean trait value of a community (Community-Weighted Mean or CWM) is expected to reflect a collective physiological response to abiotic drivers. Second, environmental constraints can limit the range of trait values that are viable in a community. Third, the relative performance and abundance of coexisting organisms can increase when their trait values are closer to some optimum (trait convergence) (Kraft and Ackerly 2010), which should entail smaller weighted variance of trait values in the community (Community-Weighted Variance or CWV). Such variation in performance and abundance can represent a better adaptation to the physical environment or greater competitive ability (Navas and Violle 2009). Conversely, trait divergence (larger CWV) is expected under the influence of limiting similarity and niche differentiation (Kraft, Valencia, and Ackerly 2008). At a large spatial scale, covariation in functional traits and environmental variables, such as climate, should reveal the imprint of macroecological filters (functional biogeography, Violle et al. 2014). Although analysing the distribution of trait values in communities reveals major drivers of their assembly, the approach is still in its infancy in microbial ecology (but see Courty et al. (2016) for fungi and Kruk et al. (2010) for phytoplankton). We applied the framework to address the imprint of niche-based processes on the functional diversity of desmid communities in Europe.

Apart from the influence of deterministic niche-based processes, historical events can determine species range limits and the structure of contemporary communities (Fukami 2015). Dispersal limitation can entail isolation-by-distance patterns of

biodiversity, where distant communities differ in composition despite similar abiotic conditions (Nekola and White 1999). Such limitation can constrain re-colonisation from former glacial refugia and affect taxonomic and functional diversity patterns at large spatial scale (Normand et al. 2011; Klüttsch, Manseau, and Wilson 2012; Ordonez and Svenning 2015). We used a network-based approach to investigate how patterns of desmid co-occurrences deviate from random at a large spatial scale (Carstensen and Olesen 2009; Carstensen, Dalsgaard, et al. 2013). Network-based analysis has been shown to be more successful in determining biogeographical regions than classically used hierarchical clustering (Bloomfield, Knerr, and Encinas-Viso 2017). This approach allows to classify both sites and taxa into groups - modules characterizing large-scale metacommunity structuring, such that the taxa of a module co-occur more often than expected by chance in the sites of the same module. "Excess" co-occurrence of taxa in modules can be the result of historical biogeography shaping geographically distinct species pools (Carstensen, Lessard, et al. 2013) or of environmental filtering of taxa with distinct ecological strategies and functional trait values in distinct environmental contexts (functional species pools, de Bello et al. 2012). We assessed the variation in functional traits of taxa and environmental conditions of sites across modules to determine the respective influences of environmental filtering and historical biogeography on large-scale patterns of desmid biodiversity. Therefore, we devised a multiscale approach to decipher (i) long-term and large-scale signatures of functional and historical biogeography in modules, and (ii) niche-based assembly dynamics shaping the trait composition of local communities.

Using a multi-scale (from local to pan-European scale) and multi-tool (trait-based community assembly analysis, network-based analysis) approach, we thus asked: Is there functional variation within and among desmid communities reflecting the role of niche-based assembly processes? At large spatial scale, are these niche-based dynamics enough to explain co-occurrence patterns? If not, what is the relative contribution of niche-based processes, dispersal limitation and historical contingencies to species pool structuring? We hypothesized that desmids with lower surface-to-volume should be better adapted to stressful environments, while smaller desmids should be more competitive, yielding variation in average values of these traits along environmental gradients. Greater abiotic

and biotic constraints should also entail a reduction of trait range and variance in communities. Furthermore, we expected distance decay of community similarity and a geographic structure of biogeographic modules reflecting limited dispersal abilities and historical legacies.

MATERIALS AND METHODS

Sampling of desmid communities

We sampled 148 desmid communities (Streptophyta, class Zygnematophyceae, order Desmidiales) distributed throughout Europe (latitudinal limits: 48.92 to 60.99 and longitudinal limits: -9.95 to 15.90, see Figure 2.1 and Table A3 in Online Resources). Desmid richness peaks in nutrient-poor and slightly acidic to neutral freshwater habitats; they are missing in limestone areas (Coesel and Meesters 2007b). Desmids are typical species of phytobenthos in peat bogs (Brook 1981). Hence, we focused our sampling on peat-forming wetlands and a wide range of climatic, pH and conductivity conditions. Moreover, we selected the main areas where desmid communities were described in Europe (basically on Atlantic side, subarctic region, central European lowland and mountain peat bogs). We sampled the upper layer of the epilimnion in 10 × 10 cm plots, immediately fixated the samples by formaldehyde, and subsequently examined under light microscope. We subsampled approximately 200 cells (from 100 to 328) per community, which we identified at the species level (374 species total). The resulting species × site matrix included the relative abundance of each species *i*, i.e., the number of cells of species *i* divided by the number of cells in the subsample (dataset is provided in Online Resources).

Environmental data

Conductivity and pH are known to be key abiotic drivers of desmid diversity (Coesel 1982; Neustupa, Černá, and Šťastný 2009). We measured both variables during sampling using a combined pH/conductivity meter WTW 340i (WTW, Germany) (for a table of environmental parameters see Table A3, Online Resources).

Large-scale bioclimatic variation was characterized using the WorldClim database (Hijmans et al. 2005) (2.5 arc-minutes resolution). We reduced the dimensionality of bioclimatic data by performing a principal component analysis (see Online Resources, Fig A1). The main gradient expressed by the first principal component (PC1) explained 61.6% of the bioclimatic variability, and was positively correlated with precipitation, and negatively with annual range and seasonality of temperature, reflecting a gradient from continental to oceanic climate. PC2 explained 20.9% of the variation and was positively correlated with annual mean temperature and isothermality. We used PC1 and PC2 site scores, pH and conductivity values to compute Euclidean environmental distances among communities.

Desmid trait measurements

We estimated two key morphological traits of desmids: biovolume (volume of the cell) and surface-to-volume ratio (S/V). We used existing data set created by (Neustupa, Černá, and Šťastný 2011) and followed their algorithm to complete information for all species. Desmids have elaborous cell shapes with incisions, lobes, and branchings, therefore it is necessary to adjust computed characteristics of general objects, which are commonly used in biovolume calculations of other photoautotrophs (Hillebrand et al. 1999). First, we calculated volume and surface of standard geometrical object characterizing species' general shape with dimensions obtained from the published literature (mean values for length, width, and thickness). Secondly, we measured frontal area and perimeter of species from microphotograph or where not available illustration from the published literature using the Fiji software (Schindelin et al. 2012). We used this projected area and perimeter to derive actual volume and surface of desmid cell following the protocol developed by Neustupa et al. (2011, 2013) (for details of computations see Online Resources). Desmids have few colonial, filament forming genera. In our samples, we identified 10 filamentous species. We estimated volume and surface for individual cells in filaments and calculated number of cells in filaments. Further in analyses, we treated them as individual cells. The traits were not directly measured in situ; therefore, they represent average volume and surface of the species and neglect intraspecific variability. Trait data were available for 352 species out of 374 and were missing only for some rare species (trait values available in Online Resources, Table A3).

Data analysis

Species turnover

We estimated the Sørensen index of taxonomic dissimilarity between communities (Sørensen 1948) (SorIndex hereafter). We analysed the changes in SorIndex with geographical and environmental distances using Mantel and partial Mantel tests (Legendre and Legendre 1998).

Metacommunity modules

A set of communities (a metacommunity) can be represented as a bipartite network (a.k.a. metacommunity network), in which species and sites are nodes, and the links are the occurrences of species in sites (Carstensen and Olesen 2009; Carstensen et al. 2012). Modularity analysis allows delineating species pools (or modules) based on the distribution of occurrences in this network (Carstensen, Dalsgaard, et al. 2013). The modules are sub-networks including (i) species that tend to co-occur more often, and (ii) related sites showing more similar composition. Each species and site are thus ascribed to a module so that the modules represent spatially delimited species pools (Carstensen et al. 2012). The modules are robust to sampling heterogeneity and richness variation because they are defined by reference to a null model conserving community richness and species frequencies.

We used the Louvain algorithm (Blondel et al. 2008) to perform a modularity analysis of the sampled desmids metacommunity, and only species with more than 2 occurrences were included. To assess the significance of the decomposition into modules, we calculated the null distribution of the Q statistics of modularity by permuting 999 times the species x site matrix using a swap algorithm (Gotelli and Entsminger 2001; Gotelli and Entsminger 2003). In addition, we calculated two parameters representing the way each node was connected to the modules: the coefficient of participation (c) represents the frequency of links of a node to other modules compared to its own module, while the within-module degree (z) represents the relative number of links of the node to other nodes from the same module (Guimerà and Amaral 2005; Carstensen et al. 2012). In other words, sites and species with low c and high z values were more "typical"

representatives of their module. Species with high c were ecologically more generalist and widespread, and sites with high c included a mix of taxa from different ecological and biogeographical contexts.

To check the robustness of the decomposition into modules, we also performed an alternative delineation based on ‘assemblage dispersion field’ (Lessard et al. 2012). This probabilistic approach considers that sites with more similar species composition are more likely to provide immigrants to a recipient community. The probability that a given site is included in a species pool is equal to the proportion of species it shares with the recipient community. For each community, we checked whether the source communities defined by dispersion field were more likely to be drawn from the same module as the recipient community. We used ‘assemblage dispersion field’ to define species pools in following analyses.

Trait-based community assembly

We calculated trait mean, variance and range values for every community. Traits were log-transformed before computing these metrics. Trait range (max - min) was calculated for each community, without considering species abundances. Community-level trait Mean (CWM) and Variance (CWV) were weighted by the relative abundance of species occurring in the community (Enquist et al. 2015) as:

$$\text{Eqn. 2} \quad \text{CWM} = \sum_{i=1}^S A_i \times t_i$$

$$\text{Eqn. 3} \quad \text{CWV} = \sum_{i=1}^S A_i \times (t_i - \text{CWM})^2$$

Where t_i is the log-transformed trait value and A_i the relative abundance of species i , and S is the number of species in a given community.

We performed Kruskal-Wallis tests to compare the trait range, CWM and CWV values of sites among modules.

To test whether the metrics of trait variation (range and CWV) in local desmid communities significantly deviated from random community assembly, we carried out two types of null models (Bernard-Verdier et al. 2012; Taudiere and Violle 2015) For each

community, the first null model (NM1) randomly assembled null communities from source communities defined by assemblage dispersion field, while keeping community richness unchanged (Lessard et al. 2012). Reduction in the trait range of local communities was expected under the influence of environmental filtering, compared to null values under NM1. The second null model (NM2) shuffled species abundances within communities. This model thus kept the range of trait values fixed, while changing the distribution of relative abundances in a community. Whenever some optimal trait values conferred greater performance and abundance (trait convergence), or greater abundance was only possible for more dissimilar trait values (limiting similarity yielding trait divergence), we expected a deviation of the community weighted variance (CWV) from null values under NM2 (Bernard-Verdier et al. 2012). Each null distribution was calculated based on 999 randomizations.

We calculated Standardized Effect Size (SES) (Gotelli and McCabe 2002) to quantify the deviation of an observed value from null values as:

$$\text{Eqn. 3} \quad \text{SES} = (I_{\text{observed}} - I_{\text{null}}) / SD_{\text{null}}$$

Where I_{observed} is the observed value of a trait-based statistic, I_{null} is the mean value of the statistic for null communities and SD_{null} is the corresponding standard deviation. Therefore, an observed value that did not differ from the null expectation had an SES value of 0. We performed a Wilcoxon signed ranked test to assess any deviation of SES values from zero (either positively or negatively).

Using the *plsreg1* function of the *plsdepot* R package (Sanchez 2012), we performed Partial Least Square Regression (PLS) of the trait-based community statistics (CWM, SES of range, and SES CWV) according to the environmental variables. PLS derives from predictors a set of orthogonal latent variables which maximize the explained variance in the dependent variable (Carrascal, Galván, and Gordo 2009). We used the full set of bioclimatic variables, conductivity, and pH as predictors. We standardized all predictors prior computation. We selected the number of latent variables for PLS based on cross-validation (Leave-one-out cross-validation). We chose the lowest number of latent variables with highest cumulative Q^2 (cross-validated R^2). For all models, this

corresponded to two latent variables. All the analyses were performed using R (ver. 3.0.3; R Development Core Team).

RESULTS

Species turnover along geographical and environmental gradients

Rarefied richness to minimum sample size ($N = 100$) ranged between 1.87 and 42.2. It was positively correlated with latitude (Spearman $\rho = 0.55$, $P < 0.001$) and negatively with longitude (Spearman $\rho = -0.56$, $P < 0.001$). We detected significant species turnover related to both environmental (Mantel $r = 0.20$, $P < 0.001$) and geographical (Mantel $r = 0.18$, $P < 0.001$) distances. Partial Mantel test showed a significant distance-decay of community similarity apart from the influence of environmental distances (partial Mantel $r = 0.19$; $P = 0.001$).

Metacommunity modules

The metacommunity network was significantly modular (Q statistics of modularity = 0.29, $P < 0.001$). The first five main modules each included more than 8 communities, summing to 103 communities. The other smaller modules, together 11, included too few communities and were excluded from the detailed description. Based on the c - z metrics, Table A1 in Online Resources shows the most typical sites and species of the modules and their basic ecological requirements. The first five main modules showed distinct geographical (Figure 2.1, Figure 2.2a), environmental (Figure 2.2b) and functional separation (Figure 2.2c), for the distribution of all 11 modules (see Online Resources, Fig. A2).

