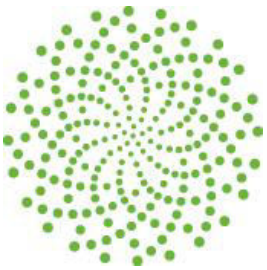
On the Origin of Cryptic Species:

Insights from the *Stygocapitella* species complex

José Cerca

UiO : Universitetet i Oslo



Thesis submitted for the degree of Philosophiae Doctor

Natural History Museum
Faculty of Mathematics and Natural Sciences
University of Oslo

2019

"The beauty and brilliancy of this insect are indescribable, and none but a naturalist can understand the intense excitement I experienced when I at length captured it. On taking it out of my net and opening the glorious wings, my heart began to beat violently, the blood rushed to my head, and I felt much more like fainting than I have done when in apprehension of immediate death. I had a headache the rest of the day, so great was the excitement produced by what will appear to most people a very inadequate cause."

Alfred Russel Wallace

"Em cada esquina um amigo, em cada rosto igualdade."
(In each corner a friend, in each face equality)

José Afonso – Zeca

"Não sou nada.
Nunca serei nada.
Não posso querer ser nada.
À parte disso, tenho em mim todos os sonhos do mundo."
(I am nobody.
I will never be anything.
I cannot desire to be anything.
Other than this, I hold every dream in the world.)

Fernando Pessoa

Contents

Acknowledgments

I belong to a lineage from Portugal's rural interior. Growing up in an isolated city in the interior of the country, I never had many goals or ambitions. As a 17-year-old I took a 'career and intelligence test' which pointed out that I had an IQ far lower than the world average. Going to University was not a tough choice though – everyone else did it, and it was only natural that I followed the herd. My first choice was Sports' science, but by the time I had made my choice I had already missed the mandatory physical exams. Back then, I had a supportive biology teacher who helped me understand that I was passionate about biology. I went to University to study biology with no concrete goal in mind, but knew I would be happy becoming a biology high-school teacher. During my period at the University of Coimbra I met incredible people who helped me, stimulated me, and trained me – I developed a liking for plant taxonomy and eventually did a MSc in pollinator ecology. During those years I took a liking to student politics and debating which helped me mature. It was not until I read for my MSc thesis that I understood that I felt like a fish out of water doing ecology. During that period, I heard a talk of Rosemary Gillespie (ESEB 2013) on adaptive radiation of spiders which helped me understand I was truly passionate about evolutionary biology. Following my MSc, I was unemployed for a year. I felt undone and spent my time trying to read up on evolutionary biology. I read Nosil's Ecological Speciation and Schulter's The Ecology of Adaptive Radiation, which made me understand – that's where I want to go. I want to work with evolution and its interface with ecology and morphology and other domains. Having a background in taxonomy and in ecology, in a time where evolutionary biology was being transformed by the 'genomics-revolution', I could not find a position. I had no network, no support or advice, and did not even understand what I had to do (or the skills to learn) to secure a position. My year of unemployment (note, I took some not-so-relevant jobs) started weighting and defeating me. Nobody was willing to take someone without a skillset in bioinformatics, let alone without much genetics-lab experience. I applied to nearly 40 positions before I took my current position in Oslo.

This background fuels my ambition to work as hard as I can to prove my abilities to myself and to the world. But it also highlights the role of those who believed in me and helped me become who I am. To you, I could not be more grateful. There is not a single day that goes by that I do not acknowledge how lucky I am in having found something that I am so passionate about, and for having had the support to mature and to navigate my way to evolutionary biology. I was lucky I had food, a roof, a bed and a peaceful environment to grow. That being said, I do not think acknowledgements should be laid in paper. Acknowledgements should be done on a daily basis, embodied through caring actions and words, smiles and celebrating each other's success. I thus write these words for personal memories, but also to make sure that in the midst of all our cultural differences, you understand how deeply grateful I am to all of you.

First, and foremost, I am thankful to my main supervisor **Torsten H. Struck**. I cannot thank you enough for believing in my potential from the beginning. For accepting that you had to train me and that you had to be patient. You invested an incredible amount of time in me – helping me in the lab, discussing

PCR-gel results as the photography came out, letters of recommendation, grant proposals, in the field, in the terminal, in manuscripts, research ideas, talks and posters. There are many lessons I have learnt from you, but most importantly, you have taught me to always have a high ethical standard – that piece of rigor that separates excellent science from shenanigans. I hope one day I become a scientist like you and make you proud that I came from your laboratory. To **Mark Ravinet**, for all your advice and support. Hearing how you faced the adversities of your career, and your advice on navigating through graduate school made me spend 4 years admiring you. To **Lutz Bachmann**, for hours and hours of advice – which made me think twice, and perhaps even become less reckless. To **Hugo de Boer** for all your time, advice and encouragement. Your ambition and strategy-minded thinking will always be a reference (and a lesson) to me. To **Dimitar Dimitrov** for your friendship, advice, collaboration and mentorship. It was truly great to have regular lunches with you, as well as to learn from you. To **Mike Nowak**, for always having an open door. To **Günter Purschke** for all your support as my co-advisor and expertise in invertebrate morphology. To **Mark Blaxter** and **Julian Catchen** for receiving me and treating me as one of your own. I hope to carry a bit of Torsten, MarkR, Lutz, Hugo, Mike, Dimitar, Günter, MarkB, Julian with me.

To everyone who has helped me building up this thesis. This includes the three lab warlocks **Lisbeth**, **Jarl Andreas** and **Audun** for your kind support in laboratory work. To **Christian Meyer** for helping in sampling and photographing worms. To those who provided me with laboratory space, funding or help in obtaining specimens including **Andrew Mackie**, **Claudia Miller**, **Christer Erséus**, **Gustav Paulay**, **Henning Reiss**, **Inês Modesto**, **Lis Lindal Jørgensen**, **Natalyia Budaeva**, **Nicola Pennisi**, **Tim Worsfold**, **Vasily Radashevsky**, and relevant funding bodies.

To my kind and supportive girlfriend **Tora Kjærnes Knutsen**, who I want to spend my life next to. For all your love and comprehension (i.e. wasted summer holidays, lonely weeks and weekends). À minha **mãe** e ao meu **pai**, porque apesar de pensarmos de forma diferente, eu sempre vos amei. Aos **meus avós**, que estariam em lágrimas por terem criado 'um doutor'. Ao meu irmão **Luis Nuno** por tanto ser besta como bestial, mas por estar sempre presente quando preciso. To my **close friends** who kept me going and made me constantly feel **saudade** (Wikipedia translation: 'deep emotional state of nostalgic or profound melancholic longing for an absent something or someone that one cares for and/or loves (…). One English translation of the word is missingness, although it might not convey the feeling of deep emotion attached to the word "saudade"'). It is not easy to fight alone and I felt the weight of distance was counteracted by your messages and caring support. Thank you urso-**Manso** (por estares sempre presente quando preciso, e por juntos sermos as duas maiores, mas mais felizes, bestas do mundo), **Ruizinho** (possas – porque não conseguiria ser feliz sem mandar postas contigo!), **Eli** (por seres um amor e um grande apoio; já sabes, se o Rui não der..), **Ivo** (o iron-palhaço que me deu sempre casa e apoio sempre que precisei – mesmo quando não tinha tempo), **Rocha** (pelo constante apoio, desde que nascemos e até aos nossos últimos dias), **Chichorro** (pelo apoio e entendimento nesta jornada de doutoramento), **Pardal** (por estares constantemente preocupada comigo), **Maria Palma** (por teres sempre uma palavra carinhosa),

MafaldaJ (por me teres acompanhado desde sempre e me entenderes como ninguém), **Paula** e **Pedro** (olhem que vou precisar de espaço no vosso casamento – obrigado por estarem sempre presentes), **MafaldaM** (por me diagnosticares que sou obcessivo-compulsivo, hiperactivo e ter um défice de atenção – e por aturares toda uma pancada nestes anos), **Lucas** (por partilharmos ambição e 'background' e crescermos lado-a-lado), **David** (pela nossa amizade de infância e por ter orgulho das tuas causas), **Tiago** da Rapoula (possas, tu sabes – sem ti eu não seria eu), **Tiago** orelhas (por estares sempre presente), restantes ursos do **C.d.B.** (porque posso estar no cú do mundo, mas jamais terei um grupo de amigos como vocês), **Carolina** e **Bárbara Xavier** (bem, não sei. A Bárbara não entrou no parlamento, portanto não sei se quero continuar a ser vosso amigo.. pelo vosso apoio!), **Nuno** do museu (por te lembrares sempre de mim e me quereres sempre bem), **Biotecnólogos da Floresta** (por quererem sempre o meu bem), **Inês Modesta** (pela amizade e por aturares com cada coisa), 'mana' **Bea** (pelo teu entendimento, apoio e carinho), **Deisy** (por todo o teu apoio e entendimento), **Fátima** (for your support), **Nicolas** (for constant support and friendship), and **Cindy** (how would I have made it without you in the past two years? You were one of my most fundamental pillars and I have no words to say how much your friendship means to me). Your love, friendship, charisma, and kindness kept me running. Finally, to **Luisa Queiroz**, **António Pereira Coutinho**, **Paulo Ramos**, **Isabel Costa**, and **Rubén Torices** for having believed in me when nobody else did, for being an instrumental part of the story above, and for helping me become who I am today.

To my **Norwegian friends**. **Ann-Helén**, for all your comradeship and understanding of my world. Thank you for having regular lunches and drinking coffee with me – You know I enjoyed every second of it, and missed having you around every time I left Oslo. The experience at the museum would not have been the same without you. To **Siri**, for being overwhelmingly present, positive and kind. My four years in Oslo would not have been the same without you. You have become one of my best friends and strongest source of support in these years. To **Trude**, for being always being supportive, and for hearing my constant rants and complaints in coffee breaks during the last three years. For becoming one of my best friends and such an important support in this four-year journey. To **Silje**, for being contagiously positive, encouraging and always being able to cheer me up. To the four of you, I really hope our time in Oslo is not over. To **Vincent**, **Irene**, **Brecht**, **Marius** and **Abel** for all the good moments in the museum, to **Aleks**, **Sigrid** and **Sergey** for the great times in Oslo. To **Luís Morgado** for being my best friend in Oslo. You always knew how I felt, and always knew what to say, possibly due to our similar worldview, upbringing in Portugal, and ambition – como é que um gajo sobrevive em Oslo sem ti, pá?

Finally, to all kind people I met throughout my four-year journey in meetings, conferences, courses and seminars. **Alexandra**, **Lene**, **Marie**, **Marco** and **Kerry**, thank you for chatting regularly with me, trying to meet outside conferences, in between flights and courses. To my friends in Illinois and Edinburgh including **Angel**, **George** and your friends, **Niraj**, **Chris** and **Jane**, **Andrea**, **Jessica**. You made my time in Edinburgh and Illinois worthwhile.

'Em cada esquina, um Amigo' (*tr.* In each corner, a friend)

To all of you, thank you..

Manuscripts included as part of this thesis

1. T. H. Struck, J. L. Feder, M. Bendiksby, S. Birkeland, **J. Cerca**, V. I. Gusarov, S. Kistenich, et al. 2018. Finding evolutionary processes hidden in cryptic species. *Trends in Ecology & Evolution*: 3, 33: 153–163.
2. **J. Cerca**, G. Purschke, and T. H. Struck. 2018. Marine connectivity dynamics: clarifying cosmopolitan distributions of marine interstitial invertebrates and the meiofauna paradox. *Marine Biology* 165: 123.
3. **J. Cerca**, C. Meyer, D. Stateczny, D. Siemon, J. Wegbrod, G. Purschke, D. Dimitrov, and T. H. Struck. Deceleration of morphological evolution in a cryptic species complex and its links to paleontological stasis. *Accepted in Evolution.*
4. **J. Cerca**, C. Meyer, G. Purschke, and T. H. Struck. Delimitation of cryptic species drastically reduces the geographical of marine interstitial ghost-worms (*Stygocapitella*; Annelida, Sedentaria). *Minor revision submitted – Accepted in Molecular Phylogenetics and Evolution*
5. **J. Cerca**, M. Ravinet, M. Nowak, T. H. Struck. Big data in biodiversity: genome-level data (ddRADseq) suggests a complex evolutionary history in a morphologically similar cryptic species complex, which is not revealed by few molecular markers. *manuscript format*

Appendices including non-peer reviewed replies and book chapters

1. T. H. Struck, J. L. Feder, M. Bendiksby, S. Birkeland, **J. Cerca**, V. I. Gusarov, S. Kistenich, et al. 2018. Cryptic Species – More Than Terminological Chaos: A Reply to Heethoff. *Trends in Ecology & Evolution*: 3, 33: 310-312.
2. T. H. Struck, **J. Cerca**, 2019. Cryptic Species and Their Evolutionary Significance. *eLS* 1-9.

Summary

The revolution of DNA sequencing in biology unveiled unrecognised genetic diversity in previously established species – cryptic species. Recent evidence suggests that cryptic species may represent an important, yet overlooked, component of biodiversity. Despite this, current definitions of cryptic species have led to the interpretation that this unrecognised diversity stems from artefacts from classifying and delimiting species, that is, deficiently delimited species. This view has fuelled the interpretation that cryptic species do not exist in nature.

As part of this thesis, I challenge this view by suggesting that cryptic species are morphologically identical or quasi-identical species. I provide a framework to identify cryptic species, specifically focused on teasing apart 'taxonomic artefacts' and morphologically identical species (i.e. cryptic species). This framework involves a two-step process consisting of a regular species delimitation, followed by a rigorous investigation of morphological divergence (Struck et al., 2018b, 2018a; Struck and Cerca, 2019).

I apply this framework to the *Stygocapitella* species complex (*manuscripts 3-5*). Species belonging to the *Stygocapitella* species complex were estimated to have diverged ~250 millions of years ago, despite being morphologically very similar (Struck et al., 2017). As part of my thesis, I have collected *Stygocapitella* from every continental coast in the Northern Hemisphere and studied genetic and morphological divergence among these populations. I find several reproductively isolated *Stygocapitella* species to be morphologically similar, some living in sympatry. Comparing to closely related annelid taxa, I show that *Stygocapitella* is morphologically slow evolving (*manuscript 3*). Based on genetic and morphological divergence, I describe 8 new *Stygocapitella* species (*manuscript 4*) and discuss the implications of the detection of cryptic species in biogeography, evolutionary biology, paleontology and systematics (*manuscripts 3-5*). I optimized and applied a whole-genome amplification protocol together with double-digestion Restriction Associated DNA sequencing (ddRADseq), showing that three morphologically similar *Stygocapitella* have a complex demographic history (*manuscript 5*). Finally, I provide a literature survey which demonstrates that the discovery of cryptic species in the meiofauna leads to the reduction of the distribution of the originally described species (Cerca et al., 2018).

My thesis broadly shows that cryptic species represent an important, yet overlooked component of biodiversity. Deceleration of morphological evolution has the potential to bridge the gap between paleontological stasis and extant cryptic species complexes. I find that failing to detect cryptic species results in the overlook biogeographic breaks and in the inflation of species' distributions. I discuss the importance of understanding and describing cryptic species in evolutionary biology, systematics, paleontology and biogeography.

Introduction

Biological diversity

Describing and delimiting biodiversity is one of the most arduous tasks in biology, due to the diversity and complexity of life. Facing severe underfunding, the resources and funding needed to collect, describe and preserve organisms jeopardizes our ability to understand natural history biodiversity. However, facing global biodiversity losses, environmental changes and human destruction of habitats, studying and cataloguing biodiversity is of fundamental importance and urgency as we will leave a less diverse planet to future generations.

The species is the fundamental unit to characterize, delimit and understand biodiversity, serving as a pillar to obtain general inferences on patterns and processes in various disciplines including ecology, evolution, and biogeography. Biodiversity research is currently undergoing major changes and progress, driven by the recent revolutions of DNA barcoding and High-Throughput Sequencing. DNA barcodes, that is a species-specific DNA identifier, have become a standardized and practical approach to delimit species. Each species is customarily associated with a "DNA barcode", which allows a fast, and somehow reliable identification of a species, without requiring much taxonomic expertise. However, the application of this process has revealed a hidden layer of diversity within previously established species.

Biological diversity and cryptic species

The upheaval of DNA sequencing in biology uncovered unrecognised genetic diversity in previously established species – cryptic species (Bickford et al., 2007; Fišer et al., 2018; Struck et al., 2018a). Cryptic species have been found in all major biological groups (Hawksworth and Lücking, 2017; Leasi and Norenburg, 2016; Payo et al., 2013; Singer et al., 2018; Surveswaran et al., 2018; Wada et al., 2013), including well studied groups such as primates (Hotaling et al., 2016), amphibia, reptiles and crustaceans (Pérez-Ponce de León and Poulin, 2016). In some cases, the discovery of cryptic species involved more than ten overlooked lineages, some of them occurring in sympatry (Kon et al., 2007). Estimations of cryptic species in the sea include numbers as high as 9,000 – 35,000 species (Appeltans et al., 2012), hence potentially representing a substantial, yet hidden, fraction of biodiversity.

Cryptic species were defined as "two or more distinct species that are erroneously classified (and hidden) under one species name." (Bickford et al., 2007). This definition has been widely accepted and cited, having become the most commonly used definition. However, it has recently prompted criticism because it focuses on the taxonomic history of the species complex (Korshunova et al., 2019, 2017). For example, rates of cryptic species are expected to be higher in groups whose 'taxonomic schools' favoured a conservative approach to delimitating species (traditionally called the "lumpers"), as opposed to those prone to splitting species (the "splitters") (Endersby, 2009). In this way, rates and the occurrence of cryptic species could reflect a taxonomic artefact, rather than an underlying biological phenomenon,

leading to the opinion that the term cryptic species should be avoided, or used as a temporary formalization of the problems associated with delineation of species complexes (Korshunova et al., 2019, 2017).

An opposing view is to consider cryptic species as the outcome of biological processes leading to slow morphological evolution (Chenuil et al., 2019; Fišer et al., 2018). Biological species are expected to remain morphologically similar under 'morphological stasis', in scenarios of stabilizing selection for morphology (Futuyma, 2010; Hansen and Houle, 2004), hybridization (Futuyma, 2010), due to particularities of their ecosystems and habitats (Sheldon, 1996), or facing constraints to evolution (Futuyma, 2010; Hansen and Houle, 2004; Maynard Smith et al., 1985). In such scenarios, species are expected to remain similar due to the extrinsic (i.e. ecology, habitat, biotic and abiotic interactions) and intrinsic properties (i.e. developmental constraints, genetic constraints) of their biology, in opposition to being taxonomic artefacts. However, morphological stasis has received attention mostly in the paleontological literature as part of the theory of punctuated equilibrium (Eldredge and Gould, 1972). As a result, morphological stasis has received attention mostly from theoretical models, paleontological data and commentaries (Estes and Arnold, 2007; Futuyma, 2005; Hansen, 1997; Hansen and Houle, 2004; Voje et al., 2018), leaving a gap between these and extant taxa (but see Wada et al., 2013; Swift et al., 2016).

The interstitial environment and cryptic meiofaunal species

A group of organisms with a particularly high incidence of cryptic species is the meiofauna living in interstitial sediments (Giere, 2009; Jörger and Schrödl, 2013). 'Meiofauna' usually refers to organisms living in the interstitial environment or the space available between sand grains, and is defined by sizes approximately between 22 μm and 1000 μm. These organisms were first described in the 19th century (Dujardin, 1851; Lovén, 1844), yet their diversity remained unappreciated for decades. Today we know that sediments in beaches are inhabited by a bewildering diversity of animal groups, with 23 out of the 34 metazoan phyla having meiofaunal representatives and four animal phyla being exclusively meiofaunal (Gnathostomulida, Kinorhyncha, Loricifera and Micrognathozoa) (Giere, 2009). The meiofauna is usually considered as distinct from micro- and from macro-fauna, being its own independent ecological evolutionary unit (Giere, 2009). Its adaptations to the spatially restricted interstitial environment are its most remarkable and distinctive set of traits. The 'meiofaunal syndrome' (Brenzinger et al., 2013; Jörger et al., 2014) describes the general uniform, elongated, worm-like body shape and overall simplified external organization with adhesive structures, a set of traits well-adapted to life in the sediment (Giere, 2009).

The small size of the meiofauna and the absence of pelagic larvae have led biologists to describe these organisms as sedentary and having limited dispersal capacities (Danielopol and Wouters, 1992; Giere, 2009; Sterrer, 1973). This contrasts with their wide distribution ranges, often encompassing whole continental coastlines, being amphi-oceanic or even cosmopolitan (Cerca et al., 2018; Gerlach, 1977; Giere, 2009; Jörger et al., 2012; Kajihara et al., 2015; Sterrer, 1973; Westheide, 1977; Westheide and Rieger, 1987)

– a contradiction which became known as the 'meiofauna paradox' (Cerca et al., 2018; Giere, 2009). Recent evidence suggests that the presence of cryptic species (i.e. hidden diversity) may lead to the inflation of species distribution (Derycke et al., 2013; Jörger and Schrödl, 2013; Leasi and Norenburg, 2014). In such scenario, species living in separate coastlines would represent different species, but would have been identified as a single cosmopolitan species (Westheide, 2005, 1991).

The *Stygocapitella* study system

The *Stygocapitella* (Annelida: Orbiniidae) genus comprises 3 species of interstitial annelids (Figure 1). The genus was originally described by Knöllner (1934), along with the formal description of the species *Stygocapitella subterranea* based on individuals collected from Baltic coastline of Germany. *Stygocapitella subterranea* was posteriorly reported in Sweden, the Mediterranean (French and Tunisian coastlines), the Black Sea (Romanian coast), in both coastlines of North America, and in New Zealand (Purschke et al., 2019; Riser, 1980; Westheide, 2008, 1990), becoming recognized as a cosmopolitan distributed species. However, the application of RAPDs uncovered a phylogeographic pattern, with phylogenetic analyses separating specimens from the Atlantic from those from the Pacific, and further breaking specimens from the distinct North Atlantic coastlines (Schmidt and Westheide, 2000). Struck *et al.* (2017) described two new *Stygocapitella* species based on specimens from South Africa and Australia, suggesting that individuals from different coastlines represent cryptic species, and estimated that the time of divergence among some cryptic species might be as old as 290 MY. These evidences suggest that the lineages found by Schmidt and Westheide (2000) could potentially represent cryptic species, and the 'cosmopolitan' distribution of *Stygocapitella* could be a result from considering multiple cryptic species as a single species. Additionally, Struck *et al* (2017) reported that these three species were morphologically quasi-identical, only distinct by the presence-absence of specific chaetae. *Stygocapitella* thus stands out as an excellent study system to understand rates of morphological and genetic evolution, as well as to understand the impacts of cryptic species for systematics, biogeography and evolutionary biology.

Objectives and major questions addressed

The main objective of this thesis is to understand whether cryptic species are a mere taxonomic artefact, or whether they are by-products of biological phenomena. To do so, I propose:

Objective 1 A new framework to delimit cryptic species (Struck et al., 2018a, 2018b);

Objective 2 The application of this framework to the *Stygocapitella* species complex;

Objective 3 The investigation of rates of morphological and genetic evolution in *Stygocapitella* using phylogenetic and population genetic tools (*manuscripts 3-5*).

Objective 4 To fully understand the impact of cryptic species in various disciplines of biology, a literature survey to understand the incidence of cryptic species in meiofauna, and its contribution to the 'meiofauna paradox' (Cerca et al., 2018).

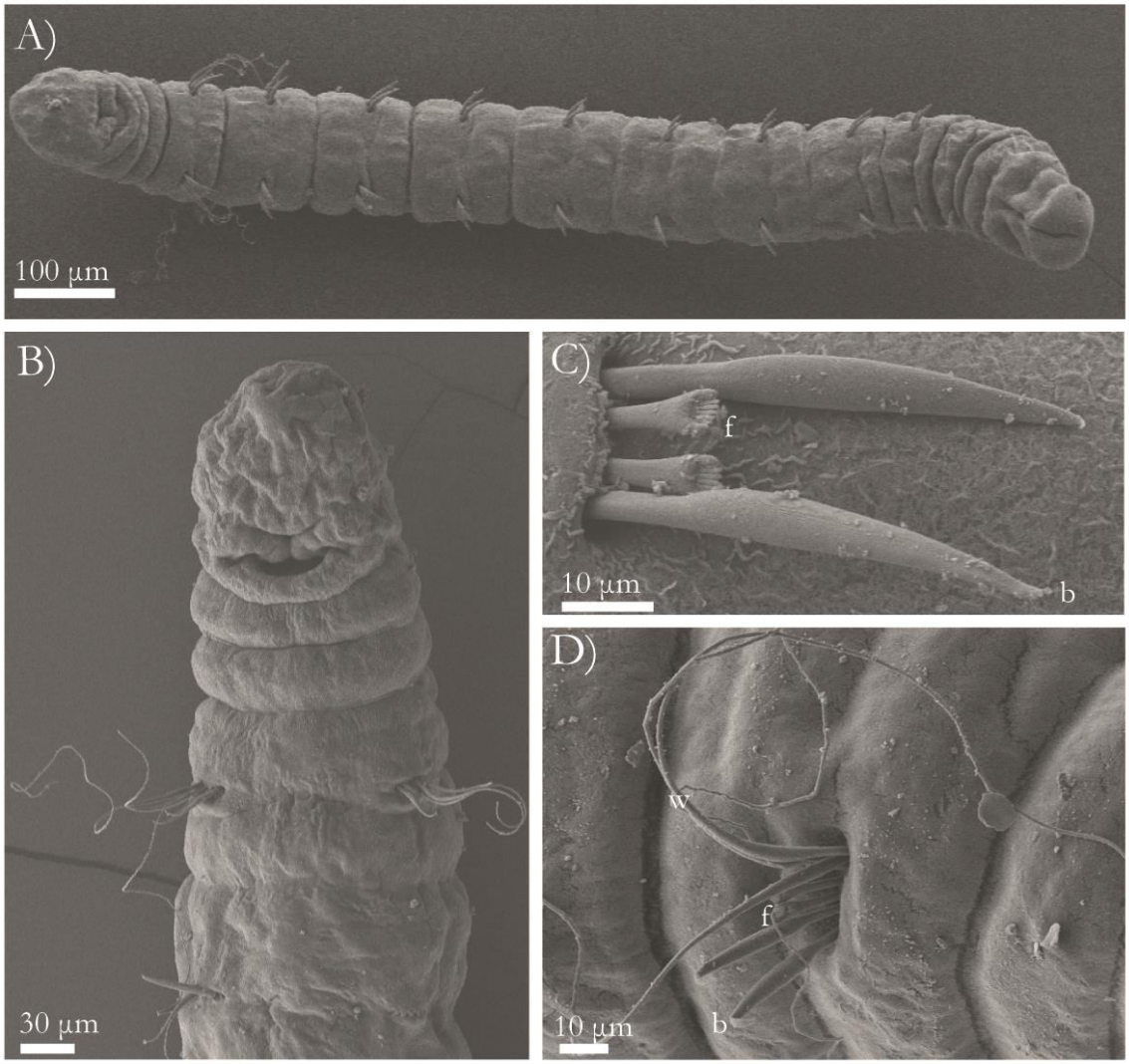


Figure 1. Scanning Electron Microscopy of *Stygocapitella* spp. including (A) photos of a complete organism, (B) the prostomium, (C) the chaetal pattern found in every *Stygocapitella* species in segments 3-10, and (D) the characteristic whip-like chaeta. Magnification is provided for each photo. **(A) Whole organism photograph of *Stygocapitella furcata sp. nov.*** from the 4th of July Beach, Friday Harbor (WA), USA. **(B) Prostomium and first two segments of *Stygocapitella berniei sp. nov.*** from Roche Harbor, Friday Harbor (WA), USA**. (C) Abdominal chaetal pattern of *Stygocapitella josemariobrancoi sp. nov.*** consisting of a bilimbate (b), two forked (f), and a bilimbate chaetae. This pattern is common to the 3rd-10th segment of every *Stygocapitella* species. Photograph from a specimen from Gravesend, UK. **(D) First segment chaetae of *Stygocapitella josemariobrancoi sp. nov.*** with a chaetal pattern consisting of two whip-like chaetae (w), two forked chaetae (f), and two bilimbate chaetae (b). Specimen from Plymouth, UK.

Methods and materials

To meet the proposed objectives, I have conducted two literature reviews (Cerca et al., 2018; Struck et al., 2018a) and analysed empirical data (*manuscripts 3-5*). We conducted literature reviews to understand the impact of cryptic species in various disciplines, justify the need for the new framework, and present a novel framework. The empirical data included morphological, genetic and genomic data and focused on the *Stygocapitella* genus (Figure 1). My results reveal the usefulness and practicality of the established theoretical framework.

Literature review on cryptic species as part of *manuscript 1* (Struck et al., 2018a)

On June 17 2016 we searched for 'cryptic sp*' and downloaded a list of all papers in the ISI Web of Science database. In total, we obtained a list of 6,002 papers from which we scored 606 papers (10%). For each paper, we scored the use of molecular data (i.e. whether it was used or not; whether it included mitochondrial, plastid or nuclear data; the number of obtained loci; whether genome level data had been obtained; quantification of genetic divergence; the use of an outgroup taxa; the use of molecular data; the use fossil-calibration), morphological data (including if it was statistically analyzed and if differences in morphology were found), the use other phenotypic data, the definition of cryptic species, taxa, and whether any species had been described.

Literature review on the meiofauna paradox as part of *manuscript 2* (Cerca et al., 2018)

We did a literature review to understand whether and how the presence and the discovery of cryptic species influenced the distribution of meiofaunal taxa. This survey was carried on June 6th 2018, using the following search terms in ISI Web of Knowledge: "(meiofauna* OR meiobenth* OR Gnathostomulida OR Kinorhyncha OR Loricifera) AND (marine OR Atlantic OR Pacific OR Indian OR Arctic OR Antarctic OR "Southern Ocean") AND (molecular OR cryptic OR paradox OR taxonom* OR dispersal OR phylo* OR biogeo* OR distribut*)". This yielded a total of 1,069 publications, from which we were unable to obtain 16, either due to paywalls (8), being meeting abstracts (3), not being found in google, google scholar or research gate (5). We downloaded and analyzed the abstract and main results of the remaining 1,053 publications, which resulted in the removal of 302 publications because they were written in a language other than English, did not focus primarily on metazoan-meiofaunal taxa, marine areas or did not present new data (i.e. reviews, perspectives and methods papers). The complete list of papers is provided in Supplementary Table 1 as part of Cerca *et al.* (2018).

In the remaining 752 papers, we scored discipline (i.e. Ecology, Evolution, Biogeography, Taxonomy, Development, Physiology, Review or Perspective, Paleontology), taxa (i.e. Phylum and Species), the use of molecular and morphological data, occurrence of cryptic and pseudo-cryptic species (i.e. if these species had been formally described and the number of species), difficulties of taxonomical characterization (i.e. low-morphology problem), distribution range before and after the study, geographical

area studied (i.e. the nearest continent or coastline; including Africa, Antarctica, Australia, Asia, Europe, North and Center America, and South America), sediment depth (supra-littoral, intertidal, subtidal from the low water line to a depth of 200 m, or deep sea below 200 m), occurrence of pelagic larvae, description of the habitat, and the approach to understand dispersal (i.e. experimental or descriptive). To score these fields we defined a specific set of rules which included: (i) we only registered changes in species distributions (before and after study) only if the study provided unambiguous information about the species distribution before and after the study; (ii) when a study focused on more than 2 genera or species, we registered species as NA; (iii) we only considered the use of morphological methods when a study analyzed morphological traits, and not only use it as a means of species identification; (iv) range distribution was considered "local" if it was only known from a restricted area (e.g., coastline of one country), "regional" if present at an entire coastline (e.g., all Europe), "amphi-oceanic" if present in both sides of an ocean and "cosmopolitan" if present in more than one ocean; (v) "phylum" included every phyla studied, even if only one individual from a particular phylum was mentioned; (vi) in micro- and mesocosms, we considered the geographical area with respect to where sediments or water samples were collected; (vii) when a new species was described, regional range was recorded as "NA" before the study and local, regional, amphi-oceanic, or cosmopolitan afterwards.

Fieldwork

Stygocapitella specimens are usually found above the high-water line of stable, sheltered gravel or sandy beaches. We selected sampling sites based on previous records available to us, and by inspecting promising beach areas using google maps (Figure 2 A-G). Upon selection of a sampling site, we dug a hole every meter from the high-water line to the foot of the dune (Figure 2 H). In each hole, we dug to about 1 meter deep and collected samples every 15 cm height (volume of 375 cm^3), until approximately 60-75 centimetres depth. Interstitial invertebrates, including *Stygocapitella*, were anesthetized and extracted from the sediment using the $MgCl_2$ method and sorted under a dissecting microscope (Figure 2 I) (Westheide and Purschke, 1988). *Stygocapitella* is easily identified by the presence of two whip-like chaetae in its first segment. After identification, specimens were preserved either for molecular biology or morphological analyses, either by preservation in 70% ethanol or in SPAFG following Westheide and Purschke (1988). A total of 33 sites spanning every coastline in the Northern Hemisphere were included for this study (Figure 2 A-G).