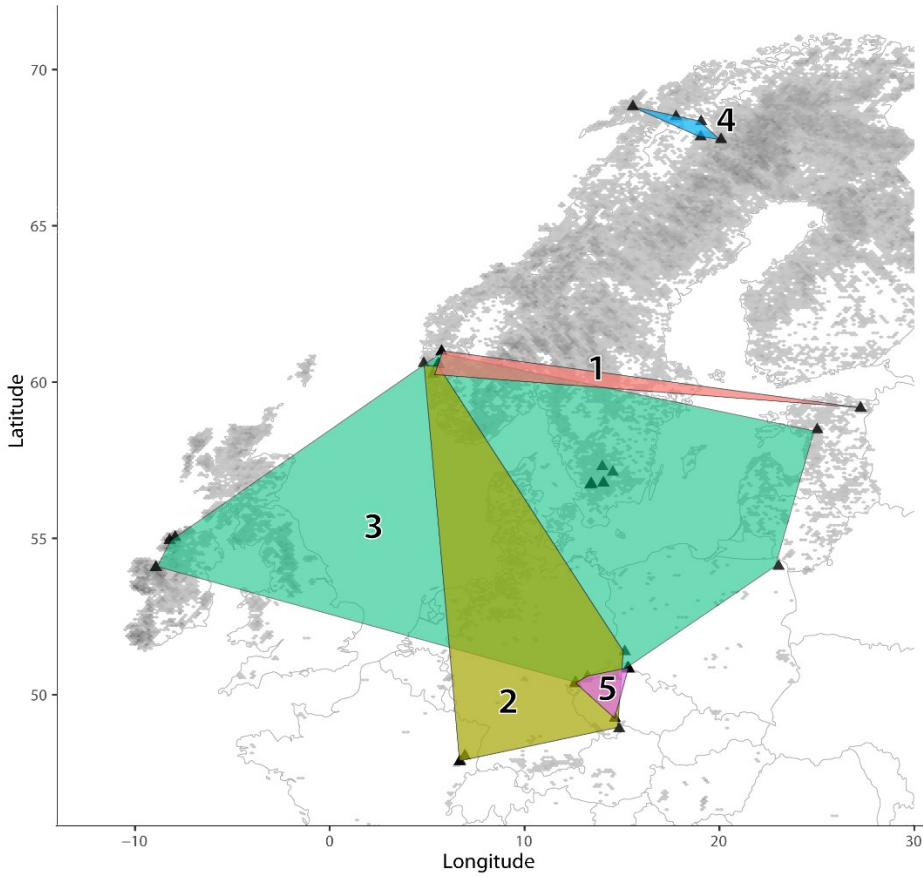


Figure 2.1 Geographical distribution of five main modules. Triangles are representing communities typical for modules, based on their c - z coefficients (c value below the third quartile and z value above the first quartile). Polygons represent minimal convex hulls enclosing modules

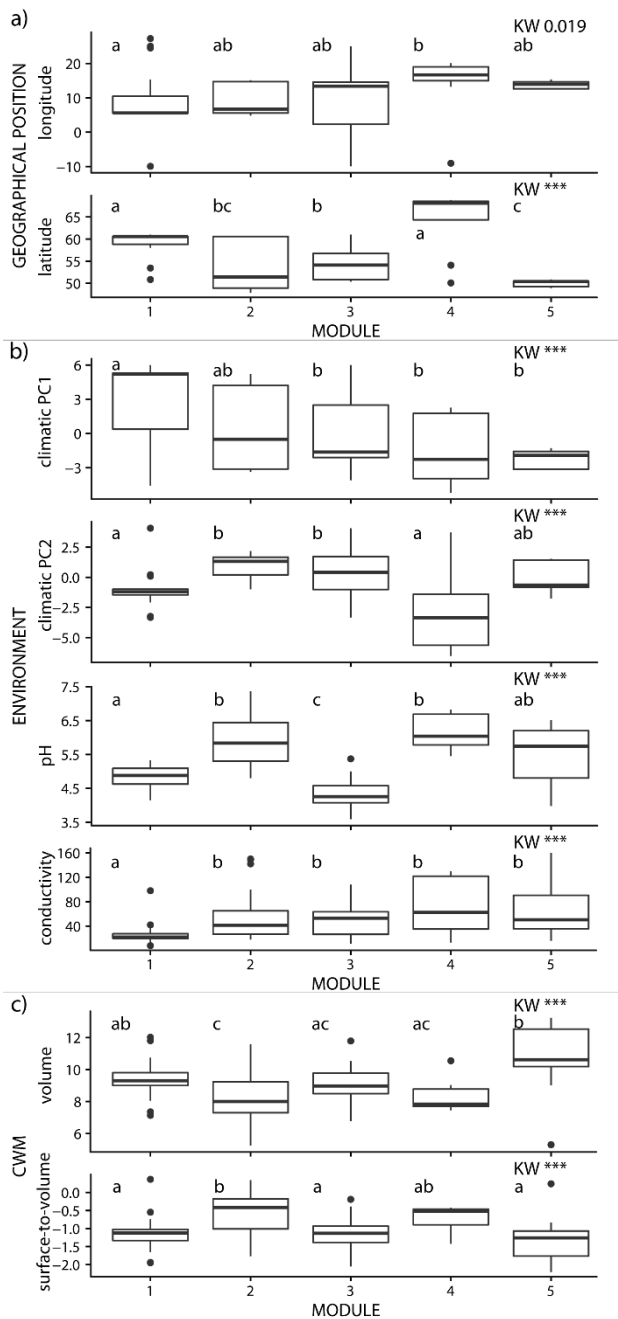


Figure 2.2 Variation among five main modules of a) the latitudinal and longitudinal position of the sites of the module; b) their local environmental characteristics and c) of community weighted means (CWM) for volume and surface to volume ratio. Values for traits were log-transformed prior analysis. Statistics for Kruskal-Wallis test (KW) and groups of pairwise comparison based on Tukey's test are indicated. $N = 103$

Modules 2 and 4 were similar in terms of local environment (higher pH and conductivity - Figure 2.2b, Tukey's test $P > 0.05$) (Tukey's test $P = 0.0015$), and functional composition (see Figure 2.2c, for the distribution of all 11 modules see Online Resources, Fig. A2). They displayed lower CWM volume (Tukey's test $P > 0.05$) and higher CWM

S/V (Tukey's test $P > 0.05$, Figure 2.2c), and the most typical species of these modules were described in the literature as indicators of the mesotrophic environment (Table A1; Online Resources). However, module 4 was mainly confined to extreme North, while module 2 was in mid-latitude (Figure 2.1 and Figure 2.2a; comparison of distribution Tukey's test: latitudinal $P = 0.015$; longitudinal $P = 0.068$). This was reflected also in their climatic differences: modules 2 and 4 were similar in climatic PC1 (Tukey's test $P = 0.6198$) but differed in climatic PC2 (Tukey's test $P = 0.0015$); module 4 had a lower annual mean temperature. Modules 1 and 3 were similar in terms of local environment and functional composition, with lower pH (Figure 2.2b), higher CWM volume and lower CWM S/V (Figure 2.2c) compared to modules 2 and 4. They included species associated with oligotrophic habitats (Table A1; Online Resources). While their spatial distribution broadly overlapped, module 3 showed broader longitudinal amplitude (Figure 2.1). Module 5 included communities with a broad range of pH values but with restricted geographical distribution to Central Europe and narrow climatic range. This module was also functionally distinct with large CWM of volume. See Online Resources Table A2 for complete results of pairwise comparisons between modules, and Fig A3, PCA analysis of environmental variables showing the environmental envelopes of modules.

We tested whether the most probable source communities defined in assembly dispersion fields (ADF) belonged or not to the same module as the recipient site. We found that the average probability of communities forming ADF pools was significantly higher for communities of the same module of the recipient community than for communities of other modules (Wilcoxon signed rank test: $P < 0.001$). Therefore, the delineation of network-based modules and of ADF pools proved consistent.

Independently of the environmental variation within modules, we found significant distance decay of species composition within each of the four first modules (modules 1-4 Mantel $P < 0.05$, module 5 $P > 0.05$).

Trait-based assembly processes

The Partial Least Square Regressions (PLS) allowed identifying two latent predictors underlying most of the variation in community-level trait metrics (Figure 2.3). The first latent variable was generally related to a climatic gradient from oceanic to continental

climate, correlated negatively with bioclimatic variables characterizing temperature; except mean annual temperature (bio2, bio5, bio8) and temperature seasonality (bio7, bio4) and positively with variables related to rainfall amounts (bio12, bio13, bio14, bio16, bio17, bio18, bio19). The second latent variable of PLS was conversely related to mean annual temperature and to precipitation seasonality.

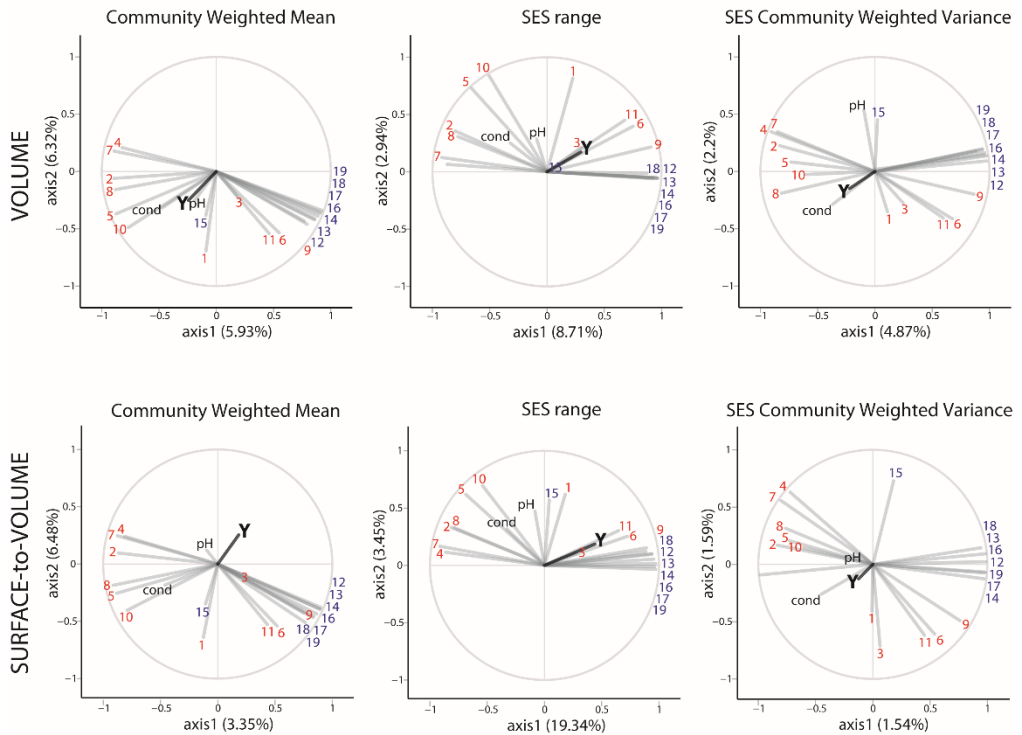


Figure 2.3 Correlation circles of Partial Least Square Regression analysis of community-level trait metrics. First two latent variables (axis) are shown. Axis are oriented that, the first one corresponds to the most dominant climatic gradient ranging from continental (high temperature seasonality (4) and temperature annual range (7)) to oceanic climate (high annual precipitation (12)). The second axis was correlated with mean annual temperature (1) and precipitation seasonality (15). Predicted variables are marked Y. $N = 148$. Codes for explanatory variables as follows: cond – conductivity, bioclimatic variables (from WorldClim database) are colour coded those linked with Precipitation (Precip.) are italicized and blue. Variables linked with Temperature (Temp.) are red: 1 Annual Mean Temperature, 2 Mean Diurnal Temp. Range, 3 Isothermality, 4 Temperature Seasonality, 5 Max. Temp. of the Warmest Month, 6 Min. Temp. of the Coldest Month, 7 Temperature Annual Range, 8 Mean Temp. of the Wettest Quarter, 9 Mean Temp. of the Driest Quarter, 10 Mean Temp. of the Warmest Quarter, 11 Mean Temp. of the Coldest Quarter, 12 Annual Precip., 13 Precip. of the Wettest Month, 14 Precip. of the Driest Month, 15 Precip. Seasonality, 16 Precip. of the Wettest Quarter, 17 Precip. of the Driest Quarter, 18 Precip. of the Warmest Quarter, 19 Precip. of the Coldest Quarter

CWM values of cell volume and surface-to-volume varied with climate and to some extent with conductivity. Larger species were found at higher conductivity and in oceanic climate sites with higher annual mean temperature (Figure 2.3). Conversely, surface-to-volume ratio did not vary along the continental-oceanic climatic gradient but was negatively correlated to the axis related to mean annual temperature. We did not find any influence of pH on CWM for the two studied traits. We generally detected a significantly smaller range of trait values in local communities, based on null model NM_1 (Wilcoxon test $P < 0.001$). While the standard effect size (SES) of range for surface-to-volume ratio significantly deviated from a random situation for only one community, up to 32% of the communities deviated from random for the SES of the range of cell volume (SES higher than 1.96 or lower than -1.96). The SES of trait range increased along the first axis of PLS from oceanic to continental climate (Figure 2.3, volume: 1. PLS axis $R^2 = 8.71$, 2. axis $R^2 = 2.94$ %, surface-to-volume: 1. PLS axis $R^2 = 19.34$, 2. axis $R^2 = 3.45$), although being steadily negative.

Based on null model NM_2 , CWV values were generally lower than expected by chance (Wilcoxon test towards lower values $P < 0.001$; for volume and surface-to-volume), while only four communities had significantly higher (SES > 1.96) variance of cell volume than expected by chance and only two for the surface-to-volume ratio. The SES of CWV also responded to environmental variation, namely, CWV for volume - more convergent communities (lower SES) occurred in oceanic climates (Figure 2.3). SES of CWV for surface-to-volume ratio was explained by PLS only marginally, two latent predictors explained only 3.13 % of the variance.

DISCUSSION

Disentangling the influence of community assembly dynamics at multiple spatial and temporal scales is a long-standing goal of macroecology and biogeography. The relative importance of biogeography, environmental filtering and dispersal limitation in microorganisms has been long debated (Baas Becking 1934; Finlay 2002; Green and Bohannan 2006; Martiny et al. 2006; Hanson et al. 2012). Here we used an original combination of network-based and trait-based approaches to tease apart the influences of niche-based assembly dynamics and historical biogeography on desmid diversity in Europe. We found that (i) morphological functional traits of desmids vary according to environmental conditions across communities at broad scale, (ii) species pools identified from the network-based analysis show specific environmental, functional and geographical structuring apart from the broad-scale trait-environment relationships, (iii) there is a distance decay of similarity in community composition apart from the influence of species pools and environmental variation, and (iv) the range and variance of trait values in communities reflect influences of environmental filtering and competition during community assembly. These results thus suggest the joint influence of environmental filtering, dispersal limitation and large-scale biogeographical history in the functional and taxonomic diversity of desmids.

Broad-scale trait-environment relationships

Under the original hypothesis proposed by Baas Becking (1934), weak dispersal limitation entails that "everything is everywhere, but, the environment selects". In this perspective, environmental gradients should determine species turnover and variation in functional community composition at a broad scale (Violle et al. 2014). We found changes in community-level mean values (CWM) of the desmids' volume and surface-to-volume depending on environmental conditions: cells were larger under a continental climate, as well as in habitats characterized by higher conductivity (Figure 2.3). Kruk et al. (2016) similarly showed that phytoplankton cell size decreases towards warmer tropical regions. However, in our study, the strength of these trait-environment relationships was quite weak (PLS volume: $r^2 = 0.122$ and surface-to-volume: $r^2 = 0.098$), indicating that other

biogeographical and ecological processes should determine the variation of community composition.