DNA extraction and Sanger sequencing as part of *manuscripts 3-5*

A detailed account of the molecular methods can be found in *manuscripts 3-4*. We extracted DNA from individual *Stygocapitella* specimens either using a phenol-chloroform or a column-based (E. Z. Kit) approach. We used standard PCR amplification together with Sanger sequencing to sequence the mitochondrial genes Cytochrome c oxidase subunit I (COI) and 16S ribosomal RNA (16S), and the nuclear genes 18S ribosomal RNA (18S) and Internal transcribed spacer-1 (ITS1). Nuclear markers and

COI were amplified using the QIAGEN® Multiplex PCR Kit (Qiagen, Hilden, Germany) in a solution containing multiplex mix, Q-solution, forward and reverse primer, genomic DNA and deionized water. 16S was amplified using a solution which included H_2O, 10x PCR Buffer I (with $MgCl_2$ added), BSA, dNTPs, forward and reverse primer and amplitaq gold. For COI, we used LCO1490-JJ and HCO2198-JJ as primers (Astrin and Stüben, 2008), and for 18S 18e (Hillis and Dixon, 1991) and 18R1779 (Struck et al., 2002). For ITS1, we used the species-specific primers Stygo_ITS1_F and Stygo_ITS1_R, and for 16S 16SarL (Palumbi et al., 1991) and 16S_AN-R (Zanol et al., 2010). Exceptionally, we used polyLCO and polyHCO (Lobo et al., 2016) to amplify COI in Atlantic-American populations. Purified PCR fragments using a 10x dilution of a phosphatase-exonuclease mix were Sanger-sequenced. The 18S fragment required internal sequencing primers, which included 18r, 18L (Hillis and Dixon, 1991), 18F997 (Struck et al., 2002) and 18SF3_Stygo (Struck et al., 2017). After sequencing, sequences were assembled using Geneious

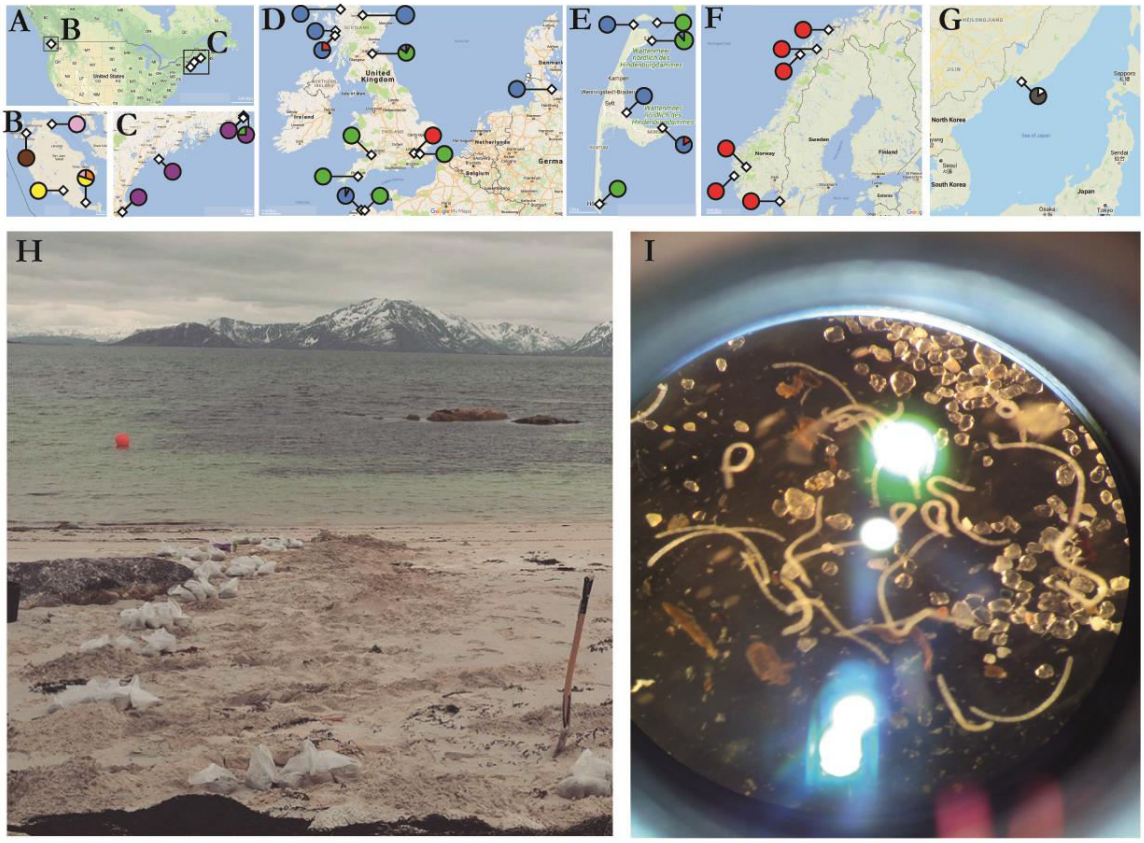


Figure 2. Sampling locations (A-G), a sampling site (H), and view through the dissecting microscope (I). Sampling locations in North America include the (A) North American Pacific coastline, in specific the (B) San Juan Island (WA, USA), and the (C) Atlantic coastline of the USA (ME, MA). Sampling locations in Europe include the (D) UK, France and Germany, (E) the island of Sylt in Germany, (F) and Norway and Sweden. Sampling in (G) Far-East Russia was done in Volchanets (Russia). For GPS coordinates see Supplementary table 1 included as part of *manuscript 4*. Circles denote lineages collected: Orange – *Stygocapitella furcata* sp. nov.; Yellow – *Stygocapitella* Spec. A (undescribed); Brown – *Stygocapitella berniei* sp. nov.; Pink – *Stygocapitella americae* sp. nov.; Purple – *Stygocapitella westheidei* sp. nov.; Red – *Stygocapitella zecai* sp. nov.; Blue – *Stygocapitella subterranea*; Green – *Stygocapitella josemariobrancoi* sp. nov.; Grey – *Stygocapitella budaevae* sp. nov.; White – *Stygocapitella pacifica* sp. nov.. Circles with multiple colors identify sympatric areas. (H) Sampling site in Andøya (Vesterålen), Northern Norway. (I) Meiofauna under the stereomicroscope.

(v6.8.1). The ends of sequences were first automatically, and then manually trimmed to remove primer sequences. Each consensus sequence was visually checked and blasted using NCBI database.

Sanger sequencing as part of *manuscripts 3-5*

We assembled sequenced Forward and Reverse reads into high-quality sequences using Geneious (v6.8.1). After assembling, each sequence was visually checked, and sequences of poor quality were discarded, and ends with poor quality trimmed. Sequences were then BLASTED using the NCBI database to guarantee that no contamination occurred in the dataset. Sequence alignment was done using mafft v7.310 (Katoh and Standley, 2013). COI, 16S and 18S sequences were aligned with a maximum of 1,000 iterations and using the local pair alignment algorithm (Katoh and Standley, 2013). ITS1 sequences required a different approach due to the occurrence of multiple gaps and tandem repeats (sequences ranged from 750 – 1600 bp). As we were not able to align the full ITS1 data, we removed sequences longer than 1100 bp. Removal of these sequences allowed aligning the remaining sequences using the global pair alignment algorithm as part of mafft, which accounts for gap-rich sequences.

Double Digestion Restriction Associated Digestion sequencing (ddRADseq) as part of *manuscript 5*

To obtain a genome-level dataset we combined a whole-genome amplification approach (WGA) (Golombek et al., 2013) with a Double Digestion Restriction Associated Digestion sequencing protocol (ddRADseq) (Peterson et al., 2012). Building up on results from *manuscripts 3-4*, we selected specimens from three morphologically-similar cryptic species occurring in the Atlantic, which form a monophyletic clade (*Stygocapitella subterranea*, *S. josemariobrancoi* sp. nov., *S. westheidei* sp. nov.). A WGA approach was necessary due to the low DNA concentration yielded by the extractions, and permitted increasing the amount of genomic DNA of the organisms. To amplify the DNA, we used the Illustra GenomiPhi HY DNA Amplification Kit (GE Healthcare Life Science) and followed the manufacturer's instructions. This method essentially relies on the activity of the Phi29 DNA polymerase in combination with random sequence hexamer-primers. This polymerase synthesizes DNA in an isothermal process, thus increasing the amount of DNA in a sample.

The amplified genomic DNA was then normalized and used for a ddRAD library preparation. First, we digested the DNA using the restriction enzymes Pst-I HF and MseI, using a mix of the Cutsmart buffer, enzymes and DNA. We purified the product using ampure-beads to remove enzymes and salts, and eluted DNA in purified water. Second, we ligated adaptors to the digested DNA, using a DNA-ligase, ligase buffer and illumina adapters with barcodes. Third, we ran a size-selection step using Blue Pippin using the BDF2010 (100-600 bp cassette) selecting for fragments sized between 300-600 bp. Fourth, we did a library amplification step by doing 18 PCR cycles. These samples were then sent for Illumina Sequencing on an Illumina Hi-Seq 6000.

Morphology data collection as part of *manuscripts 3-4*

We quantified morphological divergence using morphological measurements based on light microscopy photos. First, we fixed individuals in the field in 70% ethanol and photographed these at 10X amplification in the laboratory. Whole-organism photographs were stitched together using photoshop and we measured body length and width, prostomium length and width, and pygidium length and width using ImageJ. We measured these traits in a total of 133 individuals including 11 specimens of *Stygocapitella minuta* and 10 specimens of *S. australis* (Struck et al., 2017), and 112 from *S. subterranea*. However, we split *Stygocapitella subterranea* into nine different new species, and measurements from 112 specimens included six from an unassigned pacific clade, seven individuals from *S. berniei* sp. nov., seven individuals from *S. americae* sp. nov., nine from *S. westheidei* sp. nov., 14 from *S. zecai* sp. nov., 24 from *S. subterranea*, 30 from *S. josemariobrancoi* sp. nov., eight from *S. budaevae* sp. nov. and two from *S. pacifica* sp. nov..

To complement morphological measurements, we looked for morphological differences in detail using Scanning Electron Microscopy (SEM). Specimens were rinsed in phosphate buffer, then treated with a buffered 1% OsO_4 solution for one hour at ambient temperature, and dehydrated in a graded ethanol series starting in 30% ethanol and finishing in 100% ethanol. Dehydrated specimens were then critically-point-dried with CO_2, mounted on aluminium stubs and sputter-coated with platinum. Based on the photographs obtained using a Zeiss Auriga field emission SEM we determined number of segments with chaetae (chaetiger) and the type and number of chaetae in each chaetiger.

To understand the rates of morphological evolution within *Stygocapitella*, we compared these with those from closely related groups (Struck et al., 2015). We selected 12 species from Orbiniidae and 11 Nerillidae, for which morphological and molecular datasets exist (Bleidorn et al., 2009; Struck et al., 2015; Worsaae, 2005; Zrzavý et al., 2009), and which have a similar degree of genetic divergence (Struck et al., 2015). The integration of this data led to a morphological data matrix consisting of 32 species (nine from *Stygocapitella* (including the newly described species), 11 from Nerillidae, and 12 from Orbiniidae) and a total of 75 morphological characters.

Phylogenetic and molecular clock analyses as part of *manuscript 3-5*

We downloaded Orbiniidae outgroup sequences from GenBank. These, together with the *Stygocapitella* data, were aligned using mafft as described above. After alignment, we concatenated the dataset using FASconCAT (Kück and Meusemann, 2010), and ran a partitioned Maximum Likelihood (ML) using IQ-tree (Chernomor et al., 2016; Hoang et al., 2017; Nguyen et al., 2015), defining an automatic determination of the best substitution model for each gene independently. To obtain complementary evidence, we ran a Bayesian tree using BEAST v2 using COI and 18S (Bouckaert et al., 2014). To do so, we used IQ-tree's ModelFinder to determine which substitution models best fit the dataset (Kalyaanamoorthy et al., 2017). After model selection, we fit TNe+I for 18S and TIM+F+I+G4 for COI (F= Empirical base frequencies; I = Invariable sites; G = Gamma model). A relaxed, log-normal

clock was applied with a substitution rate of 0.0001425 for 18S (Struck et al., 2017) and 0.0176 for COI (Lehmacher et al., 2016). A birth-death model was applied and a MCMC chain run for 100,000,000 generations sampling every 100,000 generation. Convergence was confirmed using Tracer (Rambaut et al., 2007). A Maximum Credibility Consensus Tree was obtained using TreeAnnotator, with a 10% burnin (Bouckaert et al., 2014). Finally, to verify the congruence in the dataset we obtained maximum likelihood trees for each gene separately.

Haplotype network as part of *manuscript 4*

We built haplotype networks of COI, 16S, 18S and ITS1 using TCS (Clement et al., 2000). We calculated a 95% connection limit to partition the dataset and considered gaps as "fifth state" to account for indels. Graphical representation was done with tcsBU (Múrias Dos Santos et al., 2015) and using Adobe Illustrator.

Species delimitation methods as part of *manuscript 4*

We adopted several approaches to do species delimitation following best practices (Carstens et al., 2013). These include a General Mixed Yule Coalescent (GMYC) model (ran online at https://species.h-its.org/gmyc/; (Fujisawa and Barraclough, 2013)), a Bayesian Poisson tree processes (bPTP) model (ran online at https://species.h-its.org/ptp/; (Zhang et al., 2013), a 16S- and COI-based 95% connection limit using TCS (Clement et al., 2000), a posterior cut-off at 0.9 following the generated Bayesian tree and a bootstrap cut-off of 95% after the ML tree. To understand how well morphology is able to tease species apart we followed a "morphological species concept", focusing on delimiting species based on the presence of certain chaetal types on the 1st, 2nd, 3rd and consecutive segments – the only diagnostic features we were able to obtain in our data.

Morphological data analysis as part of *manuscripts 3-4*

Using the morphological data of *Stygocapitella*, we used general linear models (GLM), Least Square Means Analyses (Lenth, 2013), PCA and Multidimensional Morphological Disparity (MMD) indices (Struck et al., 2017) to quantify morphological differences. For each measurement, we fit a GLM model, using measurement as the dependent variable and the assigned "species" as a categorical and independent variable. Because GLM models do not allow assessing differences between categorical variables (i.e. "species" in this analysis), we used a Least Square Means approach that provides pairwise statistical comparisons between the categorical variables. We conducted a principal component analyses using all 6 measurements, using the function prcomp included in R's stats-package (R Core Team, 2013). Finally, we applied the MMD index as done in (Struck et al., 2017). This index quantifies the total difference between two individuals, across the totality of all considered principal components. Plotting of results was done using the ggplot2 package (Wickham, 2016) and the Hmisc package (Harrell Jr and Many Others, 2019).

Using the data comprising *Stygocapitella*, Nerillidae, and Orbiniidae, we did a PCA using the "FactoMineR" package (Lê et al., 2008), obtained the values from the first 18 principal components, which together explain 99.07% of the variation, and determined MMD indices (Struck et al., 2017). We tested whether MMD indices were statistically different between *Stygocapitella*, Nerillidae and Orbiniidae using Tukey's HSD and pairwise students' T tests.

To fully contrast morphological and molecular evolution, we compiled a dataset of 18S sequences for the *Stygocapitella* species, together with those from Orbiniidae and Nerillidae. 18S is the slowest evolving gene in the dataset, being thus ideal to analyse distantly related lineages. Based on this dataset, we reconstructed a Maximum Likelihood tree using IQ-Tree (as described above), and obtained pairwise genetic distances between species using MEGA (Kumar et al., 2016). Pairwise MMD indices were plotted against the corresponding pairwise genetic distances using the the R package "ggplot2" (Wickham, 2016), and fitting a 'loess smoothed fit regression' including confidence regions. Lastly, we mapped the morphological characters on the ML tree using Mesquite version 3.51 with the ML reconstruction option. We counted the number of changes occurring at each branch, and plotted these on the ML tree to quantify the number of total changes per branch.

ddRADseq data analysis as part of *manuscript 5*

We obtained a total of 1,277,919,764 reads as a result of two Illumina sequencing lanes (including forward and reverse reads). Using the "process radtags" script, included as part of the STACKS pipeline v2.2 (Rochette et al., 2019; Rochette and Catchen, 2017), we quality-checked reads, discarding those of poor quality. From the original pool of reads, "process radtags" discarded 107,830,588 due to ambiguous barcode (8.4 %), 802,222 due to low quality (>0.1%), and 270,174,154 due to ambiguous RAD-tag drops (21.1%), retaining 899,112,800 reads (70.4%). Retained reads were then assembled to stacks (ustacks), which were used to build a catalog (cstacks) for variant calling (sstacks), we then transposed the data so it is oriented by locus (tsv2bam) and re-called variants in the whole data (gstacks). Following best practices (Paris et al., 2017), we selected stacks with, at the most, 3 variants (single nucleotide polymorphisms; SNPs). To improve the number of retrieved loci and reduce missingness, we ran STACKS for each population independently. This allowed reducing phylogenetic distance and teasing apart between allelic drop-out due to phylogenetic distance as opposed to drop-out due to poor library preparation (Maurstad *et al*, *in prep*). Finally, we obtained variant-call format files and fasta-files for data analysis using the "populations" module included as part of STACKS, restricting variants to being present in at least 50 % of a population (-r 50) and being present in at least 8 populations (-p 8).

Phylogenetic analysis as part of *manuscript 5*

The fasta consisting of the complete radseq locus, comprising 4,737 loci from 70 individuals (12 individuals were removed based on missingness – see below on population genomics) were used in a phylogenetic analysis. These loci were concatenated to a super-matrix consisting of 1,487,496 bp using

FASconCAT (Kück and Meusemann, 2010), which was used to build a phylogenetic tree IQ-tree (Chernomor et al., 2016; Nguyen et al., 2015). We defined an automatic determination of the best substitution model for each locus independently, using default values (Hoang et al., 2017). To guarantee that clades were not grouped following patterns of missing data, we obtained the % of missing data using Base Composition Calculation (BaCoCa) (Kück and Struck, 2014), and plotted these values along the tree using the R package "ape" (Paradis and Schliep, 2018).

Population genomics as part of *manuscript 5*

We obtained a variant-call format file (.vcf) with single nucleotide polymorphisms (SNPs) from STACKS. We pruned polymorphisms based on coverage (minimum depth of 10, maximum depth of 100) and minimum allele frequency (removing variants with > 0.05 %) using vcftools v0.1.13 (Danecek et al., 2011). After this, we removed 12 individuals which had missingness values above 90% (i.e. <10% of the SNPs present) using vcftools and pruned the dataset for linkage by writing a Unix script to keep a single variant per RAD locus. Pruning the data resulted in a dataset of 3,428 SNPs. We analysed the partitioning of genetic variation using PCA, a Multidimensional Scaling (MDS) and STRUCTURE (Falush et al., 2003). As a complement to the phylogenetic analysis, we did a network analysis of the dataset using SplitsTree v4 based on the variant files (Huson and Bryant, 2006).

We analysed the demographic history of the three species (including *Stygocapitella westheidei* sp. nov., *S. subterranea* and *S. josemariobrancoi* sp. nov.) using Site-frequency-spectrum (SFS) coalescent-based simulations as implemented in fastsimcoal v2.6 (Excoffier et al., 2013). This method allows modelling arbitrary scenarios, and provides estimates of several parameters including population size, migration matrixes, time of coalescence in number of generations, population growth and recession rates, which can explain the observed SFS. To implement these simulations, we used the obtained phylogeny, with *S. subterranea* and *S. westheidei* sp. nov. as sister species, and *S. josemariobrancoi* sp. nov. as the sister to these two. Given the phylogeny, we defined models such as no gene flow, ancient gene flow (between *S. josemariobrancoi* sp. nov. and the common ancestor of *S. subterranea* and *S. westheidei* sp. nov.), constant gene flow (between any branch), geographic geneflow (similar as the ancient geneflow, but with modern gene flow between the sympatric *S. subterranea* and *S. josemariobrancoi* sp. nov.), or modern geneflow (gene flow between the three species), modern gene flow between *S. josemariobrancoi* sp. nov. and *S. subterranea*, modern gene flow between *S. josemariobrancoi* sp. nov. and *S. westheidei* sp. nov., and modern gene flow between *S. westheidei* sp. nov. and *S. subterranea*. Each model was run for 10,000 iterations, and the best fitting model was evaluated using a likelihood approach. The obtained parameters associated with the best model were then assessed by means of 100 bootstrap replicates.

Main findings and Discussion

Summary of main findings

This thesis provides a framework to delimit cryptic species, and describes the deceleration of morphological evolution in the *Stygocapitella* cryptic species complex. Building upon the developed framework, I provide evidence for the impact of cryptic species in marine biogeography, with reference to the 'meiofauna paradox'. I find that missing cryptic species can obscure biogeographic breaks and inflate species' distributions.

Focusing on the *Stygocapitella* species complex, I delimit 8 new species, some being morphologically identical. Despite the infered time of divergence of ~270 MY (but notice the confidence interval) of the species complex, very few morphological differences occur in this species complex, thereby being one of the most prominent cases of morphological deceleration in a cryptic species complex. Using genome-level data I found a degree of admixture between species in the Atlantic, potentially due to incomplete lineage sorting, ancient admixture or symplesiomorphic evolution.

For the rest of this discussion I will refer to cryptic species as morphologically identical or quasi-identical species, and distinguish these from poorly delimited species (taxonomic artefacts).

A new framework to understand and delimit cryptic species

The most commonly used definition of cryptic species states that "[cryptic species are] two or more distinct species that are erroneously classified (and hidden) under one species name" (Bickford et al., 2007). As I have suggested as part of Struck *et al.* (2018a, 2018b) and Struck and Cerca (2019), this definition does not separate poorly delimited species (i.e. due to overlooked characters) from morphologically similar species. This separation is of fundamental importance to understand morphological similarity and the deceleration of morphological evolution in nature. For example, clear morphological differences were identified between populations of the *Marphysa sanguinea* complex (Elgetany et al., 2018). Populations are genetically and morphologically distinct, and it was recently concluded that these represent, in fact, different cryptic species given its degree of genetic divergence (Elgetany et al., 2018). Strictly under the definition of Bickford *et al.*, these are two distinct species that were erroneously classified under a single species name (Bickford et al., 2007), and therefore, they have to be classified as cryptic species, despite clear-cut (yet overlooked) morphological differences (Elgetany et al., 2018). On the other hand, morphologically similar *Polygordius* species cannot be considered cryptic species because their original description was based on the continental coastline populations occurred (Ramey-Balci et al., 2012). Using genetic and morphological assessments, Ramey-Balci *et al* have confirmed that the originally described species are indeed genetically distinct and adults are morphologically identical (Ramey-Balci et al., 2012). However, strictly under the Bickford *et al.* definition, this complex of morphologically similar species could not be considered as cryptic species, because they were not 'under one species name'

in the past (Ramey-Balcı et al., 2018). This exposes the arbitrary nature of this definition, and invites for a reassessment of the definition of cryptic species (Struck et al., 2018a, 2018b; Struck and Cerca, 2019).

The separation between species complex which are morphologically identical (i.e. cryptic species), from those which have originated from 'taxonomic artefacts' is not possible when the focus of the delimitation lies in the taxonomic history (Cerca et al., 2018). 'Taxonomic artefacts' are defined as poorly delimited species included as part of a species complex. I have shown as part of Cerca *et al.* (2018), that problems in species delimitation may stem from various causes, including: (i) sloppy taxonomic practices; (ii) the use of taxonomic keys from European and American regions in developing countries (Hutchings and Kupriyanova, 2018); (iii) development of new methods, which uncover previously concealed traits; and (iv) the low morphology problem, that is, the absence of traits with systematic value (Klautau et al., 1999; van Oppen et al., 1996). In any case, the distinction between morphologically similar species, or those resulting from taxonomic artefacts is an important distinction to understand biodiversity, but also to understand underlying evolutionary phenomena (see below) (Pante et al., 2015; Struck et al., 2018a).

The suggested framework to identify cryptic species included as part of Struck *et al.* (2018a, 2018b) and Struck and Cerca (2019), consists in a two-step model. First, a species delimitation step should be done, benefiting from all sources of data including behaviour, morphology, physiological and molecular data when relevant or possible. This is not different from any regular species delimitation step, benefiting from scrupulous practice and the inclusion of various sources of data. The second step consists in diagnosing the 'cryptic' status, that is, in showing that species are morphologically more similar than one would expect, given the time of divergence. This step requires estimates of the time of divergence and genetic divergence, as provided by molecular clock and fossil data approaches. Estimates from closely related taxa (i.e. outgroups) will benefit this comparative outlook by providing potential traits which should warrant special attention, such as those under selection or divergence.

While the application of this framework allows distinguishing between taxonomic artefacts from lineages which are morphologically similar, it also encourages better taxonomic practices for species delimitation and systematics in general (Struck et al., 2018a, 2018b; Struck and Cerca, 2019). Indeed, I have found that many cryptic species are identified without clarity (Struck et al., 2018a). In a survey consisting of 606 papers, I have found that only 14% of the papers included an explicit definition of cryptic species; a majority (84.2%) of studies use molecular data, but a substantial part used only a single DNA-marker (35.5%), and less than half (42.7%) included morphological data.

Nonetheless, this proposed framework has met criticism (Heethoff, 2018; Korshunova et al., 2019). Critics have suggested that the discovery of cryptic species results from the 'incompatibility of species 'complexes' in applied taxonomy' (Heethoff, 2018), being an artificial blend between morphological and genetic species concepts (Heethoff, 2018). Other sources of criticism have suggested that the degree of morphological similarity remains ambiguous and that this approach requires a very

scrupulous and detailed systematic approach (Korshunova et al., 2019), and hence cryptic species should be considered as a temporary problem of the taxonomy of a species complex. I have responded to these criticisms by highlighting evidence of morphologically similar species to occur in nature (Struck and Cerca, 2019), by pointing out Heethoff's misconceptions in mixing the grey-zone of speciation and recently-distinguished species (Struck et al., 2018b), as well as by uncovering the underlying mechanisms of deceleration of morphological evolution after scrupulous taxonomy (*manuscripts 3-4*).

Deceleration of morphological evolution

Deceleration of morphological evolution occurs when two closely-related lineages remain morphologically similar after speciation (Struck et al., 2018a; *manuscript 3*). For instance, when contrasting morphological and genetic evolution (Figure 3), four potential scenarios are identified: 1) cases of fast-paced morphological evolution in recently divergent lineages, such as adaptive radiation and character displacement (orange area in Figure 3); 2) cases where morphological disparity follows genetic divergence and vice-versa (light green area in Figure 2); 3) cases where speciation has occurred recently, and morphological differences have not accumulated, but are likely to accumulate (dark green area in Figure 2); and 4) cases where morphological evolution is clearly deccelerated, as expected in cryptic species (blue area in Figure 2) (Struck et al., 2018a).

Multiple causes can underlie the deceleration of morphological divergence. On one hand, speciation is not necessarily accompanied by morphological divergence, being potentially guided by differences in behaviour, immunity or physiology (Lee and Frost, 2002a; Novo et al., 2012, 2010; Struck et al., 2018a). On the other hand, species can remain morphologically similar due to stabilizing selection (Charlesworth et al., 1982; Futuyma, 2010; Hansen and Houle, 2004), niche conservatism and tracking (Futuyma, 2015, 2010), fluctuating ecological conditions (Futuyma, 2015, 2010, 1987; Sheldon, 1996; Smith et al., 2011), lack of new ecological interactions (Nordbotten and Stenseth, 2016), constraints (Charlesworth et al., 1982; Futuyma, 2010; Hansen and Houle, 2004; Maynard Smith et al., 1985; Smith et al., 2011; Wagner and Schwenk, 2000), recurrent bottlenecks (Futuyma, 2010), physiological or behavioural adaptation (Futuyma, 2010; Lassance et al., 2019; Lee and Frost, 2002b), and the influence of particular environments and environmental conditions (Futuyma, 2010, 1987; Giere, 2009; Gueriau et al., 2016; Westheide, 1977; Westheide and Rieger, 1987). Importantly, many of these scenarios have been discussed in terms of paleontological stasis, but a thorough investigation of the mechanisms and extent of these factor still remains elusive (*manuscript 3*).

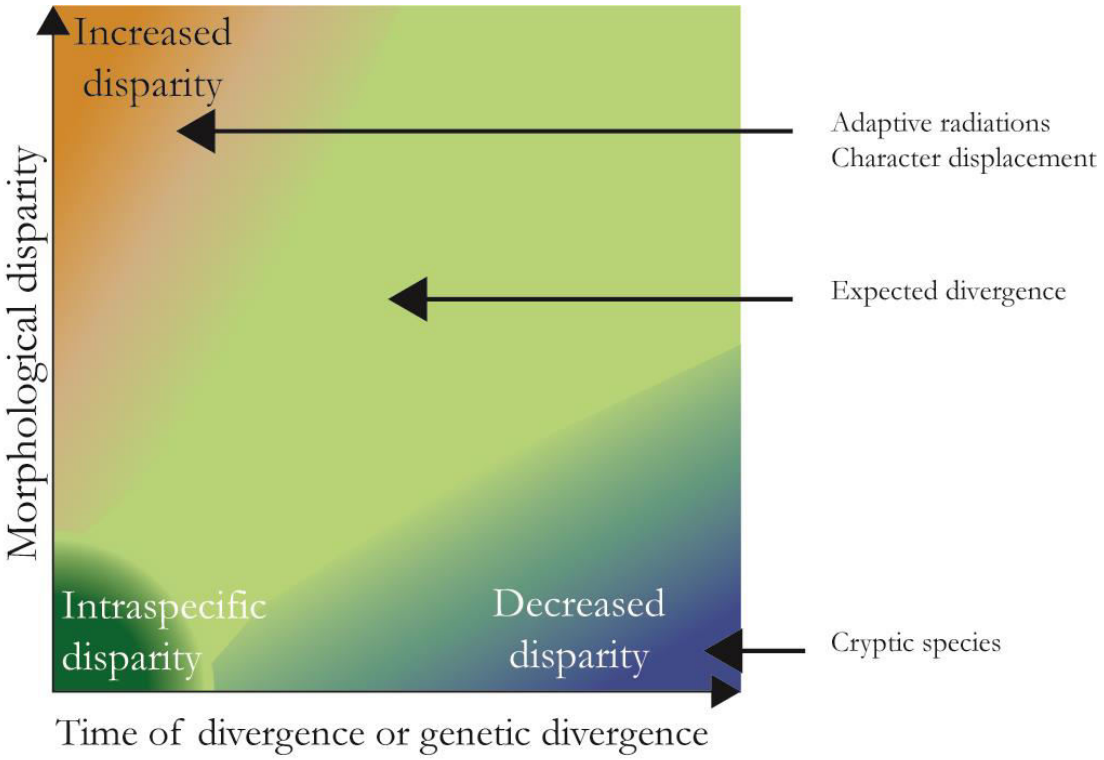


Figure 3. Conceptual framework for understanding genetic divergence and morphological disparity, following (Struck et al., 2018a). The x-axis represents the time of divergence between taxa since their most recent common ancestor given by estimates of genetic divergence. The y axis represents morphological disparity. Intraspecific variation (i.e. polymorphism) within a species is depicted by the dark green area in the lower left corner of the figure. Morphological disparity between taxa relative to sister species is expected to increase proportionately with divergence time (light green area). Morphological disparity could increase at a significantly higher rates in cases such as adaptive radiations (orange area in the upper left corner of the figure). Alternatively, morphological disparity could be substantially lower than expected over time (blue area in the lower right corner), the hallmark of cryptic species.

Four ways to become cryptic

I have suggested four scenarios to underlie cryptic species, including recent divergence (Panel A in Figure 4), convergent evolution (Panel B, Figure 4), parallel evolution (Panel C, Figure 4) and morphological stasis (Panel D, Figure 4). These are discussed below.

Recent divergence without morphological differentiation (Panel A, Figure 4) include species which have only recently diverged, but remain morphologically similar (Struck et al., 2018a; Struck and Cerca, 2019). One example is the *Anopheles* species complex, where the "M" and "S" forms were recognised as being at an early stage of ecological speciation (Reidenbach et al., 2012). These two forms explore different habitats, with the M-form mainly exploiting stable larval habitats with many stressors, and the S-form exploiting unpolluted, predator-free ephemeral habitats associated with rainfall (Reidenbach et al., 2012). This is an extremely relevant example of a cryptic species delimitation-problem extending outside fundamental fields of biology because not every morphologically-similar species is

capable of transmitting malaria (Erlank et al., 2018). Other cases of recent speciation without morphological evolution could result from selection acting upon behavioural immunological, physiological, reproductive traits (Struck et al., 2018a).

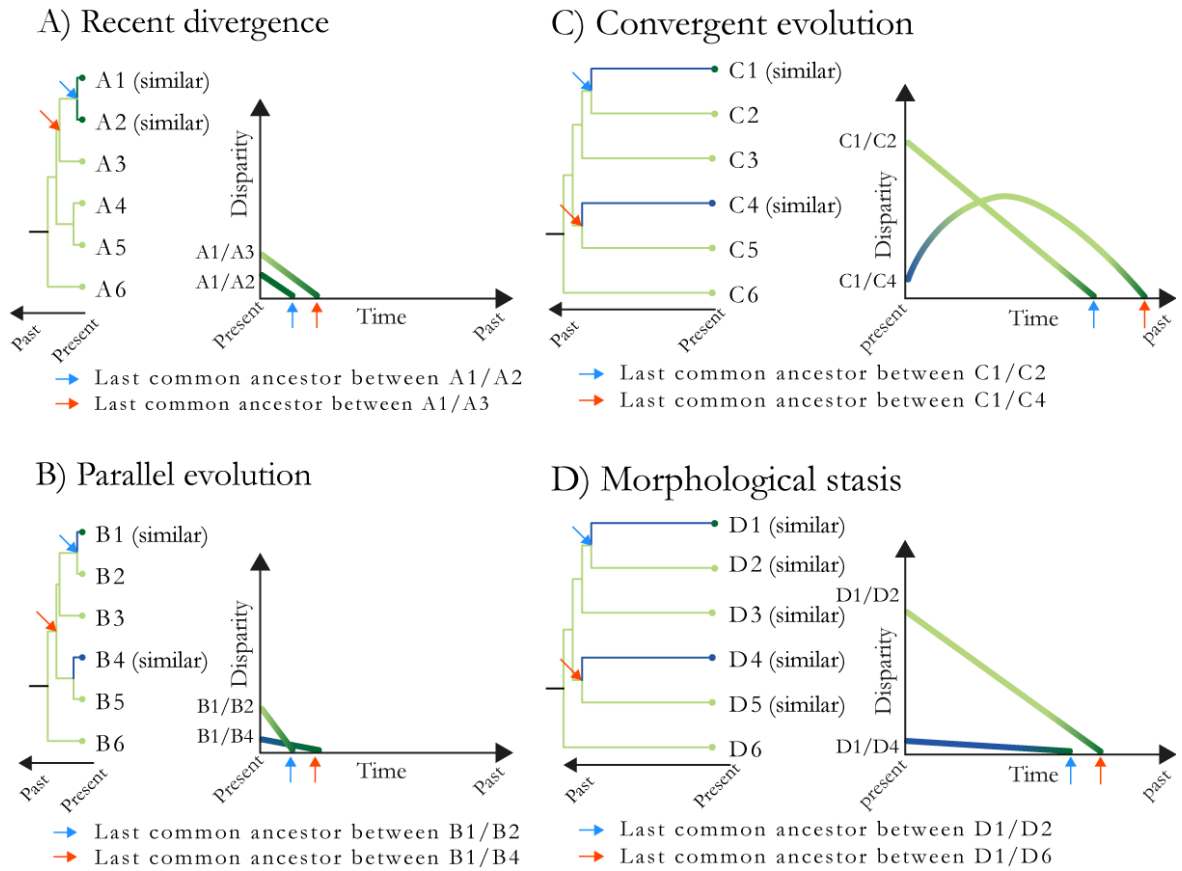


Figure 4. Expected signatures of recent divergence (A), parallel evolution (B), convergent evolution (C) and morphological stasis (D) that may lead to cryptic species. The colours of lines in phylogenies and graphs correspond to the different areas in Figure 3. Species with similar morphotypes are denoted by '(similar)'. Panels to the left (cladogram) denote the phylogenetic relationships among taxa, while the panels to the right (Disparity as a function of time graphic) depict the evolution of morphological disparity through time for pairs of cryptic and non-cryptic species (e.g., A1/ A2 vs. A1/A3). **(A) Recent divergence:** cryptic species have only recently diverged from each other, being thus closely related. However, the rate of morphological disparity is not necessarily substantially different from that for non-cryptic species. **(B) Parallel evolution:** cryptic species are not sister species, however the rate of morphological disparity for non-cryptic species is much greater than that for cryptic species. Disparity between non-cryptic species evolved from the dark to the light green area, disparity between cryptic species progressed into the dark blue area of Figure 3. **(C) Convergent evolution:** cryptic species are not closely related to each other. Initially, morphological disparity for cryptic species can accumulate in a manner similar to that for non-cryptic species. However, at some point, morphological disparity decreases for the cryptic species. Hence, in their past the level of disparity of the cryptic species was first within the light green area of Figure 3, but then evolved toward the dark blue area associated with the low level of disparity of cryptic species. **(D) Stasis:** the cryptic species are closely related to each other or are part of a species complex which diverged a long time ago. In comparison with non-cryptic species, the rate of morphological change is substantially reduced. Image adapted from (Struck et al., 2018a).

The second and third discussed scenarios include evolutionary convergence and parallelism (Panel B & C, Figure 4) (Struck et al., 2018a; Struck and Cerca, 2019; Swift et al., 2016). The distinction between convergence and parallelism comes from considering an 'ancestral set of traits', that is, if the ancestral set of traits is different for a given species complex, it is considered a case of convergence, otherwise it is a case of parallelism (Struck et al., 2018a; Swift et al., 2016). The *Mastigias* species complex (Scyphozoa) is an example where parallelism and convergence occur (Swift et al., 2016). Different species with an 'oceanic phenotype' have independently colonized several marine salt water lakes, which have formed as a result of rising sea levels after the last glacial maxima (Swift et al., 2016). Modern species found in these lakes have evolved a 'lake-phenotype'. Parallelism in this system occurs when a group of 'lake-phenotype' species share a common ancestor with the same 'oceanic-phenotype' species, whereas convergence occurs when two 'lake-phenotype' have different 'oceanic-phenotype' ancestors (Struck et al., 2018a; Swift et al., 2016).

The fourth scenario proposed for the evolution of cryptic species is morphological stasis (Panel D, Figure 4), wherein closely related species display severely decelerated rates of morphological evolution throughout a long period (Struck et al., 2018a; Swift et al., 2016; Wada et al., 2013). One clear-cut example of cryptic species under stasis include the *Cavernacmella* snails (Wada et al., 2013). Several *Cavernacmella* species remained morphologically identical for the last 3 million years. However, several lineages have accumulated differences in shell morphology only in the last few thousands of years (Wada et al., 2013). Morphologically divergent lineages have colonized and inhabit limestone outcrop groups (Wada et al., 2013). This highlights the role of ecology in driving shifts of morphological evolution (i.e. acceleration and deceleration), as in cases of adaptive radiation (Gillespie, 2004; Losos, 2010). The *Stygocapitella* species complex is another potential case of stasis (see below) (Struck et al., 2017).

Strictly speaking, the cases of recent divergence, parallel and convergent evolution do not contribute to our understanding of morphological deceleration. In recent divergence morphological change can be relatively dormant and lag genetic divergence. In parallel and convergent evolution morphological disparity has occurred in the past. Despite this, these cases indicate that reduced morphological disparity and the lack of morphological evolution are distinct features of cryptic species.

Incidence of cryptic species in meiofauna and the 'meiofauna paradox'

The 'meiofauna paradox' describes the inconsistency between the wide distribution and the presumed limited dispersal abilities of interstitial invertebrates (Cerca et al., 2018; Giere, 2009). This is best discussed in two separate components: dispersal abilities and distribution (Cerca et al., 2018). I have demonstrated that there is paramount experimental evidence for meiofaunal dispersal (Arroyo et al., 2006; Boeckner et al., 2009; Callens et al., 2012; Commito and Tita, 2002; Cristoni et al., 2004; Cuvelier et al., 2014; De Meester et al., 2015, 2012; Fonsêca-Genevois et al., 2006; Gallucci et al., 2008; Gobin and Warwick, 2006; Guilini et al., 2011; Gwyther and Fairweather, 2005; Hooper and Davenport, 2006;

Junkins et al., 2006; Lins et al., 2013; Mcfarlane et al., 2013; Mevenkamp et al., 2016; Pugh, 1996; Schratzberger et al., 2000; Teasdale et al., 2004; Thistle, 2003; Thomas and Lana, 2011; J. Ullberg and Ólafsson, 2003; Jörgen Ullberg and Ólafsson, 2003). However, this evidence is in clear contrast with historically defined hypotheses and ideas which have shaped the literature, yet that lack evidence (Christiansen and Fenchel, 1979; Danielopol and Wouters, 1992; Kieneke et al., 2012; Sterrer, 1973; Westheide and Hass-Cordes, 2001).

With regards to 'distribution', I found that there is a high incidence of cryptic species, which interferes with the estimation of meiofaunal species' distribution range (Cerca et al., 2018). When analysing the literature (752 papers), I found that the discovery of cryptic species, led to species distribution rantes to remain unchanged in 40 papers, increase in 25, and decrease in 22. However, when analysing based on the number of species, 82 species remained unchanged, 112 increased their distribution, and 160 decreased their distribution (Cerca et al., 2018). The decrease of the distribution range is linked to the discovery of cryptic species, which were typically localized to a given area. Nemertea was the phylum where the discovery of cryptic species had a bigger impact. 72 species were reported with decreased distributions, 0 with increased distributions and 13 with unchanged distributions (Leasi and Norenburg, 2016, 2014; Tulchinsky et al., 2012). Chaetognatha, Chordata, Cnidaria, Gnathostomulida, Hemichordata, Echinodermata, Entoprotoa, Loricifera, Priapulida and Tardigrada had 0 species with decreased distributions (Cerca et al., 2018). However, these numbers are likely to reflect sampling efforts and the application of molecular tools to species identification and delimitation. For instance, from the aforementioned list of phyla without decreases in range distribution, only 7 studies applied molecular tools (2 studies in Gnathostomulida, 1 study in Priapulida, and 4 studies in Tardigrada).

While very few papers (61, <10%) applied molecular tools, those which did found a high number of cryptic species. This is in line with previous suggestions that the meiofauna has an high incidence of cryptic species (Fontaneto et al., 2015; Jörger and Schrödl, 2013; Leasi et al., 2013). While I could not distinguish which particular cases result from taxonomic artefacts or due to biological phenomena, I have suggested that high numbers are likely to stem from high rates of morphological stasis, likely due to stabilizing selection on morphology as a result of the restricted space available in the interstium (Schmidt and Westheide, 2000; Sterrer, 1973; Westheide and Rieger, 1987), but also due to the natural degree of convergence resulting from simplified external appendices, elongated worm-like body shape and external adhesive structure – the 'meiofaunal syndrome' (Brenzinger et al., 2013; Jörger et al., 2014).

Finally, I have suggested a roadmap for future meiofauna research on the meiofauna paradox (Cerca et al., 2018). I suggested that future research should: (i) focus on understanding morphological similarity, including (ii) the rates of stasis and convergence and (iii) on the selective pressures leading to it; (iv) understand dispersal capacities of meiofaunal taxa both empirically and experimentally; (v) understand the factors underlying the distribution of a species or group of species, with particular focus on life-history and contribution of specific traits; (vi) have a strong taxonomic component, focusing on describing new

species using morphology and molecular data; and (vii) using metabarcoding and metatranscriptomic tools to understand whether species are present in the water column, including the stage of development in which dispersal occurs (i.e. using transcriptomic data).

Finally, I have highlighted the *Stygocapitella* cryptic species complex as a promising study system to address questions on species distributions and morphological similarity. Considering the existence of multiple and world-wide records (Riser, 1984; Schmidt and Westheide, 2000), as well as evidence for potential morphological deceleration (Struck et al., 2017).

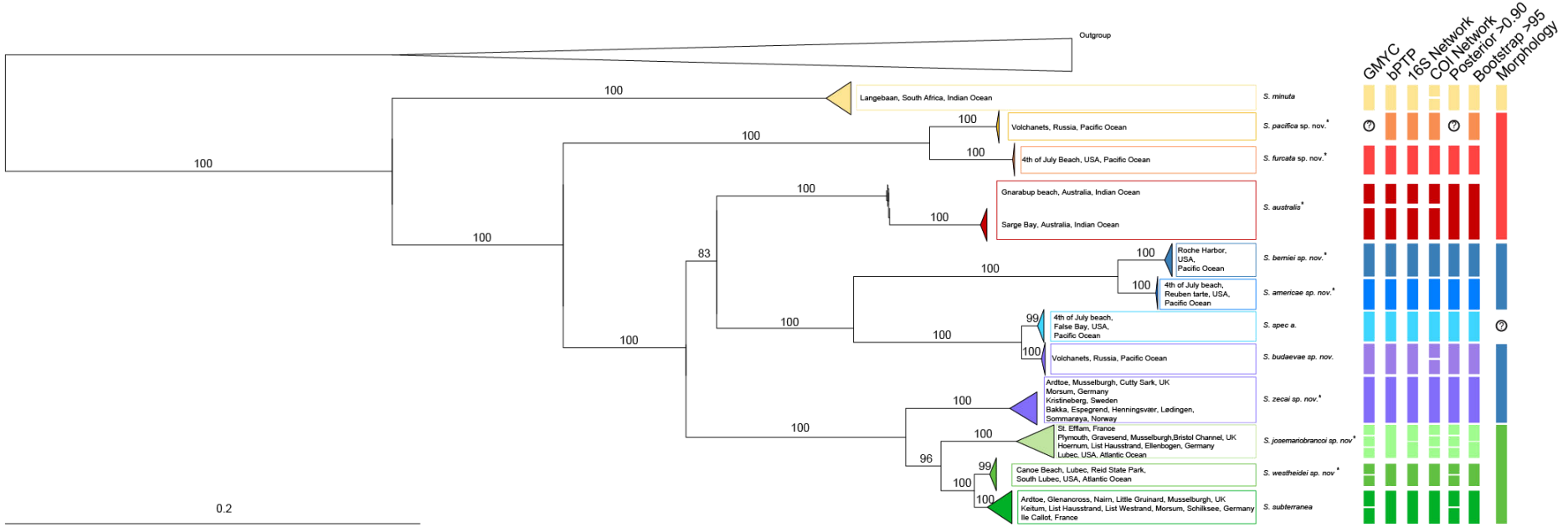


Figure 5. Maximum likelihood phylogeny and species delimitation included as part of *manuscript 4*. The phylogenetic tree was obtained using a partitioned dataset including COI, 16S, 18S and ITS1 sequences. Bootstrap support is included for each branch. Substitutions per site are shown in the scale bar. Species names and sampling locations are included after each edge. Species delimitation analysis include: bPTP (Bayesian Poisson Tree Processes), 16S network, COI network, posterior probabilities of >0.90, Generalized Mixed Yule Coalescent Approach (GMYC), bootstrap support > 95 and morphology (chaetal composition). Question marks highlight cases where models did not run: because I had only one specimen for the final Bayesian analysis, I was unable to obtain posterior probabilities and to run GMYC (question mark in GMYC and posterior columns); question mark in morphology indicates the species, for which I was unable to obtain Scanning Electron Microscopy photographs. Species followed by an asterisk (*) represent species, which were previously considered as *S. subterranea* (*sensu lato*)

.

The *Stygocapitella* species complex – application of the framework to delimit cryptic species

We have proposed a two-step framework to identify and cryptic species, consisting of a custom species delimitation, followed by the identification of the cryptic status (Struck et al., 2018a, 2018b; Struck and Cerca, 2019). The *Stygocapitella* genus has three described species including *S. subterranea* (cosmopolitan species), *S. australis* (found in Australia) and *S. minuta* (found in South Africa) (Knöllner, 1934; Purschke et al., 2019; Schmidt and Westheide, 2000; Struck et al., 2017). I have collected *Stygocapitella* in multiple coastlines (Figure 2) and studied morphological and genetic divergence. The application of several complementary approaches to delimit *Stygocapitella* species, as suggested by best practices (Carstens et al., 2013), including genetic (haplotype networks, Generalized Mixed Yule Coalescent Approach, Bayesian Poisson Tree Processes, Maximum Likelihood, Posterior probability) and morphological data has led us to suggest 8 new species. Based on this, and on formal action to describe these species (*manuscript 4*), I will refer to the 8 new *Stygocapitella* species after their new taxonomic identities: *S. pacifica* sp. nov., *S. furcata* sp. nov., *S. berniei* sp. nov., *S. americae* sp. nov., *S. budaevae* sp. nov., *S. zecai* sp. nov., *S. josemariobrancoi* sp. nov., and *S. westheidei* sp. nov.. One further species remains undescribed due to the lack of type-material to describe the species, and will hence be referred to as *Stygocapitella* spec. A. To avoid over splitting, I have opted for a conservative approach to delimitate species, that is, for a given clade, if one approach suggested this as a single species, while other approaches as more than one species, I regarded this as a single species. Finally, I applied a monophyly-criterium, that is, recognized species had to be retrieved as monophyletic, and strongly supported in phylogenetic reconstructions.

In total, I used 353 specimens from 33 sites to obtain genetic data for *Stygocapitella*. This dataset consisted of 332 16S, 273 COI, 125 18S and 177 ITS1 sequences. Generally, the approaches to delimitate the *Stygocapitella* species were concordant (Figure 5), suggesting at the most 16 species (GMYC algorithm), and at least 13 (bPTP algorithm). Every approach using genetic data suggests that that *S. americae* sp. nov., *S. berniei* sp. nov., *S. furcata* sp. nov., *S. pacifica* sp. nov., *S.* spec. A, and *S. zecai* sp. nov. all represent single taxonomic units. *Stygocapitella minuta* and *S. budaevae* sp. nov. are considered as single species in every genetics-based approach, with the exception of the COI haplotype network which suggest the presence of two species in each of these lineages (Figure 5). GMYC, bPTP, and network approaches suggest that *S. australis* represent two separate species, yet I take no taxonomic action as any potentially delimitated species could be paraphyletic (Figure 5).

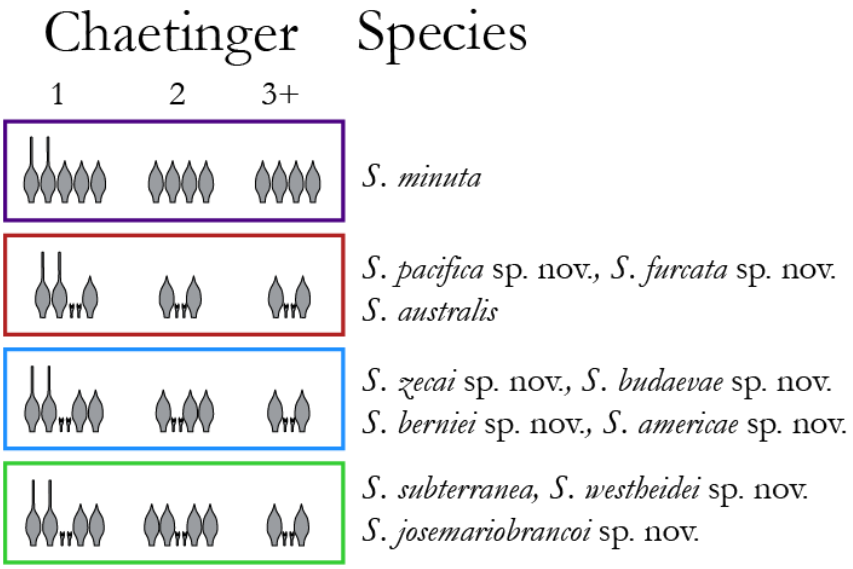


Figure 6. Four *Stygocapitella* morphotypes based on chaetal pattern and composition. Species are given on the right of each morphotype. Chaetal composition is given for the first (1), second (2) and third and consecutive (3+) chaetae. The first morphotype is composed by two whip-like chaetae and three bilimbate chaetae in the first chaetinger and four bilimbates in the second chaetinger (purple), and is found in *S. minuta*. The second morphotype consists of two whip-like, two forked and one bilimbate chaetae in the first chaetinger, and one bilimbate, two forked and one bilimbate chaetae in the second chaetiger (red). It is present in *S. pacifica* sp. nov., *S. furcata* sp. nov., *S. australis*. The third morphotype consist of two whip-like, two forked and two bilimbate chaetae in the first chaetinger and two bilimbate, two forked and two bilimbate chaetae in the second chaetiger (blue). It is present in *Stygocapitella berniei* sp. nov., *S. americae* sp. nov., *S. budaevae* sp. nov., *S. zecai* sp. nov. The fourth morphotype is identified by two whip-like, two forked and two bilimbate chaetae in the first chaetinger, and one bilimbate chaetae, two forked and two bilimbate chaetae in the second chaetiger (green). This morphotype is present in *S. josemariobrancoi sp. nov.*, *S. westheidei sp. nov.*, and *S. subterranea*.

After species delimitation (Figure 5), one should provide estimates of morphological evolution by focusing on detecting differences between species (Figures 6-9). After analyzing Scanning Electron Microscopic photos, I found four morphotypes (Figures 6-7), based on the only variable external characters found in *Stygocapitella*, that is, the chaetae present in chaetingers (Figure 6). I will refer to different morphotypes as the purple, red, blue and green morphotype, as shown in Figures 6 and 7. The red phenotype is composed of two whip-like, two forked and one bilimbate chaetae in the first chaetinger, and one bilimbate, two forked and one bilimbate chaetae in the second chaetiger. It is present in species from the Pacific and Indic Oceans including *S. pacifica* sp. nov., *S. furcata* sp. nov., *S. australis*. The blue morphotype is composed by two whip-like, two forked and two bilimbate chaetae in the first chaetinger and two bilimbate, two forked and two bilimbate chaetae in the second chaetiger. It is present in species living in the Pacific Ocean including *S. berniei* sp. nov., *S. americae* sp. nov., *S. budaevae* sp. nov., and in the Atlantic species *S. zecai* sp. nov. The green morphotype is identified by two whip-like, two forked and two bilimbate chaetae in the first chaetinger and one bilimbate chaetae, two forked and two bilimbate chaetae in the second chaetiger. It is found in the Atlantic species *S. josemariobrancoi* sp. nov., *S. westheidei* sp. nov., and *S. subterranea*. I did not find any diagnostic trait within morphotypes (Figure 7), and quantitative morphological measurements are unable to separate species in morphotype (Figures 8-9).

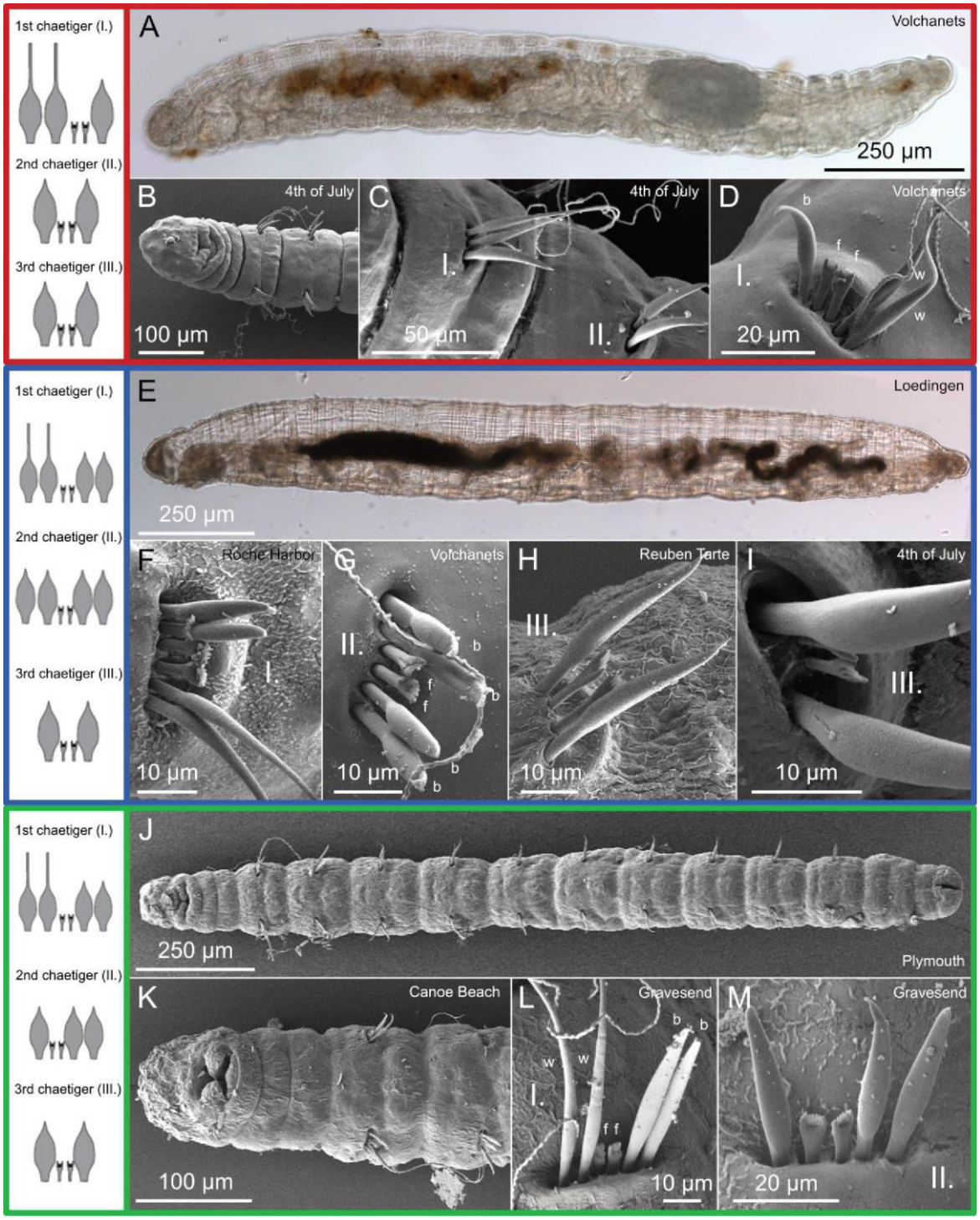


Figure 7. Scanning electron microscopy and light microscopy images of *Stygocapitella* individuals. The three morphotypes are represented in different colors (red, blue and green boxes) and shown on the left pannels; A) Light microscopy photograph of *S. pacifica* sp. nov.. B & C) SEM images of *S. furcata* sp. nov. with first (I.) and second (II.) chaetae bearing chaetiger. D) 1st chaetiger of *S. pacifica* sp. nov. with 2 whip-like (w), two forked (f) and 1 bilimbate (b) chaetae. E) Light microscopy photograph of *S. zecai* sp. nov.. F) 1st chaetiger of *S. berniei* sp. nov. with 2 whip-like, 2 forked and 2 bilimbate chaetae. G) 2nd chaetiger of *S. budaevae* sp. nov. with 2 bilimbate (b), 2 forked (f) and 2 bilimbate (b) chaetae. H) 3rd chaetiger of *S. americae* sp. nov.. I) 3rd chaetiger of *Stygocapitella* from 4th of July Beach. J) SEM images of whole *S. josemariobrancoi* sp. nov.. K) Anterior end of *S. westheidei* sp. nov.. L & M) First two chaetigers of *S. josemariobrancoi* sp. nov.. 1st chaetiger with 2 whip-like (w), 2 forked (f) and 2 bilimbate (b) chaetae. 2nd chaetiger with 1 bilimbate (b), two forked (f) and 2 bilimbate (b) chaetae.

I explored quantitative differences by measuring body length and width, prostomium length and width, and pygidium length and width (Figure 8). General Linear Models demonstrate significant differences in measurements: body length (Likelihood Ratio Test scaled dev. = 124.72, p <0.001), body width (LRT scaled dev. = 118.3, p <0.001), prostomium length (LRT scaled dev. = 76.5, p <0.001), prostomium width (LRT scaled dev. = 122.8, p < 0.001), pygidium length (LRT scaled dev. = 45.4, p < 0.001), and pygidium width (LRT scaled dev. = 140.5, p < 0.001). Results from the morphological measurements show that pairwise differences in body length between species are also roughly reflected in the remaining five measurements (Figure 8) and thus, I will concentrate on body length in the following section. *S. minuta* is significantly shorter in body length (mean 1046.88; SD 76.29) than every other species, with the exception of *S. pacifica* sp. nov. (mean 1261.47; SD 52.21) and *S. budaevae* sp. nov. (mean 1368.49; SD 164.53). For the red morphotype (Figure 6), *S. pacifica* sp. nov. is smaller than *S. australis* (mean 1933.61; SD 218.01), but this difference is not statistically significant. For blue morphotype (Figure 6), *S. budaevae* sp. nov. has the shortest body length, followed by *S. berniei* sp. nov. (mean 2006.98; SD 436.99), whereas *S. americae* sp. nov. (mean 2550.4; SD 229.74) has the largest body length, followed by *S. zecai* sp. nov. (mean 2238.23; SD 322.48). For the green morphotype (Figure 6), *S. josemariobrancoi* sp. nov. (mean 2409.7; SD 472.15) is clearly the longest species, while *S. subterranea* (mean 1703.7; SD 380.68) and *S. westheidei* sp. nov. (mean 1820.2; SD 293.68) have overlapping body lengths. Accordingly, *S. josemariobrancoi* sp. nov. is significantly different from *S. subterranea* and *S. westheidei* sp. nov., but the latter two are not separated from each other.

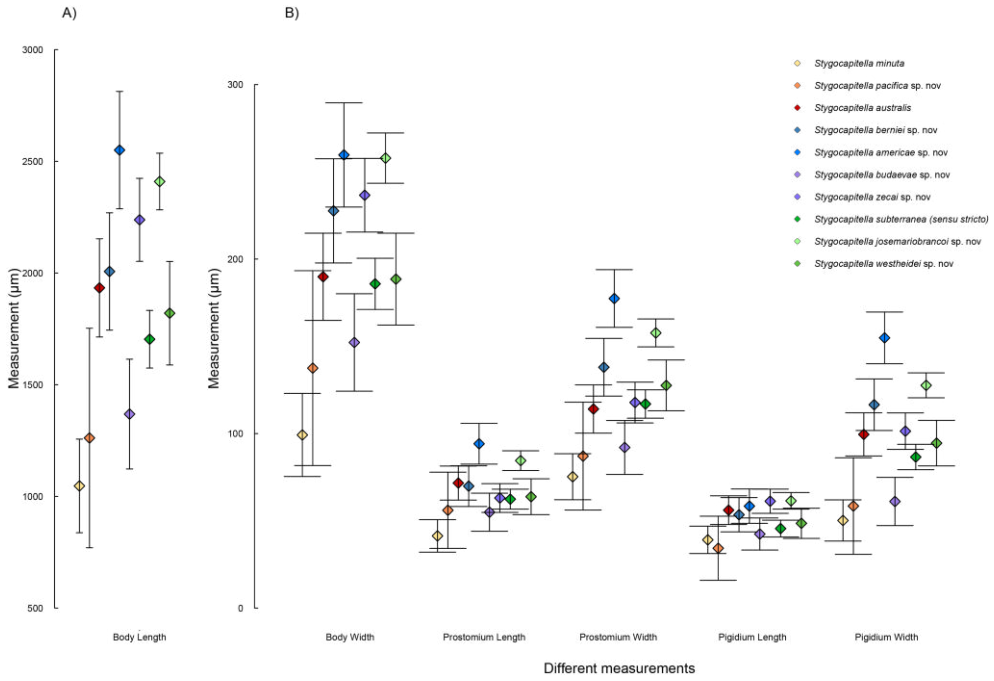


Figure 8. Morphometric analysis of *Stygocapitell*a. Panel A displays body length measurements (µm) and panel B) displays body width, prostomium length and width, and pygidium length and width (µm). Each species is displayed with different colours (see legend on the top-right corner). *Stygocapitella subterannea* is labeled as *sensu stricto* due to the revised status (see *manuscript 4*).

Using principal component analyses, the first principal component separates only *S. minuta* from the remaining species (PC1 explains 75.4% of the variance; Figure 9A). The second principal component explained 11.9 % of the variance, and the third axis explained 5.4 %. Neither the 2nd, neither the 3rd axis separated any of the species (data for the third component is not shown). When considering variance within morphotypes, the results are slightly more informative. In the red morphotype, *S. pacifica* sp. nov. is separated from *S. australis* based on the first principal component (Figure 9B). However, *S. pacifica* sp. nov. is only represented by two specimens and more data is needed for a reliable species separation. In the blue morphotype, the first principal component separates *S. budaevae* sp. nov. from the remaining species (Figure 9C). *S. zecai* sp. nov. is separated from *S. americae* sp. nov. based on the first two principal components, but both overlap substantially with *S. berniei* sp. nov.. For the green morphotype all three species overlap substantially, and they cannot be separated based on the PCA analysis (Figure 9D). This is not due to the lack of data as these are the three species with highest number of sampled specimens.

In sum, genetic evidence suggests that the *Stygocapitella* genus is composed of at least 12 species. However, only four morphotypes, based on chaetal number and composition, exist.

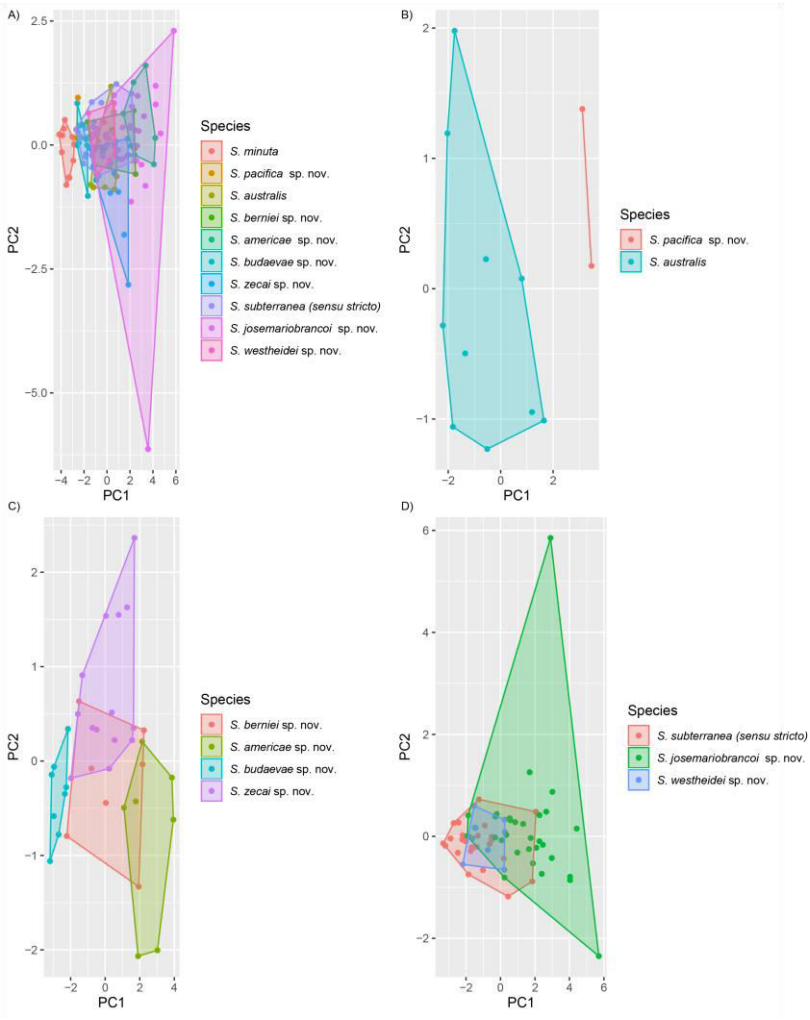


Figure 9. Principal component analysis comprising morphological measurements of *Stygocapitella*. Every panel displays the first two principal components (PC1-PC2). Panel A) displays all species. Panel B) displays the red morphotype, Pannel C) the blue morphotype and D) the green morphotype.

The *Stygocapitella* species complex – application of the framework to understand species' distributions

The splitting of *S. subterranea* into nine new species with substantialy decreased distributions suggests that overlooking cryptic species leads to an inflation of the distribution of marine organisms (*manuscript 4*; Knowlton, 2000, 1993), being in line with evidence from the survey carried for meiofaunal taxa (Cerca et al., 2018). *Stygocapitella subterranea* was previously described as a cosmopolitan species (Riser, 1984; Schmidt and Westheide, 2000), yet the described species are present only in one coastline (Figures 2A-G, 5). The only exception is *S. josemariobrancoi*, which is present in the North American coastline of the Atlantic Ocean and the European coastline. However, given the mitochondrial similarity of specimens found at both coastlines, I suspect that these might represent a recent human translocation (Radziejewska et al., 2006).

***Stygocapitella* species complex – morphological deceleration and stasis**

I contrasted morphological disparity in a dataset comprising *Stygocapitella*, Nerellidae and Orbiniidae (Figures 10-11). Mapping of 75 morphological characters on a maximum likelihood tree revealed that morphological evolution in *Stygocapitella* is relatively slower to that of nerillids and orbiniids (Figure 10). In a total of 16 branches, only 4 have morphological changes in *Stygocapitella*, while in nerillids and orbiniids, only 1 and 3 branches have no differences, respectively. In *Stygocapitella*, there is an average of 0.5 changes per branch, whereas in nerillids, there is an average of 4.1 morphological changes per branch, and only six branches showed three or less changes. In orbiniids, there is an average of 2.8 changes per branch, eight branches only have one to three morphological changes, and 10 branches have four to eight changes (Figure 10).

Decomposition of morphological variance in a principal component analysis (Figure 11A) shows that Nerillidae, Orbiniidae and *Stygocapitella* are clearly separated from each other (PC1 and PC2 together explain 64.4% of the variance). Clearly, Nerillidae and Orbiniidae occupy a substantially larger morphospace area than *Stygocapitella* (Figure 11A). Multidimensional morphological disparity (MMD) shows that this difference is not restricted to the first principal components, but holds up for the first 18 principal components (*manuscript 4*). MMD indices of *Stygocapitella* had a mean of 1.14 (Figure 11B), with a standard deviation of 1.28. The MMD indices of Nerillids had a mean of 7.21 with a standard deviation of 2.76. For orbiniids the mean was 7.46 with a standard deviation of 3.29 values (Figure 11B). Boxplot representations of the MMD distribution show that the quartiles of *Stygocapitella* do not overlap with the quartiles belonging to Nerillidae and Orbiniidae, while they overlap completely (Figure 11B). Tukey's HSD and students' T-tests show that morphological disparity in *Stygocapitella* is significantly lower in comparison to both Nerillidae and Orbiniidae ($p < 0.000001$), while there is no significant difference between Nerillidae and Orbiniidae (Tukey's HSD: $p = 0.7343163$; students' T: $p = 0.45$).