Biogeographical and environmental segregation of species pools

The analysis of modularity structure revealed strong geographical, environmental and functional structuring among groups (modules) of desmid communities. The five primary modules represented distinct environmental contexts and sets of functional traits, revealing functional species pools (de Bello et al. 2012), but with further spatial segregation of modules covering similar environments, revealing biogeographic species pools (Carstensen et al. 2013b).

The most important natural divisions in peat lands are reflected in pH (Wheeler and Proctor 2000), with a significant contribution of conductivity (Hájek et al. 2006). It is in accordance with the distribution of modules, which largely differed in pH values and to some extent conductivity. Patterns of desmid taxonomic diversity and co-occurrence in modules thus reflected environmental filtering. This filtering was further demonstrated in trait distribution within modules. Specifically, we found communities with higher CWM of volume and small CWM of surface-to-volume in modules characterized by low pH. Previous research already showed that biovolume of desmid communities in Central Europe responded to pH (Neustupa, Veselá, and Šťastný 2013). In acidic conditions, maintaining neutral cytosolic pH is highly demanding (Gerloff-Elias, Spijkerman, and Pröschold 2005). Consistent with physiological expectations, desmids show lower surface-to-volume in more acidic conditions, which can limit the influx of H⁺ ions (Černá and Neustupa 2009). Therefore, low pH condition may select for species with low surface-to-volume (Černá and Neustupa 2009).

In addition, geographical segregation of modules 1/3 and 2/4 in similar environmental contexts is consistent with an influence of historical biogeography. Geographical separation of modules can be related to previously described biogeographic groups of desmids (atlantic-subarctic, circumpolar, and continental) (Heimans 1969; Coesel 1996; Coesel and Krienitz 2008). Our results support segregation of regional species pools within Europe apart from environmental differences, which could have emerged as a result of historical legacies. They could be caused by Pleistocene glaciation

and lack of suitable conditions for desmids at glacial times over most of Europe. Glacial refugia may have existed on the Atlantic side of Europe, where we found the higher overall richness of desmid communities. The presence of relict habitats and refugia in Western Europe would have allowed subsequent recolonization from West. The presence of refugia has already been suggested in south-western Ireland for desmids (Jurdíková et al. 2014) as well as for land plants (Sinclair, Morman, and Ennos 1998) and macroalgae (Provan, Wattier, and Maggs 2005). Desmids are closely associated with *Sphagnum* habitats (Brook 1981) for which (Szövényi et al. 2007) evidenced refugia along the Atlantic coast of Europe. Current high-latitude distribution of module 4 could also be considered a relict of former widespread distribution of arctic-alpine taxa. Moreover, the module 5 geographically restricted within a wide range of environmental condition supports the hypothesis about different origins of desmid species pools. The role of Pleistocene glaciation creating refugia, with recolonization afterward, and relict distribution of former widespread taxa, is well acknowledged in terrestrial organisms (Normand et al. 2011), but this is less known in aquatic ecosystems (but see Brochmann et al. 2003). Interpreting higher Western species richness as a testimony of former refugia is still under debate. Environmental conditions over the Atlantic side are overall suitable for desmids due to the mild climate, little anthropogenic disturbance, a wide range of habitats and their high connectivity (John and Williamson 2009). Therefore, current biodiversity patterns and variation of richness could reflect a variation in habitat quality (e.g., related to disturbance regimes), this possible influence should be explored in the future.

Role of dispersal limitation

Within modules, partial Mantel tests evidenced a distance decay of community similarity that was not explainable by environmental variation. In a hierarchical conception of ecological processes, such residual pattern of isolation-by-distance can reflect the influence of dispersal limitation (Freestone and Inouye 2006). Assessing dispersal limitation is not trivial given the dispersal ability of microbial species (Martiny et al. 2006), but the patchy nature of desmid habitats is likely to limit the exchanges among communities at large scale. The hierarchy of spatial structure among and within modules could then reflect a multiscale influence of dispersal limitation on

metacommunity dynamics (Huth et al. 2015), from long-term migration limitation in former refugia, expressed by spatial patterns of module distribution, to more recent effect of dispersal limitation within pools.

Niche-based assembly processes constrain local functional composition

The variation of functional traits within the community is expected to reflect the influence of niche-based community assembly processes, such as environmental filtering and niche differentiation (Ackerly and Cornwell 2007; Kraft, Valencia, and Ackerly 2008). The constraints exerted by assembly processes can be assessed through the Standard Effect Size (SES) of trait range and trait variance in communities, quantifying the deviation from a null model without such constraints (Bernard-Verdier et al. 2012). SES of trait range and SES of CWV were lower than expected by null models, indicating overall trait range reduction and functional convergence in communities, the degree of which varied with climate (Figure 2.3).

We found trait range reduction for both volume and surface-to-volume ratio at community-level under continental climate. Trait range reduction is a signature of habitat filtering that excludes individuals with phenotypes not able to thrive under certain conditions (Enquist et al. 2015). Our results support the idea that more continental and low precipitation climate represents severe environmental conditions ('stronger filtering') for desmid establishment and persistence. It echoes previous research, showing that low temperatures create constraints on desmid growth. Epipellic algae occurrence has already been shown to be correlated with temperature (Špačková et al. 2009), and desmids show nearly no growth at temperatures below 15 °C (Coesel and Wardenaar 1990). Under continental climate, growing season is shorter and local abundances are reduced by winter freezing. However, Svoboda, Kulichová, and Štátný (2013) showed that such freezing has no effect on species composition of desmid communities. Reduced trait ranges and shift in CWM could illustrate the ability of large cells with smaller surface-to-volume to better survive and thrive under highly seasonal climate with low precipitation.

Conversely, we found smaller CWV (functional convergence) of biovolume in high precipitation and low seasonality climate. Opposite trends for trait range and variance of

biovolume along the climate gradient is consistent with distinctive abiotic and biotic drivers along the gradient. The more stressful environment can entail filtering and trait range reduction at one extreme of the gradient, while more favourable environment can be related to more competitive interactions at the other extreme. Relatively stable and humid climate can, therefore, represent favourable, highly competitive climate, without strong influences of environmental filtering. We found smaller cells in those 'milder' environments. Smaller cells have generally higher surface-to-volume, high nutrient uptake efficiencies and higher growth (Nielsen et al. 1996; Passy 2007). Consequently, larger cells would survive better but display lower growth rates (Reynolds 1987). Functional convergence, more pronounced under maritime climate, could thus be explained by the exclusion of species having large cell sizes, and slower growth, which appears to be disadvantaged in highly competitive environments. In this perspective, functional convergence can be due to the competitive dominance of certain phenotypes (Chesson 2000; Mayfield and Levine 2010), while trait range reduction can simultaneously represent an influence of abiotic constraints (see also Bernard-Verdier et al. (2012) for similar findings in plant communities). In the other perspective, decreased trait variance could also reflect functional redundancy in more stable environments, as it was shown for phytoplankton in tropical lakes (Kruk et al. 2016). These findings emphasize the entangled influence of niche-based processes on the assembly of desmid communities.

Limitations and perspectives

The studied morphological traits varied along environmental gradients and showed non-random distribution. As hypothesized, biovolume and surface-to-volume could be related to varying physiological responses and community assembly of desmids depending on local environmental conditions. Simple morphological traits are thus likely to provide relevant proxies for the ability of desmids to survive and coexist in response to abiotic and biotic drivers. Cell volume-environment gradient in desmid communities was reported so far at smaller scales (Neustupa, Veselá, and Šťastný 2013) and we here highlighted its ecological meaning over broad-scale environmental variation. Although there is apparent determinism of environment on community-level trait distribution, the explanatory power of environmental variables remained low using PLS models. Unexplained variability could be connected to some other unmeasured environmental

characteristics, e.g. nutrient availability, the limnological status of locality, and to dispersal and migration limitation (Ordonez and Svenning 2015), stochasticity (Segura et al. 2010) or biotic interactions with other organismal groups (Kruk et al. 2016).

Interestingly, pH did not explain variation in community-level trait values (CWM), even though it clearly separated modules 1-5 with different CWM of surface-to-volume values. Communities outside modules 1-5 probably represent a more heterogeneous concept, therefore they bring stochasticity, blur the results, and enhance the role of climatic variables. Furthermore, volume and surface-to-volume probably do not capture all ecological strategies of species. Additional functional traits should also be considered to represent other aspects of desmid ecology susceptible to influence their survival, reproduction, and dispersal. For instance, physiological properties could provide complementary information. It was already shown that two desmid species with similar size and surface-area can have different affinities for phosphorus uptake (Spijkerman and Coesel 1996). Creating a global database of microbial (algal) traits is necessary for more detailed studies of microbial functional ecology (Litchman and Klausmeier 2008). Such databases have recently emerged (see (Edwards, Klausmeier, and Litchman 2015; Klais et al. 2017), however, there is need to broaden their focus for microbes outside phytoplankton.

A single-cell trait-based approach can be very useful in future microbial ecological studies (especially in protists with large body sizes and therefore more reliable and straightforward trait estimation). In addition, the role of intraspecific trait variation in community assembly is now largely recognized in macroorganisms (de Bello et al. 2011; Violle et al. 2012). In desmids, Černá and Neustupa (2009) experimentally confirmed that species can to some extent modify their shape in response to changing pH. Neustupa, Šťastný, and Hodač (2008) also showed changing shape with temperature, although changes were smaller than interspecific shape variations. Accounting for intraspecific variation and direct measuring of biovolume could increase the explanatory power of trait-gradient analyses (Siefert et al. 2015). Our study assumed that interspecific were larger than intraspecific differences, a hypothesis that we suggest testing in future studies (see Kazakou et al. (2014) for a test in plants).

Overall, the combined application of network theory and trait-based community analysis to the distribution of desmid communities provides unique insights into the patterns and drivers of desmid diversity at multiple scales. In turn, it can be considered as a fruitful study case for the relevance of this approach to community ecology and biogeography. Importantly, the application of network theory to different taxonomic groups would be a valuable approach to functional biogeography that aims at integrating multitrophic and multifaceted biodiversity (Violle et al. 2014).

Acknowledgments

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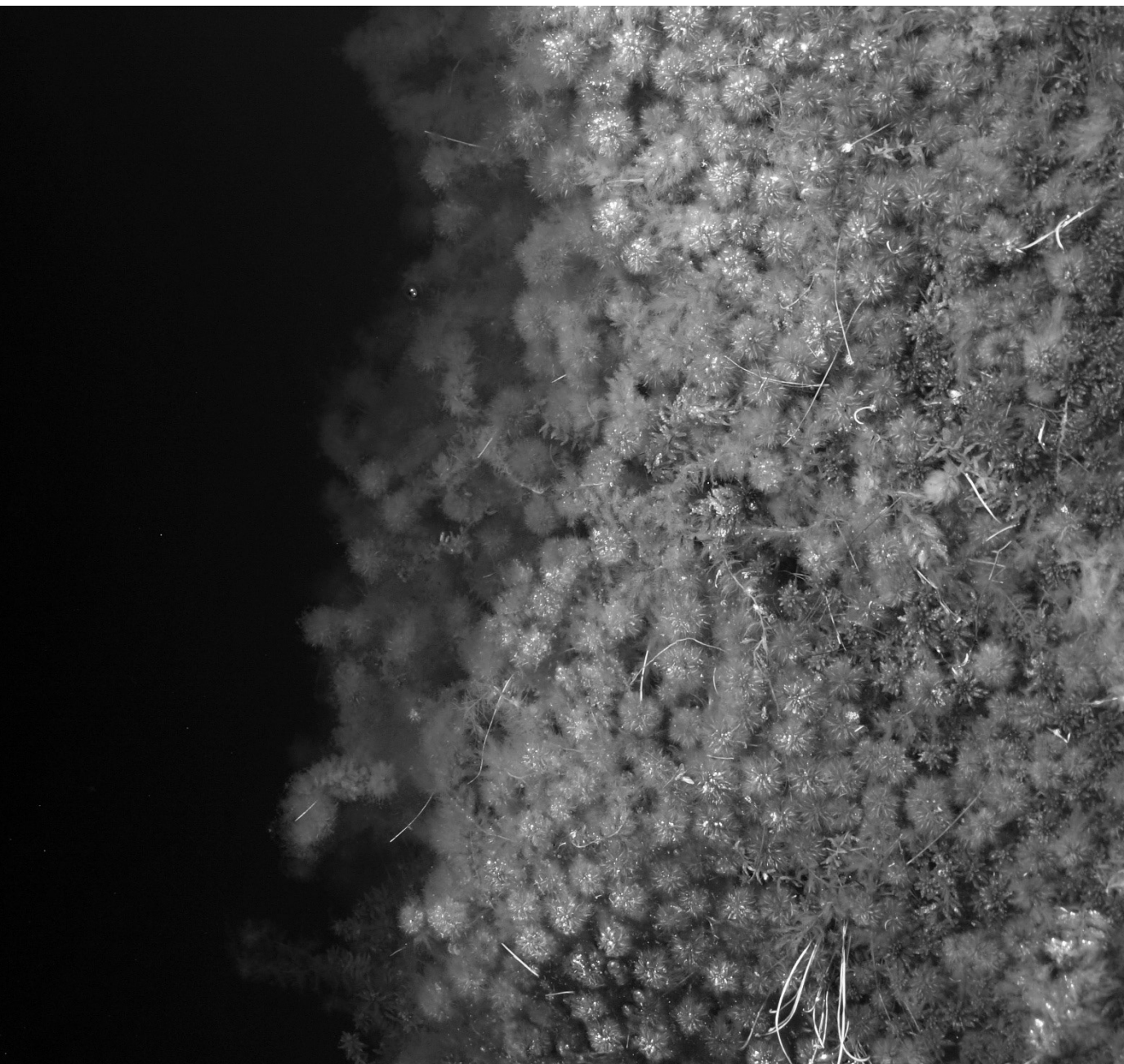
Supplementary material

Supplementary material is available with online version of this article at <https://doi.org/10.1007/s00442-018-4074-x>



3. Niche conservatism, environmental filtering, and competition in freshwater green algae: insights from community phylogenetics

Helena Bestová and Pavel Škaloud



ABSTRACT

Complex interactions of processes structure diversity at several spatial and temporal scales. The diversity of desmids, unicellular green algae, displays large regional differences in Europe and locally covaries with pH and conductivity. We used information about species evolutionary relatedness as an additional diversity facet and aim at understanding ecological and evolutionary processes forming desmid communities in Europe.