Plotting of MMD indices against pairwise genetic distances, in these groups (Figure 11C) indicates that lower MMD values in *Stygocapitella* are not an artefact of the arbitrary above species taxonomical ranks (i.e. *Stygocapitella* is a genus; Nerillidae and Orbiniidae are families). In *Stygocapitella*, MMD indices remain between 0 and 1 until a pairwise genetic distance of 0.025. These values increase slightly above 4 only at higher molecular distances. In clear contrast, MMD indices in Nerillidae and Orbiniidae vary between 5 and 10 at relatively shallow genetic distances of <0.01 (Figure 11C). Morphological disparity in these lineages remains at high levels with increasing genetic distances and only a few outliers display disparity values as low as *Stygocapitella*.

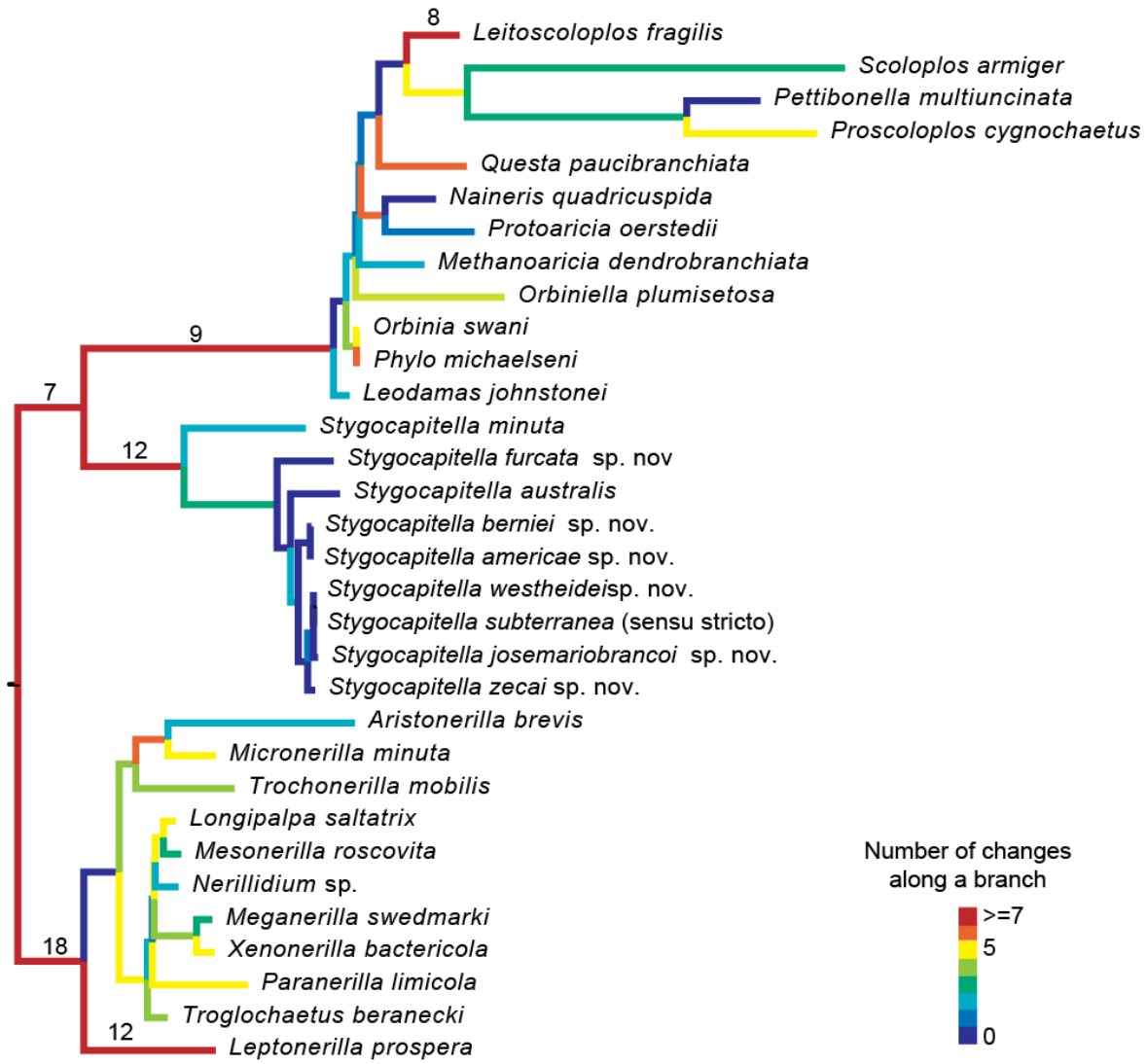


Figure 10. Mapping of character evolution in *Stygocapitella* (Parergodrilidae), Orbiniidae and Nerillidae. Tree topology is based on a 18S ML phylogeny (*manuscript 3*). The number of changes along branches are based on a matrix comprising 75 morphological characters, and are portrayed in different colours (see left-bottommost corner). Zero corresponds to no change occurring along the branch. The number of changes along a branch >7 is shown on top of the branch.

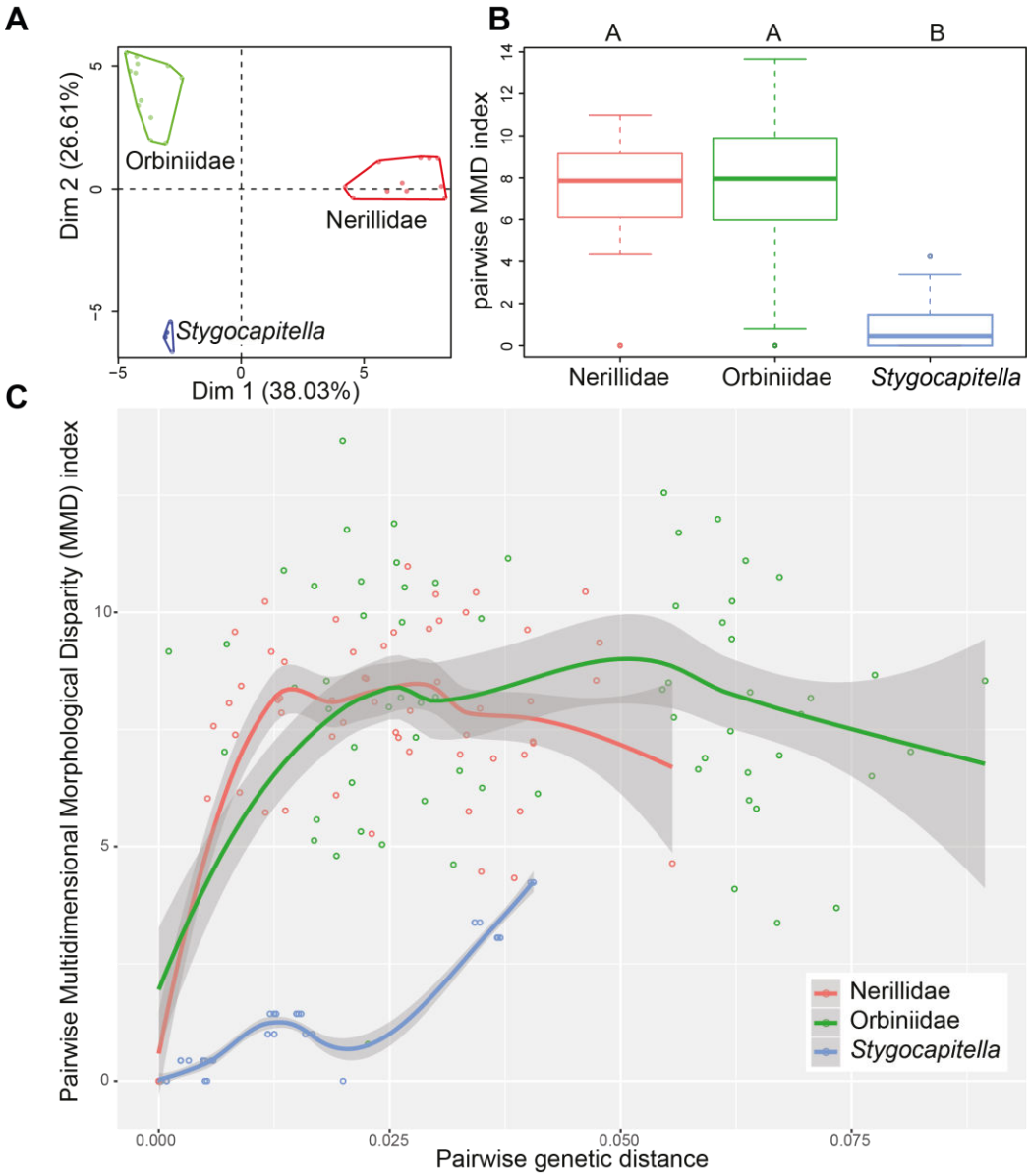


Figure 11. Principal Component (PC) analysis and Multidimensional Morphological Disparity (MMD) index results among *Stygocapitella*, Orbiniidae and Nerillidae. A) PC analysis of the 75 morphological characters. The first PC explains 38.03% of the variation and the second explains 26.61%. B) Pairwise differences in the MMD index. Outliers are represented by single dots above or below the confidence intervals. Groups which are not significantly different are signed by same letter (top of the box plot). C) Plotting of the pairwise MMD indices against the pairwise genetic distance in 18S shows that at relatively big genetic distances, *Stygocapitella* displays a lower morphological disparity than the remaining taxa.

I plotted an ancestral state reconstruction on a time-calibrated tree using time estimates based on a molecular clock analysis (Figure 12). These results suggest that *Stygocapitella* spp. Started to diverge about ~275 million years ago. Two morphologically similar species, namely *S. australis* and *S. furcata* sp. nov. have potentially diverged 140 million years ago. The blue morphotype is likely to have appeared about 18 MY ago (confidence intervals range between 5 – 37 MY) (Figure 12) and corresponds to *S. subterranea*, *S. westheidei* sp. nov. and *S. josemariobrancoi* sp. nov.. The green morphotype was reconstructed as the ancestral

condition for a clade comprising *S. zecai* sp. nov., *S. berniei* sp. nov., and *S. americae* sp. nov, as well as for *S. subterranea*, *S. westheidei* sp. nov. and *S. josemariobrancoi* sp. nov. (Figure 12). The age of divergence for this clade (also including *S. spec. A*, which no morphological data was obtained) was estimated to be about 64 MY (33 – 104 MY) ago (Figure 12). Finally, the red morphotype was reconstructed as the ancestral state for the whole radiation, except for *S. minuta*, and it was dated at ca. 140 MY (75 – 205 MY) (Figure 12). The age for *Stygocapitella* spp. was dated at about 275 MY (124 – 438 MY), being congruent with previous estimates on a substantially smaller dataset based only on 3 species (*S. subterranea*, *S. minuta* and *S. australis*) and on 18S (Struck et al., 2017) which estimated an age of 270 MY for the whole complex and 83 MY for the split of *S. australis* from *S. subterranea*. When considering the 95% confidence interval (not shown in Figure 12), the red morphotype has been maintained for at least 75 MY, and the green for at least 33 MY and the blue for at least 5 MY. Long-term morphological stasis is evident (*manuscript 3*).

Morphological stasis is defined by the retention of a given ancestral character state over an extended period (Struck et al., 2018a). Retention of a given ancestral character, and the reduction of variation can potentially be achieved by scenarios of niche conservatism and tracking (Futuyma, 2015, 2010), fluctuating ecological conditions (Futuyma, 2015, 2010, 1987; Sheldon, 1996; Smith et al., 2011), stabilizing selection (Charlesworth et al., 1982; Futuyma, 2010; Hansen and Houle, 2004), constraints (Charlesworth et al., 1982; Futuyma, 2010; Hansen and Houle, 2004; Maynard Smith et al., 1985; Smith et al., 2011; Wagner and Schwenk, 2000), the influence of particular environments and environmental conditions (Futuyma, 2010, 1987; Giere, 2009; Gueriau et al., 2016; Westheide, 1977; Westheide and Rieger, 1987), recurrent bottlenecks (Futuyma, 2010), physiological or behavioural adaptation (Futuyma, 2010; Lassance et al., 2019; Lee and Frost, 2002b), and lack of new ecological interactions (Nordbotten and Stenseth, 2016). Below, we consider a potential scenario to explain stasis in *Stygocapitella*. However, we warrant data including ecological, developmental, genome-level to potentially confirm any scenario.

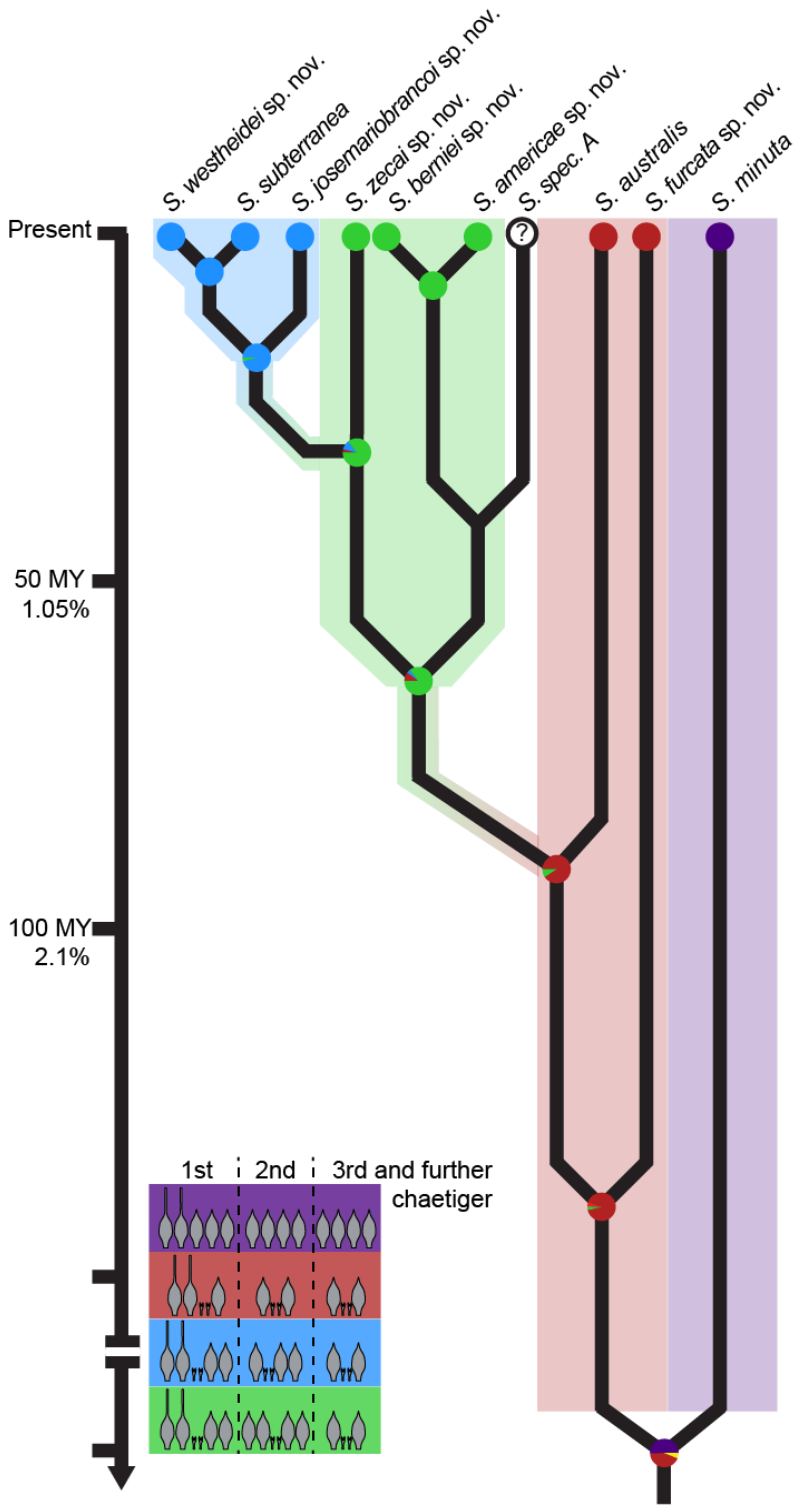


Figure 12. Tree-like representation of the evolution of the four *Stygocapitella* morphotypes. Circles in the tree nodes follow differences at the chaetigers, as displayed in Figure 6. Node-colour at terminal branches represent morphological assignment of the studied species, whereas nodes in intermediate positions were obtained based on an ancestral character reconstruction. Time at the y axis is based on the molecular clock analyses. Morphological transitions between morphotypes are shown by transitioning colours. Tree topology is based on a ML phylogeny (Figure 5). Pie charts at nodes denote ancestral state reconstructions using a ML approach. Percentages refer to genetic divergence in 18S (0.0002127/MY).

It has been suggested that stasis can result from a combination of niche conservatism, niche tracking and the occurrence of fluctuating ecological dynamics (Futuyma, 2010; Lindholm, 2014; Sheldon, 1996). In such a scenario, species are expected to (i) remain in the same environment; (ii) be able to track new areas of that given environment; and (iii) fluctuations in abiotic conditions could occur in short-term, but being stable on the long term. The *Stygocapitella* genus seems to have remained for an extended period in sandy beaches, considering the combination of times of divergence (Figure 12), and the fact that the most distantly related species live in beaches. This is in line with the argumentation stating that the colonization of the space between the space grains requires a high degree of specialization, which could 'lock' interstitial organisms to this environment (Westheide, 1987). This is indeed one of the defining features of the meiofauna, that is the 'meiofauna syndrome', which describes organisms as having an array of convergent features including small body sizes, flat and broad or vermiform-elongated body shapes (Brenzinger et al., 2013; Cerca et al., 2018; Jörger et al., 2014). The idea behind niche tracking is that species are able to persist in their own niche, and remain under the same selective pressures (Futuyma, 2010; Gueriau et al., 2016; Hansen and Houle, 2004; Smith et al., 2011). Given the relative stability of interstitial habitats over geological times, the interstitial realm could represent a "hyperstable niche" (Appeltans et al., 2012; Hansen and Houle, 2004) or one where the core sets of environmental conditions are always present somewhere (Giere, 2009; Noodt, 1974). This is observed on our data as I find multiple instances of dispersal, and the maintenance of wide distributions in different *Stygocapitella* species, potentially suggesting a strong dispersal capacity (Figures 5,12-13; see below). For instance, *S. subterranea* and *S. josemariobrancoi* sp. nov. have wide distributions ranging from Scotland, to Germany and France, with haplotypes occurring over long distances (Figure 13). The phylogeny of *Stygocapitella* displays a biogeographic signal related to oceanic water bodies, with several oceanic transitions being observed (Figure 5). This is in line with evidence for wide distributions of meiofaunal nemerteans (Leasi and Norenburg, 2016, 2014), xenacoelomorphans (Meyer-Wachsmuth et al., 2014), nematodes (Derycke et al., 2008) and molluscs (Jörger et al., 2012). Finally, fluctuations in ecological conditions are expected to promote stasis by selecting 'morphologically-idle' species (Sheldon, 1996). Fluctuating conditions seem to occur rapidly in the sediments (pH, salinity and moisture), yet conditions have been potentially similar for millions of years (Giere, 2009; Noodt, 1974). The interstitial habitat could, thus, be considered as constantly changing, yet as a long term stable environment (Giere, 2009; Westheide, 1977; Westheide and Rieger, 1987), as suggested by the "plus ça change, plus c'est la même chose" model (French for 'The more it changes, the more it is the same') (Sheldon, 1996). The combination of these three scenarios may explain stasis in interstitial organisms and in *Stygocapitella*, but further data should be collected to substantiate these conclusions.

Stygocapitella species complex – evolutionary history

Stygocapitella species maintain wide distributions suggesting wide dispersal capacities, with no association between population structure and geography. For example, *S. zecai* sp. nov. is distributed from Northern Norway to England, and one of its 16S haplotypes is shared among specimens from Henningsvær and Lødingen (Northern Norway), Ardtoe (Western Scotland) and Cutty Sark (England) suggesting that no population structure occurs for about ~2000 km distance (Figure 13). *Stygocapitella subterranea* and *S. josemariobrancoi* sp. nov. have wide distributions ranging from Scotland, Germany and France, with a similar pattern of haplotypes occurring over long distances (Figure 13). *Stygocapitella westheidei* sp. nov. has one single 16S haplotype along the entire North-western Atlantic coastline in the USA, spanning ca. 450 km (Figure 13).

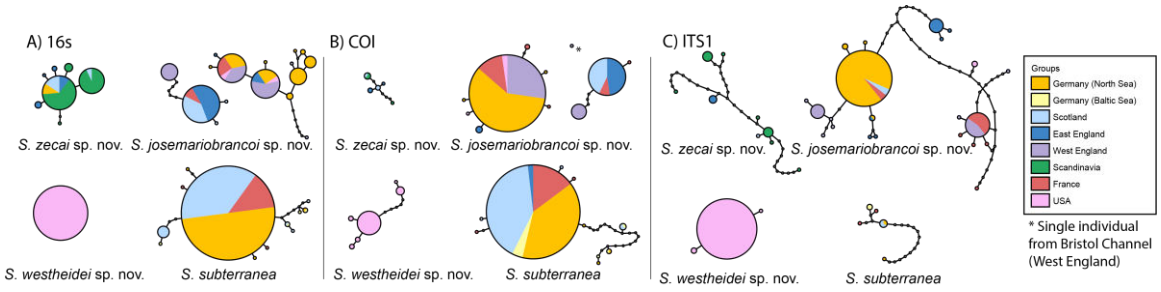


Figure 13. Haplotype networks of the species occurring in the Atlantic Ocean (*S. zecai* sp. nov., *S. subterranea*, *S. westheidei* sp. nov., *S. josemariobrancoi* sp. nov.). Data includes (A) the mitochondrial 16S and (B) COI, and the nuclear gene ITS1. Haplotype sp. nov. networks are colored based on countries and regions.

The phylogeny of *Stygocapitella* displays a biogeographic signal related to the oceans. Northern Atlantic species form a monophyletic group (Figure 5). However, the North-American distributed *S. westheidei* sp. nov. is nested within the remaining Atlantic species which occur in Europe. The Northern Atlantic group is closely related to the species present in the Indo-Pacific Oceans, indicating that a transition from the Indo-Pacific to the Atlantic has occurred only once. Interestingly, I find evidence for two groups of sister species occurring in opposite sides of the Northern Pacific Ocean, with *S. furcata* sp. nov. and *S. pacifica* sp. nov., as well as *Stygocapitella* spec. A and *S. budaevae* sp. nov. occurring in Northern America and Russia, respectively. This suggests that the ancient lineages of each pair speciated allopatrically following vicariance or could transverse the Pacific Ocean (Figure 5). The Australian species, *S. australis*, is closely related to the Northern Pacific ones, while *S. minuta*, from South Africa, is the first to branch off in the phylogenetic tree (Figure 5). This suggests that at least two equatorial transitions must have occurred.

Two major hypotheses have been suggested to explain the distribution of meiofaunal groups. These include the 'strict vicariance hypothesis', which states that meiofaunal taxa are poor dispersers, and the 'long-distance dispersal hypothesis' which states that meiofaunal taxa are indeed capable of dispersing. Evidence gathered from my thesis is congruent with a previous analysis (Struck et al., 2017), which

together suggest that a strict vicariance hypothesis does neither fit the observed distribution patterns nor the phylogeny of *Stygocapitella*. I find evidence for several events of long-distance dispersal (Westheide, 1991, 1977), which have an important role in establishing new populations across oceans and spreading along coastlines (Derycke et al., 2008; Schmidt and Westheide, 2000).

Two specimens collected in Lubec (Maine, USA) were identified as *S. josemariobrancoi* sp. nov.

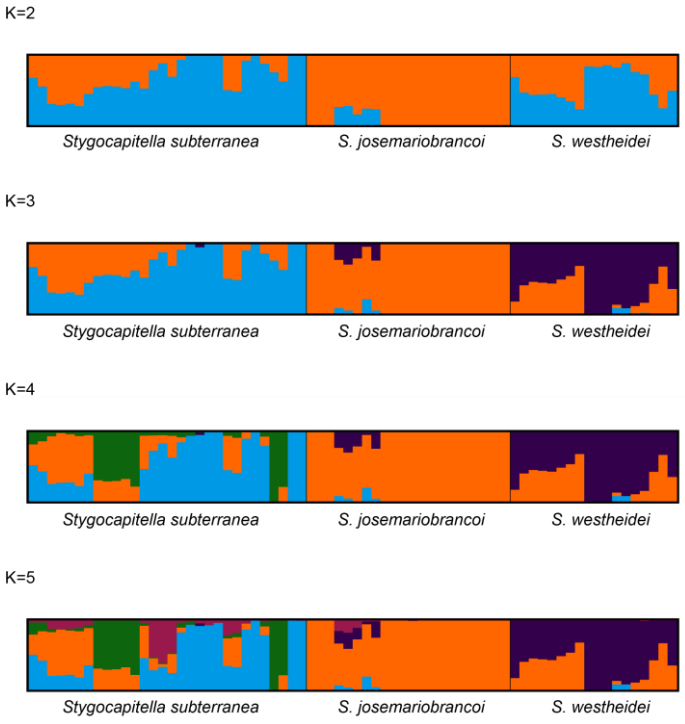


Figure 14. Structure analysis of 3,428 single-nucleotide-polymorphisms (SNPs) based on the ddRAD dataset. Different K's represent different number of clusters fit in different analyses. *Stygocapitella subterranea*, *S. josemariobrancoi* and *S. westheidei* are consecutively plotted from left to right. K=3 was the best supported K (*manuscript 5*).

using molecular tools (Figure 2A, 5). This species is elsewhere present in Northern Europe. The specimens from Lubec share a 16S haplotype with specimens from England, France and Germany, suggesting a very recent dispersal event possibly due to trans-Atlantic trade.

However, the application of double digestion Restriction-Associated DNA Digestion Sequencing (ddRAD) together with a whole genome amplification (WGA) approach, suggest shared genetic variation among *S. stubterranea*, *S. westheidei*, and *S. josemariobrancoi* (Figures 14-16). While WGA includes a certain amount of bias in the form of palindromes (Warris et al., 2018) and missingness (de Medeiros and Farrell, 2018), these challenges are surmounted by stringent filtering of the data (de Medeiros and Farrell, 2018; *manuscript 5*). Evidence from shared genetic variation comes from structure analyses (Figure 14), the phylogenomic tree (Figure 15) and coalescent simulations based on the Site-Frequency-Spectrum (Figure 16). Strictly spoken, the phylogenomic-based tree analysis shows that the species are non-monophyletic

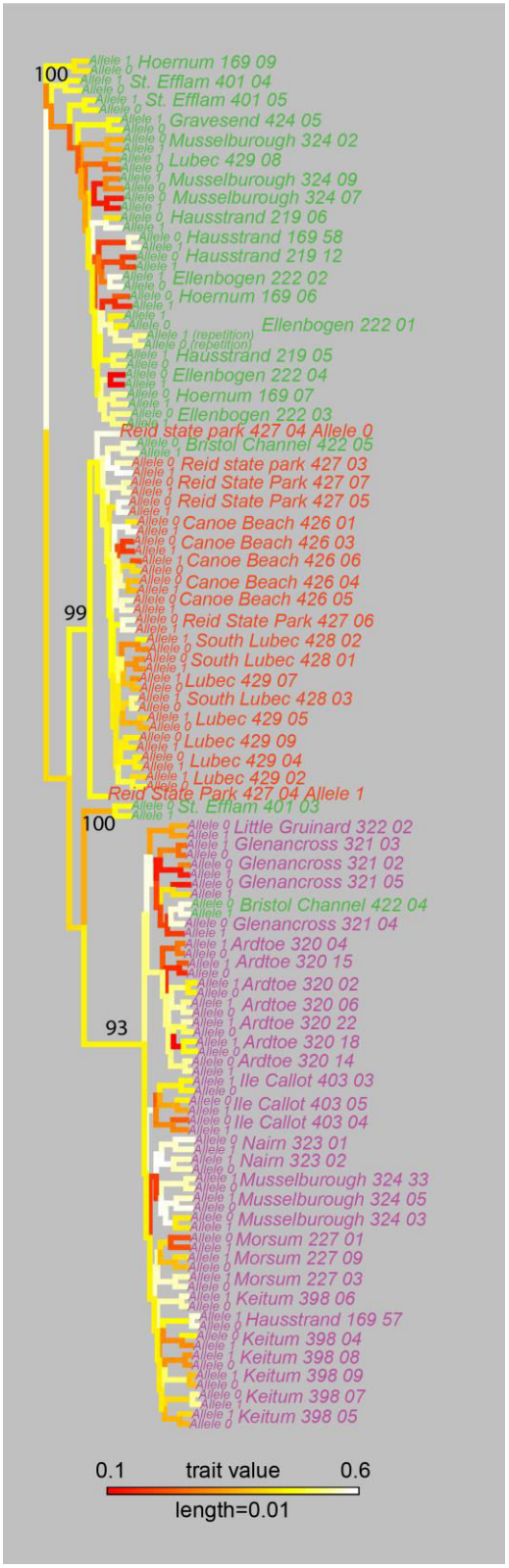


Figure 15. Phylogenomic tree consisting of 4,737 RADseq locus. Bootstrap support for the three species are given on top of the branches. Colours follow species with *Stygocapitella subterranea* being represented in purple, *S. josemariobrancoi* in green, and *S. westheidei* inorange. Colours on the tree topology represent missingness (10% missing data in red; 60% missing data in white). The sample Ellenbogen222_01 was included twice to test for data quality.

(Figure 15). In this tree, specimens from *S. josemariobrancoi* sp. nov. form a monophyletic clade, but some individuals are nested in the branches of *S. subterranea* and *S. westheidei* sp. nov. (Figure 15). Genome-level data thus suggests that some degree of admixture, incomplete lineage sorting or symplesiomorphic variation. This is confirmed in demographic analyses (Figure 16). The most supported demographic scenarios include the "Ancient gene flow", the "No Gene Flow" and the "Modern Geneflow between *S. subterranea* and *S. westheidei* sp. nov." (modern refers to currently existing lineages). When evaluating parameter files for these three scenarios, ancient geneflow suggests that the coalescent events have occurred 1,130,483 generations ago (*S. subterranea*, *S. westheidei* sp. nov.) and 12,816,687 generations ago (*S. josemariobrancoi* sp. nov., and the most common ancestor branch between *S. subterranea* and *S. westheidei* sp. nov.). The "No gene flow scenario" suggests that the coalescence have occurred 205,937 generations ago and 7,482,922 generations ago, and the "Modern Geneflow between *Stygocapitella subterranea* and *S. westheidei*" suggest these have occurred 3,759 and 31,853 generations ago. Considering a generation time of 1 year in *Stygocapitella* (Günter Purschke, *pers. comm.*), the "Ancient gene flow" scenario is in agreement with molecular clock approaches (*manuscript 4*).

These results suggest that selected markers might limit the study of the evolutionary history of a given clade but

cryptic species in the *Stygocapitella* can likely be determined by a combination of mitochondrial and nuclear markers (*manuscript 5*). However, complex demographic histories, likely comprising incomplete lineage sorting, admixture between ancestral lineages, or symplesiomorphies are overlooked by a limited number of selected markers and are only revealed by thousands of individual markers. This is in agreement with the evidence that suggests that the application of a limited amount of markers, typically mitochondrial markers can overlook deep splits in lineages (Dincă et al., 2019; Giska et al., 2015; Hinojosa et al., 2019), introgression (Toews and Brelsford, 2012), and incomplete lineage sorting (Toews and Brelsford, 2012).

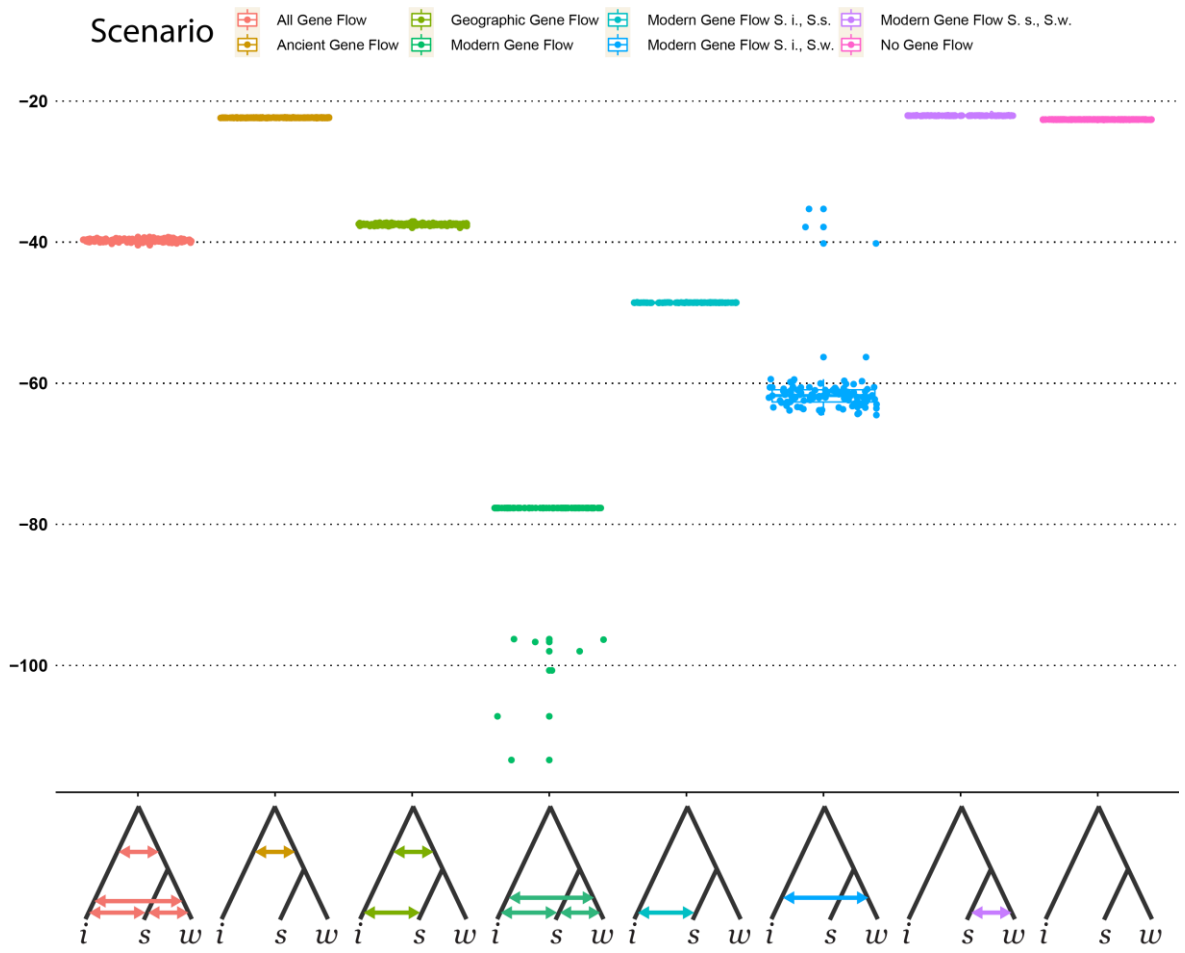


Figure 16. Demographic scenarios used for coalescent simulations based on the Site Frequency Spectrum. In the X axis, different scenarios are modelled. In the Y axis, the likelihood of each scenario is displayed. Modelled gene flow is given by arrows as displayed in the cladograms bellow each scenario. From left to right, scenarios include 1) all gene flow (gene flow between all lineages); 2) ancient gene flow (gene flow in the ancestral lineage of *S. josemariobrancoi* sp. nov., *S. subterranea* and *S. westheidei* sp. nov.); 3) geographic gene flow (geneflow between potentially para/sympatric lineages; 4) modern gene flow (between all currently existing lineages); 5) gene flow between *S. josemariobrancoi* sp. nov. and *S. subterranea*; 6) geneflow between *S. josemariobrancoi* sp. nov., *S. westheidei* sp. nov.; 7) geneflow between *S. subterranea*, *S. westheidei* sp. nov.; 8) no gene flow.