We analyzed phylogenetic and taxonomic diversity of 102 sampled communities. We quantified the relative importance of spatial and environmental factors on diversity turnover (β -diversity). We used a null model to determine whether local communities are non-randomly phylogenetically structured and tested phylogenetic signal in environmental preferences of species. We related local diversity (α -diversity) to environmental gradients.

Taxonomical and phylogenetic turnover in desmids were dominantly driven by environmental differences between sites, moreover, there was no spatial phylogenetic turnover. Locally, communities were significantly phylogenetically structured, but the pattern changed along the pH gradient. In low pH, communities were composed of closely related species, whereas in high pH the species were distantly related. This corresponded with ecological niche conservatism. Moreover, we found phylogenetic signal in pH and climatic requirements. pH was the sole best predictor of phylogenetic diversity and taxonomic diversity responded to pH and climate.

On a regional scale, European desmids are of single evolutionary origin, without the signature of allopatric speciation, and turnover in species as well in lineages is originating mostly in environmental heterogeneity. pH-related change in the phylogenetic structure together with the phylogenetic signal in environmental preferences testifies that dominant driver of local community assembly is changing along pH. Low pH represents environmental filter and after relaxation of this filter, in less acidic sites, communities are shaped by competition. By using supplementary diversity facet, we gained comprehensive pictures of desmid diversity drivers from species sorting and niche conservatism to local environmental filtering and competition.

INTRODUCTION

Ecological and evolutionary processes interact to shape diversity (Ricklefs 1987; HilleRisLambers et al. 2012). Understanding those processes is a long-standing goal of community ecology. Especially when distinguishing between ecological and evolutionary drivers, taxonomic identity of species is only a little informative (Cadotte, Albert, and Walker 2013). Taxonomic β -diversity, species turnover among communities, has been frequently used to quantify the relative importance of dispersal (neutral dynamics and mass effect) and environmental processes (species sorting) (Cottenie 2005; Leibold et al. 2004). However, such turnover can result not only from present-day ecological processes, but also from past evolutionary processes such as niche adaptations, allopatric speciation or historical biogeography (Saladin et al. 2019; Leibold et al. 2010). Phylogeny provides information about shared evolutionary history (Graham and Fine 2008). Phylogenetic β -diversity, turnover of lineages, can separate ecological and evolutionary processes (Jin, Cadotte, and Fortin 2015; Graham and Fine 2008; Leibold et al. 2010). For example, Leibold et al. (2010) showed, using phylogenetic diversity, distinct pattern for two co-existing copepods groups. Diversity of calanoids was strongly influenced by historical biogeography, contrastingly daphniids showed niche divergence linked to the environment with no signature of past geographical separation (Leibold et al. 2010).

Moreover, patterns of evolutionary relatedness within communities may be informative of local assembly processes, especially competition and environmental filtering (Webb et al. 2002). Phylogeny can be considered as a proxy to functional similarity of species (Srivastava et al. 2012; Cadotte, Cardinale, and Oakley 2008). Competition is hypothesized to be stronger between closely related, functionally similar species (Cahill et al. 2008). The dominant effect of competition, therefore, should result in phylogenetically overdispersed communities composed of distantly related species (Webb et al. 2002; Cavender-Bares et al. 2009). Conversely, the influence of environmental filter should promote co-occurrence of closely related species sharing functional characteristics making them successful in present environment. Such communities are phylogenetically underdispersed (Webb et al. 2002; Cavender-Bares et al. 2009). However, this initial simplistic view has been challenged because

underdispersion can arise also from competition (Mayfield and Levine 2010). Crucial traits may be evolutionary labile (Losos 2008). Moreover, biotic and abiotic processes are not mutually exclusive and their simultaneous action can result in random patterns (Gerhold et al. 2015). However a change in phylogenetic pattern linked with environmental gradients, can help to formulate a hypothesis about the mechanisms driving this change (Cadotte and Tucker 2017). In line with this reasoning, Helmus et al. (2007) showed a pH-related change of phylogenetic structure in fish communities, supporting the role of low pH as an environmental filter.

Indeed, phylogenetic diversity has been already used to decipher community assembly in the freshwater realm. For instance, it was used to unveil an imprint of colonisation history and habitat filtering in French fish communities (Blanchet et al. 2014), environmental filtering determining the structure of testate amoeba communities (Singer et al. 2018), and combined roles of competition and environmental filtering in lake littoral macroinvertebrate (Heino and Tolonen 2017). However, the parallel use of phylogenetic alpha and beta diversity to gain a full picture of local and regional processes remains rarely applied (but see (Blanchet et al. 2014; Strecker and Olden 2014)).

In the presented study, we focused on processes driving diversity patterns in desmids (Desmidiaceae, Zygnematophyceae, Streptophyta), a group of green algae often dominating in peatland phytobenthos communities. Their diversity displays substantial regional differences within Europe, linked with high species richness in oceanic regions (Bestová et al. 2018; Coesel and Krienitz 2008). Locally, desmid diversity covaries with pH (Neustupa, Veselá, and Šťastný 2013; Neustupa, Černá, and Šťastný 2009; Coesel 2001) and conductivity (Mataloni 1999). However, processes underlying those diversity patterns in desmid communities remain poorly explained. For example, higher regional richness in oceanic regions could be caused by evolutionary factors such as speciation or lower extinction (Graham and Fine 2008; Gerhold et al. 2015; Pavoine and Bonsall 2011), ecological factors such as higher regional connectivity and habitat availability (Monteiro, Paiva, and Peres-Neto 2017), large scale environmental variation (Blanchet et al. 2014) or historical biogeographical (Leibold et al. 2010). Lower local diversity can be the outcome of environmental stress (Passy et al. 2017), isolation (Lindström and Langenheder 2012) or low regional diversity (Arnan, Cerdá, and Retana 2017). Investigating both

phylogenetic and taxonomic diversity and focusing on both β -diversity and α -diversity components, can shed light on processes forming contemporary desmid communities. Indeed, the first effort to use evolutionary relationships in desmid communities was done by Coesel (1982) who described a shift in species:genus ratio linked with vegetation succession and changes of habitat stability. However, species:genus ratio is a very coarse measure, especially when taking into account that many desmid genera are in fact polyphyletic (Gontcharov and Melkonian 2011). Here we took advantage of the availability of sequence data and built solid phylogeny of desmids. This enabled to address ecological and evolutionary drivers of taxonomic and phylogenetic diversity across four important desmid regions within Europe, spanning a broad gradient of pH, conductivity and habitat connectivity.

METHODS

Community sampling

We sampled and analyzed 102 benthic desmid communities from freshwater wetlands in Europe. Samples were taken from a wide range of environmental conditions (pH and conductivity) and several regions are known to be different in their taxonomic richness (Czech Republic, Southern Sweden, Southern Norway, and Ireland) and habitat connectivity (

Figure 3.1). Samples of the upper layer of phytobenthos were taken by syringe from 10*10 cm quadrates. Sampling quadrates (communities) were located at least 30 m from each other. We immediately fixed samples with formaldehyde (2% final concentration), and later examined them under a light microscope (Olympus, CX31). We identified a total of 200 desmid cells from each sample.

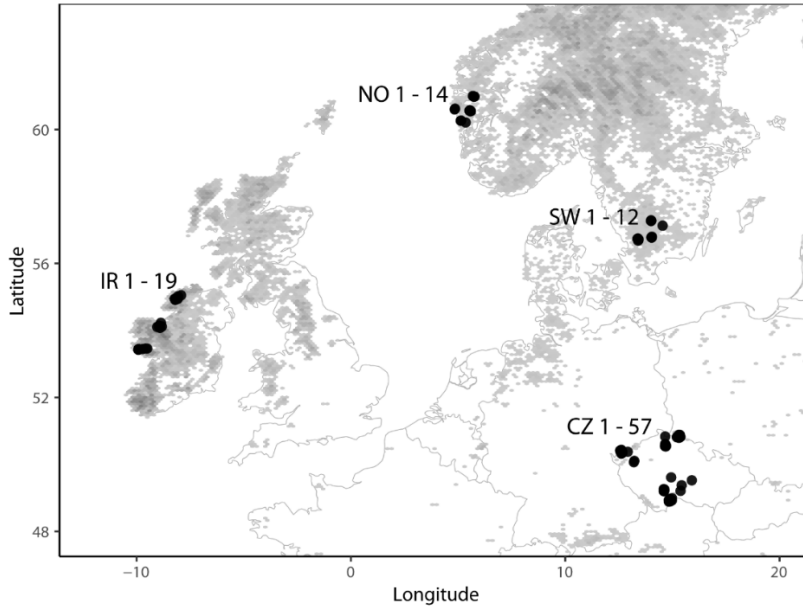


Figure 3.1 Maps of sampled communities with a density of peatland habitats

Environmental characteristics

We directly measured conductivity and pH at the site by pH/conductivity meter WTW 340i (WTW, Germany). We obtained set of 19 bioclimatic variables from WorldClim database with 2.5 arc-min resolution (Hijmans et al. 2005) and reduced their dimensionality by PCA (see PCA biplot in Supplementary material Figure S 3.1) and used first two PCA axes for further analysis. PC₁ was representing oceanic to the continental climatic gradient (ranging from high precipitation and low-temperature seasonality to high-temperature seasonality and lower precipitation) and PC₂ was reflecting a gradient in mean annual temperature and isothermality. Those two axes explained 62.9 and 21.47% of climatic variability, respectively. Lastly, we estimated habitat connectivity of sampling sites. We defined it as the total area of peat lands, inland marches and water bodies in the buffer zone of 10 km around the sampling site. Data of peat lands, marches, and water bodies distribution were obtained from CORINE land cover 2006 database (Bossard, Feranec, and Otahel 2000) using clc2006_412, clc2006_411, clc2006_512 raster data with resolution 100 m × 100 m.

Phylogeny reconstruction

We used the chloroplast-encoded *rbcL* gene for reconstruction of the phylogenetic relationship among desmid species. We downloaded most of the sequences from the GenBank database. We cultured abundant taxa with missing sequence and we extracted DNA according (Škaloud et al. 2011). We performed PCR reaction following (Gontcharov and Melkonian 2011) and purified PCR products were sequenced by Macrogen Corp. in Seoul, Korea. This resulted in an alignment of 385 unique sequences in total. We selected the appropriate evolutionary model for subsequent phylogenetic analysis by the Akaike information criterion with PAUP/MrModeltest (Nylander 2004). We used the maximum-likelihood criterion implemented in program GARLI 2.0 (Zwickl 2006) with GTR+ Γ +I model of substitution to infer phylogenetic tree. We estimated branch lengths from substitution rates. We used *Netrium digitus*, *Netrium oblongum*, and *Spirogyra pratensis* as an outgroup to root the phylogeny. We added taxa with missing phylogenetic information to their congeners as a polytomy, based on already published phylogenies for other genes (Gontcharov and Melkonian 2010; Gontcharov, Marin, and Melkonian 2003) or morphological similarities. We added polytomies by merge script from phyloGenerator v1.2 (Pearse and Purvis 2013).

β -diversity

We quantified taxonomical and phylogenetic turnover between communities. We used Sørensen dissimilarity index for taxonomical β -diversity (Sørensen 1948) and phylogenetic Sørensen, a fraction of shared phylogenetic branch length to describe evolutionary dissimilarity (Bryant et al. 2008). PhyloSor quantifies the fraction of shared phylogenetic branch length between samples. We estimated the amount of variance explained by connectivity, environmental and spatial factors by variation partitioning based on distance-based redundancy analysis (dbRDA) (Legendre and Anderson 1999). Environmental distance matrices were created as Euclidean distances of scaled values of pH, conductivity and climatic PC₁, PC₂. We defined a spatial structure in communities by distance-based MEM - Moran's eigenvector maps (Dray, Legendre, and Peres-Neto 2006). Prior to the variance partitioning, we calculated dbRDA for connectivity, spatial and environmental matrices separately. When the full model was significant, we selected

only the best explaining environmental variables and MEMs by stepwise selection procedure. We carried out analysis is package *vegan* 2.5 – 2 (Oksanen et al. 2018).

α -diversity

We calculated two facets of diversity: i) taxonomic diversity (TD) defined as species richness, number of species (taxonomical units) in each community, and ii) phylogenetic diversity (PD) based on metric Phylogenetic Species Variability developed by (Helmus et al. 2007). Phylogenetic species variability is defined as the variance of hypothetical trait evolving under Brownian motion among all species in a community. It is calculated from the variance-covariance matrix, it ranges from 0 to 1 and it is mathematically independent of species richness (Helmus et al. 2007). Communities with completely unrelated species, whose overall community phylogenies are star-like, will have high phylogenetic diversity and PD equal to 1. This metric is statistically independent of a number of species in communities (Helmus et al. 2007).

Diversity and environment

The number of sampled communities differed between regions, therefore we performed sample-based rarefaction to evaluate if we are capturing differences of taxonomic richness between regions. We fitted generalized linear models (GLM) to link observed diversity patterns with environmental gradients. TD was best fitted by negative binomial error term and PD with Gaussian error term. We fitted following environmental predictors: pH, conductivity, first and second climatic PCA axis, habitat connectivity, and regional identity. Full models included their interactions as well. All continuous predictors were standardized to z-scores prior analysis. Best models were selected based on their Akaike's information criterion (Yamaoka, Nakagawa, and Uno 1978).

Null models

We compared calculated PD with the distribution of PDs of null communities to test whether the communities are significantly phylogenetically structured. We used randomization proposed by (Gotelli 2000). We reshuffled species identities preserving species richness of each community, null model labeled “richness” in R package *picante* (Kembel et al. 2013). This model tests if species of community represent non-random samples from species pool (Helmus et al. 2007). We performed 1000 permutations to define the distribution of mean PDs of null communities. And tested the mean PD value of real communities against this mean null distribution. We also divided communities on highly acidic (pH < 5) and slightly acidic (pH > 5), following division of Coesel (2001) and tested them separately against same mean null distribution. We performed those analyses with R package *picante* 1.7 (Kembel et al. 2013).

Phylogenetic conservatism of environmental niche

We tested phylogenetic conservatism in environmental niche preferences. We defined niche preference of each species based on environmental values of communities it was present. We calculated mean, minimal, the maximal value of an environment where each species was present and value where species had a maximal abundance for environmental parameters. We tested phylogenetic conservatism by Pagel’s λ (Pagel 1999). We used a phylogenetic tree from our phylogenetic reconstruction. Branch lengths of this tree were estimated from substitution rates because lack of fossil records does not allow to time-calibrate desmid phylogenies. We also repeated the analysis with all branch lengths set to equal. This allowed us to test purely topology and prevent error caused by incorrect branch length estimation (Münkemüller et al. 2012). We performed those analyses with R package *phylosignal* 1.2 (Keck et al. 2016).

All statistical analyses were carried out using R Statistical Software (R Core Team 2014).

RESULTS

Overall, 308 taxa belonging to the order Desmidiales were recognised in 102 sampled communities. Conductivity of sampled communities ranged from 8 to 217 $\mu\text{S}/\text{cm}$ and pH from 3.9 to 7.6. Conductivity and pH were correlated (Pearson's correlation $r = 0.276$; P -value < 0.01). Climate was correlated with habitat connectivity (with PC1 $r = -0.46$; P -value < 0.001 , with PC2 $r = -0.68$; P -value < 0.001). Regions differed in their environmental characteristics according to series of Kruskal-Wallis tests (KW) (pH: KW P -value = 0.003; conductivity: KW P -value < 0.001 ; habitat connectivity: KW P -value < 0.001 ; climatic PC1: KW P -value < 0.001 ; climatic PC2: KW P -value < 0.001 ; see Figure S 3.3 in Supplementary material). Taxonomic diversity (TD defined as species richness) varied among regions (KW P -value < 0.001). Although number of samples from regions varied, this did not cause the differences (see sample-based rarefaction curves Figure S 3.2 in Supplementary material). Ireland and Norway had higher TD (rarefied TD to minimal sample size ($n = 12$): Czechia = 65.27; Ireland = 164.52; Norway = 144.92; Sweden = 39). Phylogenetic diversity (PD) and TD were not correlated (Spearman $\rho = 0.12$ P -value = 0.204). There was no difference in PD between regions (KW P -value = 0.33).

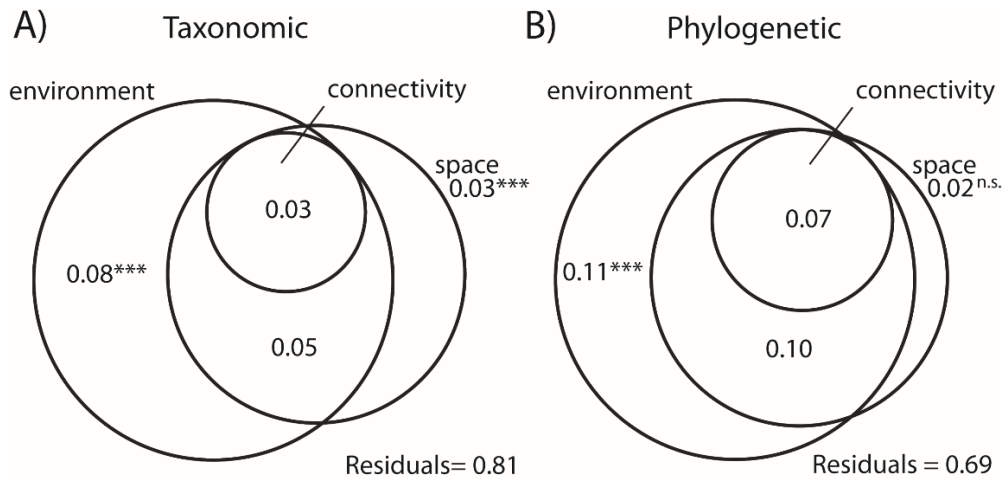
β -diversity

Figure 3.2 Taxonomic and phylogenetic turnover is mainly linked with environmental differences between communities. Results of variance partitioning, values indicate fractions of A) taxonomic and B) phylogenetic β -diversity.

The biggest proportion of variance in β -diversity was explained by environmental differences between sites (all environmental variables – pH, conductivity, climatic PC₁, and PC₂ – were selected into the best model) (Figure 3.2). Purely spatial factors explained only a very small percentage of variability in taxonomic β -diversity and were non-significant in phylogenetic β -diversity. Spatially structured environment explained 17% in phyloSor and 8% in Sorensen dissimilarity between sites. Pure habitat connectivity did not contribute to any variability, but it shared a minor proportion with the environment and spatial factor for both types of diversity.

Diversity and environment

GLM models show that different environmental predictors influence TD and PD. PD was best predicted solely by pH (Table 3.1). The best fit model for TD contained pH and climatic factors. Both TD and PD increased with increasing pH. Additionally, TD increased towards oceanic climate (negatively correlated with PC₁) and towards warmer sites with higher isothermality (negatively correlated with PC₂). Habitat connectivity, regional identity, and conductivity were not selected by any of the best models.

Table 3.1 Results of generalized linear models to explain variation on taxonomical and phylogenetic diversity, best-selected models shown. We present Estimates for Gaussian GLM and Incidence Rate Ratios (exponentiated estimates) for negative binomial distribution GLM for each predictor, its confidence intervals (CI) and *P*-value.

<i>Predictors</i>	TD			PD		
	<i>Incidence Rate Ratios</i>	<i>CI</i>	<i>P</i> -value	<i>Estimates</i>	<i>CI</i>	<i>P</i> -value
(Intercept)	13.24	12.21 – 14.36	<0.001	0.45	0.42 – 0.48	<0.001
pH	1.30	1.20 – 1.40	<0.001	0.08	0.05 – 0.10	<0.001
climatic PC 2	0.77	0.71 – 0.83	<0.001			
climatic PC 1	0.54	0.51 – 0.59	<0.001			

Null models

All together communities were randomly phylogenetically structured. Mean phylogenetic diversity was within 95 % of means for null communities (mean PD observed = 0.4503; 2.5 % - 97.5 % quantiles of null distribution 0.4135 - 0.4665) (Figure 3.3). We divided data set to communities with low pH ($\text{pH} \leq 5$) and high pH ($\text{pH} > 5$). Low pH communities were significantly underdispersed (mean PD = 0.393), composed of more closely related species. On the other hand, high pH communities were significantly overdispersed (mean PD = 0.531), composed of distantly related species. Communities of both groups do not represent random samples from species pool (all species observed in this study).

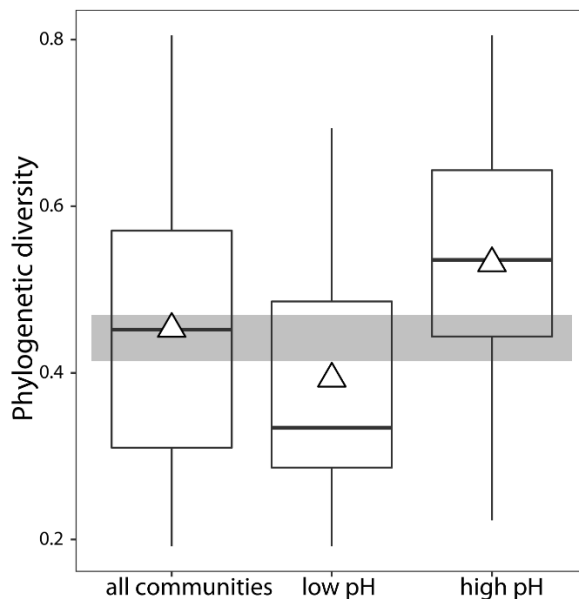


Figure 3.3 Communities in low pH are phylogenetically underdispersed and in higher pH overdispersed. We show the distribution of phylogenetic diversity and its comparison to the null model. Data are shown for all communities and divided into two groups - communities with $\text{pH} < 5$ and $\text{pH} > 5$. Grey ribbon represent 2.5 % to 97.5 % quantiles of means of null distributions. Triangles are means of observed distribution. Triangle below grey ribbon signifies phylogenetic underdispersion, triangle above phylogenetic overdispersion. The box indicates the inter-quartile range, the line in the box is median, whiskers spread to $1.5 \times$ inner-quartile range and points represent outliers.

Phylogenetic conservatism of environmental niche

We found statistically significant levels of phylogenetic signal in the ecological niche of pH, conductivity and climatic axis 1 (Table 3.2). Closely related species shared environmental preferences. Results presented in Table 2 are based purely on the topology of the tree (branch length set equal), for results including branch, length see Table S 3.1 in Supplementary material.

Table 3.2 Summary table of a test of phylogenetic signal in the ecological niche of species. For each environmental value mean, minimal and maximal value where species was present was tested together with value in which species reached maximal abundance. Values of Pagel's λ and respective P -values are presented. Significant values are in bold.

	pH		conductivity		climatic PC1		climatic PC2	
	λ	P -value	λ	P -value	λ	P -value	λ	P -value
mean	0.61	0.001	0.51	0.002	0.72	0.001	0.00	1.000
minimal	0.30	0.014	0.31	0.051	0.59	0.001	0.00	1.000
maximal	0.06	0.110	0.04	0.265	0.39	0.010	0.09	0.176
max. abundance	0.55	0.001	0.39	0.261	0.73	0.001	0.13	0.332

DISCUSSION

The diversity of European desmids is known for their Atlantic-subarctic vs. continental aspect (Coesel and Krienitz 2008), however, drivers underlying this pattern have not been yet identified. We found that only a limited fraction of taxonomic turnover was caused purely by spatial factors. The most of taxonomical differences between communities were indeed linked with environmental variation (in pH, conductivity, and climate). This indicates that species sorting dominates over neutral dynamics which would have resulted in strong spatial structure (Leibold et al. 2004). Species sorting seems to be common among metacommunities of passively dispersing species, such as freshwater algae, provided that immigration to communities is sufficient (Cottenie 2005). Habitat connectivity should represent an even better estimate of dispersal ability than a spatial structure on its own (Monteiro, Paiva, and Peres-Neto 2017). Therefore, we expected isolated habitats to have distinct species composition (Heino et al. 2015) or well-connected habitats to have reduced influence of species sorting due to a large

immigration (Lindström and Langenheder 2012). Surprisingly the only effect of connectivity on β -diversity we found was shared with a spatially structured environment. Phylogenetic turnover was also dominantly linked with ecological differences of communities. This was accompanied by a phylogenetic signal in pH, conductivity, and especially climatic niche linked with an oceanic-continental gradient. Together it shows the importance of evolutionary niche adaptations, where distinct lineages have conserved environmental niches (Jin, Cadotte, and Fortin 2015). Moreover, the pure spatial structure in phylogenetic turnover was non-significant. Even though the unexplained variation was high (81% for taxonomic and 69% for phylogenetic turnover), this variability could be attributed to unmeasured environment, which would only strengthen the role of species sorting (Cottenie 2005) and niche conservatism (Saladin et al. 2019). Niche conservatism, especially in climatic preferences, can explain a higher number of endemic species described in Atlantic flora of European desmids (Coesel and Krienitz 2008). On a regional scale, our results showed that European desmids are of single evolutionary origin, without the signature of allopatric speciation, and turnover in species as well in lineages is originating in large-scale environmental heterogeneity.

The dominant influence of deterministic niche-based processes was also reflected in α -diversity of desmid communities. TD and PD, although uncorrelated, both responded to variation in pH. The positive covariance of pH and TD of desmid communities has been long known (e.g. Mataloni 1999). However, the connection between pH and evolutionary diversity of desmid assemblages is a novel result and brings insight into processes acting in local communities. Our null model analysis showed that on both sides of pH gradient communities represented non-random draws from species pool in respect to their phylogeny. Niche-based assembly processes determined the structure of local communities (Kembel 2009; Cavender-Bares et al. 2009). But the dominant process changed along the pH gradient. In low pH, closely related species coexist, conversely communities in higher pH were composed of distantly related species. We also found that evolutionary relatedness was positively correlated with pH niche similarity. The most plausible hypothesis, therefore, is that low pH acted as an environmental filter selecting for closely related species, that share some characteristics favoring them in hostile conditions (Webb et al. 2002). In localities with higher pH environmental filtering is relaxed and communities were driven by limiting similarity and

competitive exclusion eliminated similar species (Kembel 2009). In fact, pH as a determinant of assembly processes altering the phylogenetic composition of communities has been shown for other aquatic organisms (Helmus et al. 2007; Ren et al. 2015).

A low pH causes several problems for organisms including increased metal toxicity, reduced bicarbonate availability (Greenwood and Lowe 2006), and acidification of cytoplasm (Gimmler 2001). Moreover, low pH does not have act as a strict filter eliminating species who cannot survive. Our data represent the realized niche of organisms. Therefore the effect of pH could be also indirect, mediated by changes in interactions with other species (Aguilar-Trigueros, Rillig, and Ballhausen 2017). It is also reflected in lower intrinsic growth rates of non-adapted species, which could lead to their competitive exclusion (Cadotte and Tucker 2017). Indeed growth-rate pH covariance in desmids has been already been shown (Coesel 1993). Moreover, Černá and Neustupa (2009) demonstrated morphological adaptations, when desmids reacted to acidic conditions by changing their shapes and lowering surface-to-volume ratios. In fact, the response to pH could be only with difficulties linked to specific traits in desmids. The surface-to-volume ratio could be a good candidate, but functional patterns in the surface-to-volume ratio in desmid communities were shown to respond more to a climate than to pH (Bestová et al. 2018). We argue that in the case of desmids evolutionary relatedness is a good representation of pH niche (Mouquet et al. 2012).

Taxonomic diversity increased with pH and in a milder climate. Highly acidic communities had low PD but also lower TD. This is in agreement with the hypothesis that environmental stress causes a decrease in TD, as it was shown on diatoms and fish (Passy et al. 2017). Additionally, to the dominant influence of pH, local TD was higher in milder oceanic climates with higher mean annual temperatures and isothermality. Desmid diversity and number of endemic species are generally higher in warmer regions (Coesel 1996). However, the cause of this pattern is unknown. PD did not vary with climate, therefore the increase in TD could not be linked with higher speciation. Winter freezing could represent stress that lowers taxonomical diversity. The species composition of desmids assemblages did not change on the short temporal scale (Svoboda, Kulichová, and Šťastný 2013), but winter freezing decreases abundances.

Higher TD in freshwater algae could be also caused by higher habitat availability (Telford, Vandvik, and Birks 2006; Vyverman et al. 2007). Connectivity, which we took as a proxy of habitat availability, was indeed positively correlated with climate but it was not included in best fit models of α -diversities. Higher TD could be also a signature of historical legacies, where Atlantic sites could have served as refugia during the ice age and retained their higher richness into present days (Jurdíková et al. 2014; Bestová et al. 2018). Indeed, refugia are associated with hotspots of diversity for example for plants (Médail and Diadema 2009). In the presented study, we are not able to reveal the mechanism linked with higher TD in milder climates, and it remains an interesting direction for future research. However, we show that the most important local environmental driver is pH. Processes such as habitat acidification could possibly lead to change in community assembly mechanism linked with change in the evolutionary structure of the desmid community and decrease in taxonomic diversity.