The importance of 'cryptic biodiversity'

The detection of cryptic species has been raising in recent years with the advent and application of DNA barcoding (Struck et al., 2018a). Some estimations suggest that there are around 9,000-36,000

cryptic species in the seas, comprising a total of 3-12% of life in oceans (Appeltans et al., 2012). Understanding which groups are more prone to have decelerated morphologies and hence more overlooked species becomes thus important in a time of global extinctions and human-induced climatic changes. This hidden layer of biodiversity is likely to affect ecological parameters such as community composition (Chenuil et al., 2019), the determination of species' evolutionary history (Hinojosa et al., 2019), estimates of paleontological rates of evolution (Alizon et al., 2008), estimation of biogeographic breaks (Weber et al., 2019), and in species' conservation (Bernardo, 2011). Future works should focus on quantifying the numbers of cryptic species by habitats and in different branches in the tree of life (Pérez-Ponce de León and Poulin, 2016), and should focus on detecting cryptic species in lineages affected by extinction and in habitats facing fragmentation, destruction or climate change.

Conclusions

Biological forms have evolved to occupy nearly every environment in our planet, displaying an astonishing diversity of forms, traits and strategies. Morphological diversity has inspired generations of biologists, being at the heart of the passion we hold for biology. 'Evolvability' (i.e. the ability to evolve new traits) is seen as a measure of biological success, and as a desired feature of any system (Weiss, 2011). All of this has rendered ideas of 'invariability', 'deceleration' or 'stasis' as dubious or as bad observations. However, the occurrence of cryptic species is becoming more relevant and more prominent in recent years (Struck et al., 2018a). Cryptic species potentially represent an important component of biodiversity, and warrant further attention.

As part of this thesis, I have provided a framework to understand and identify cryptic species. This framework focuses on the biological history of the species complex, rather than on human-error (i.e. taxonomic artefacts). With reference to the 'meiofauna paradox', I have provided an example of how overlooking cryptic species can bias estimations of species distribution and confound species' biogeography. Focusing on the *Stygocapitella* cryptic species complex, I have shown that it has decelerated rates of morphological evolution relatively to closely related taxa. The description of 8 new species has reduced the distribution of *Stygocapitella subterranea* from cosmopolitan to only being present in the European coastlines. Some of these species are morphologically undisguisable using quantitative tools (i.e. Scanning Electron Microscopy), or various measurements. Furthermore, genomic data suggests that a complex of morphologically similar *Stygocapitella* species have a potentially complex demographic history.

Future research should focus on quantifying the occurrence of cryptic species in in different lineages of the tree of life. It should also further our knowledge on the mechanisms leading to the deceleration of morphological evolution and, in particular, the extreme example of stasis. These approaches will benefit from the integration of decades of research of paleontological stasis, together with modern genomic tools including population genomics, phylogenomics and comparative genomics.

References

Alizon, S., Kucera, M., Jansen, V., 2008. Competition between cryptic species explains variations in rates of lineage evolution. Proc. Natl. Acad. Sci. U. S. A. 105, 12382–12386. https://doi.org/10.1073/pnas.0805039105

Appeltans, W., Ahyong, S.T., Anderson, G., Angel, M. V., Artois, T., Bailly, N., Bamber, R., Barber, A., Bartsch, I., Berta, A., Błazewicz-Paszkowycz, M., Bock, P., Boxshall, G., Boyko, C.B., Brandão, S.N., Bray, R.A., Bruce, N.L., Cairns, S.D., Chan, T.Y., Cheng, L., Collins, A.G., Cribb, T., Curini-Galletti, M., Dahdouh-Guebas, F., Davie, P.J.F., Dawson, M.N., De Clerck, O., Decock, W., De Grave, S., De Voogd, N.J., Domning, D.P., Emig, C.C., Erséus, C., Eschmeyer, W., Fauchald, K., Fautin, D.G., Feist, S.W., Fransen, C.H.J.M., Furuya, H., Garcia-Alvarez, O., Gerken, S., Gibson, D., Gittenberger, A., Gofas, S., Gómez-Daglio, L., Gordon, D.P., Guiry, M.D., Hernandez, F., Hoeksema, B.W., Hopcroft, R.R., Jaume, D., Kirk, P., Koedam, N., Koenemann, S., Kolb, J.B., Kristensen, R.M., Kroh, A., Lambert, G., Lazarus, D.B., Lemaitre, R., Longshaw, M., Lowry, J., MacPherson, E., Madin, L.P., Mah, C., Mapstone, G., McLaughlin, P.A., Mees, J., Meland, K., Messing, C.G., Mills, C.E., Molodtsova, T.N., Mooi, R., Neuhaus, B., Ng, P.K.L., Nielsen, C., Norenburg, J., Opresko, D.M., Osawa, M., Paulay, G., Perrin, W., Pilger, J.F., Poore, G.C.B., Pugh, P., Read, G.B., Reimer, J.D., Rius, M., Rocha, R.M., Saiz-Salinas, J.I., Scarabino, V., Schierwater, B., Schmidt-Rhaesa, A., Schnabel, K.E., Schotte, M., Schuchert, P., Schwabe, E., Segers, H., Self-Sullivan, C., Shenkar, N., Siegel, V., Sterrer, W., Stöhr, S., Swalla, B., Tasker, M.L., Thuesen, E. V., Timm, T., Todaro, M.A., Turon, X., Tyler, S., Uetz, P., Van Der Land, J., Vanhoorne, B., Van Ofwegen, L.P., Van Soest, R.W.M., Vanaverbeke, J., Walker-Smith, G., Walter, T.C., Warren, A., Williams, G.C., Wilson, S.P., Costello, M.J., 2012. The magnitude of global marine species diversity. Curr. Biol. 22, 2189–2202. https://doi.org/10.1016/j.cub.2012.09.036

Arroyo, N.L., Aarnio, K., Bonsdorff, E., 2006. Drifting algae as a means of re-colonizing defaunated sediments in the Baltic Sea. A short-term microcosm study. Hydrobiologia 554, 83–95. https://doi.org/10.1007/s10750-005-1008-5

Astrin, J.J., Stüben, P.E., 2008. Phylogeny in cryptic weevils: Molecules, morphology and new genera of western Palaearctic Cryptorhynchinae (Coleoptera: Curculionidae). Invertebr. Syst. 22, 503–522. https://doi.org/10.1071/IS07057

Bernardo, J., 2011. A critical appraisal of the meaning and diagnosability of cryptic evolutionary diversity, and its implications for conservation in the face of climate change. Clim. Chang. Ecol. Syst. 380–438. https://doi.org/10.1017/CBO9780511974540.019

Bickford, D., Lohman, D.J., Sodhi, N.S., Ng, P.K.L., Meier, R., Winker, K., Ingram, K.K., Das, I., 2007. Cryptic species as a window on diversity and conservation. Trends Ecol. Evol. 22, 148–155. https://doi.org/10.1016/j.tree.2006.11.004

Bleidorn, C., Hill, N., Erséus, C., Tiedemann, R., 2009. On the role of character loss in orbiniid phylogeny (Annelida): Molecules vs. morphology. Mol. Phylogenet. Evol. 52, 57–69. https://doi.org/10.1016/j.ympev.2009.03.022

Boeckner, M.J., Sharma, J., Proctor, H.C., 2009. Revisiting the meiofauna paradox: Dispersal and colonization of nematodes and other meiofaunal organisms in low- and high-energy environments. Hydrobiologia 624, 91–106. https://doi.org/10.1007/s10750-008-9669-5

Bouckaert, R., Heled, J., Kühnert, D., Vaughan, T., Wu, C.H., Xie, D., Suchard, M.A., Rambaut, A., Drummond, A.J., 2014. BEAST 2: A software platform for bayesian evolutionary analysis. PLoS Comput. Biol. 10, 1–6. https://doi.org/10.1371/journal.pcbi.1003537

Brenzinger, B., Haszprunar, G., Schrödl, M., 2013. At the limits of a successful body plan - 3D microanatomy, histology and evolution of *Helminthope* (Mollusca: Heterobranchia: Rhodopemorpha), the most worm-like gastropod. Front. Zool. 10, 37. https://doi.org/10.1186/1742-9994-10-37

Callens, M., Gheerardyn, H., Ndraro, S.G.M., De Troch, M., Vanreusel, A., 2012. Harpacticoid copepod colonization of coral fragments in a tropical reef lagoon (Zanzibar, Tanzania). J. Mar. Biol. Assoc. United Kingdom 92, 1535–1545. https://doi.org/10.1017/S0025315411001597

Carstens, B.C., Pelletier, T.A., Reid, N.M., Satler, J.D., 2013. How to fail at species delimitation. Mol. Ecol. 22, 4369–4383. https://doi.org/10.1111/mec.12413

Cerca, J., Purschke, G., Struck, T.H., 2018. Marine connectivity dynamics: clarifying cosmopolitan distributions of marine interstitial invertebrates and the meiofauna paradox. Mar. Biol. 165, 123. https://doi.org/10.1007/s00227-018-3383-2

Charlesworth, B., Lande, R., Slatkin, M., 1982. A Neo-Darwinian commentary on macroevolution. Evolution (N. Y). 36, 474–498.

Chenuil, A., Cahill, A.E., Délémontey, N., Luc, E.D.S. du, Hadrien, Fanton, 2019. Problems and questions posed by cryptic species. A framework to guide future studies. pp. 77–106. https://doi.org/10.1007/978-3-030-10991-2

Chernomor, O., Von Haeseler, A., Minh, B.Q., 2016. Terrace aware data structure for phylogenomic inference from supermatrices. Syst. Biol. 65, 997–1008. https://doi.org/10.1093/sysbio/syw037

Christiansen, F.B., Fenchel, T.M., 1979. Evolution of marine invertebrate reproductive patterns. Theor. Popul. Biol. 16, 267–282. https://doi.org/10.1016/0040-5809(79)90017-0

Clement, M., Posada, D., Crandall, K.A., 2000. TCS: a computer program to estimate gene genealogies. Mol. Ecol. 9, 1657–1660. https://doi.org/10.1046/j.1365-294x.2000.01020.x

Commito, J.A., Tita, G., 2002. Differential dispersal rates in an intertidal meiofauna assemblage. J. Exp. Mar. Bio. Ecol. 268, 237–256. https://doi.org/10.1016/S0022-0981(01)00386-0

Cristoni, C., Colangelo, M.A., Ceccherelli, V.U., 2004. Spatial scale and meiobenthic copepod recolonisation: Testing the effect of disturbance size in a seagrass habitat. J. Exp. Mar. Bio. Ecol. 298, 49–70. https://doi.org/10.1016/j.jembe.2003.08.005

Cuvelier, D., Beesau, J., Ivanenko, V.N., Zeppilli, D., Sarradin, P.M., Sarrazin, J., 2014. First insights into macro- and meiofaunal colonisation patterns on paired wood/slate substrata at Atlantic deep-sea hydrothermal vents. Deep. Res. Part I Oceanogr. Res. Pap. 87, 70–81. https://doi.org/10.1016/j.dsr.2014.02.008

Danecek, P., Auton, A., Abecasis, G., Albers, C.A., Banks, E., DePristo, M.A., Handsaker, R.E., Lunter, G., Marth, G.T., Sherry, S.T., McVean, G., Durbin, R., 2011. The variant call format and VCFtools. Bioinformatics 27, 2156–2158. https://doi.org/10.1093/bioinformatics/btr330

Danielopol, D.A.N., Wouters, K., 1992. Evolutionary (Paleo)biology of marine interstitial ostracoda. Geobios 25, 207–211.

de Medeiros, B.A.S., Farrell, B.D., 2018. Whole genome amplification in double-digest RAD-seq results in adequate libraries but fewer sequenced loci. PeerJ. https://doi.org/10.7717/peerj.5089

De Meester, N., Derycke, S., Moens, T., 2012. Differences in time until dispersal between cryptic species of a marine nematode species complex. PLoS One 7, 1–8. https://doi.org/10.1371/journal.pone.0042674

De Meester, N., Derycke, S., Rigaux, A., Moens, T., 2015. Active dispersal is differentially affected by inter- and intraspecific competition in closely related nematode species. Oikos 124, 561–570. https://doi.org/10.1111/oik.01779

Derycke, S., Backeljau, T., Moens, T., 2013. Dispersal and gene flow in free-living marine nematodes. Front. Zool. 10, 1. https://doi.org/10.1186/1742-9994-10-1

Derycke, S., Remerie, T., Backeljau, T., Vierstraete, A., Vanfleteren, J., Vincx, M., Moens, T., 2008. Phylogeography of the *Rhabditis* (Pellioditis) marina species complex: Evidence for long-distance dispersal, and for range expansions and restricted gene flow in the northeast Atlantic. Mol. Ecol. 17, 3306–3322. https://doi.org/10.1111/j.1365-294X.2008.03846.x

Dincă, V., Lee, K.M., Vila, R., Mutanen, M., 2019. The conundrum of species delimitation: a genomic perspective on a mitogenetically super-variable butterfly. Proc. Biol. Sci. 286.

Dujardin, F., 1851. Sur un petit animal marin, l'Echinodère, formant un type intermédiaire entre les Crustacés et les Vers. Ann Sci Nat Zool. 3, 158–160.

Eldredge, N., Gould, S.J., 1972. Punctuated Equilibria: An alternative to phylogetic gradualism, in: Schopf, T.J.M. (Ed.), Models in Paleobiology. Freeman, Cooper and Co., San Francisco., pp. 82–115.

Elgetany, A.H., El-Ghobashy, A.E., Ghoneim, A.M., Struck, T.H., 2018. Description of a new species of the genus marphysa (Eunicidae), Marphysa aegypti sp.n., based on molecular and morphological evidence. Invertebr. Zool. 15, 71–84. https://doi.org/10.15298/invertzool.15.1.05

Endersby, J., 2009. Lumpers and splitters: Darwin, Hooker, and the search for order. Science (80-.). 326, 1496–1499. https://doi.org/10.1126/science.1165915

Erlank, E., Koekemoer, L.L., Coetzee, M., 2018. The importance of morphological identification of African anopheline mosquitoes (Diptera: Culicidae) for malaria control programmes. Malar. J. 17, 1–7. https://doi.org/10.1186/s12936-018-2189-5

Estes, S., Arnold, S.J., 2007. Resolving the paradox of stasis: models with stabilizing selection explain evolutionary divergence on all timescales. Am. Nat. 169, 227–244. https://doi.org/10.1086/510633

Excoffier, L., Dupanloup, I., Huerta-Sánchez, E., Sousa, V.C., Foll, M., 2013. Robust Demographic Inference from Genomic and SNP Data. PLoS Genet. 9. https://doi.org/10.1371/journal.pgen.1003905

Falush, D., Stephens, M., Pritchard, J.K., 2003. Inference of population structure using multilocus genotype data: Linked loci and correlated allele frequencies. Genetics 164, 1567–1587. https://doi.org/10.1111/j.1471-8286.2007.01758.x

Fišer, C., Robinson, C.T., Malard, F., 2018. Cryptic species as a window into the paradigm shift of the species concept. Mol. Ecol. 27, 613–635. https://doi.org/10.1111/mec.14486

Fonsêca-Genevois, V. da, Somerfield, P.J., Neves, M.H.B., Coutinho, R., Moens, T., 2006. Colonization and early succession on artificial hard substrata by meiofauna. Mar. Biol. 148, 1039–1050. https://doi.org/10.1007/s00227-005-0145-8

Fontaneto, D., Flot, J.F., Tang, C.Q., 2015. Guidelines for DNA taxonomy, with a focus on the meiofauna. Mar. Biodivers. 45, 433–451. https://doi.org/10.1007/s12526-015-0319-7

Fujisawa, T., Barraclough, T.G., 2013. Delimiting species using single-locus data and the generalized mixed yule coalescent approach: A revised method and evaluation on simulated data sets. Syst. Biol. 62, 707–724. https://doi.org/10.1093/sysbio/syt033

Futuyma, D., 2005. Evolution, Evolution. Sinauer Associates, Inc, Sunderland, MA. https://doi.org/10.1007/s13398-014-0173-7.2

Futuyma, D.J., 2015. Can modern evolutionary theory explain macroevolution?, in: Macroevolution. pp. 29–86. https://doi.org/10.5860/choice.192898

Futuyma, D.J., 2010. Evolutionary constraint and ecological consequences. Evolution (N. Y). 64, 1865–1884. https://doi.org/10.1111/j.1558-5646.2010.00960.x

Futuyma, D.J., 1987. On the role of species in anagenesis. Am. Nat. 130, 465–473.

Gallucci, F., Moens, T., Vanreusel, A., Fonseca, G., 2008. Active colonisation of disturbed sediments by deep-sea nematodes: Evidence for the patch mosaic model. Mar. Ecol. Prog. Ser. 367, 173–183. https://doi.org/10.3354/meps07537

Gerlach, S.A., 1977. Means of meiofauna dispersal., in: The Meiofauna Species in Time and Space. Mikrofauna Meeresbod. pp. 89–103.

Giere, O., 2009. Meiobenthology: the microscopic motile fauna of aquatic sediments, 2nd ed. Springler-Verlag, Berlin Heidelberg. https://doi.org/10.1016/0022-0981(94)90135-X

Gillespie, R., 2004. Community assembly through adaptive radiation in Hawaiian spiders. Science (80-.). 303, 356–360.

Giska, I., Sechi, P., Babik, W., 2015. Deeply divergent sympatric mitochondrial lineages of the earthworm Lumbricus rubellus are not reproductively isolated. BMC Evol. Biol. 15, 217. https://doi.org/10.1186/s12862-015-0488-9

Gobin, J.F., Warwick, R.M., 2006. Geographical variation in species diversity: A comparison of marine polychaetes and nematodes. J. Exp. Mar. Bio. Ecol. 330, 234–244. https://doi.org/10.1016/j.jembe.2005.12.030

Golombek, A., Tobergte, S., Nesnidal, M.P., Purschke, G., Struck, T.H., 2013. Mitochondrial genomes to the rescue – Diurodrilidae in the myzostomid trap. Mol. Phylogenet. Evol. 68, 312–326.

Gueriau, P., Rabet, N., Clément, G., Lagebro, L., Vannier, J., Briggs, D.E.G., Charbonnier, S., Olive, S., Béthoux, O., 2016. A 365-million-year-old freshwater community reveals morphological and ecological stasis in branchiopod crustaceans. Curr. Biol. 26, 383–390. https://doi.org/10.1016/j.cub.2015.12.039

Guilini, K., Soltwedel, T., van Oevelen, D., Vanreusel, A., 2011. Deep-sea nematodes actively colonise sediments, irrespective of the presence of a pulse of organic matter: Results from an in-situ experiment. PLoS One 6. https://doi.org/10.1371/journal.pone.0018912

Gwyther, J., Fairweather, P.G., 2005. Meiofaunal recruitment to mimic pneumatophores in a cool-temperate mangrove forest: spatial context and biofilm effects. J. Exp. Mar. Bio. Ecol. 317, 69–85. https://doi.org/10.1016/j.jembe.2004.11.012

Hansen, T.F., 1997. Stabilizing selection and the comparative analysis of adaptation. Evolution (N. Y). 51, 1341–1351.

Hansen, T.F., Houle, D., 2004. Evolvability, stabilizing selection, and the problem of stasis, in: Pigliucci, M., Preston, K. (Eds.), Phenotypic Integration: Studying the Ecology and Evolution of Complex Phenotypes. Oxford University Press, New York, pp. 130–154.

Harrell Jr, F.E., Many Others, contributions from, 2019. Hmisc: Harrell Miscellaneous. R package.

Hawksworth, D.L., Lücking, R., 2017. Fungal diversity revisited : 2.2 to 3.8 million species. Microbiol. Spetrum 5, 1–17. https://doi.org/10.1128/microbiolspec.FUNK-0052-2016.Correspondence

Heethoff, M., 2018. Cryptic species – Conceptual or terminological chaos? A response to Struck et al. Trends Ecol. Evol. 33, 2018. https://doi.org/10.1016/j.tree.2018.02.006

Hillis, D.M., Dixon, M.T., 1991. Ribosomal DNA: Molecular evolution and phylogenetic inference. Q. Rev. Biol. 66, 411–453.

Hinojosa, J.C., Koubínová, D., Szenteczki, M.A., Pitteloud, C., Dincă, V., Alvarez, N., Vila, R., 2019. A mirage of cryptic species: Genomics uncover striking mitonuclear discordance in the butterfly

Thymelicus sylvestris . Mol. Ecol. 1–12. https://doi.org/10.1111/mec.15153

Hoang, D.T., Chernomor, O., von Haeseler, A., Quang Minh, B., Sy Vinh, L., 2017. Ufboot2: Improving the ultrafast bootstrap approximation 35, 518–522. https://doi.org/10.5281/zenodo.854445

Hooper, G.J., Davenport, J., 2006. Epifaunal composition and fractal dimensions of intertidal marine macroalgae in relation to emersion. J. Mar. Biol. Assoc. United Kingdom 86, 1297–1304. https://doi.org/10.1017/S0025315406014329

Hotaling, S., Foley, M.E., Lawrence, N.M., Bocanegra, J., Blanco, M.B., Rasoloarison, R., Kappeler, P.M., Barrett, M.A., Yoder, A.D., Weisrock, D.W., 2016. Species discovery and validation in a cryptic radiation of endangered primates: Coalescent-based species delimitation in Madagascar's mouse lemurs. Mol. Ecol. 25, 2029–2045. https://doi.org/10.1111/mec.13604

Huson, D.H., Bryant, D., 2006. Application of phylogenetic networks in evolutionary studies. Mol. Biol. Evol. 23, 254–267. https://doi.org/10.1093/molbev/msj030

Hutchings, P., Kupriyanova, E., 2018. Cosmopolitan polychaetes – fact or fiction? Personal and historical perspectives. Invertebr. Syst. 32, 1–9. https://doi.org/https://doi.org/10.1071/IS17035

Jörger, K.M., Neusser, T.P., Brenzinger, B., Schrödl, M., 2014. Exploring the diversity of mesopsammic gastropods: how to collect, identify, and delimitate small and elusive sea slugs? Am. Malacol. Bull. 32, 290–307. https://doi.org/10.4003/006.032.0205

Jörger, K.M., Norenburg, J.L., Wilson, N.G., Schrödl, M., 2012. Barcoding against a paradox? Combined molecular species delineations reveal multiple cryptic lineages in elusive meiofaunal sea slugs. BMC Evol. Biol. 12, 245. https://doi.org/10.1186/1471-2148-12-245

Jörger, K.M., Schrödl, M., 2013. How to describe a cryptic species? Practical challenges of molecular taxonomy. Front. Zool. 10, 59. https://doi.org/10.1186/1742-9994-10-59

Junkins, R., Kelaher, B., Levinton, J., 2006. Contributions of adult oligochaete emigration and immigration in a dynamic soft-sediment community. J. Exp. Mar. Bio. Ecol. 330, 208–220. https://doi.org/10.1016/j.jembe.2005.12.028

Kajihara, H., Ikoma, M., Yamasaki, H., Hiruta, S.F., 2015. *Trilobodrilus itoi* sp. nov., with a re-description of *T. nipponicus* (Annelida: Dinophilidae) and a molecular phylogeny of the genus. Zoolog. Sci. 32, 405–417. https://doi.org/10.2108/zs140251

Kalyaanamoorthy, S., Minh, B.Q., Wong, T.K.F., Von Haeseler, A., Jermiin, L.S., 2017. ModelFinder: Fast model selection for accurate phylogenetic estimates. Nat. Methods 14, 587–589. https://doi.org/10.1038/nmeth.4285

Katoh, K., Standley, D.M., 2013. MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. Mol. Biol. Evol. 30, 772–780. https://doi.org/10.1093/molbev/mst010

Kieneke, A., Martínez Arbizu, P.M., Fontaneto, D., 2012. Spatially structured populations with a low level of cryptic diversity in European marine Gastrotricha. Mol. Ecol. 21, 1239–54. https://doi.org/10.1111/j.1365-294X.2011.05421.x

Klautau, M., Russo, C.A.M., Lazoski, C., Boury-esnault, N., Thorpe, J.P., Sole-cava, A.M., Klautau, M., Russo, C.A.M., Lazoski, C., Boury-esnault, N., John, P., Solt-cava, A.M., 1999. Does cosmopolitanism result from overconservative systematics? A case study using the marine sponge *Chondrilla nucula*. Evolution (N. Y). 53, 1414–1422.

Knöllner, F., 1934. *Stypocapitella subterranea* nov.gen. nov.spec. Schriften der Naturwissenschaftlichen Vereins für Schleswig-Holstein 20, 468–472.

Knowlton, N., 2000. Molecular genetic analyses of species boundaries in the sea. Hydrobiologia 420, 73–90. https://doi.org/10.1023/A:1003933603879

Knowlton, N., 1993. Sibling species in the sea. Annu. Rev. Ecol. Syst. 24, 189–216.

Kon, T., Yoshino, T., Mukai, T., Nishida, M., 2007. DNA sequences identify numerous cryptic species of the vertebrate: A lesson from the gobioid fish *Schindleria*. Mol. Phylogenet. Evol. 44, 53–62. https://doi.org/10.1016/j.ympev.2006.12.007

Korshunova, T., Martynov, A., Bakken, T., Picton, B., 2017. External diversity is restrained by internal conservatism: New nudibranch mollusc contributes to the cryptic species problem. Zool. Scr. 46, 683–692. https://doi.org/10.1111/zsc.12253

Korshunova, T., Picton, B., Furfaro, G., Mariottini, P., Pontes, M., Prkić, J., Fletcher, K., Malmberg, K., Lundin, K., Martynov, A., 2019. Multilevel fine-scale diversity challenges the 'cryptic species' concept. Sci. Rep. 9, 6732. https://doi.org/10.1038/s41598-019-42297-5

Kück, P., Meusemann, K., 2010. FASconCAT: Convenient handling of data matrices. Mol. Phylogenet. Evol. 56, 1115–1118. https://doi.org/10.1016/j.ympev.2010.04.024

Kück, P., Struck, T.H., 2014. BaCoCa - A heuristic software tool for the parallel assessment of sequence biases in hundreds of gene and taxon partitions. Mol. Phylogenet. Evol. 70, 94–98. https://doi.org/10.1016/j.ympev.2013.09.011

Kumar, S., Stecher, G., Tamura, K., 2016. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. Mol. Biol. Evol. 33, 1870–1874. https://doi.org/10.1093/molbev/msw054

Lassance, J.M., Svensson, G.P., Kozlov, M. V., Francke, W., Löfstedt, C., 2019. Pheromones and barcoding delimit boundaries between cryptic species in the primitive moth genus *Eriocrania* (Lepidoptera: Eriocraniidae). J. Chem. Ecol. 45, 429–439. https://doi.org/10.1007/s10886-019-01076-2

Lê, S., Josse, J., Husson, F., 2008. FactoMineR: An R Package for Multivariate Analysis. J. Stat. Softw. 25, 253–8. https://doi.org/10.1016/j.envint.2008.06.007

Leasi, F., Norenburg, J.L., 2016. At least some meiofaunal species are not everywhere. Indication of geographic, ecological and geological barriers affecting the dispersion of species of *Ototyphlonemertes* (Nemertea, Hoplonemertea). Mol. Ecol. 25, 1381–1397. https://doi.org/10.1111/mec.13568

Leasi, F., Norenburg, J.L., 2014. The necessity of DNA taxonomy to reveal cryptic diversity and spatial distribution of meiofauna, with a focus on Nemertea. PLoS One 9. https://doi.org/10.1371/journal.pone.0104385

Leasi, F., Tang, C.Q., De Smet, W.H., Fontaneto, D., 2013. Cryptic diversity with wide salinity tolerance in the putative euryhaline Testudinella clypeata (Rotifera, Monogononta). Zool. J. Linn. Soc. 168, 17–28. https://doi.org/10.1111/zoj.12020

Lee, C.E., Frost, B.W., 2002a. Morphological stasis in the *Eurytemora affinis* species complex (Copepoda: Temoridae). Hydrobiologia 480, 111–128. https://doi.org/10.1023/A:1021293203512

Lee, C.E., Frost, B.W., 2002b. Morphological stasis in the Eurytemora affinis species complex (Copepoda: Temoridae), in: Hydrobiologia. pp. 111–128. https://doi.org/10.1023/A:1021293203512

Lehmacher, C., Ramey-balci, P.A., Wolff, L.I., Fiege, D., 2016. Ultrastructural differences in presumed photoreceptive organs and molecular data as a means for species discrimination in Polygordius (Annelida, Protodriliformia , Polygordiidae). Org. Divers. Evol. https://doi.org/10.1007/s13127-016-0272-8

Lenth, R. V., 2013. Lsmeans: Least-squares means. R package version 1.10-4. http://CRAN.R-

project.org/package=lsmeans [WWW Document].

Lindholm, M., 2014. Morphologically conservative but physiologically diverse: The mode of stasis in Anostraca (Crustacea: Branchiopoda). Evol. Biol. 41, 503–507. https://doi.org/10.1007/s11692-014-9283-6

Lins, L., Vanreusel, A., van Campenhout, J., Ingels, J., 2013. Selective settlement of deep-sea canyon nematodes after resuspension - an experimental approach. J. Exp. Mar. Bio. Ecol. 441, 110–116. https://doi.org/10.1016/j.jembe.2013.01.021

Lobo, J., Teixeira, M.A.L., Borges, L.M.S., Ferreira, M.S.G., Hollatz, C., Gomes, P.T., Sousa, R., Ravara, A., Costa, M.H., Costa, F.O., 2016. Starting a DNA barcode reference library for shallow water polychaetes from the southern European Atlantic coast. Mol. Ecol. Resour. 16, 298–313. https://doi.org/10.1111/1755-0998.12441

Losos, J.B., 2010. Adaptive Radiation, Ecological Opportunity, and Evolutionary Determinism. Am. Nat. 175, 623–639. https://doi.org/10.1086/652433

Lovén, S., 1844. Chaetoderma, ett nytt masksläkte n.g. Öfvers K. Vetenskaps-Akad Förh 1, 116+pl.112.

Maynard Smith, J., Burian, R., Kauffman, S., Alberch, P., Campbell, J., Goodwin, B., Lande, R., Raup, D., Wolpert, L., 1985. Developmental constraints and evolution. Q. Rev. Biol. 60, 265–287.

Mcfarlane, C.B.A., Drolet, D., BArbeau, M.A., Hamilton, D.J., Ollerhead, J., 2013. Dispersal of marine benthic invertebrates through ice rafting. Ecology 94, 250–256.

Mevenkamp, L., Van Campenhout, J., Vanreusel, A., 2016. Experimental evidence for selective settlement of meiofauna from two distinct environments after sediment suspension. J. Exp. Mar. Bio. Ecol. 474, 195–203. https://doi.org/10.1016/j.jembe.2015.10.005

Meyer-Wachsmuth, I., Curini Galletti, M., Jondelius, U., 2014. Hyper-cryptic marine meiofauna: Species complexes in Nemertodermatida. PLoS One 9. https://doi.org/10.1371/journal.pone.0107688

Múrias Dos Santos, A., Cabezas, M.P., Tavares, A.I., Xavier, R., Branco, M., 2015. TcsBU: A tool to extend TCS network layout and visualization. Bioinformatics 32, 627–628. https://doi.org/10.1093/bioinformatics/btv636

Nguyen, L.T., Schmidt, H.A., Von Haeseler, A., Minh, B.Q., 2015. IQ-TREE: A fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Mol. Biol. Evol. 32, 268–274. https://doi.org/10.1093/molbev/msu300

Noodt, W., 1974. Anpassungen an interstielle Bedingungen: ein faktor in der evolution höherer taxa der Crustacea. Faun.-ökol. Mitt 4, 445–452.

Nordbotten, J.M., Stenseth, N.C., 2016. Asymmetric ecological conditions favor Red-Queen type of continued evolution over stasis. Proc. Natl. Acad. Sci. U. S. A. 113, 1847–52. https://doi.org/10.1073/pnas.1525395113

Novo, M., Almodóvar, A., Fernández, R., Trigo, D., Dáaz-Cosán, D.J., Giribet, G., 2012. Appearances can be deceptive: Different diversification patterns within a group of mediterranean earthworms (Oligochaeta, Hormogastridae). Mol. Ecol. 21, 3776–3793. https://doi.org/10.1111/j.1365-294X.2012.05648.x

Novo, M., Almodóvar, A., Fernández, R., Trigo, D., Díaz Cosín, D.J., 2010. Cryptic speciation of hormogastrid earthworms revealed by mitochondrial and nuclear data. Mol. Phylogenet. Evol. 56, 507–512. https://doi.org/10.1016/j.ympev.2010.04.010

Palumbi, S., Martin, A., Romano, S., McMillan, W.O., Stice, L., Grabowski, G., 1991. The simple fool's guide to PCR, version 2.

Pante, E., Puillandre, N., Viricel, A., Arnaud-Haond, S., Aurelle, D., Castelin, M., Chenuil, A., Destombe, C., Forcioli, D., Valero, M., Viard, F., Samadi, S., 2015. Species are hypotheses: avoid connectivity assessments based on pillars of sand. Mol. Ecol. 24, 525–544. https://doi.org/10.1111/mec.13048

Paradis, E., Schliep, K., 2018. Ape 5.0: An environment for modern phylogenetics and evolutionary analyses in R. Bioinformatics 35, 526–528. https://doi.org/10.1093/bioinformatics/bty633

Paris, J.R., Stevens, J.R., Catchen, J.M., 2017. Lost in parameter space: a road map for stacks. Methods Ecol. Evol. 8, 1360–1373. https://doi.org/10.1111/2041-210X.12775

Payo, D.A., Leliaert, F., Verbruggen, H., D'hondt, S., Calumpong, H.P., De Clerck, O., 2013. Extensive cryptic species diversity and fine-scale endemism in the marine red alga Portieria in the Philippines. Proc. R. Soc. B Biol. Sci. 280, 20122660–20122660. https://doi.org/10.1098/rspb.2012.2660

Pérez-Ponce de León, G., Poulin, R., 2016. Taxonomic distribution of cryptic diversity among metazoans: not so homogeneous after all. Biol. Lett. 12, 20160371. https://doi.org/10.1098/rsbl.2016.0371

Peterson, B.K., Weber, J.N., Kay, E.H., Fisher, H.S., Hoekstra, H.E., 2012. Double digest RADseq: An inexpensive method for de novo SNP discovery and genotyping in model and non-model species. PLoS One 7. https://doi.org/10.1371/journal.pone.0037135

Pugh, P.J.A., 1996. Using Artificial Substrata to Monitor How Cryptofaunal Acari Colonize Littoral Algae on Sub-antarctic South Georgia. Acarologia 37, 188–200.

Purschke, G., Böggemann, M., Westheide, W., 2019. Parergodrilidae Reisinger, 1925, in: Purschke, G., Böggemann, M., Westheide, W. (Eds.), Annelida, Volume 1: Annelida Basal Groups and Pleistoannelida, Sedentaria. De Gruyter, Berlin, Boston, pp. 237–250.

R Core Team, 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/. [WWW Document].

Radziejewska, T., Gruszka, P., Rokicka-Praxmajer, J., 2006. A home away from home: A meiobenthic assemblage in a ship's ballast water tank sediment. Oceanologia 48, 259–265.

Rambaut, A., Drummond, A.J., Suchard, M.A., 2007. Tracer v1.6.

Ramey-Balci, P., Fiege, D., Purschke, G., 2012. Polygordiida: Polygordiidae Czerniavsky, 1881., in: Handbook of Zoology. Berlin, Boston.

Ramey-Balcı, P., Fiege, D., Struck, T.H., 2018. Molecular phylogeny, morphology, and distribution of Polygordius (Polychaeta: Polygordiidae) in the Atlantic and Mediterranean. Mol. Phylogenet. Evol. 127, 919–930. https://doi.org/10.1016/j.ympev.2018.06.039

Reidenbach, K.R., Neafsey, D.E., Costantini, C., Sagnon, N., Simard, F., Ragland, G.J., Egan, S.P., Feder, J.L., Muskavitch, M.A.T., Besansky, N.J., 2012. Patterns of Genomic Differentiation between Ecologically Differentiated M and S Forms of Anopheles gambiae in West and Central Africa. Genome Biol. Evol. 4, 1202–1212. https://doi.org/10.1093/gbe/evs095

Riser, N.W., 1984. General observations on the intertidal interstitial fauna of New Zealand. Tane 30, 239–250.

Riser, N.W., 1980. The aberrant polychaete *Stygocapitella* from some American beaches. Wasmann J. Biol. 38, 10–17.

Rochette, N., Rivera-Colón, A., Catchen, J.M., 2019. Stacks2: Analytical methods for paired-end sequencing improve RADseq-based population genomics. Mol. Ecol. https://doi.org/10.1111/mec.15253

Rochette, N.C., Catchen, J.M., 2017. Deriving genotypes from RAD-seq short-read data using Stacks. Nat.

Protoc. 12, 2640–2659. https://doi.org/10.1038/nprot.2017.123

Schmidt, H., Westheide, W., 2000. Are the meiofaunal polychaetes *Hesionides arenaria* and *Stygocapitella subterranea* true cosmopolitan species? - results of RAPD-PCR investigations. Zool. Scr. 29, 17–27. https://doi.org/doi:10.1046/j.1463-6409.2000.00026.x

Schratzberger, M., Rees, H.L., Boyd, S.E., 2000. Effects of simulated deposition of dredged material on structure of nematode assemblages - the role of burial. Mar. Biol. 136, 519–530.

Sheldon, P.R., 1996. Plus ça change - A model for stasis and evolution in different environments. Palaeogeogr. Palaeoclimatol. Palaeoecol. 127, 209–227. https://doi.org/10.1016/S0031-0182(96)00096-X

Singer, D., Kosakyan, A., Seppey, C.V.W., Pillonel, A., Fernández, L.D., Fontaneto, D., Mitchell, E.A.D., Lara, E., 2018. Environmental filtering and phylogenetic clustering correlate with the distribution patterns of cryptic protist species. Ecology 99, 904–914. https://doi.org/10.1002/ecy.2161

Smith, K.L., Harmon, L.J., Shoo, L.P., Melville, J., 2011. Evidence of constrained phenotypic evolution in a cryptic species complex of agamid lizards. Evolution (N. Y). 65, 976–992. https://doi.org/10.1111/j.1558-5646.2010.01211.x

Sterrer, W., 1973. Plate tectonics as a mechanism for dispersal and speciation in interstitial sand fauna. Netherlands J. Sea Res. 7, 200–222.

Struck, T.H., Cerca, J., 2019. Cryptic species and their Evolutionary significance. eLS 1–9. https://doi.org/10.1002/9780470015902.a0028292

Struck, T.H., Feder, J.L., Bendiksby, M., Birkeland, S., Cerca, J., Gusarov, V.I., Kistenich, S., Larsson, K.-H., Liow, L.H., Nowak, M.D., Stedje, B., Bachmann, L., Dimitrov, D., 2018a. Finding evolutionary processes hidden in cryptic species. Trends Ecol. Evol. 1–11. https://doi.org/10.1016/j.tree.2017.11.007

Struck, T.H., Feder, J.L., Bendiksby, M., Birkeland, S., Cerca, J., Gusarov, V.I., Kistenich, S., Larsson, K.-H.H., Liow, L.H., Nowak, M.D., Stedje, B., Bachmann, L., Dimitrov, D., 2018b. Cryptic Species – More Than Terminological Chaos: A Reply to Heethoff. Trends Ecol. Evol. 33, 310–312. https://doi.org/10.1016/j.tree.2018.02.008

Struck, T.H., Golombek, A., Weigert, A., Franke, F.A., Westheide, W., Purschke, G., Bleidorn, C., Halanych, K.M., 2015. The evolution of annelids reveals two adaptive routes to the interstitial realm. Curr. Biol. 1–7. https://doi.org/10.1016/j.cub.2015.06.007

Struck, T.H., Koczula, J., Stateczny, D., Meyer, C., Purschke, G., 2017. Two new species in the annelid genus *Stygocapitella* (Orbiniida, Parergodrilidae) with comments on their biogeography. Zootaxa 4286, 301–332. https://doi.org/10.11646/zootaxa.4286.3.1

Struck, T.H., Westheide, W., Purschke, G., 2002. Progenesis in Eunicida ("Polychaeta," Annelida) - Separate evolutionary events? Evidence from molecular data. Mol. Phylogenet. Evol. 25, 190–199. https://doi.org/10.1016/S1055-7903(02)00231-2

Surveswaran, S., Gowda, V., Sun, M., 2018. Using an integrated approach to identify cryptic species, divergence patterns and hybrid species in Asian ladies' tresses orchids (Spiranthes, Orchidaceae). Mol. Phylogenet. Evol. 124, 106–121. https://doi.org/10.1016/j.ympev.2018.02.025

Swift, H.F., Daglio, L.G., Dawson, M.N., 2016. Three routes to crypsis: stasis, convergence, and parallelism in the *Mastigias* species complex (Scyphozoa, Rhizostomeae). Mol. Phylogenet. Evol. 99, 103–115. https://doi.org/10.1016/j.ympev.2016.02.013

Teasdale, M., Vopel, K., Thistle, D., 2004. The timing of benthic copepod emergence. Limnol. Oceanogr. 49, 884–889.

Thistle, D., 2003. Harpacticoid copepod emergence at a shelf site in summer and winter: Implications for hydrodynamic and mating hypotheses. Mar. Ecol. Prog. Ser. 248, 177–185. https://doi.org/10.3354/meps248177

Thomas, M.C., Lana, P.C., 2011. A new look into the small-scale dispersal of free-living marine nematodes. Zoologia 28, 449–456. https://doi.org/10.1590/S1984-46702011000400006

Toews, D.P.L., Brelsford, A., 2012. The biogeography of mitochondrial and nuclear discordance in animals. Mol. Ecol. 21, 3907–3930. https://doi.org/10.1111/j.1365-294X.2012.05664.x

Tulchinsky, A.Y., Norenburg, J.L., Turbeville, J.M., 2012. Phylogeography of the marine interstitial nemertean *Ototyphlonemertes parmula* (Nemertea, Hoplonemertea) reveals cryptic diversity and high dispersal potential. Mar. Biol. 159, 661–674. https://doi.org/10.1007/s00227-011-1844-y

Ullberg, J., Ólafsson, E., 2003. Effects of biological disturbance by *Monoporeia affinis* (Amphipoda) on small-scale migration of marine nematodes in low-energy soft sediments. Mar. Biol. 143, 867–874. https://doi.org/10.1007/s00227-003-1139-z

Ullberg, Jörgen, Ólafsson, E., 2003. Free-living marine nematodes actively choose habitat when descending from the water column. Mar. Ecol. Prog. Ser. 250, 141–149. https://doi.org/10.3354/meps260141

van Oppen, M.J.H., Klerk, H., Olsen, J.L., Stam, W.T., 1996. Hidden diversity in marine algae: some examples of genetic variation below the species level. J. Mar. Biol. Assoc. United Kingdom 76, 239–242.

Voje, K.L., Starrfelt, J., Liow, L.H., 2018. Model adequacy and microevolutionary explanations for stasis in the fossil record. Am. Nat. 191, 000–000. https://doi.org/10.1086/696265

Wada, S., Kameda, Y., Chiba, S., 2013. Long-term stasis and short-term divergence in the phenotypes of microsnails on oceanic islands. Mol. Ecol. 22, 4801–10. https://doi.org/10.1111/mec.12427

Wagner, G.P., Schwenk, K., 2000. Evolutionarily stable configurations: Functional integration and the evolution of phenotypic stability, in: Hecht, M.K., Macintyre, R.J., Clegg, M.T. (Eds.), Evolutionary Biology. Springer US, Boston, MA, pp. 155–217.

Warris, S., Schijlen, E., van de Geest, H., Vegesna, R., Hesselink, T., Te Lintel Hekkert, B., Sanchez Perez, G., Medvedev, P., Makova, K.D., de Ridder, D., 2018. Correcting palindromes in long reads after whole-genome amplification. BMC Genomics 19, 798. https://doi.org/10.1186/s12864-018-5164-1

Weber, A.A.-T., Stöhr, S., Chenuil, A., 2019. Species delimitation in the presence of strong incomplete lineage sorting and hybridization. Mol. Phylogenet. Evol. 131, 240218. https://doi.org/10.1101/240218

Weiss, A.M., 2011. The evolution of evolution: reconciling the problem of stability. Evol. Biol. 38, 42–51. https://doi.org/10.1007/s11692-010-9099-y

Westheide, W., 2008. Polychaetes: Interstitial families., 2nd Editio. ed. Field Studies Council, Shrewsbury, 169 pp.

Westheide, W., 2005. Meiofauna geographic distribution: vicariance and dispersal. Meiofauna Mar. 14, 201–207.

Westheide, W., 1991. The meiofauna of the Galapagos: a review, in: Mathew J. James (Ed.), Galápagos Marine Invertebrates. Springler, New York, pp. 37–69.

Westheide, W., 1990. Polychaetes: interstitial families. Keys and notes for the identification of the species., Universal. ed.

Westheide, W., 1987. Progenesis as a principle in meiofauna evolution. J. Nat. Hist. 21, 843–854. https://doi.org/10.1080/00222938700770501

Westheide, W., 1977. The geographical distribution of interstitial polychaetes. Mikrofauna Meeresb. 61, 287–302.

Westheide, W., Hass-Cordes, E., 2001. Molecular taxonomy: description of a cryptic *Petitia species* (Polychaeta : Syllidae) from the island of Mahe (Seychelles, Indian Ocean) using RAPD markers and ITS2 sequences. J. Zool. Syst. Evol. Res. 39, 103–111.

Westheide, W., Purschke, G., 1988. Organism processing, in: Higgins, R.P., Thiel, H. (Eds.), Introduction to the Study of Meiofauna. Smithsonian Institution Press, Washington, pp. 146–160.

Westheide, W., Rieger, R.M., 1987. Systematics of the amphiatlantic *Microphthalmus listensis* species-group (Polychaeta: Hesionidae): facts and concepts for reconstruction of phylogeny and speciation. Zeitschrift für Zool. Syst. und Evol. 25, 12–39.

Wickham, H., 2016. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag, New York.

Worsaae, K., 2005. Phylogeny of Nerillidae (Polychaeta, Annelida) as inferred from combined 18S rDNA and morphological data. Cladistics 21, 143–162. https://doi.org/10.1111/j.1096-0031.2005.00058.x

Zanol, J., Halanych, K.M., Struck, T.H., Fauchald, K., 2010. Phylogeny of the bristle worm family Eunicidae (Eunicida, Annelida) and the phylogenetic utility of noncongruent 16S, COI and 18S in combined analyses. Mol. Phylogenet. Evol. 55, 660–676. https://doi.org/10.1016/j.ympev.2009.12.024

Zhang, J., Kapli, P., Pavlidis, P., Stamatakis, A., 2013. A general species delimitation method with applications to phylogenetic placements. Bioinformatics 29, 2869–2876. https://doi.org/10.1093/bioinformatics/btt499

Zrzavý, J., Říha, P., Piálek, L., Janouškovec, J., 2009. Phylogeny of Annelida (Lophotrochozoa): Total-evidence analysis of morphology and six genes. BMC Evol. Biol. 9, 1–14. https://doi.org/10.1186/1471-2148-9-189

Manuscripts 1-5

Manuscript 1

Finding evolutionary processes hidden in cryptic species

Trends in Ecology & Evolution

CellPress REVIEWS

Opinion

Finding Evolutionary Processes Hidden in Cryptic Species

Torsten H. Struck,[1,*] Jeffrey L. Feder,[2] Mika Bendiksby,[1,3] Siri Birkeland,[1] José Cerca,[1] Vladimir I. Gusarov,[1] Sonja Kistenich,[1] Karl-Henrik Larsson,[1] Lee Hsiang Liow,[1,4] Michael D. Nowak,[1] Brita Stedje,[1] Lutz Bachmann,[1] and Dimitar Dimitrov[1,5]

Cryptic species could represent a substantial fraction of biodiversity. However, inconsistent definitions and taxonomic treatment of cryptic species prevent informed estimates of their contribution to biodiversity and impede our understanding of their evolutionary and ecological significance. We propose a conceptual framework that recognizes cryptic species based on their low levels of phenotypic (morphological) disparity relative to their degree of genetic differentiation and divergence times as compared with non-cryptic species. We discuss how application of a more rigorous definition of cryptic species in taxonomic practice will lead to more accurate estimates of their prevalence in nature, better understanding of their distribution patterns on the tree of life, and increased abilities to resolve the processes underlying their evolution.

Highlights

Current definitions of cryptic species are inconsistent and can lead to biased estimates of species diversity.

Cryptic species are often implied to represent taxa displaying low phenotypic disparity in relation to divergence time, but this relationship is usually not formally quantified.

Here we propose a quantitative framework, which provides a formal characterization of the intuitive concept of cryptic species.

The proposed framework facilitates understanding of evolutionary processes leading to and resulting from cryptic species and provides a basis for estimates and modeling of occurrences of cryptic species across taxa and environments.

The framework fosters a shift from pattern- to process-driven research concerning cryptic species.

Cryptic Species – Taxonomic Oddities or Biologically Relevant Entities?

'Cryptic species' is a common and increasingly used term that refers to taxa that cannot readily be distinguished morphologically, yet evidence indicates they are on different evolutionary trajectories (Box 1). While researchers may not be able to visually recognize cryptic species as different species, the organisms can. Cryptic species are found on all major branches of the tree of life and probably represent a significant portion of undiscovered biodiversity [1–4]. As such, cryptic species might significantly add to our understanding of biodiversity, calling for increased conservation efforts [2,4–9]. Cryptic species are also important because they serve as an intellectual bridge connecting the study of taxonomy and phylogenetic pattern with ecosystems functioning, evolutionary processes, and macroevolutionary trends, including speciation, **parallelism** (see Glossary), **convergence**, and **stasis**. However, problems with the definition, among others the linkage to the species' taxonomic nomenclature history, and inconsistencies in the use of the term 'cryptic species' make it difficult to draw firm conclusions about their prevalence in nature and their implications for ecology and evolution.

Here, we discuss the general problem of defining cryptic species based on a literature survey that revealed the wide latitude in what researchers call cryptic species. Some authors have even suggested considering cryptic species as a temporary formalization problem of species delineation, rather than as a natural phenomenon [10]. To help mitigate the problem, we propose a more rigorous, multidimensional, and interdisciplinary approach for cryptic species. The approach focuses on better quantifying the extent of phenotypic **disparity** of taxa compared with the degree to which they have genetically diverged and exchanged genes (have evolved reproductive isolation). Standardizing the delineation of cryptic species will facilitate investigations into several outstanding questions concerning their biological significance (see Outstanding Questions). It will also lead to a better characterization and

[1]Natural History Museum, University of Oslo, 0318 Oslo, Norway
[2]Department of Biological Sciences, University of Notre Dame, Notre Dame, IN 46556, USA
[3]NTNU University Museum, Norwegian University of Science and Technology, 7491 Trondheim, Norway
[4]Centre for Ecological & Evolutionary Synthesis (CEES), Department of Biosciences, University of Oslo, 0316 Oslo, Norway
[5]Current address: Center for Macroecology, Evolution and Climate, Natural History Museum of Denmark, University of Copenhagen, Copenhagen, Denmark

Box 1. Cryptic Species: History and Definitions

The English clergyman William Derham reported cryptic species in the avian genus *Phylloscopus* as early as 1718 [58]. Cryptic species have thus been recognized for several hundred years. In the last few decades the number of publications referring to cryptic species has increased dramatically (Figure IA), likely due to more researchers in the field and the increased use of genetic methods to distinguish taxa (Figure IB and, for example, [5,10]). However, criteria used in the literature to designate taxa as cryptic have often been vague and nonuniform. In the few cases where an explicit definition has been stated, the wording is often similar to that of Bickford *et al.* [5]: Cryptic species are 'two or more distinct species that are erroneously classified (and hidden) under one species name'. This taxonomy-based definition is often elaborated upon to highlight that cryptic species are morphologically indistinguishable [5,35]. Others have included an additional requirement of genetic divergence or distinctiveness between cryptic species ([15]; see Supplemental Table S4 online for a list of definitions). How genetically diverged populations must be to be considered cryptic species is usually not specified, but one can assume that this will be of the same magnitude as for non-cryptic species (e.g., a certain barcode gap) [5]. By contrast, several definitions seem to mostly follow trends and concepts related to the research topic of the paper or field of the researcher. For example, in speciation research, definitions tend to highlight reproductive isolation and the biological species concept [37]. Mayr [59], for instance, defined cryptic species as 'morphologically similar or identical natural populations that are reproductively isolated'. Other terms such as 'semi-cryptic', 'pseudo-cryptic', 'sibling', and 'hyper-cryptic' indicating different degrees of 'crypticity' have also been proposed [10], complicating the debate of the biological relevance of cryptic species. Regardless, our literature survey (Box 2) revealed that many cryptic species have been defined based on molecular data and taxonomic history, with little regard for actually quantifying morphological disparity.

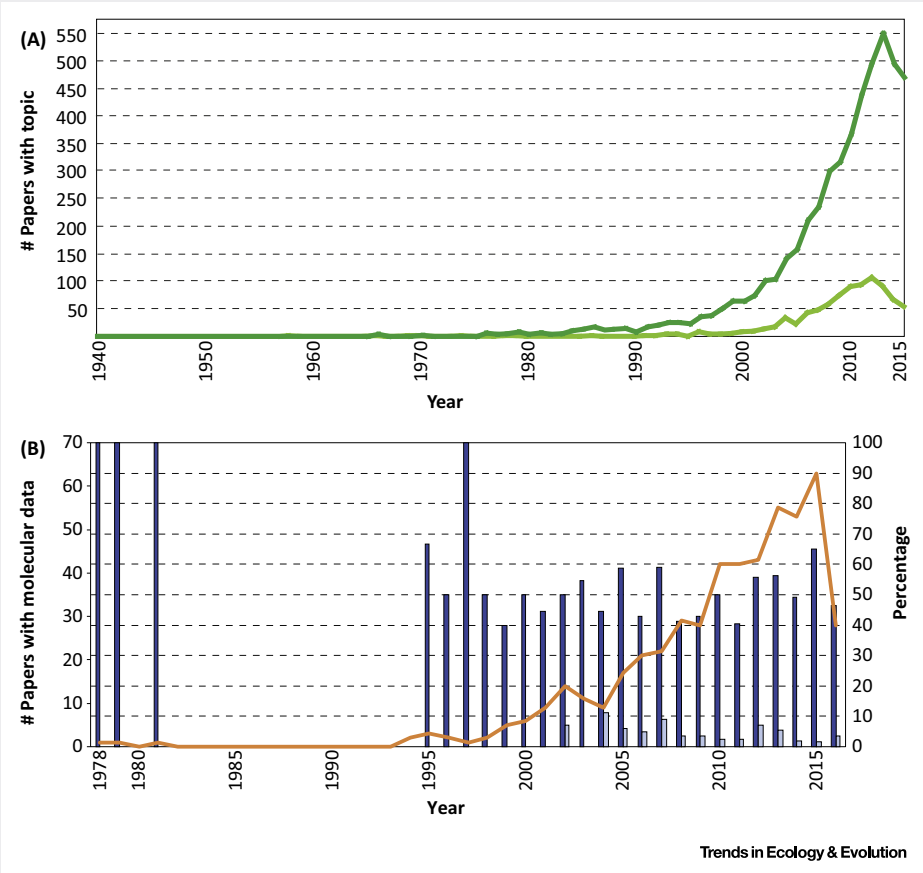


Figure I. Scientific Publications on the Subject of Cryptic Species since 1940. (A) The number of papers found with the search term 'cryptic speci*' (dark green line) and 'cryptic speciation' (light green). Of note is the marked increase in publications since 1990. (B) The number of papers included in the literature survey (Box 2) that included molecular data in the study (orange line) is also increasing similar to the overall numbers in A. Dark blue bars indicate the percentages of molecular papers that analyzed more than one genetic marker and light blue bars indicate studies based on genomic data. Note that these percentages are not increasing through time.

*Correspondence:
t.h.struck@nhm.uio.no (T.. Struck).

understanding of the different types of cryptic species, from recently diverged to phylogenetically distant taxa. In doing so, conclusions concerning (i) evolutionary parallelism, convergence, and stasis; (ii) the role that cryptic species play in ecosystem functioning; and (iii) factors initiating and contributing to speciation can be more confidently accepted.

The Problem of Definition in Theory and Practice

Cryptic species have generated both taxonomic and evolutionary ambiguity. A frequently cited definition of cryptic species [5] describes them as two or more distinct species that were earlier classified as one. Hence, cryptic species are defined based only on their taxonomic nomenclature history. However, this is unsatisfactory because various biological factors or taxonomic artifacts might result in erroneous species lumping. In addition, it offers no guidance for how morphologically similar or by how many characters species should differ to be considered as cryptic. Moreover, one of the longest and most contentious debates in evolution concerns what constitutes a species. If biologists cannot even agree on what to consider different species, then how can we reach consensus on what represents cryptic species?

Our literature survey of 606 studies indicates that the lack of philosophical clarity translates into a serious empirical problem in the operational designation of cryptic species (Box 2, see Supplemental Material and Tables S1–S4 online). For example, 47% of them, even though claiming cryptic species status for taxa, presented no phenotypic data, while 25.3% reported at least one trait differing between cryptic species. Thus, morphological similarity is subjectively evaluated and rarely quantified to address how similar cryptic species are [11–13]. Moreover, nonmorphological phenotypes, such as behavior, were seldom considered (Box 2). In this regard, cryptic species designation was often pattern driven with a focus on morphological characters discriminating taxa and little else. When several phenotypic traits were assessed, analyses seldom extended to species beyond the focal cryptic species. This is relevant because rates of morphological evolution for cryptic 'ingroup' taxa should be substantially (statistically) reduced compared with non-cryptic taxa to be considered cryptic.

Glossary

Convergence: independent evolution of a derived character state between taxa from different ancestral traits [41].
Disparity: the morphological or phenotypic difference between taxa [60].
Most recent common ancestor (MRCA): the last ancestor genetically shared by a group of individuals.
Parallelism: independent evolution of a character state in different taxa from a similar and shared ancestral trait [41].
Pattern-driven research: research focusing on the detection of biological patterns in empirical data.
Process-driven research: research focusing on the underlying processes generating observed patterns.
Stasis: retention of the same ancestral character state over an extended period [41].
Symplesiomorphy: character state of the MRCA present in descendant taxa.

Box 2. Characteristics of Published Studies of Cryptic Species

Our literature survey was based on the ISI Web of Science 'Life Sciences' database, using the search term 'cryptic speci*' for 'Topic' on June 17, 2016. The initial search returned 6002 entries (see Supplemental Table S1 online), from which approximately 15% were discarded as they were either not research papers, did not use our search term in a taxonomic context, or were not written in English. From the remaining publications, 606 were randomly chosen (see Supplemental Table S2 online) and assessed according to (i) how cryptic species were defined; (ii) whether and which types of genetic markers were scored; (iii) the analyses conducted; and (iv) the conclusions that could be drawn (see Supplemental Material and Table S3 online for additional details). For these 606 papers, 72.4% involved animals, 7.5% plants, 10.1% fungi, and 6.4% other groups, including protozoans. Only 14.0% of the studies explicitly referred to a specific definition of the term 'cryptic species', indicating the degree of subjectivity in the field. Moreover, according to the Code, species – including cryptic ones – are only valid when accompanied by a formal description. However, only 19.3% of the studies provided such formal descriptions. This low number can be indicative of uncertainties of the species status, ignorance of taxonomic practice, or that the species were formally described elsewhere.

The majority of studies (84.2%) provided molecular data, but many (35.5%) used only one locus. In comparison, only 42.7% of the studies included explicit analyses of morphological data and 23.9% other phenotypic traits. Overall, 56.6% of the studies targeted mitochondrial loci and 52.6% nuclear markers. Of the studies using nuclear data, 48.3% contained results for multiple loci. Very few studies included genome-scale data (3.1%). The relative numbers of studies with more than one marker or genomic data have not increased in recent years (see Figure IB in Box 1). Most studies (73.9%) provided an estimate of genetic divergence of some form (e.g., distance estimates or phylograms) and included congeneric species in the comparison (61.4%). However, only 16.0% of the studies applied genetic dating methods to estimate the time to the MRCA and only 4.3% used fossil calibrations.

The genetic data provided in the surveyed studies were also of limited utility in cryptic species delineation. Of the 606 studies, 35.5% based cryptic species designation on only a single molecular marker, most often from the plastid or mitochondrion, and lacked information on phenotypic disparity. Only 15.4% of the surveyed studies combined different types of molecular markers with morphological and/or other phenotypic data, and compared genetic divergence of the cryptic taxa with other congeneric non-cryptic species. The results show that there is remarkable inconsistency in the operational designation of 'cryptic species' [5,14,15] and huge variation in the applied analytical rigor [11,16–21]. Taxonomic practice for identifying cryptic species thus requires attention if the term is to be useful for comparative studies.

With recent advances in high-throughput DNA sequencing, visualization/microscopy, and statistical analytical tools, there are no technological or methodological impediments restricting higher standards in the empirical investigation of cryptic species [22,23]. This is important as informed estimates of species diversity and speciation rates are crucial for understanding evolutionary processes and ecosystem functioning, and for developing effective conservation strategies and sustainable usage of ecosystem services [2,4–9]. Cryptic species are one component of these estimates. Estimates of cryptic biodiversity based on vague definitions are of little help and, like undiscovered species or lack of species lists, will be counterproductive. For example, in ecology and conservation research, cryptic species are usually taken at face value based on the original reports. In particular, studies investigating patterns of cryptic species distribution across habitats, taxonomic groups, or life history strategies are often based on meta-analyses [5,24–28]. Given the shaky foundation in which cryptic species appear to be subjectively defined, it is difficult to place much confidence in the conclusions drawn from such meta-analyses. Sympatric cryptic species might, for example, contradict the ecological paradigm of competitive exclusion [29,30], but based on the current state it remains difficult to decide whether this is specifically or generally true. Similar considerations apply to studies of parallelism, convergence, and stasis. Without better standardization of the designation of cryptic species including details about phenotypic variation, levels of genomic differentiation, and divergence times, it remains difficult to make proper inference about evolutionary processes. Such standardizations as suggested herein will substantially improve comparability across lineages, as taxonomic nomenclature traditions are replaced with studies quantifying variation in a similar manner within and across groups.

The Conceptual Framework

Accurate **pattern-** and **process-driven research** on cryptic species is possible. However, to accomplish this, a sound and consistent foundation for defining cryptic species is needed. We do not pretend to solve the cryptic species problem completely here, but offer a conceptual framework to alleviate the problem by combining phenotypic disparity and genetic divergence. The latter serving as a proxy for reduced gene flow and an estimate of the time since divergence from the **most recent common ancestor (MRCA)**. By doing so, we emphasize the importance of reduced gene flow between taxa and the establishment of reproductive isolation between sexually reproducing populations relative to the extent to which they have changed in morphological and other phenotypic characters. As we explain later, this approach facilitates studies of parallelism, convergence, and speciation. The proposed framework provides a yardstick for the standardization of cryptic species descriptions without getting too entangled in the issue of species concepts. We concentrate on sexually reproducing organisms, for which a metric of gene flow and divergence time versus phenotypic disparity are key considerations.

Our conceptual framework highlights two important elements for defining cryptic species (Figure 1). First, species have to be distinguishable, for example, as statistically separable

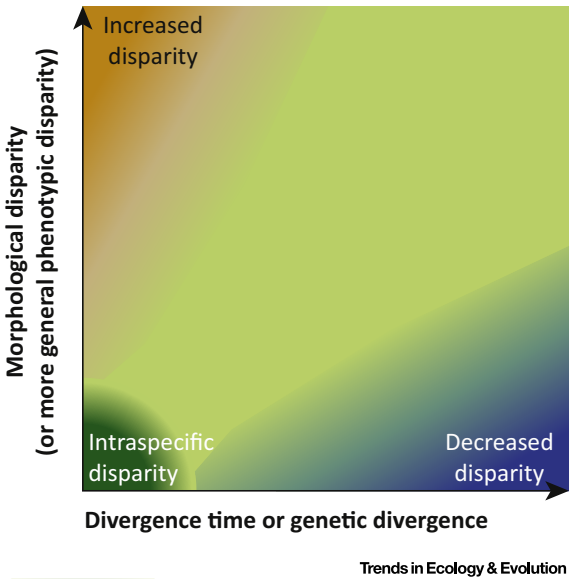


Figure 1. Our Conceptual Framework for Cryptic Species. The *x* axis represents the time of divergence between taxa since their most recent common ancestor approximated by genetic divergence. The *y* axis represents phenotypic (morphological) disparity. Intraspecific variation (polymorphism) within a taxon is depicted by the dark green area in the lower left corner of the figure. The null hypothesis is that morphological disparity between taxa relative to sister species should increase proportionately with divergence time (light green area). However, morphological disparity could increase at a significantly higher rate than the null expectation due to, for example, a recent adaptive radiation (orange area in the upper left corner of the figure). Alternatively, morphological disparity could also be substantially lower than expected over time (blue area in the lower right corner), the hallmark of cryptic species.

and diverged genotypic clusters of individuals (reflecting reproductive isolation) that do not form diagnostic morphological clusters. Although estimates of reproductive isolation in nature are only truly possible for taxa that geographically overlap, data from laboratory crosses, when technically feasible, and other information can be used to help gauge the level of gene flow and reproductive isolation. One major consideration is the time point when diverging populations are considered as being genetically and reproductively distinguishable species (e.g., [31–35]), as this will affect conclusions about recently diverged species. Consequently, cases, where populations exhibit sufficient gene flow to not cluster distinctively using methods like STRUCTURE or genetic network analyses, should be considered, if at all, as races or ecotypes [34,36], rather than cryptic species [37].

Second, the temporal dimension of cryptic species should be recognized by their showing of statistically lower degrees of phenotypic (or more specifically morphological) disparity than non-cryptic relatives given similar divergence time estimates from their MRCAs (Figure 1). By placing morphological disparity directly in relation to time (genetic divergence), recognition of cryptic species can become divorced from taxonomic nomenclature traditions based on the numbers of previously recognized species (e.g., lumpers vs. splitters), and debates about levels of 'crypticity' [10] more nuanced.

Although these two components of defining cryptic species seem self-evident, they are seldom adequately performed to allow for quantitative comparisons. For example, the temporal dimension is frequently ignored [38–40] and, of the 606 studies in our survey, only 3.3% and 4.5% of the reported divergence events could confidently be regarded as young or old,

respectively. For accurately determining genetic divergence, genome-wide sequence data are highly preferred for any group of taxa. However, very few studies applied genome-scale data (Box 2) [18,23]. Uniparentally inherited markers, such as the mitochondrial cytochrome oxidase subunit I gene (COI) – the target marker for DNA barcoding in animals – do not provide a comprehensive assessment of gene flow and reproductive isolation. There are several examples of high genetic divergence in COI that reflect deep population structure rather than species differences [18].