In conclusion, our study documented that phylogenetic diversity can be used as supplementary diversity facets for desmid communities and it brings additional insights into drives of diversity.

Acknowledgments

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Supplementary material

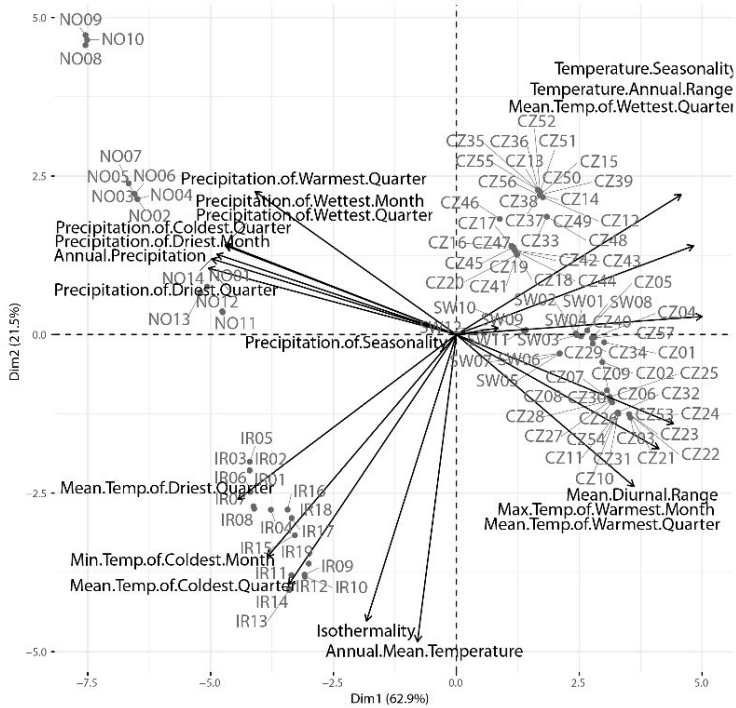


Figure S 3.1 Principal component analysis of desmid communities, based on 19 bioclimatic variables obtained from the WorldClim database, first two PC axis displayed

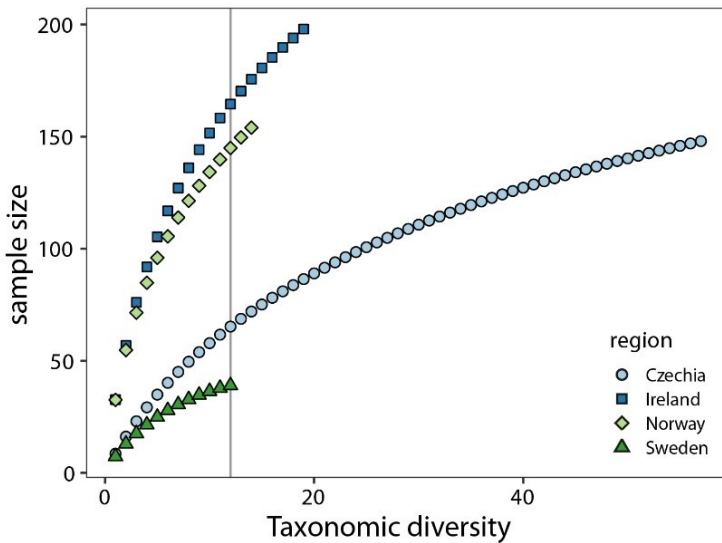


Figure S 3.2 Sample-based rarefaction curves of taxonomic diversity of regions.

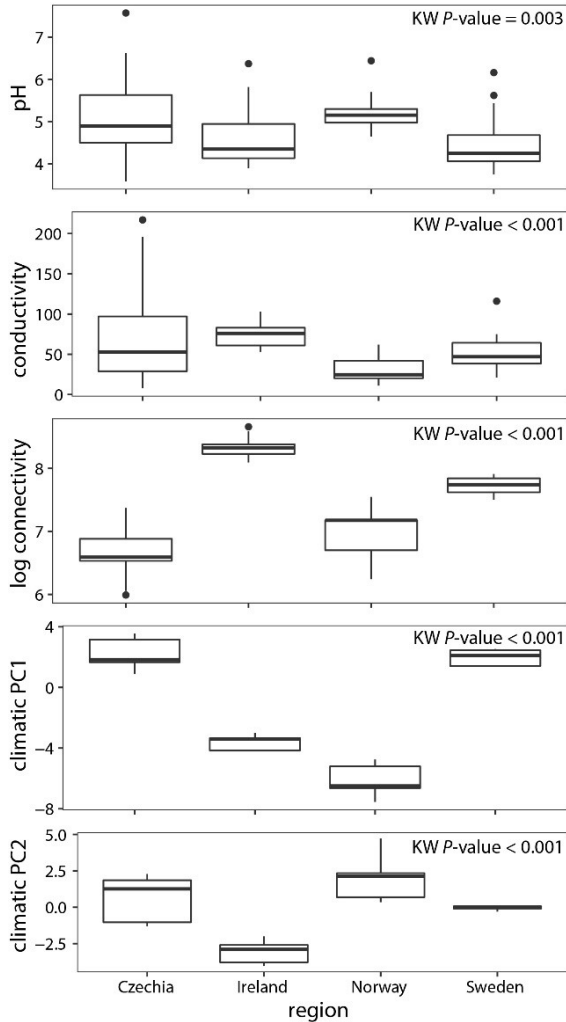
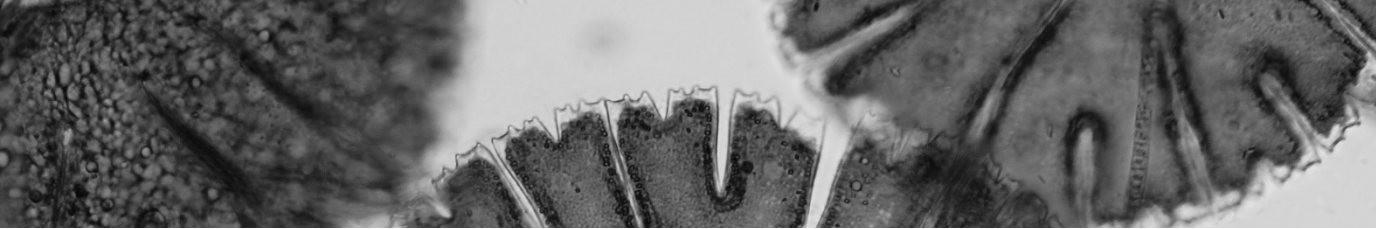


Figure S 3.3 Environmental variation of regions. The boxplot indicates the inter-quartile range, the line in the box indicates median, whiskers spread to 1.5x inner-quartile range and circles represent outliers.

Table S 3.1 Summary table of a test of phylogenetic signal in the ecological niche of species. For each environmental value mean, minimal and maximal value where species was present was tested together with value in which species reached maximal abundance. Values of Pagel's λ and respective P-values are presented. Significant values are in bold.

	pH		conductivity		climatic PC1		climatic PC2	
	λ	P-value	λ	P-value	λ	P-value	λ	P-value
mean	0.29	0.003	0.23	0.340	0.36	0.011	0.00	1.000
minimal	0.33	0.002	0.19	0.543	0.29	0.011	0.36	0.215
maximal	0.26	0.030	0.22	1.000	0.37	0.084	0.00	1.000
max. abundance	0.05	0.072	0.03	0.263	0.05	0.123	0.00	1.000



4. Biological scaling in green algae: the role of cell size and geometry

Helena Bestová, Jules Segrestin, Klaus von Schwartzberg, Pavel Škaloud, Thomas Lenormand and Cyrille Violle



ABSTRACT

According to Metabolic Scaling Theory (MST), the relationship between population growth rate and body size is predicted to follow a universal quarter-power scaling. MST hypothesizes limitations of resource-transport networks in organisms and predicts their optimization into fractal-like structures. However, the universality of metabolic scaling has been challenged, particularly across transitions from bacteria to protists to multicellulars. The population growth rate of unicellulars should be constrained by external diffusion, ruling nutrient uptake, and internal diffusion, responsible for nutrient distribution. Both constraints intensify with increasing size possibly leading to shifting in scaling exponent. We focused on unicellular green alga *Micrasterias*. Their large size and fractal-like morphology make these species a candidate group between unicellulars and multicellulars in the evolution of allometry. We tested MST predictions using original measurements of growth rate, size, and morphology-related traits. We showed that growth scaling of *Micrasterias* does not differ from MST predictions in accordance with constraints by internal diffusion transport. Cell fractality and density decrease led to a proportional increase in surface area with body mass overcoming external constraints. Complex allometric optimizations enable *Micrasterias* to maintain quarter-power scaling of populations growth rate even with a large unicellular plan. Overall, our findings support pervasive stabilizing selection for biological scaling laws.

Keywords: allometry, population growth rate, morphological optimization, metabolic scaling theory, protists, green algae

INTRODUCTION

The Metabolic Scaling Theory (MST) states that the pace of organismal processes, captured by population growth rate (μ_{max}) in particular, is tightly linked to body size (M) (Brown et al. 2004), following power-law functions such as:

$$\text{Eqn. 4} \quad Y = Y_0 M^b$$

where Y represents metabolic rate or growth rate, M organism's body mass, Y_0 a normalization constant and b the scaling exponent. MST predicts b to be proportional to $\frac{1}{4}$ (G. B. West, Brown, and Enquist 1997; G. B. West, Enquist, and Brown 1999; Savage, Deeds, and Fontana 2008). This “quarter-power” allometric scaling, also known as Kleiber's law, contradicts former intuitions stating that the scaling of metabolic processes should be proportional to $\frac{1}{3}$, reflecting the outcome the allometric scaling between volume and surface area (Banavar et al. 2010). According to MST, population growth rate (μ_{max}) should scale with body size with an allometric coefficient equal to $-\frac{1}{4}$ (Fenchel 1974; Blueweiss et al. 1978; Savage, Gillooly, Brown, et al. 2004). Based on the hypothesis that allometric relationships reflect optimal phenotype resulting from natural selection and constrained by physical laws (Enquist, Tiffney, and Niklas 2007), a universal metabolic scaling across a wide range of organisms would indicate the existence of common constraints and optimal body plan. However, DeLong et al. (DeLong et al. 2010) highlighted changes in the allometric scaling of metabolic rate and μ_{max} along main evolutionary transitions, linked with “innovations in metabolic design”, such as cell compartmentalization and multicellularity. Their meta-analysis validated Kleiber's law for metazoans only, whereas bacteria and protists deviated from the $-\frac{1}{4}$ exponent. This finding challenged the universality of Kleiber's law and the existence of a common metabolic constraint for all organisms (G. B. West and Brown 2005). The general agreement on the form and the cause of growth rate-size relationship is still lacking, notably when navigating across the tree of life.

In protists, two types of physical constraints can regulate cell metabolism. First, external constraints govern uptake of resources from external milieu. They are linked to cell surface area that limits the pace of diffusion and the number of transport sites at the cell membrane (Yoshiyama and Klausmeier 2008; DeLong et al. 2010). Second, internal

diffusion constraints, distances and viscosity within the cytoplasm in particular, control the distribution of resources within the cell and are related to cell volume, mass, and shape (Gallet et al. 2017). The ability of intracellular diffusion to maintain a constant concentration of solutes (Beardall et al. 2009) and CO₂ (Wirtz 2011) is known to decrease with size. At the upper limit of protist size, external and internal constraints should lead to reduced metabolic efficiency (a shift in metabolic scaling) and competitive superiority of multicellulars (DeLong et al. 2010). Nevertheless, both constraints could be overcome or at least pushed further by body plan optimizations, especially by changing body geometry and diluting cell content (Okie 2013). Those changes in body plan certainly improve surface to active cell volume ratio (Okie 2013; Niklas 1994a), but it is little known to what extent such allometric adjustments influence the scaling of organismal processes.

In multicellular organisms, the fractal resource transportation networks were proposed as an optimization strategy that overcomes internal distribution constraints and leads to optimal quarter-power scaling (G. B. West, Enquist, and Brown 1999). The fractal networks are typically missing in unicellulars, but they strikingly resemble star-shaped morphology of streptophyte green algae genus *Micrasterias* (Streptophyta, Desmidiaceae, Zygnematophyceae). The extraordinary diversity of sizes and shapes within a single genus (Fig. 1) and among species with a similar ecology offers a unique opportunity to examine the role of body plan optimization, especially fractality. Neustupa (Neustupa 2015) reported elevated surface-area to volume allometry of *Micrasterias* and showed that cell ramification compensates for large cell volume. However, it remains unclear whether the variation in phenotype is linked with variation in process-based allometry. In this study, we carried out an experiment investigating growth allometries of 24 species of *Micrasterias* with a large range of sizes spanning two orders of magnitude and morphological complexity. We experimentally measured population growth rate and analyzed its size dependency using size measurements (volume and body mass). To understand the constraints that govern the observed allometric relationships we also measured morphological traits related to body plan optimization. Specifically, we quantified the increase in surface area due to fractality and flattening of the cell. We investigated the role of morphology, more precisely the effect of body plan optimization on deviation from growth rate scaling and shifts in surface and body mass allometries.

METHODS

Assessment of species growth rate

We grew monocultures of 24 strains of the genus *Micrasterias* (Figure 4.1).