More importantly, to identify and quantify species that are cryptic from those that are not, detailed information about phenotypic disparity has to be related to genetic divergence, levels of gene flow, and reproductive isolation. Therefore, population to species-level morphological variation needs to be explicitly quantified to measure morphological disparity among cryptic species and their relatives as, for example, done in [41–43]. Available species descriptions can provide a good starting point for such morphological comparisons, providing information on both discrete and continuous characters. Depending on the data, appropriate methods for the quantification of morphological variation are available, including geometric morphometrics [44], landmark-free approaches such as the generalized Procrustes surface analysis [45], and multivariate analysis like nonmetric multidimensional scaling [46]. These methods and clustering, principal component, and discriminant function analyses should be employed to assess whether populations can be statistically distinguished from another or not. In addition, statistical tools like disparity through time plots [47] allow for testing whether morphological disparity between hypothesized cryptic taxa is significantly lower than expected given a null random walk expectation of drift. Tests of rate variation (e.g., variance ratio test) among hypothesized cryptic and non-cryptic lineages can also indicate whether morphological and other phenotypic traits (e.g., those related to behavior, life history, and physiology) deviate significantly from neutral expectation to statistically support cryptic species status for taxa. Note that hybridization has the potential to complicate analyses by reducing phenotypic disparity below levels seen for allopatric or completely reproductively isolated populations. However, it can generally be expected that proportional reductions in the level of genomic divergence would compensate for this and help to maintain the standardization of cryptic species delineation.

Currently, there are no studies that adhere completely to the proposed framework. There are several examples, however, where most of the requirements are fulfilled, for example, in studies of unicellular eukaryotes [48], cnidarians [41], annelids [42], mollusks [43], vertebrates [46], and plants [49]. However, the primary focus of these studies has been to find diagnostic characters. Phenotypic disparity was usually not cast in relation to other non-cryptic taxa and/or genetic divergence. One reason for this is that detailed examination of phenotypic and genetic variation in a comparative context, as proposed here, is time-consuming and not practical for projects whose primary focus is not the delineation of cryptic species (but then they should also refrain from assigning them). However, accurate rather than quick science is what should be aimed for, and when conducted properly, the proposed framework will provide the rigor to move beyond suggestive evidence to full and more standardized recognition of cryptic species.

Evolutionary Processes and Cryptic Species

Given a standardized and more accurate characterization of cryptic species, it is possible to examine their ecological and evolutionary implications in greater depth and with more confidence. For example, one question of interest is the extent to which cryptic species represent recently diverged versus more distantly related taxa. Other questions concerning evolutionary processes like parallelism, stasis, and convergence that are often considered primarily with respect to single traits [50–52] could also be extended to investigate whole phenotypes by

more robust analysis of cryptic species. In this regard, underlying selective regimes might be expected to be more pronounced or generally constrained to impact the entire (or nearly entire) suite of phenotypic traits [53], to which the term 'cryptic speciation' has been misleadingly applied in recent years (Figure IA). We examine these questions in the following section.

Recent Divergence

In this case, hypothesized cryptic species are sister taxa or members of a species complex with short divergence times, which are too recent for substantial morphological differences to accumulate [37,54,55] (Figure 2A). In many of these instances, the rate of accumulation of morphological disparity might actually not differ significantly from older non-cryptic species (Figure 2A). In speciation research it is commonly assumed that in the early stages of speciation selection acts largely on physiological, immunological, reproductive, or behavioral traits rather than on morphology [16,17,19]. Hence, for very young species, similarity in morphology might not be unexpected and it could take additional time to visually observe differences between taxa [10]. However, recently diverged taxa showing significantly lower rates of morphological disparity might be constrained by stabilizing selection and represent early stages of stasis.

Parallelism

Cryptic species that evolved by parallelism are not sister taxa, but are phylogenetically separated from each other to such a degree that their similarity can no longer be considered **symplesiomorphic**, but rather independently evolved from morphologically similar ancestors (Figure 2B). In comparison to more closely related and younger non-cryptic species, morphological disparity changes less as the cryptic species evolve from one similar morphotype to another similar one (Figure 2B). However, if the evolution of the new morphotype in one lineage precedes the other lineage in time, morphological disparity will first increase and then decrease again (similar to the plot in Figure 2C). Regardless, ancestral character state reconstructions are important to distinguish between recent divergence, convergence, or parallelism, and to assess and test rates of morphological change. Swift *et al*. [41], for example, showed that similar morphologies for lake species evolved by parallelism in closely related scyphozoan species. Confirmation of parallelism begs the question of whether similar morphotypes evolved due to intrinsic (e.g., developmental or genetic constraints) or extrinsic factors (e.g., deterministic environmental pressures) confining the available morphospace to only one selectively advantageous solution.

Convergence

In this case, cryptic species are not closely related and their morphological similarity results from independent evolution of morphologically dissimilar ancestors (Figure 2C). At early stages of divergence, cryptic and non-cryptic species pairs are expected to show similar rates of morphological differentiation. However, at some point in time the cryptic species pairs would begin to converge morphologically (Figure 2C). Convergence as a mechanism for cryptic species is rare, but has been reported in the deep sea [56]. In contrast to parallelism, intrinsic factors are expected to be less important for convergence than extrinsic ones, as convergent evolution is assumed to have started from different genetic and developmental backgrounds.

Stasis

Under stasis, cryptic species are sister taxa or members of a complex that retain a high degree of morphological similarity over extended periods (Figure 2D). Hence, symplesiomorphies prevail for millions of years, and significantly longer than expected by random drift. For example,

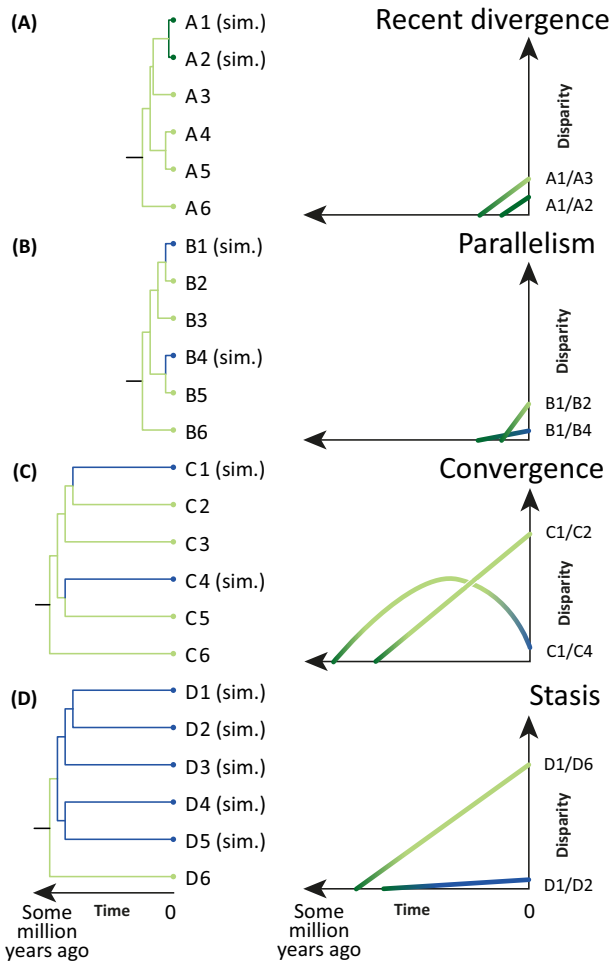


Figure 2. Expected Signatures of Four Evolutionary Processes That Can Lead to Cryptic Species. The colors of lines in phylogenies and graphs correspond to the different areas in Figure 1 and species with similar (identical) morphotypes denoted with 'sim.'. Panels on the left denote the phylogenetic relationships among taxa, while the panels to the right depict the evolution of morphological disparity through time for pairs of cryptic and non-cryptic species (e.g., A1/A2 vs. A1/A3). (A) Recent divergence: cryptic species are very closely related and only recently diverged from each other. However, the rate of morphological disparity is not necessarily substantially different from that for non-cryptic species and, as such, these taxa may not actually represent cryptic species. The supposed cryptic species might indeed be on a trajectory, which with time might lead from the borders of the dark green area to the light green area in Figure 1. (B) Parallelism: the cryptic species are not very closely related to each other and the rate of morphological disparity for non-cryptic species is much greater than that for cryptic species. While disparity between non-cryptic species evolved from the dark to the light green area, disparity between the cryptic species progressed into the dark blue area of Figure 1. (C) Convergence: the cryptic species are also not closely related to each other. Initially, morphological disparity for cryptic species can change in a manner similar to that for the non-cryptic species pair. However, at some point, morphological disparity decreases for the cryptic species, while continuing to increase between non-cryptic taxa. Hence, in their past the level of disparity of the cryptic species was first within the light green area of Figure 1, but then evolved toward the dark blue area associated with the low level of disparity of cryptic species. (D) Stasis: the cryptic species are closely related to each other or are part of a species complex and diverged a long time ago. In comparison with non-cryptic species, the rate of morphological change is substantially reduced, as cryptic species evolved from the dark green to the dark blue area of Figure 1.

one cryptic complex of annelid worms has been shown to display little morphological variation over tens of millions of years [42]. The lack of morphological diversification could result from low standing genetic variation and/or developmental constraints on the morphospace [5,57]. It is also possible that the ecology of taxa showing stasis has remained relatively constant through time and strong stabilizing selection has retained a common, shared morphology. This raises the question of whether cryptic species tend to be ecological generalists versus specialists, the answer to which might hinge on how common adaptation to different environments underlies speciation and depends on morphological change.

Concluding Remarks

Current research practices regarding cryptic species require change. There is much insight to be gained by standardizing and increasing the rigor in the way that cryptic species are defined and studied. Current practices, however, do not allow firm conclusions to be made concerning the number and significance of cryptic species in nature or the evolutionary processes associated with them. Indeed, given the results of our literature survey it is likely that many reported cryptic species should not be considered as such. Consequently, there is a need for careful re-analyses of many proposed cryptic species complexes with more rigorous criteria to better assess their true prevalence in nature. We propose an interdisciplinary approach that involves combining comprehensive data on genomic and phenotypic traits to statistically test for significant differences in rates of phenotypic disparity (e.g., morphological disparity) between cryptic and non-cryptic species. This approach will standardize the designation of cryptic species in the literature for taxonomic and comparative purposes; eliminate the history of taxonomic nomenclature as a consideration; and enable meta-analyses based on comparisons involving taxa categorized as displaying similar versus differing levels of disparity, periods of divergence, and degree of reproductive isolation. Adopting the approaches we advocate will provide a more sound basis for policy making in conservation biology and make it possible to address a number of questions involving evolutionary parallelism, convergence, and stasis associated with cryptic species (see Outstanding Questions), helping to reveal the biological meaning hidden in cryptic species. Conducted across lineages, general principles and accurate predictions, for example, to what extent cryptic species prevail in certain groups or are affected by climate change can be deduced.

Outstanding Questions

What is the general relationship between phenotypic disparity and reproductive isolation and genetic divergence through time?

Do thresholds of phenotypic disparity indicating the presence of cryptic species exist or is the relationship a continuum, with taxa lying in the tail of the distribution warranting cryptic species status?

Which methods for assessing phenotypic disparity and their significance are most universally applicable and most powerful with regard to discerning cryptic species?

Is it possible to establish an *a priori* best-practice strategy for defining cryptic species across a broad range of diverse taxonomic groups?

Are there more cryptic species in certain branches of the tree of life, among taxa with certain life histories (e.g., generalists vs. specialists), or in certain habitats?

Which cryptic species are the results of recent speciation, parallelism, convergence, or stasis, and how common are they?

What are the relevant intrinsic and extrinsic factors affecting morphological evolution and to what degree do they affect the phenotypic landscape of cryptic species?

Acknowledgments

We would like to thank the Natural History Museum of the University of Oslo for funding and Sarah Samadi and Gene Hunt for discussion on the subject. We also appreciate the comments of three anonymous reviewers. This is NHM Evolutionary Genomics Lab contribution No. 7.

Supplemental Information

Supplemental information associated with this article can be found online at https://doi.org/10.1016/j.tree.2017.11.007.

References

1. Jörger, K. and Schrödl, M. (2013) How to describe a cryptic species? Practical challenges of molecular taxonomy. *Front. Zool.* 10, 59
2. Pante, E. *et al.* (2015) Species are hypotheses: avoid connectivity assessments based on pillars of sand. *Mol. Ecol.* 24, 525–544
3. Loxdale, H.D. *et al.* (2016) Known knowns and unknowns in biology. *Biol. J. Linn. Soc.* 117, 386–398
4. Nygren, A. (2013) Cryptic polychaete diversity: a review. *Zool. Scr.* 43, 172–183
5. Bickford, D. *et al.* (2007) Cryptic species as a window on diversity and conservation. *Trends Ecol. Evol.* 22, 148–155
6. Alizon, S. *et al.* (2008) Competition between cryptic species explains variations in rates of lineage evolution. *Proc. Natl. Acad. Sci. U. S. A.* 105, 12382–12386
7. Nadler, S.A. and Perez-Ponce de Leon, G. (2011) Integrating molecular and morphological approaches for characterizing parasite cryptic species: implications for parasitology. *Parasitology* 138, 1688–1709
8. Boykin, L.M. *et al.* (2012) Species delimitation and global biosecurity. *Evol. Bioinform.* 8, 1–37
9. Krug, P.J. *et al.* (2013) Integrative species delimitation in photosynthetic sea slugs reveals twenty candidate species in three nominal taxa studied for drug discovery, plastid symbiosis or biological control. *Mol. Phylogenet. Evol.* 69, 1101–1119

10. Korshunova, T. *et al.* (2017) External diversity is restrained by internal conservatism: new nudibranch mollusc contributes to the cryptic species problem. *Zool. Scr.* 46, 683–692
11. Wu, Z.-Z. *et al.* (2014) Sequence analysis of mitochondrial ND1 gene can reveal the genetic structure and origin of *Bactrocera dorsalis* s.s. *BMC Evol. Biol.* 14, 55
12. Sanchez, G. *et al.* (2016) Evaluation of the 5′ end of the 16S rRNA gene as a DNA barcode marker for the Cephalopoda. *Fish Sci.* 82, 279–288
13. Schmidt, R.C. *et al.* (2016) High levels of endemism in sucker-mouth catfishes (Mochokidae: Chiloglanis) from the Upper Guinean forests of West Africa. *Mol. Phylogenet. Evol.* 100, 199–205
14. Wang, Y. *et al.* (2014) Morphology, molecular genetics, and bioacoustics support two new sympatric *Xenophrys* toads (Amphibia: Anura: Megophryidae) in Southeast China. *PLoS One* 9, e93075
15. Van Campenhout, J. *et al.* (2016) Transcription, signaling receptor activity, oxidative phosphorylation, and fatty acid metabolism mediate the presence of closely related species in distinct intertidal and cold-seep habitats. *Genome Biol. Evol.* 8, 51–69
16. Bensch, S. *et al.* (2004) Linkage between nuclear and mitochondrial DNA sequences in avian malaria parasites: multiple cases of cryptic speciation? *Evolution* 58, 1617–1621
17. Damm, S. *et al.* (2010) An integrative approach to species discovery in odonates: from character-based DNA barcoding to ecology. *Mol. Ecol.* 19, 3881–3893
18. Giska, I. *et al.* (2015) Deeply divergent sympatric mitochondrial lineages of the earthworm *Lumbricus rubellus* are not reproductively isolated. *BMC Evol. Biol.* 15, 217
19. Derycke, S. *et al.* (2016) Coexisting cryptic species of the *Litoditis marina* complex (Nematoda) show differential resource use and have distinct microbiomes with high intraspecific variability. *Mol. Ecol.* 25, 2093–2110
20. Karanovic, T. *et al.* (2016) Cryptic species or inadequate taxonomy? Implementation of 2D geometric morphometrics based on integumental organs as landmarks for delimitation and description of copepod taxa. *Syst. Biol.* 65, 304–327
21. Razkin, O. *et al.* (2017) Species delimitation for cryptic species complexes: case study of *Pyramidula* (Gastropoda, Pulmonata). *Zool. Scr.* 46, 55–72
22. Richards, S. (2015) It's more than stamp collecting: how genome sequencing can unify biological research. *Trends Genet.* 31, 411–421
23. Janzen, D.H. *et al.* (2017) Nuclear genomes distinguish cryptic species suggested by their DNA barcodes and ecology. *Proc. Natl. Acad. Sci. U. S. A.* 114, 8313–8318
24. Pfenninger, M. and Schwenk, K. (2007) Cryptic animal species are homogeneously distributed among taxa and biogeographical regions. *BMC Evol. Biol.* 7, 121
25. Poulin, R. and Pérez-Ponce de León, G. (2017) Global analysis reveals that cryptic diversity is linked with habitat but not mode of life. *J. Evol. Biol.* 30, 641–649
26. Perez-Ponce de Leon, G. and Poulin, R. (2016) Taxonomic distribution of cryptic diversity among metazoans: not so homogeneous after all. *Biol. Lett.* 12, 20160371
27. Adams, M. *et al.* (2014) Global biodiversity assessment and hyper-cryptic species complexes: more than one species of elephant in the room? *Syst. Biol.* 63, 518–533
28. Skoracka, A. *et al.* (2015) Cryptic speciation in the Acari: a function of species lifestyles or our ability to separate species? *Exp. Appl. Acarol.* 67, 165–182
29. Chesson, P. (1991) A need for niches. *Trends Ecol. Evol.* 6, 26–28
30. Gause, G.F. (1934) *The Struggle for Existence,* Hafner Publishing Company
31. Norris, R.D. and Hull, P.M. (2012) The temporal dimension of marine speciation. *Evol. Ecol.* 26, 393–415
32. The Marie Curie SPECIATION Network (2012) What do we need to know about speciation? *Trends Ecol. Evol.* 27, 27–39
33. De Queiroz, K. (2007) Species concepts and species delimitation. *Syst. Biol.* 56, 879–886
34. Roux, C. *et al.* (2016) Shedding light on the grey zone of speciation along a continuum of genomic divergence. *PLoS Biol.* 14, e2000234
35. Bernardo, J. *et al.* (2011) A critical appraisal of the meaning and diagnosability of cryptic evolutionary diversity, and its implications for conservation in the face of climate change. In *Climate Change, Ecology and Systematics* (Hodkinson, T.R., ed.), pp. 380–438, Cambridge University Press
36. Sukumaran, J. and Knowles, L.L. (2017) Multispecies coalescent delimits structure, not species. *Proc. Natl. Acad. Sci. U. S. A.* 114, 1607–1612
37. Reidenbach, K.R. *et al.* (2012) Patterns of genomic differentiation between ecologically differentiated M and S forms of *Anopheles gambiae* in West and Central Africa. *Genome Biol. Evol.* 4, 1202–1212
38. Harder, A.M. *et al.* (2016) Diversity and distribution within the sea spider genus *Pallenopsis* (Chelicerata: Pycnogonida) in the Western Antarctic as revealed by mitochondrial DNA. *Polar Biol.* 39, 677–688
39. Sauvage, T. *et al.* (2016) A metabarcoding framework for facilitated survey of endolithic phototrophs with tufA. *BMC Ecol.* 16, 8
40. Williams, J.T. and Viviani, J. (2016) *Pseudogramma polyacantha* complex (Serranidae, tribe Grammistini): DNA barcoding results lead to the discovery of three cryptic species, including two new species from French Polynesia. *Zootaxa* 4111, 15
41. Swift, H.F. *et al.* (2016) Three routes to crypsis: stasis, convergence, and parallelism in the *Mastigias* species complex (Scyphozoa, Rhizostomeae). *Mol. Phylogenet. Evol.* 99, 103–115
42. Struck, T.H. *et al.* (2017) Two new species in the annelid genus *Stygocapitella* (Orbiniida, Parergodrilidae) with comments on their biogeography. *Zootaxa* 4286, 301–332
43. Wada, S. *et al.* (2013) Long-term stasis and short-term divergence in the phenotypes of microsnails on oceanic islands. *Mol. Ecol.* 22, 4801–4810
44. Bookstein, F.L. (1991) *Morphometric Tools for Landmark Data. Geometry and Biology,* Cambridge University Press
45. Pomidor, B.J. *et al.* (2016) A landmark-free method for three-dimensional shape analysis. *PLoS One* 11, e0150368
46. Shirley, M.H. *et al.* (2014) Rigorous approaches to species delimitation have significant implications for African crocodilian systematics and conservation. *Proc. Biol. Sci.* 281, 20132483
47. Harmon, L.J. *et al.* (2003) Tempo and mode of evolutionary radiation in iguanian lizards. *Science* 301, 961–964
48. Krenek, S. *et al.* (2015) New *Paramecium* (Ciliophora, Oligohymenophorea) congeners shape our view on its biodiversity. *Org. Divers. Evol.* 15, 215–233
49. Vigalondo, B. *et al.* (2015) Unmasking cryptic species: morphometric and phylogenetic analyses of the Ibero-North African *Linaria incarnata* complex. *Bot. J. Linn. Soc.* 177, 395–417
50. Huang, S. *et al.* (2015) Convergence, divergence, and parallelism in marine biodiversity trends: integrating present-day and fossil data. *Proc. Natl. Acad. Sci. U. S. A.* 112, 4903–4908
51. Hunt, G. *et al.* (2015) Simple versus complex models of trait evolution and stasis as a response to environmental change. *Proc. Natl. Acad. Sci. U. S. A.* 112, 4885–4890
52. Ralph, P.L. and Coop, G. (2015) Convergent evolution during local adaptation to patchy landscapes. *PLoS Genet.* 11, e1005630
53. Futuyma, D.J. (2010) Evolutionary constraint and ecological consequences. *Evolution* 64, 1865–1884
54. Knowlton, N. (1993) Sibling species in the sea. *Annu. Rev. Ecol. Syst.* 24, 189–216
55. Gustafsson, A.L.S. *et al.* (2014) Genetics of cryptic speciation within an arctic mustard, *Draba nivalis*. *PLoS One* 9, e93834
56. Vrijenhoek, R.C. (2009) Cryptic species, phenotypic plasticity, and complex life histories: assessing deep-sea faunal diversity

with molecular markers. *Deep Sea Res. Part II Top. Stud. Oceanogr.* 56, 1713–1723

57. Appeltans, W. *et al.* (2012) The magnitude of global marine species diversity. *Curr. Biol.* 22, 2189–2202
58. Winker, K. (2005) Sibling species were first recognized by William Derham (1718). *Auk* 122, 706–707
59. Mayr, E. (1970) *Populations, Species, and Evolution: An Abridgment of Animal Species and Evolution,* Belknap Press of Harvard University Press
60. Wills, M.A. *et al.* (2001) Morphological disparity: a primer. In *Fossils, Phylogeny, and Form: An Analytical Approach* (Adrain, J.M., ed.), pp. 55–144, Kluwer Academic/Plenum Publishers

Supplementary data for manuscript 1

The supplementary data for this manuscript can be found online in
https://doi.org/10.1016/j.tree.2017.11.007

Manuscript 2

Marine connectivity dynamics: clarifying cosmopolitan distributions of marine interstitial invertebrates and the meiofauna paradox

Marine Biology (2018) 165:123
https://doi.org/10.1007/s00227-018-3383-2

STUDENT REVIEW

Marine connectivity dynamics: clarifying cosmopolitan distributions of marine interstitial invertebrates and the meiofauna paradox

José Cerca[1] · Günter Purschke[2] · Torsten H. Struck[1]

Received: 25 September 2017 / Accepted: 2 July 2018

Abstract

Many interstitial species were first described as widely distributed, often cosmopolitan or amphi-oceanic, contrasting with descriptions of a sedentary life style and the general absence of pelagic dispersal stages. These inconsistencies became known as the "meiofauna paradox". In this review, we present a literature review investigating these inconsistencies and address the assumptions of the meiofauna paradox. We break the paradox down to two aspects including species distribution and dispersal. Focusing on distribution, we demonstrate that wide distributions are seldom given and false records likely stem from biological phenomena like stasis or recent speciation. These phenomena account for morphological similarity, ultimately represented by the pronounced occurrence of cryptic species with restricted distribution ranges. Additionally, taxonomic artefacts such as the erroneous application of taxonomic keys contribute to the report of widely distributed species. Considering dispersal, we point out the mismatch between traditional assumptions of meiofaunal sedentarism and growing experimental and empirical evidences suggesting higher dispersal potential. These evidences include not only indications for dispersal by pelagic stages, but further consider ecological and life-history traits in shaping distribution ranges. We conclude that the meiofauna paradox sensu stricto most likely does not exist and provide a roadmap for future research, suggesting a focus on morphological similarity and marine connectivity. Meiofaunal research should concentrate on evolutionary factors resulting in morphological similarity, improving the taxonomic resolution of species complexes and conducting more sophisticated experimental experiments to meiofaunal dispersal. In all cases, meiofaunal research will benefit from high-throughput sequencing such as genome scanning approaches, metagenomics or metatranscriptomics.

Introduction

Few environments would seem more homogeneous and lifeless than an extensive area of sandy sediments. Accordingly, the interstitium or the space between the sand grains was overlooked as a potential source of biological diversity for a long time. The first meiofaunal organisms were described in the 19th century (e.g., Lovén 1844; Dujardin 1851) and while this diversity was recognized it was not further considered (Giard 1904). The first naturalist who began to uncover this diversity was Remane (1933). He studied the fauna of the so-called coastal groundwater of sandy beaches by digging holes into the sand and collecting floating animals from the accumulating brackish ground water using small landing nets—the common collecting method in those times. Thus, owing to an error-prone sampling strategy, meiofauna organisms were assumed to only inhabit the groundwater. Later on, evidence accumulated suggesting that these organisms in fact inhabited the spaces between the sand grains in areas of moist sand and that the coastal groundwater itself only contained very few individuals, if any. These new findings initiated an intensive phase in meiofaunal research, leading to thousands of publications in many fields of zoology (for reviews see Higgins and Thiel 1988; Giere 2009) and uncovered an astonishing diversity, whereby a mere teaspoon of marine sediment or of sand in a beach could yield a

Responsible Editor: S. Connell.

Reviewed by Undisclosed experts.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00227-018-3383-2) contains supplementary material, which is available to authorized users.

✉ José Cerca
jose.cerca@gmail.com

[1] Frontiers of Evolutionary Zoology Research Group, Natural History Museum, University of Oslo, Oslo, Norway

[2] Fachbereich Biologie/Chemie, Universität Osnabrück, Osnabrück, Germany

Published online: 10 July 2018

bewildering biological diversity (Fig. 1; Fenchel 1978; Giere 2009). Today we know that marine sediments anywhere in between the supralittoral to the deep sea are inhabited by a considerably diverse meiofaunal diversity. The fauna living in the space between sand grains is also generally known as interstitial fauna (Giere 2009), and the terms meiofauna and interstitial species are often used synonymously in the literature. In this way, although officially classified by sizes passing through sieves ranging from 22–44 µm to 500–1000 µm (Giere 2009; Zeppilli et al. 2015), these ranges represent just a convenient (yet arbitrary) definition. For instance, several interstitial species are considerably larger than 1000 µm such as the well-known annelid *Polygordius* and hence, strictly spoken, do not belong to the meiofauna. On the other hand, some meiofauna are not strictly interstitial, as they burrow through the sediment due to the small open space available. They are nonetheless referred to as interstitial (e.g., some *Nerillidae* species (Annelida) living in muddy sediments). In this review, we concentrate on both marine meiofaunal and interstitial species and consider them synonymously as it is the case in the literature. In the following sections, we refer to them as meiofauna for consistency with the literature, where this term is more commonly used than interstitial.

Out of the approximately 34 metazoan phyla, 23 have at least some meiofaunal representatives, and four, namely Gnathostomulida, Kinorhyncha, Loricifera and Micrognathozoa, are exclusively meiofaunal (Fenchel 1978; Sands et al. 2008; Giere 2009; Zeppilli et al. 2015; Figs. 1a–r, 2), but so far, no marine representatives are known for Micrognathozoa. Meiofauna is usually considered as an independent ecological evolutionary unit (Giere 2009) and its adaptation to the spatially restricted interstitial environment is the group's most prominent and distinctive feature. Indeed, the meiofauna's unique type of form has been coined the "meiofaunal syndrome" (Brenzinger et al. 2013; Jörger et al. 2014), which is generally characterized by an uniform, elongated, worm-like body shape and usually simplified external organization with adhesive structures for attachment to sand grains (Giere 2009). Hence generally, on first sight their appearance seems often to be that of simple-bodied organisms.

The combination of small size and the absence of pelagic larvae in some species have led meiofauna biologists to describe these organisms as sedentary (i.e., limited dispersal capacities) and to suggest severely restricted distribution rates (Giere 2009). At the same time, a substantial number of species were described with distribution ranges encompassing whole continental coast lines, amphi-oceanic, or even cosmopolitan (Sterrer 1973; Gerlach 1977; Westheide 1977, 2005; Westheide and Rieger 1987; Giere 2009; Jörger et al. 2012). This contradiction became known as the "meiofauna paradox" (Sterrer 1973; Gerlach 1977; Westheide 1977; Boeckner et al. 2009; Giere 2009). Several alternative dispersal hypotheses were suggested to account for this inconsistency. For instance, dispersal models considering either stepping stone, or infrequent occasions of long-distance transport of a few individuals (such as bird-mediated dispersal, rafting on drifting material or recent accidental dispersal by humans) were suggested (Gerlach 1977; Westheide 1991; von Soosten et al. 1998). Alternatively, vicariance-driven hypotheses focusing on Pangea's division and subsequent continental drift (i.e., successive vicariance events) have been put forward to account for the meiofauna paradox (Sterrer 1973). Part of this discussion considers vicariance and dispersal as mutually exclusive. Following Giere (2009), this paradox can be summarized into two questions: (1) "Why are so many meiofaunal taxa from distant areas so similar despite their limited means of dispersal?" and (2) "How can meiofauna have bridged oceans and occupied distinct shores in the absence of large populations and competitive propagative stages?".

In this review of the meiofauna paradox, we present a literature survey focusing on distribution ranges and meiofaunal dispersal and how these contribute to a modern understanding of this paradox. We consider marine metazoan species from beach shores to the deep sea. Additionally, we consider the distribution range of a species as the geographic area within which a species has genetic cohesiveness is maintained by gene flow (Klautau et al. 1999). Having Giere's (2009) questions in mind, we address this paradox into two slightly more general questions both relating to dispersal and distribution range of meiofauna species. First, we ask: "Why are so many meiofaunal taxa from distant areas so similar?". We reframe this question considering that morphological similarity across wide distribution ranges might not hinge only upon dispersal capacity, but also on other biological phenomena, as well as non-biological aspects like observers' bias. By tackling morphological similarity, we discuss that distribution ranges are often inflated as a synergetic by-product of taxonomic challenges, sampling biases and the occurrence of cryptic species. Second, we address "How can meiofauna have bridged oceans and occupied distinct shores in the absence of propagative stages?". Meiofaunal population sizes are largely unknown as their local distribution is often patchy and possibly subject to enhanced extinction–colonization dynamics (i.e., metapopulation dynamics) and hence it cannot be determined if large populations are present. Moreover, dispersal over long distances does not depend exclusively on large populations. Considering this question, we address the disparity between historical literature on dispersal and vicariance and the recent experimental and empirical evidence of meiofauna dispersal. We demonstrate that both our empirical and experimental knowledge about meiofauna dispersal is still relatively limited for general conclusions. Based on these evidences, we conclude that the meiofauna paradox in the strict sense most

Fig. 1 Meiofauna diversity shown by examples from a variety of higher taxa. Light micrographs from living animals, originals. **a** Cnidaria: *Halammohydra octopodides* Remane, 1927 (Hydrozoa). **b** Xenacoelomorpha*: Symsagittifera roscoffensis* (Graff, 1891) (Acoela, Bursalia). **c–f** Ecdysozoa. **c** *Metepsilonema hagmeieri* (Stauffer, 1924) (Cycloneuralia, Nematoda, Chromadorea). **d** An undetermined Kinorhynch (Cycloneuralia, Kinorhyncha). **e** *Batillipes mirus* Richters, 1909 (Tardigrada, Heterotardigrada). **f** *Halacarellus subterraneus* Schulz, 1933 (Arthropoda, Chelicerata, Acari). **g–r** Spiralia. **g** *Turbanella* sp. Schultze, 1853 (Gastrotricha, Macrodasyida). **h** *Dactylopodola baltica* (Remane, 1926) (Gastrotricha, Macrodasyida). **i** *Proschizorhynchus gullmarensis* Karling, 1950 (Platyhelminthes, Neoophora, Kalyptorhynchia). **k–r** Lophotrochozoa. *Prostomatella arenicola* Friedrich, 1935 (Nemertini, Monostylifera). **l** undescribed *Pholidoskepia*. **m** *Microhedyle glandulifera* (Kowalevsky, 1901) (Mollusca, Gastropoda, Opisthobranchia). **n** *Trilobodrilus axi* Westheide, 1967 (Annelida, Sedentaria, Orbiniidae). **o** *Stygocapitella subterranea* Knöllner, 1934 (Annelida, Sedentaria, Orbiniidae). **p** *Hesionides arenaria* Friedrich, 1937 (Annelida, Errantia, Phyllodocida). **q** *Protodriloides chaetifer* (Remane, 1926) (Annelida, Errantia, Protodrilida). **r** *Nerilla antennata* Schmidt, 1848 (Annelida, Errantia). Scales in **a**, **b**, **f**, **i**, **m**, **n**, **o**, **p** 250 µm; in **c**, **d**, **e**, **g**, **h**, **l** 100 µm; in **k**, **q**, **r** 500 µm

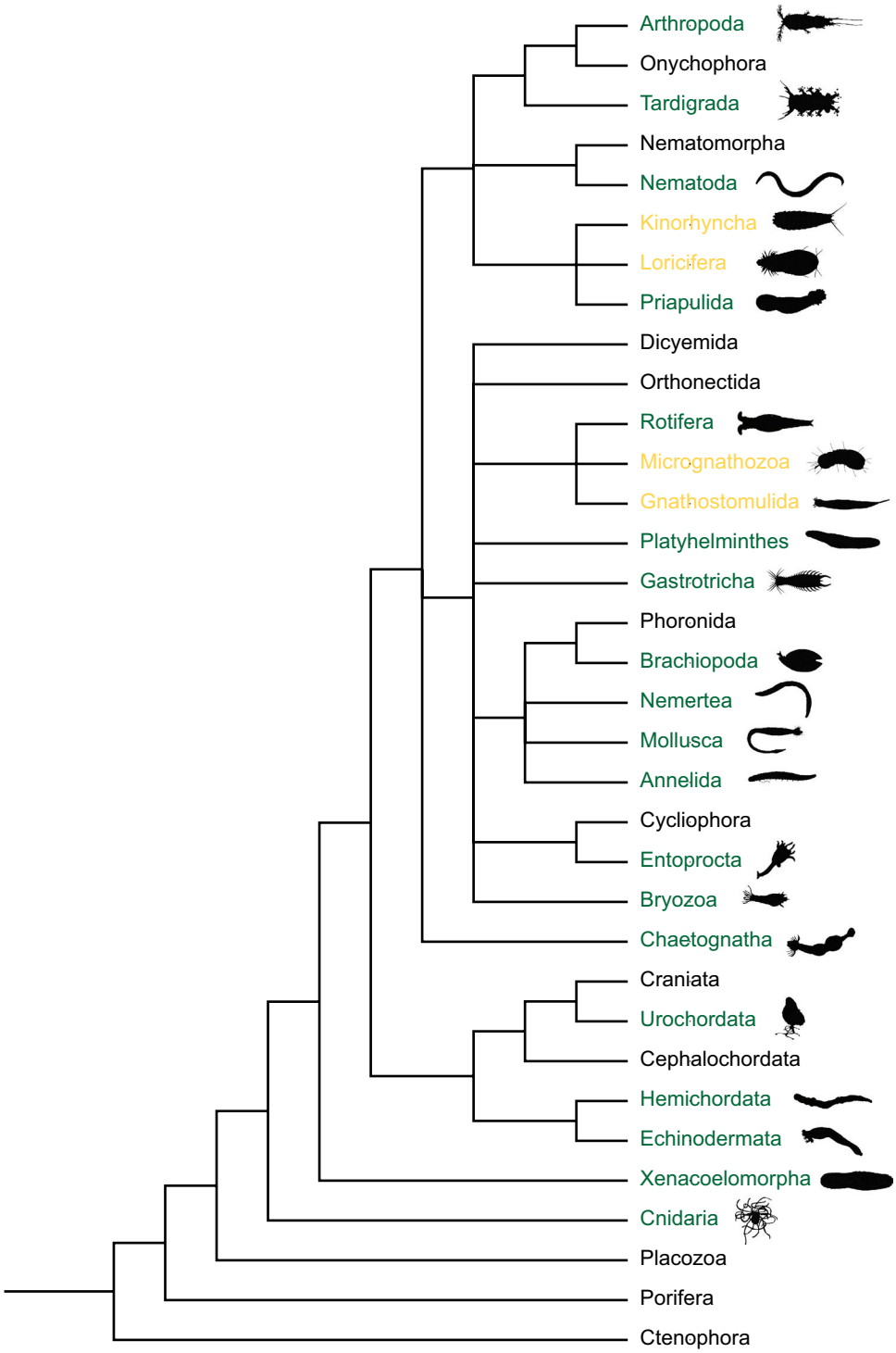


Fig. 2 Meiofaunal representatives across the animal phylogeny. Clades with meiofaunal representatives are highlighted in green. Exclusively meiofaunal clades are highlighted in yellow. Tree topology reproduced after Dunn et al. (2014)

likely does not exist and provide a roadmap for future directions of research on meiofauna dispersal and distribution.