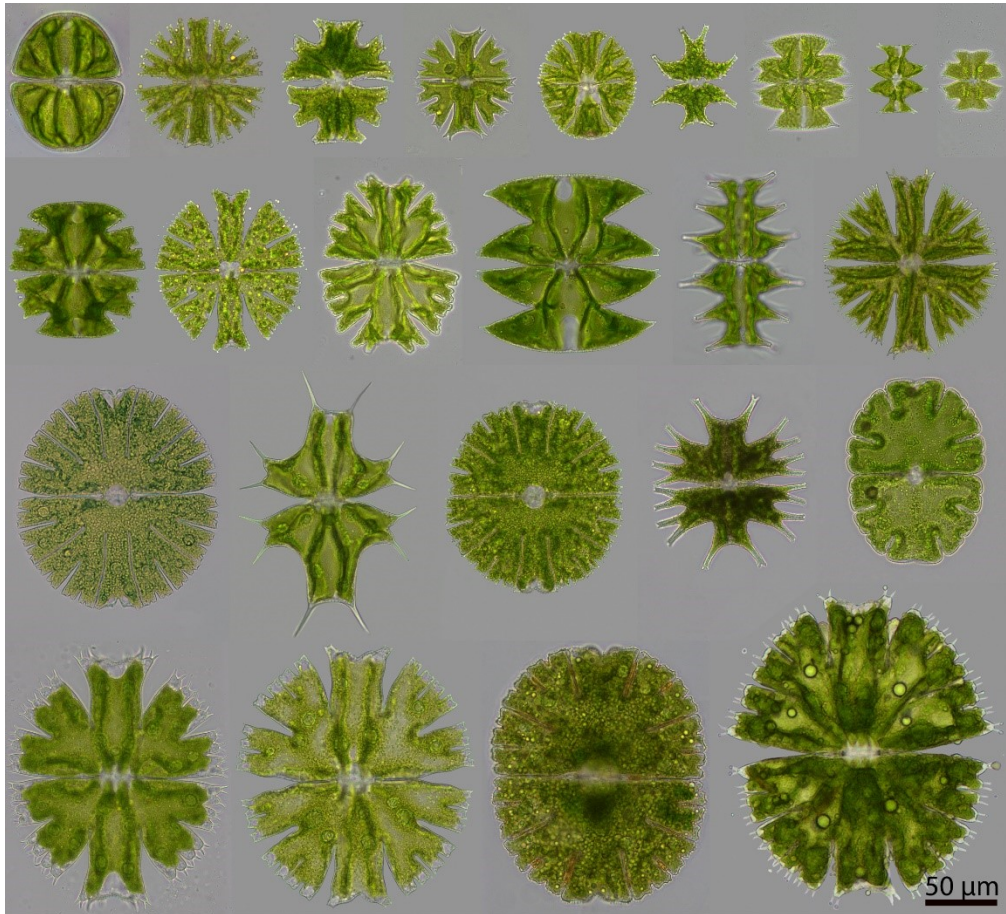


Figure 4.1 Size and morphological variability in *Micrasterias*. Microphotographs showing frontal views of the 24 strains used in the experiment. From right to left: first line *M. ralfsii*, *M. radiosa*, *M. crux-melitensis*, *M. radians*, *M. conferta*, *M. tropica*, *M. ceylanica*, *M. pinnatifida*, *M. decemdentata*; second line: *M. truncata*, *M. novae-terrae*, *M. americana*, *M. laticeps*, *M. muricata*, *M. papillifera*; third line: *M. thomasiana* (K6o2), *M. ceratofera*, *M. thomasiana* (SCVK 8), *M. furcata*, *M. jenniferi*; fourth line: *M. apiculata*, *M. rotata*, *M. denticulata*, *M. fimbriata*.

We used strains available in public culture collections MZCH-SCVK Hamburg Collection (Schwartzenberg et al. 2013) and CAUP Culture Collection Prague (Škaloud and Neustupa 2009). Details about strains used can be found in Supplementary material (Table S1).

We transferred storage cultures to fresh DY IV modified medium with pH 5.7 (botany.natur.cuni.cz/algo/caup.html), at a constant temperature of 20 °C and with constant illumination. After 9 days of accommodation, we inoculated four replicates per strain on 12-well plates to a starting concentration of 100 cells.mL⁻¹ with the total volume of 4 mL. We sealed the well plates with the Breathe-Easy sealing membrane to prevent evaporation. Every three days we sampled cultures to estimate cell abundance. We sampled 10 % of volume (400 µL) and preserved it with formaldehyde (1% final concentration). We replaced the sampled volume by fresh medium. We followed the growth of strains for 35 days. Long experimental time covered the exponential phase of growth and enabled to measure maximum population growth rates.

We estimated cell abundance in samples using Nageotte hemacytometer (Bright Line, Hauser Scientific, Horsham, PA). We regressed cell abundance (log-transformed) against time and estimated maximum population growth rate (μ_{max}) as the slope of this relationship on the linear part of the curve. We calculated the final growth rate for each strain as the maximal growth rate averaged across replicates and weighted by the goodness of fit of the linear regression on the linear part of the curve (r^2).

Measurements of cell volume and cell surface

Estimating volumes and surfaces of cells with complex shapes is not an easy task. Commonly cell shape is assigned to general geometrical shape, and volume is calculated based on the mathematical formula for this geometrical shape (Hillebrand et al. 1999). This approach would lead to inaccuracies for morphologically complex cells like *Micrasterias*. We developed a new algorithm that reduces such errors. We created a 3D model by stacking flat cross-sectional layers on each other (Fig. 2). These layers are of known thickness, area, and perimeter. When layers are thin and numerous this model accurately approximates the volume and surface of the real object. Therefore, several cell dimensions were measured on frontal and apical views of *Micrasterias* cells made by an Olympus BX51 microscope (Olympus Tokyo Japan) associated with a Canon EOS 60D. We performed image analysis by pixel counts on binary images in software Fiji (Schindelin et al. 2012) and R Statistical Software (R Core Team 2014).

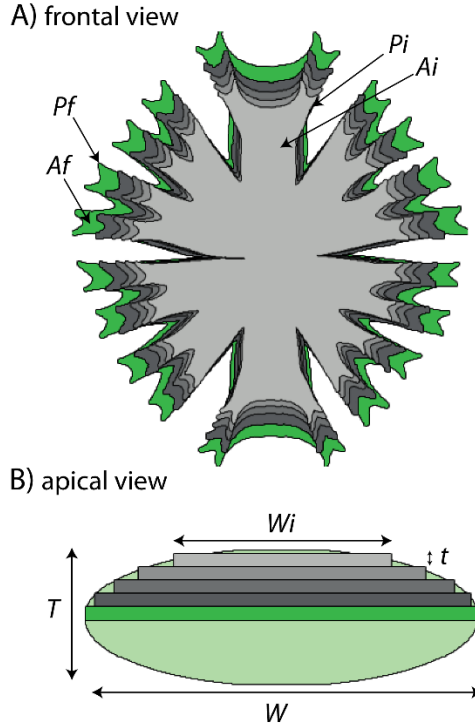


Figure 4.2 Illustration of a 3D mathematical model of cell created by stacking cross-sectional layers. A) frontal view, green layer is the frontal view of the cell B) apical view of stacked layers, green outline represents the apical view of the cell. Frontal view of the cell represents the biggest slice with the area (A_f) and the perimeter (P_f). Apical view of the cell defined maximal cell width (W) and the thickness (T). Stacked layers were of equal thickness (t) and we measured width (W_i) from which we calculated perimeter (P_i), area (A_i) and volume of each slice.

The frontal view represents the central and the largest layer of the cell. We measured the area (A_f) and the perimeter (P_f) of the frontal view (Figure 4.2). The apical view defines the maximum cell width (W) and the cell thickness (T). The picture was divided into n slices of equal thickness $t = T/n$. For each slice i , we measured the maximum slice width (W_i) from the apical cross section. W_i is always a fraction $f_i = W_i/W$ of maximum width W of the cell. For each slice i , the frontal area is therefore $A_i = A_f f_i^2$, the perimeter $P_i = P_f f_i$, the ribbon area $R_i = P_i t$, the volume $V_i = A_i t$. The total volume (V) of the cell is approximated by summing over the slices $V = \sum_{i=1}^n V_i$. Similarly, the total surface area (S) of the cell is approximated by the sum of each slice ribbon area (A_i) and twice the frontal area (A_f) (as for the surface of a coin, adding the ribbon area and the area of the two sides), $S = 2A_f + \sum_{i=1}^n R_i$.

Geometrical summaries of cell shapes

We computed different index to summarize the cell shapes. First, we used circularity

$$\text{Eqn. 5} \quad \alpha = 4\pi A_f / P_f^2$$

to measure the departure from a circular outline for the frontal face. This index ranges from 1 for a perfect circle to 0 for an infinitely elongated polygon (Osserman 1978). Second, we used degree of fractalization

$$\text{Eqn. 6} \quad \beta = S / S_{\text{ellipsoid}}$$

to measure the gain of surface area provided by cell branching. To do so, we computed the ratio of the actual cell surface (S) to the surface ($S_{\text{ellipsoid}}$) of an ellipsoid with the same volume and the same axis lengths as the observed cell. The three axis length of the observed cell are the length (L), width (W) and thickness (T). To compute $S_{\text{ellipsoid}}$, we first estimated how much these axes length need to be shrunk to keep the volume equal to the observed volume, by finding the coefficient k so that $V = \frac{4}{3}\pi \times kL \times kW \times kT$. Then we measured the surface of the resulting ellipsoid using Knud Thomsen's approximation, $S_{\text{ellipsoid}} \approx 4\pi \sqrt[3]{\frac{a^p b^p + a^p c^p + b^p c^p}{3}}$ where $p \approx 1.6075$, and where a , b and c are axis lengths (Klamkin 2006) corresponding to kL , kW and kT .

Third, we used

$$\text{Eqn. 7} \quad \gamma = S_{\text{ellipsoid}} / S_{\text{sphere}}$$

to measure the degree of cell flattening. This index indicates the surface gain obtained by cell flattening in absence of any lobulation or fractalization. To compute S_{sphere} , we simply computed the surface of the sphere that would have the same volume as the observed cell. Finally, we computed the overall gain in surface compared to a sphere with equal volume as

$$\text{Eqn. 8} \quad \delta = \beta\gamma = S / S_{\text{sphere}}$$

Measurement of cell mass

We measured cell mass from a dried weight of storage cultures grown under experimental conditions. We centrifuged four replicates of 2 mL from storage cultures at 1500 rpm for ten minutes using an Eppendorf 5424 centrifuge. We resuspended pellets in a small amount of fresh medium (approximately 200 μ L), transferred the suspension into previously weighted pressed tin capsules and dried those 48 hours at 70 °C. We placed the capsules in a desiccator to cool down at room temperature and weighted those using a Sartorius MC5 microbalance. We estimated cell weight by dividing the dry weight of pellets by the number of cells in 2 mL, previously estimated in storage cultures using a Nageotte hemacytometer. We calculated cell density as dry mass-to-volume ratio.

Statistical analysis

We used standardized major axis regression (SMA) (Warton et al. 2006) to estimate the scaling relationships between traits. SMA regressions were computed using the *smatr* R-package ver. 3.4-3 (Warton et al. 2012). To account for phylogenetic effects we performed phylogenetic standardized major axis regression using the R-package *phytools* ver. 0.6-60 (Revell 2012). For the latter, we estimated the lambda parameter by maximum likelihood. We constructed phylogenetic covariance matrix using published multi-loci phylogeny of *Micrasterias* (Škaloud et al. 2011). Slopes of phylogenetic SMA did not differ from SMA slopes (see Table S 4.3 in Supplementary material). Therefore, we further used only the results from SMA regression and take all the relationships as phylogenetically independent. We tested the correlations between SMA residuals of μ_{max} scaling and morphological and size-related traits in order to describe the influence of allometric optimization on departure from this scaling.

All statistical analyses were performed using R Statistical Software (R Core Team 2014).

RESULTS

We found that the scaling of population growth rate with body mass followed Kleiber's law. The slope of the relationship between μ_{max} and body mass was -0.25 (Figure 4.3, Table 4.1). The slope between μ_{max} and volume displayed a flatter slope -0.20, without differing from -0.25 (test for SMA slope different from -0.25; P -value = 0.16). All scaling relationships are summarized in Table 4.1.

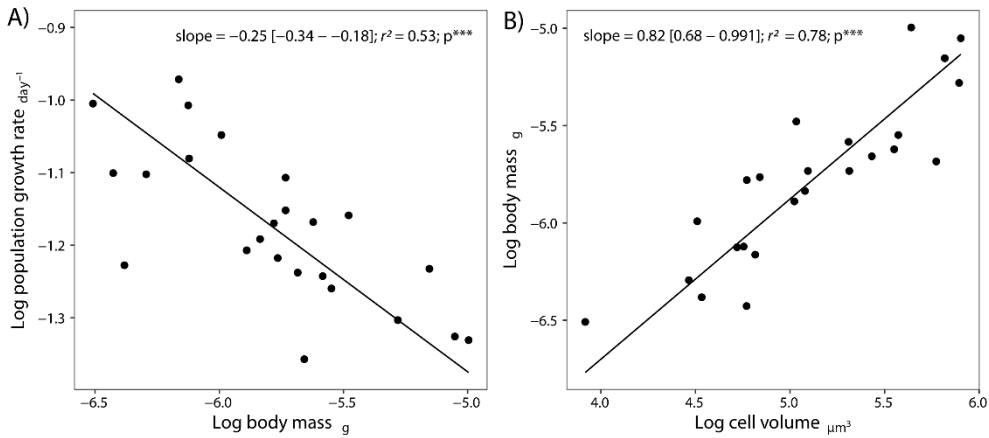


Figure 4.3 Biological scaling for the 24 studied species of *Micrasterias*. A) Population growth rate as a function of cell mass. B) Sublinear scaling of cell dry mass to volume. All variables are log-transformed. Each dot represents the average value of a given species. The black line represents SMA regression. *** P -value < 0.001

Table 4.1 Results of SMA regression of population growth rate and traits linked to body size.

		slope	[CI interval]	r^2	P -value
Growth rate	Cell mass	-0.25	[-0.34 - -0.19]	0.54	< 0.001
	Cell volume	-0.20	[-0.27 - -0.15]	0.55	< 0.001
	Cell surface	-0.27	[-0.37 - -0.20]	0.52	< 0.001
Cell mass	Cell volume	0.80	[0.64 - 0.99]	0.76	< 0.001
Cell surface	Cell volume	0.75	[0.67 - 0.84]	0.93	< 0.001
	Cell mass	0.93	[0.75 - 1.16]	0.76	< 0.001

Cell density, expressed as mass-to-volume ratio, was not correlated to the residuals of the μ_{max} vs. body mass scaling ($r = 0.17$, P -value = 0.42): lower density at a given body mass did not result in higher population growth rate. Similarly, no correlation was found between the residuals of the μ_{max} vs body mass and cell circularity ($r = -0.02$, P -value = 0.9), degree of flattening ($r = 0.11$, P -value = 0.59), degree of fractality ($r = 0.22$, P -value = 0.22), overall gain in surface ($r = 0.18$, P -value = 0.41), cell surface area ($r = 0.21$, P -value = 0.32) nor residuals of surface vs. body mass scaling ($r = 0.14$, P -value = 0.50). Therefore, higher surface area at a given cell size did not result in higher population growth rate.

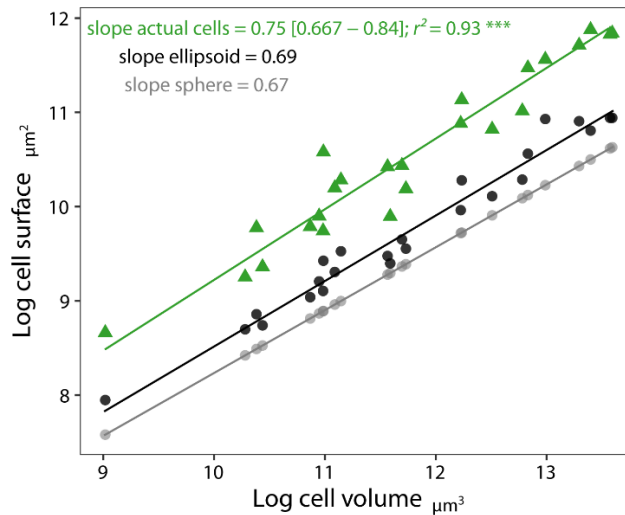


Figure 4.4 Comparison of surface area to volume scaling of *Micrasterias* cells (green triangles) and modelled theoretical shapes. Grey points represent spheres with a volume equal to actual cells. Black points represent ellipsoids with equal volume and same axis proportions (i.e. same global flattening). Only actual cells that are both flattened and with fractal-like morphology depart from Euclidean scaling. Lines represent SMA regressions. *** P -value < 0.001

We found that body mass scaled sublinearly with cell volume (SMA slope = 0.80; CI = [0.64 - 0.99]) (Table 4.1, Figure 4.3). Scaling of cell surface area to volume significantly departed from Euclidean scaling of $\frac{2}{3}$ (Figure 4.4; Table 4.1 SMA slope = 0.75; CI = [0.667 - 0.842]). Both flattening and fractality led to an increase in surface area, compared to hypothetical shapes (Figure 4.4). However, the slope of the surface to volume for an ellipsoid did not differ from $\frac{2}{3}$ (SMA slope = 0.69; CI = [0.64 - 0.75]). Only fractality elevated the slope of the surface to volume scaling from $\frac{2}{3}$ to $\frac{3}{4}$. Moreover, surface area increased isometrically with body mass (SMA slope = 0.93; CI = [0.75 - 1.16]). Residuals from surface area to volume scaling were negatively correlated with departure from cell

circularity (Pearson's $r = -0.796$, P -value < 0.001) and positively with degree of fractality (Pearson's $r = 0.73$, P -value < 0.001).

DISCUSSION

The universality of size-growth relationship is a puzzling issue, especially in face of the huge diversity of forms of organisms. Here, we focused on unicellular green alga *Micrasterias*, a genus with a large diversity of cell sizes and shapes, including diverse degrees of fractal morphology. *Micrasterias* is comparable to both small morphologically simple unicellulars and multicellular organisms with compartmentalized cells and fractal transport networks. *Micrasterias* possibly represents a key transitional group in the evolution of allometry, offering a unique opportunity to investigate geometrical constraints of cell morphology on growth rates. We measured the growth rate, size, and morphological traits of 24 *Micrasterias* species. DeLong et al. (DeLong et al. 2010) predicted that large unicellulars would have lower scaling, due to increased external and internal constraints. We found that size-dependent variation in μ_{max} of *Micrasterias* did not deviate from Kleiber's law (see Figure 4.3) (Savage, Gillooly, Woodruff, et al. 2004). We showed that each species deviation from the general Kleiber's law (residuals from μ_{max} scaling) were correlated neither with traits linked with cell shape (fractality, flattening, circularity, and overall gain in surface area) nor with cell density. We conclude that the population growth rate and its scaling are independent of the geometry of the organism, beyond the effect of body mass or volume. Phenotypic changes are more likely to affect the optimization of the scaling between surface area and mass. Such allometric optimizations should relax biophysical constraints acting on relatively large cells of *Micrasterias* and enable to reach optimal quarter-power scaling.

What are the biophysical constraints underlying $-1/4$ scaling in *Micrasterias*? If μ_{max} was limited by external diffusion constraints, it should proportionally increase with surface area. Nevertheless, we found sublinear scaling of μ_{max} with the surface area ($b = -0.26$) (Table 4.1). The quarter-power relationship between μ_{max} and body mass is consistent with resource-transport models, where the μ_{max} limitation is caused by constraints associated with nutrient transport (West, Brown, and Enquist 1997; Banavar et al. 2010). Our results therefore suggest that μ_{max} scaling is mostly governed by internal

constraints, in agreement with an experiment of Marañón et al. (Marañón et al. 2013) on phytoplankton. Although they demonstrated non-linear growth scaling, the deviation was at picoplankton size range and was caused by unbalance between nutrient uptake and requirements. The value of the scaling exponent reported for cells larger than $40 \mu\text{m}^3$ did not differ from the value observed in our experiment. Marañón et al. (2013) argued that nutrient uptake abilities of larger cells are higher than their requirements, therefore the growth of larger cells are likely to be limited by internal transport and assimilation only. The intracellular diffusion limitation is often overlooked but could represent a very important constraint in unicellular organisms (Gallet et al. 2017). It is also a good candidate for a constraint limiting the upper size of the unicellular body. It would explain why extremely large protists, such as *Halimeda* or *Caulerpa*, have siphonous body plans (large cell with multiple nuclei and cytoplasm streaming). Multinucleate cells would minimize the limiting distance from the nucleus to the periphery. Additionally, cytoplasm streaming would assist the distribution of metabolites within the cell. Our results showed that even though surface to volume ratio is typically less favourable for large sizes (Niklas and Cobb 2017), μ_{max} of *Micrasterias* is not limited by surface area, indicating the existence of effective allometric optimization strategies (Okie 2013).

The most apparent allometric optimization strategy of *Micrasterias* is cell fractality. West et al. (G. B. West, Enquist, and Brown 1999) proposed fractality as a feature elevating scaling of surface area to volume of resource-transport networks to allometrically optimal $\frac{3}{4}$. We support that fractality in *Micrasterias* arose as a mechanism increasing the scaling of the external surface area (Neustupa 2015). Indeed, flattening increased surface area but without departure from isometry of $\frac{2}{3}$. In *Micrasterias* positive surface area to volume allometry ($b = 0.75$) was reached only by fractality (see Figure 4.4). Moreover, residual variation from the surface to volume scaling was positively correlated with fractality. Species with a higher degree of fractality and departure from circular shape have an even larger increase in surface area. Interestingly Neustupa (2015) reported higher values of surface area to volume scaling ($b = 0.91$) for different set of *Micrasterias* species. The outer body fractality of *Micrasterias* differs from the self-similar organization of resource-transport networks proposed by West et al. (1997; 1999), but it functions similarly. It maximizes the exchange surface area to the volume needed to be supplied. Possibly it could also represent a branching distributional network facilitating inner diffusion

transport. The shape and its link to external diffusion have been already described (see e.g. Karp-Boss and Boss 2016; Young 2006), but the role of cell shape on internal diffusion have been overlooked and should be explored in the future (Gallet et al. 2017).

The cell size has two components: volume and mass. Our results indicate that fractality is not the only mechanism to overcome constraints imposed by size. Surface area to dry cell mass allometry was elevated even higher than $\frac{3}{4}$ to $b \approx 1$ and cell mass scaled with volume sublinearly ($b = 0.80$). Sublinear scaling of mass with volume is not new (for similar results e.g. Niklas 1994a; Menden-Deuer and Lessard 2000; Gallet et al. 2017), but it is particularly noteworthy. Many metabolic scaling studies assume size-invariant density (Banavar et al. 2010; DeLong et al. 2010) neglecting that dilution of cell content is an important allometrical optimization strategy (Okie 2013). The dilution enables an increase in size without a major increase in metabolic active volume, it decreases phosphorus and nitrogen minimal quotas (Niklas 1994b) and leads to dilution of metabolic and structural components (Okie 2013). An experimental test done by Gallet et al. (2017) showed that the evolution of bigger and faster-growing bacteria is linked to cell dilution. However, here we did not find similar results since cell density was not related to μ_{max} or its residuals.

In this study, we showed that complex morphological diversification strategies can represent “evolutionary escape route from constraints imposed on physiological functions” (Niklas and Cobb 2017). Combination of size-related shape modification and cell dilution enables *Micrasterias* not to be limited by surface area and maintain quarter-power scaling of μ_{max} . The existence of a group of large protists following Kleiber’s law would support the universal stabilizing selection towards quarter-power scaling that was suggested for all the living organisms (Niklas and Enquist 2001) or at least within the evolution of Viridiplantae (Enquist, Tiffney, and Niklas 2007).

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Supporting information

Table S 4.1 List of *Micrasterias* strains used in the study with measured growth rate and morphological traits. Strains originated from MZCH-SCVK Hamburg Collection (SCVK) and from CAUP Culture collection (K 605). α - circularity, β Degree of fractalization λ Degree of flattening δ The overall gain in surface

Taxon name	Strain number	Growth rate (day ⁻¹)	Cell volume (μm^3)	Cell surface (μm^2)	Surface-to-volume	Cell mass (μg)	Mass-to-volume	α Circularity	β Degree of fractalization	γ Degree of flattening	δ The overall gain in surface
<i>Micrasterias americana</i>	SCVK 126	0.064	119903.9	34056.8	0.28	14.6	12.2	0.113	2.19	1.33	2.92
<i>Micrasterias apiculata</i>	SCVK 247	0.059	657675.5	143829.9	0.22	70.2	10.7	0.041	2.92	1.36	3.96
<i>Micrasterias cera tofera</i>	SCVK 355	0.057	203784.8	53186.5	0.26	26.1	12.8	0.094	2.51	1.28	3.2
<i>Micrasterias ceylanica</i>	SCVK 291	0.079	58865.5	17013.9	0.29	3.7	6.4	0.22	1.89	1.24	2.34
<i>Micrasterias conferta</i>	SCVK 110	0.083	56801.7	19915.9	0.35	7.6	13.3	0.104	2	1.41	2.81
<i>Micrasterias crux-melitensis</i>	SCVK 72	0.098	52410.1	17809.1	0.34	7.5	14.3	0.178	2.11	1.25	2.65
<i>Micrasterias decemdentata</i>	SCVK 542	0.059	34090.8	11626.8	0.34	4.2	12.2	0.183	1.86	1.24	2.3
<i>Micrasterias denticulata var. angulosa</i>	SCVK 50	0.05	783735.5	136941.9	0.17	52.4	6.7	0.064	2.42	1.38	3.36
<i>Micrasterias fimbriata</i>	SCVK 41	0.047	799730.5	138629.4	0.17	88.9	11.1	0.072	2.45	1.37	3.35
<i>Micrasterias furcata</i>	SCVK 75	0.068	59033.3	39314.9	0.67	16.6	28.1	0.026	3.17	1.7	5.4
<i>Micrasterias jenneri</i>	SCVK 298	0.068	355185.3	60758.6	0.17	23.9	6.7	0.163	2.07	1.22	2.52
<i>Micrasterias lateps</i>	SCVK 121	0.044	270573.3	50029.6	0.18	22	8.1	0.156	2.03	1.23	2.49
<i>Micrasterias muricata</i>	SCVK 125	0.062	105324.9	33610.8	0.32	12.9	12.2	0.105	2.58	1.22	3.14
<i>Micrasterias novae-terrae</i>	SCVK 410	0.061	69157.9	29205.4	0.42	17.2	24.9	0.053	2.13	1.7	3.61
<i>Micrasterias papillifera</i>	SCVK 71	0.07	205598.5	68409.2	0.33	18.5	9	0.035	2.35	1.74	4.09
<i>Micrasterias pinnatifida</i>	SCVK 99	0.099	8250.1	5781.8	0.7	3.1	37.6	0.123	2.04	1.44	2.95
<i>Micrasterias radians var. borogenesis</i>	SCVK 389	0.09	32270.5	17615	0.55	10.2	31.6	0.048	2.5	1.45	3.62
<i>Micrasterias radiosa</i>	SCVK 154	0.107	65409.7	26868.2	0.41	6.9	10.5	0.053	2.44	1.41	3.45
<i>Micrasterias raffii</i>	SCVK 300	0.069	107874.4	19825.3	0.18	33.2	30.8	0.488	1.64	1.11	1.82
<i>Micrasterias rotata</i>	SCVK 26	0.058	592904.7	121959.4	0.21	20.7	3.5	0.057	2.24	1.61	3.6
<i>Micrasterias thomasiana</i>	K 605	0.047	437096.3	104979.5	0.24	101	23.1	0.045	1.88	2.02	3.8
<i>Micrasterias thomasiana</i>	SCVK 8	0.055	373495.9	95885.8	0.26	28.3	7.6	0.039	2.48	1.55	3.85
<i>Micrasterias tropica</i>	SCVK 368	0.079	29138.1	10440.5	0.36	5.1	17.4	0.254	1.74	1.32	2.3
<i>Micrasterias truncata</i>	SCVK 18	0.078	124275.3	26624.6	0.21	18.5	14.9	0.242	1.89	1.18	2.23

Table S 4.2 Results of phylogenetically informed reduced major axis regression of population growth rate and traits linked with body size. Parameter λ was estimated by ML.

		phylo RMA			OLS		
		<i>slope</i>	r^2	λ	<i>slope</i>	r^2	<i>p value</i>
<i>Growth rate</i>	<i>Cell mass</i>	-0.26	0.54	0.42	-0.25	0.54	< 0.001
	<i>Cell volume</i>	-0.24	0.397	0.57	-0.20	0.55	< 0.001
	<i>Cell surface</i>	-0.31	0.39	0.60	-0.27	0.52	< 0.001
<i>Cell mass</i>	<i>Cell volume</i>	0.87	0.70	0.35	0.80	0.76	< 0.001
<i>Cell surface</i>	<i>Cell volume</i>	0.79	0.899	0.78	0.75	0.93	< 0.001
	<i>Cell mass</i>	0.89	0.71	0.37	0.93	0.76	< 0.001

Table S 4.3 Correlations of morphological traits and residuals growth rate allometry scaling. Residuals come from mass-based and volume-based SMA regressions. None of the correlations was significant at 0.05 level

	<i>residuals of</i>			
	$\mu_{max} \sim \text{body mass}$		$\mu_{max} \sim \text{cell volume}$	
	<i>Pearson's r</i>	<i>p value</i>	<i>Pearson's r</i>	<i>p value</i>
<i>Cell density</i>	0.17	0.42	-0.39	0.056
<i>Cell surface</i>	0.21	0.32	0.34	0.096
<i>Circularity</i>	-0.02	0.9	-0.14	0.5
<i>Degree of flattening</i>	0.11	0.59	-0.05	0.82
<i>Degree of fractality</i>	0.22	0.28	0.22.	0.29

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