Literature survey: description and general results

On June 6, 2018 we searched ISI Web of Science using the following combination of search terms: "(meiofauna* OR meiobenth* OR Gnathostomulida OR Kinorhyncha OR Loricifera) AND (marine OR Atlantic OR Pacific OR

Indian OR Arctic OR Antarctic OR "Southern Ocean") AND (molecular OR cryptic OR paradox OR taxonom* OR dispersal OR phylo* OR biogeo* OR distribut*)". This search yielded 1069 publications. While we were unable to obtain 16 articles mostly due to the presence of paywalls and indexed meeting abstracts on ISI, we assessed the abstracts and results of the remaining 1053 (Supplementary Table 1). After this preliminary assessment, we excluded 302 publications because they did not focus on marine, metazoan or meiofaunal organisms or they were not written in English (for a through list of criteria see Supplementary Material). The remaining 751 contributions were scored for taxa, discipline, use of molecular or morphological methods, occurrence of cryptic and pseudocryptic species, geographical location including depth and habitat description, as well as if there was an experimental approach to test for meiofauna dispersal (for a complete list of scoring criteria see Supplementary Material).

The majority of the captured papers corresponded to taxonomic (235) or ecological studies (488; Table 1). Surprisingly, only seven studies focused specifically on the evolution of meiofaunal species (one on Annelida, two on Arthropoda, two on Kinorhyncha, one on Nematoda and one on Platyhelminthes; Table 1). Herein, we consider publications as "evolution" in the strict sense of the discipline "Evolutionary Biology" by focusing on understanding evolutionary processes such as speciation and on population genetics (i.e., performing explicit tests of demography and gene flow). This allows us to differentiate these publications from studies of other disciplines like taxonomy or systematics also addressing the species' evolution. Similarly, development (12 papers), physiology (15) and palaeontology (4) were also underrepresented. Most studies focused on nematodes and arthropods, primarily harpacticoid copepods (Table 1), reflecting their overall abundance and their availability as ecological indicators (407 out of 447 papers dealing with nematodes and 303 out of 357 of the studies on Arthropoda were ecological studies).

The uneven representation of arthropods and nematodes is not as pronounced in taxonomy or biogeography as in ecological research. In total, 48 studies focused on biogeography and 235 focused on taxonomy (Table 1). This survey was unable to detect any taxonomic or biogeographical study focusing on Chaetognatha or Echinodermata. Most taxonomic studies were performed in Arthropoda (46), Gastrotricha (50), Kinorhyncha (26), Nematoda (39), Platyhelminthes (23) and Tardigrada (21). Studies in biogeography included Annelida (7), Arthropoda (15), Chordata (1), Cnidaria (2), Gastrotricha (6), Gnathostomulida (1), Kinorhyncha (3), Loricifera (1), Mollusca (2), Nematoda (13), Nemertea (3), Platyhelminthes (5), Rotifera (3), Tardigrada (2) and Xenacoelomorpha (2). Most of the studies were performed around European (327), North and Central American (157) and Asian (141) coastlines and waters. Coastlines and waters adjacent to Antarctica (35), Africa (54), Australia (51) and South America (75) are less well-studied. Regarding depth distribution, 359 papers focused on shallow-subtidal to a depth of 200 metres, 212 focused on the deep sea (below 200 m) and 202 intertidal areas. In contrast, only 14 studies investigated species from supralittoral areas (Table 1).

Most taxonomical studies described new species (135), while relatively few, often only implicitly, reported on the distribution range of meiofaunal species (Table 2). Of these, 40 papers reported an unchanged distribution of some of the focal taxa (accounting for 82 species), 25 reported an increase of distribution (including 112 species) and 22 a decrease of distribution (including 160 species). Only 27 papers used a combination of molecular and morphological data to assess species delineation (Todaro et al. 1996, 2014; Curini-Galletti and Puccinelli 1998; Westheide and Hass-Cordes 2001; De Ley et al. 2005; Sterrer and Sørensen 2006; Suatoni et al. 2006; Leasi and Todaro 2007; Casu et al. 2009; Neusser et al. 2011; Eder et al. 2011; Kieneke et al. 2012; Jörger et al. 2012; Leasi et al. 2013; Jörger and Schrödl 2013; Rundell and Leander 2014; Di Domenico et al. 2014; Kånneby et al. 2015; Smythe 2015; Dal Zotto 2015; Kajihara et al. 2015; Karanovic et al. 2016; Sánchez et al. 2016; Tanaka and Ohtsuka 2016; Kieneke and Nikoukar 2017; Atherton and Jondelius 2018; Van Steenkiste et al. 2018), with 16 additional papers using molecular data only (Schmidt and Westheide 2000; Bhadury et al. 2006; Todaro et al. 2006; Casu and Curini-Galletti 2006; Bik et al. 2010, 2012; Gruber-Vodicka et al. 2011; Tulchinsky et al. 2012; Baldrighi et al. 2013; Yamasaki et al. 2014; Fonseca et al. 2014; Leasi and Norenburg 2014, 2016; Meyer-Wachsmuth et al. 2014; Scarpa et al. 2015; Sahraean et al. 2017). Moreover, 14 papers mentioned difficulties in morphological characterization of the considered taxa (we refer to this issue as the low-morphology problem, see below). The occurrence of cryptic or pseudocryptic species was reported in 32 papers. Finally, only 25 studies performed experimental approaches to understand meiofaunal dispersal (Supplementary Table 2).

These results point to several trends in meiofaunal research. European, North and Central American and Asian coastlines are the most well-studied, potentially as an outcome from scientific traditions in these continents. Additionally, deep-sea research is well-represented with about 20% of the works focusing on this area, yet the majority of works was still done on shallow-subtidal areas (from low-water line to 200 metres depth). Taxonomy and ecology are the most vibrant disciplines in meiofaunal works. The potential skew towards Nematoda and Arthropoda research is most pronounced in ecological surveys, while Gastrotricha, Kinorhyncha, Platyhelminthes, and Tardigrada are especially well-represented in taxonomy. Moreover,

Table 1 Number of scored papers per taxon in the literature survey

Taxon	Total number of papers	Discipline								Study area							Depth distribution			
		Ecology	Evolution	Biogeography	Taxonomy	Development	Physiology	Review or perspective	Palaeontology	Africa	Antarctica	Asia	Australia	Europe	North and Center America	South America	Supralittoral	Intertidal	Shallow-subtidal (low-water-line—200)	Deep sea (> 200 m)
Annelida	195	177	1	7	12	1	1	0	0	16	7	36	13	93	27	18	3	42	98	69
Arthropoda	357	303	2	15	46	2	4	1	2	30	18	64	19	148	71	29	4	93	148	124
Chaetognatha	2	2	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	2	0
Chordata	3	3	0	1	0	0	0	0	0	0	0	0	0	2	1	0	0	1	1	0
Cnidaria	28	25	0	2	2	0	0	0	0	1	1	5	0	14	2	3	0	5	15	10
Gastrotricha	125	74	0	6	50	2	0	0	0	6	2	20	8	66	20	18	2	27	64	31
Gnathostomulida	12	5	0	1	5	2	0	0	0	1	0	2	2	3	3	2	0	4	4	3
Hemichordata	1	0	0	0	1	0	1	0	0	0	0	0	0	0	1	0	0	0	1	0
Echinodermata	5	5	0	0	0	0	0	0	0	1	0	0	0	4	0	0	0	1	4	2
Entoprotoa	1	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0
Kinorhyncha	132	99	2	3	26	3	1	1	0	5	4	21	6	60	25	12	0	20	66	65
Loricifera	25	18	0	1	7	0	1	0	1	1	2	7	1	13	1	2	0	0	8	18
Mollusca	85	76	0	2	6	2	1	0	0	6	3	17	7	41	10	8	1	17	41	35
Nematoda	447	407	1	13	39	0	3	1	1	29	19	83	27	209	60	42	5	109	194	166
Nemertea	18	15	0	3	1	0	0	0	0	0	0	5	1	9	4	4	0	3	12	6
Platyhelminthes	129	110	1	5	23	0	1	0	0	5	6	22	8	66	16	9	2	44	63	33
Priapulida	25	21	0	0	4	0	0	0	0	1	1	4	2	13	3	1	0	4	17	8
Rotifera	37	33	0	3	3	0	1	0	0	1	1	7	2	22	9	1	2	9	12	17
Tardigrada	88	66	0	2	21	0	2	0	0	5	4	19	5	45	6	9	1	15	39	38
Xenacoelomorpha	7	1	0	2	4	0	1	0	0	0	0	1	2	5	3	0	0	0	5	0
Total	752	488	7	48	235	12	15	4	4	54	35	141	51	327	157	75	14	202	359	212

The number of papers in a category (discipline, area or depth) can add up to more than the total number as more than one subcategory could be scored (see Supplementary Tables 1 and 2 for a thorough description and the detailed dataset)

Table 2 Range change of species and occurrence of reported cryptic species in the literature survey

Taxon	Distribution range							Detection of cryptic species						
	New species[a] (papers)	Unchanged (papers)	Unchanged (species)	Increased (papers)	Increased (species)	Decreased (papers)	Decreased (species)	Molecular data (papers)	Molecular and morphological data (papers)	Low-morphology problem (papers)	Cryptic species (papers)	Cryptic species (species)	Pseudocryptic species (papers)	Pseudocryptic species (species)
Annelida	6	3	3	0	0	3	5	10	3	1	3	4	1	1
Arthropoda	25	8	14	2	6	1	2	11	2	1	3	11	0	0
Chaetognatha	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chordata	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cnidaria	1	0	0	1	1	0	0	0	0	0	0	0	0	0
Gastrotricha	30	9	12	3	19	4	9	9	5	5	4	4	3	8
Gnathostomulida	3	0	0	1	11	0	0	2	1	0	0	0	0	0
Hemichordata	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Echinodermata	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Entoprotoa	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Kinorhyncha	18	3	15	3	11	1	5	8	2	0	0	0	0	0
Loricifera	3	0	0	0	0	0	0	0	0	0	0	0	0	0
Mollusca	3	3	5	0	0	2	12	8	4	3	3	25	0	0
Nematoda	19	1	1	6	27	1	6	11	2	0	2	10	0	0
Nemertea	0	2	13	0	0	3	72	3	0	3	3	93	0	0
Platyhelminthes	12	3	4	2	7	4	18	13	5	2	4	5	2	13
Priapulida	2	0	0	1	1	0	0	1	0	0	0	0	0	0
Rotifera	0	2	4	1	1	2	21	2	2	1	2	14	0	0
Tardigrada	9	5	9	5	28	0	0	4	0	0	0	0	0	0
Xenacoelomorpha	2	1	2	0	0	1	10	2	1	1	1	20	0	0
Total	135	40	82	25	112	22	160	61	27	17	26	189	6	22

Numbers refer to either the number of papers or species as indicated in the column heading (see Supplementary Tables 1 and 2 for a thorough description and the detailed dataset; supplementary Table 2 also includes cryptic species and range changes of the different entities (OTU/MOTU/cryptic/pseudocryptic) found)

[a]New species were scored as "distribution before unknown (NA); afterwards (local/regional)" and were not included in the "increase" column

the surveyed ecological studies did not at all focus on or address geographical distribution as well as the meiofauna paradox. Hence, these studies were irrelevant with respect to the subject of this review. Given the considered studies, the incidence of cryptic species seems high and few studies have explicitly focused on uncovering the distribution of meiofaunal species (Table 2). In the next section, we discuss some of these results more exhaustively.

"Why are so many meiofaunal taxa from distant areas so similar?"

A considerably high number of meiofaunal species descriptions include distribution ranges encompassing whole continental coastlines, amphi-oceanic, or even cosmopolitan (Sterrer 1973; Gerlach 1977; Westheide 1977, 2005; Westheide and Rieger 1987; Giere 2009). While many of these descriptions were registered in early decades of meiofauna research, valuable insights provided by detailed morphological reanalyses and approaches such as molecular taxonomy and phylogeography clearly contrasted these records. Hence, Giere's first question (2009) of "similarity" between meiofaunal taxa is here considered based on biological phenomena resulting in the lack of accumulation of morphological differences between reproductively isolated species as well as difficulties in characterization and identification of meiofauna.

In recent years, the unveiling of "cryptic species" has become commonplace in meiofauna taxa, especially for those with presumed wide geographic ranges (Todaro et al. 1996; von Soosten et al. 1998; Schmidt and Westheide 2000; Rocha-Olivares et al. 2001; Casu and Curini-Galletti 2004; Derycke et al. 2005, 2006, 2012, 2013, 2016; Suatoni et al. 2006; De Meester et al. 2012; Tulchinsky et al. 2012; Jörger et al. 2012; Kieneke et al. 2012; Leasi and Norenburg 2016; Meyer-Wachsmuth et al. 2014; Van Campenhout et al. 2014; Leasi and Norenburg 2014). We consider cryptic species as species which demonstrate a high degree of molecular divergence, despite no recognizable morphological differentiation (Struck et al. 2018b). The presented literature survey showed that most of the studies using both molecular and morphological data detected either cryptic or pseudocryptic species (Table 2). In total, 189 cryptic species within Annelida, Arthropoda, Gastrotricha, Mollusca, Nematoda, Nemertea, Platyhelminthes, Rotifera and Xenacoelomorpha were recorded (Curini-Galletti and Puccinelli 1998; Schmidt and Westheide 2000; Rocha-Olivares et al. 2001; Westheide and Hass-Cordes 2001; Casu and Curini-Galletti 2006; Suatoni et al. 2006; Jouin-Toulmond and Gambi 2007; Casu et al. 2009; Leasi and Todaro 2009; Neusser et al. 2011; Kieneke et al. 2012; Tulchinsky et al. 2012; De Meester et al. 2012, 2015; Jörger et al. 2012; Leasi et al. 2013; Jörger and Schrödl 2013; Leasi and Norenburg 2014, 2016; Meyer-Wachsmuth et al. 2014; Karanovic et al. 2016; Muenter and Kieneke 2017; Sahraean et al. 2017; Kieneke and Nikoukar 2017; Van Steenkiste et al. 2018). Interestingly, the number of cryptic species is highly uneven across the studied taxa. While some studies captured an overwhelming number of cryptic species within established morphospecies or species complexes of Acoelomorpha, Nemertea, Mollusca and Rotifera (Suatoni et al. 2006; Jörger et al. 2012; Leasi et al. 2013; Leasi and Norenburg 2014, 2016; Meyer-Wachsmuth et al. 2014), other studies found only a few cryptic species within complexes of Annelida, Gastrotricha and Platyhelminthes (Schmidt and Westheide 2000; Casu et al. 2009; Kieneke et al. 2012; Kieneke and Nikoukar 2017) (Table 2). However, the uneven discovery of cryptic species does not necessarily reflect differences in occurrence of cryptic species between taxa, but rather the study's efforts. For example, the two papers addressing cryptic species in Nemertea (Leasi and Norenburg 2014, 2016) investigate several species complexes, while the ones on rotifers (Suatoni et al. 2006; Leasi et al. 2013) focus on a single species complex each (Supplementary Table 2).

Following the aforementioned definition of cryptic species, these can be considered as the result of phenomena such as recent speciation, parallelism, convergence and morphological stasis (Wada et al. 2013; Swift et al. 2016; Struck et al. 2018a, b). Several of these have been reported in meiofauna, highlighting these species as possible systems addressing questions of phenotypic conservation. Cases of recent speciation where morphological differentiation lags behind reproductive isolation have not yet been explicitly proposed in meiofauna, but numerous cases of cryptic species in our survey are likely to represent such cases (Casu et al. 2009; Jörger et al. 2012; Kieneke et al. 2012; Leasi et al. 2013; Leasi and Norenburg 2014, 2016; Meyer-Wachsmuth et al. 2014; Karanovic et al. 2016; Kieneke and Nikoukar 2017). In contrast, parallelism and convergent evolution have been explicitly suggested for some interstitial gastropods (Brenzinger et al. 2013; Jörger et al. 2014). Morphological stasis arises as the most common explicit explanation, possibly resulting from stabilizing selection on morphology due to the restricted space available in the interstitial environment (Sterrer 1973; Westheide and Rieger 1987; von Soosten et al. 1998; Schmidt and Westheide 2000; Hansen and Houle 2004; Futuyma 2010).

Although the pronounced phenotypic similarity opens venues in evolutionary research, the occurrence of cryptic species or overlooked diversity can also stem from the difficulty of characterizing and identifying meiofauna. For instance, the general paucity of traits with systematic value or inadequate morphological criteria poses a challenge to morphology-based taxonomic practices, eventually resulting in the synonymization of several species into a widely

distributed, cosmopolitan species (Sterrer 1973). This problem has been framed as the "low-morphology problem" and has been thoroughly discussed in algae and corals where molecular approaches have been suggested as a more reliable approach to species delimitation (van Oppen et al. 1996; Klautau et al. 1999). Indeed, 17 studies applying explicitly molecular and/or morphological methods directly reported or discussed issues related to difficulties of morphological-oriented practices in species delimitation in meiofauna. These studies spanned several phyla, including gastrotrichs, annelids, platyhelminths, nemerteans, molluscs, rotifers, xenacoelomorphs and arthropods (Todaro et al. 1996; Casu and Curini-Galletti 2006; Jouin-Toulmond and Gambi 2007; Casu et al. 2009; Leasi and Todaro 2009; Neusser et al. 2011; Tulchinsky et al. 2012; Jörger et al. 2012; Kieneke et al. 2012; Leasi et al. 2013; Jörger and Schrödl 2013; Meyer-Wachsmuth et al. 2014; Leasi and Norenburg 2014, 2016; Karanovic et al. 2016; Muenter and Kieneke 2017; Kieneke and Nikoukar 2017); Table 2).

Similarly, although the presence of cryptic species should be considered alongside with a strict sense of absence of morphological differentiation between species, re-analyses of meiofaunal species have uncovered overlooked morphological differences for some species (e.g., Pietsch and Westheide 1985; Westheide and Rieger 1987; Curini-Galletti and Puccinelli 1998; Rocha-Olivares et al. 2001; Casu and Curini-Galletti 2006; Jouin-Toulmond and Gambi 2007; Casu et al. 2009; Leasi and Todaro 2009; Garlitska et al. 2012; Muenter and Kieneke 2017; Struck et al. 2017). Species with morphological differences found after reinvestigations are usually named 'pseudocryptic species' (which can be considered as morphologically unrecognised species). For example, differences in setation were found within the cosmopolitan harpacticoid copepod *Nannopus palustris* (Arthropoda; Garlitska et al. 2012). Likewise, overlooked differences in the muscular system correspond with genetic differentiation within the *Xenotrichula intermedia* species complex (Gastrotricha; Leasi and Todaro 2009; Muenter and Kieneke 2017). For the annelid *Stygocapitella subterranea* complex slight differences in the chaetal composition of the first two chaetigers were re-evaluated as constituting species-specific differences in the light of molecular data and in contrast to previous conclusions (Struck et al. 2017).

The breadth of changes in distribution range before and after a study captured by the literature survey also revealed trends in meiofaunal research (Supplementary Table 3; Table 2). In the survey, we report 25 studies whose focus species increased its distribution (considering the established categories, including regional, amphi-oceanic and cosmopolitan). In common, none of these studies employed molecular approaches and relied explicitly or implicitly on morphological data only (e.g., Villora-Moreno and Grimaldi 1993; Chatterjee et al. 2000; Delogu et al. 2008; Dal Zotto and Todaro 2016; Clausen 2000; Prasath et al. 2017). In sharp contrast, studies reporting a decrease of a range distribution generally employed molecular methods (Table 2; Supplementary Tables 2–3; e.g., Curini-Galletti and Puccinelli 1998; Schmidt and Westheide 2000; Jörger et al. 2012; Leasi et al. 2013; Meyer-Wachsmuth et al. 2014; Karanovic et al. 2016; Leasi and Norenburg 2016; Kieneke and Nikoukar 2017; Sahraean et al. 2017). For example, the six cryptic species uncovered within the *Terschellingia longicaudata* species complex (Nematoda) decreased the overall distribution of a formerly cosmopolitan species to two clades occurring in Bahrain, one in Taiwan, one in the UK and Mexico (amphi-oceanic), one in the UK and one cosmopolitan. 40 studies reported unchanged distributions (Table 2). A conclusion from these results is the necessity of molecular-oriented methods in species identification. Additionally, changes in distribution rather reflected the usage of methods than taxa. Within Xenacoelomorpha, Mollusca, Nemertea, Platyhelminthes, and Rotifera, there was a tendency for decreased or unchanged distribution ranges. In contrast, for Gastrotricha, Kinorhyncha, Nematoda and Tardigrada we found an increase. Yet, this cannot be related to the taxa themselves, but rather to the methodology used. Taxa with reduced ranges are also the ones with high numbers of cryptic or pseudocryptic species (Table 2).

Added to the limited available morphological traits for species delimitation and the presence of morphologically similar species, sampling biases might also contribute to the erroneous assumption of a cosmopolitan distribution of a species. Information on species distribution is often biased by sampling localities and intensity (Leasi and Norenburg 2016; Garraffoni and Balsamo 2017; Rinaldo et al. 2017). For instance, the higher diversity of meiofaunal species from European waters likely reflects a sampling artefact due to research traditions (Fontaneto et al. 2009; Jörger et al. 2014). Due to this bias, the report of species from understudied areas is often based on the inappropriate usage of species descriptions or taxonomic keys from Europe due to lack of such information for the study area. This is a common problem in modern taxonomy and not restricted to meiofauna only. Application of keys originating from different regions is likely to result in inappropriate assignment of species (Hutchings and Kupriyanova 2018). For instance, our survey potentially captured some papers wherein British keys were used following surveys in India and Thailand (Zawierucha et al. 2013; Ansari et al. 2015a, b, 2016, 2017; Prasath et al. 2017) and, maybe not surprisingly, these suggest an increased species distribution (Supplementary Table 3).

Conclusions regarding "Why are so many meiofaunal taxa from distant areas so similar?"

Considering the prevalence of cryptic species complexes and the subsequent reduction in distribution ranges in studies employing molecular investigations, the distribution of most meiofauna species seems clearly inflated as complexes of cryptic species consists of several, independent distributions, currently interpreted as a single distribution range (Casu and Curini-Galletti 2004; Derycke et al. 2005; Andrade et al. 2011; Tulchinsky et al. 2012; Leasi and Norenburg 2014). Our survey showed that cosmopolitan or, at least, amphi-oceanic distributions of most meiofauna species seldom occur and that increases in distribution range are not supported by molecular approaches. Therefore, the taxonomic identity and assumed wide distribution ranges of many meiofauna species assumed to be examples of the 'meiofauna paradox' is not verified (Schmidt and Westheide 2000; Casu and Curini-Galletti 2006; Suatoni et al. 2006; Casu et al. 2009; Tulchinsky et al. 2012; Jörger et al. 2012; Kieneke et al. 2012; Leasi et al. 2013; Leasi and Norenburg 2014, 2016; Meyer-Wachsmuth et al. 2014; Karanovic et al. 2016; Sahraean et al. 2017; Kieneke and Nikoukar 2017). Careful reinvestigations, including detailed morphological and molecular analyses should resolve the paradox of widespread species. Hence, in the strictest sense the meiofauna paradox, that meiofauna species with limited dispersal capacities exhibit wide distribution ranges, does not seem to exist or only to a substantially lower degree than assumed before.

Nonetheless, the original observations associated with the meiofauna paradox, that widely distributed complexes of species exhibit very high degrees of morphological similarity, poses intriguing research topics. Clearly, morphological and genetic diversity seem to evolve at different paces in meiofaunal species, as suggested by the high degree of morphological conservatism. Although the provided discussions did not directly give a single and clear answer to the overall similarity between meiofaunal species complexes, it suggests that this question is indeed prominent. First, this requires that the evolutionary history and hence the taxonomy of the study system is firmly established as the basis for future research efforts. While many taxonomists have been aware of these problems, species identifications should include DNA sequences as molecular fingerprints as well as ideally the determination of the level of gene flow at the genomic level.

Additionally, besides the presence of restricted gene flow it has also to be shown that the degree of morphological similarity is as high as assumed (Struck et al. 2018b). Some degree of assumed similarity might arise from neglecting certain morphological character traits a priori (e.g., due to taxonomic tradition), that might actually help to delimitate the species and hence decrease the morphological similarity. Indeed, new developments and approaches in morphological measurements such as detailed anatomical examinations and 3D modelling, other high-resolution microscopy techniques or morphometrics might provide further resolution (Leasi and Todaro 2009; Neusser et al. 2009, 2011; Jörger et al. 2014; Struck et al. 2017). Additional and so far overlooked characters might also decrease the overall similarity in some cases (Knowlton 1993; Méndez et al. 2000; Andrade et al. 2011; Garlitska et al. 2012). Revalidation of characters in this respect could also include other phenotypic characters such as chemical traits, as most marine species rely on chemical cues for mate choice and ecological interactions (Knowlton 1993; Derycke et al. 2008) or the microbiome (Derycke et al. 2016).

If it can be shown that the homogenising effect of gene flow is not present or minimal due to the presence of reproductive isolation, the shown overall morphological or even phenotypic similarity could indicate an adaptive value of this conservatism. As suggested, phenomena such as recent speciation, parallelism, convergence and morphological stasis might account for this (Wada et al. 2013; Swift et al. 2016; Struck et al. 2018b), but further research is needed to unveil the contributions of these phenomena and the selective forces driving them as well as to determine the adaptive value of morphological conservatism (for further details please see the road map below).

"How can meiofauna have bridged oceans and occupied distinct shores in the absence of propagative stages?"

Dispersal and vicariance are generally discussed as the two major forces underlying the distribution range of meiofauna species. Both hold a fundamental role in shaping ecological and evolutionary dynamics of populations and species as they influence habitat colonization, genetic cohesion of species across space, competition and, in the case of dispersal, facilitate or hamper local adaptation (Knowlton 1993; Ronce 2007; Derycke et al. 2013; De Meester et al. 2015; Baco et al. 2016; Mevenkamp et al. 2016). Ronce (2007) defined dispersal as "any movement of individuals or propagules with potential consequences for gene flow across space". Vicariance can be regarded as the establishment of barriers, whether biotic or abiotic, to dispersal and hence gene flow.

Several lines of evidence provide support for dispersal ability in meiofauna. The presented survey included 25 works which directly tested for meiofauna dispersal with experimental approaches (Supplementary Table 2).

Generally, these experimental and empirical evidences show that certain meiofauna organisms (including annelids, arthropods, gastropods, kinorhynchs, nematodes, molluscs, platyhelminths, rotifers and tardigrades; Supplementary Table 2) are regularly found drifting in the water column, rafting on algae or ice or are able to colonize sediment traps and have a selective settlement (Pugh 1996; Schratzberger et al. 2000; Commito and Tita 2002; Thistle 2003; Ullberg and Ólafsson 2003a, b; Teasdale et al. 2004; Cristoni et al. 2004; Gwyther and Fairweather 2005; Arroyo et al. 2006; Gobin and Warwick 2006; Hooper and Davenport 2006; Junkins et al. 2006; da Fonsêca-Genevois et al. 2006; Gallucci et al. 2008; Boeckner et al. 2009; Guilini et al. 2011; Thomas and Lana 2011; Callens et al. 2012; De Meester et al. 2012, 2015; Lins et al. 2013; Mcfarlane et al. 2013; Cuvelier et al. 2014; Mevenkamp et al. 2016).

Presence in the water column can result from sediment erosion (Hagerman and Rieger 1981; Palmer 1988) or through active dispersal as a response to unexpected threats (such as predator attack), changing conditions (such as environmental deterioration, overcrowding, competition), winter migration or nocturnal emergence (Palmer and Gust 1985; Armonies 1990, 1994; Giere 2009). For example, polychaetes and harpacticoid copepods colonize nearby cages more rapidly and abundantly than those farther away (Boeckner et al. 2009). Rates of up to 80% of emergence were reported in harpacticoid copepods (Sedlacek and Thistle 2006). While in the water column, meiofauna can be transported as far as 10 kilometres by erosive tidal currents (Hagerman and Rieger 1981) and members of all meiobenthic taxa have been found in the water column (Armonies 1990). However, all these experimental studies are hampered by the fact that they could not differentiate between local recruitment and long-distance dispersal as they were based on morphological data only. Generally, the conclusions in these studies were therefore conservative and assumed that the detected meiofauna species were only locally recruited from the adjacent sediments.

Besides water column transport, meiofauna dispersal can occur by drifting macroalgae, ice, large floating islands and marine snow (microbial processes and mucus secretions; Fenchel 1978; Westheide 1991; Shanks and Walters 1997; Barnes 2002; Derycke et al. 2008; Giere 2009; de Meester et al. 2012; Tulchinsky et al. 2012; Mcfarlane et al. 2013; Mevenkamp et al. 2016). The dispersal of eggs attached to sand grains (Fenchel 1978) or "buoyant" eggs rather than individuals has also been suggested (Giere 2009; Zeppilli et al. 2011). For example, marine gastrotrichs attach their fertilized eggs directly to sand grains, making dispersal via current sediment plausible (Giere 2009; Kieneke et al. 2012). Considering the evidence for both water column transport and drift, water movements such as currents and flows could become invaluable sources of information when studying meiofauna distribution and dispersal. For example, currents influence genetic structuring in marine nematodes, where population genetic differentiation (i.e., F_{ST} values) is often uncorrelated with distance (Derycke et al. 2013). Additionally, wet ballast sand in ships potentially influences meiofaunal dispersal by human activities and could account for dispersal over hundreds of kilometres, but evidence thus far is sparse (Radziejewska et al. 2006; Giere 2009). Moreover, the possibility of stepping-stone dispersal using sea mounts has also been discussed (George and Schminke 2002; George 2013; Packmor and Riedl 2016). However, the evidence for dispersal in these studies was only indirect as they were derived from biogeographic patterns without direct testing of the means of dispersal using, for example, experimental approaches.

In contrast to all this cumulating direct or indirect evidence of dispersal, meiofaunal organisms are often considered to be one of the most sedentary of the marine faunas with virtually no capacity for dispersal (Sterrer 1973; Christiansen and Fenchel 1979; Westheide and Hass-Cordes 2001; Kieneke et al. 2012). For example, Sterrer (1973) stated that the "development, morphology and biology all seem designed to assure one thing: that the organism never leaves its interstitial environment"; while Danielopol and Wouters (1992) suggested that "they are supposed to disperse very slowly and only with or through the sediments as they have no pelagic life stages". Hence, ideas stating that meiofaunal organisms are poor dispersers influenced the general understanding, hypothesis testing and discussion of meiofaunal dispersal modes (Giere 2009). This viewpoint is further supported by the above finding that the supposed wide distribution ranges of meiofaunal are indeed the cumulated distribution ranges of species complexes and the distribution range is often substantially reduced for each species in this complex when molecular data are applied (see above, Table 2 and Supplementary Table 3).

Along this trend, antagonising views were often either dismissed, neglected or ignored (Palmer and Gust 1985) and the dispersal-distribution discussion narrowed to focus almost exclusively on the absence of pelagic larvae. This is generally in accordance with the remaining marine biology literature. Marine species without pelagic larval dispersal are generally expected to have smaller distribution ranges and higher genetic differentiation between populations than species with such stages, which are thought to ultimately connect populations at larger spatial scales and thus lowering genetic differentiation (Knowlton 1993; Kelly and Palumbi 2010; Baco et al. 2016). As a result, much research has been dedicated to understand larval developmental patterns, duration of pelagic larval stage and larval behaviour (Jokiel 1990; Bhaud and Duchêne 1995). However, evidence against the general applicability of this intuitive scheme (pelagic vs. non-pelagic) has accumulated over the

years. Several studies demonstrated cases of non-pelagic dispersed organisms with highly homogeneous haplotype networks occupying surprisingly wide ranges; on the other hand, other studies reported pelagic dispersed species with clear population structuring, for example, due to local settlement of larva within in the vicinities of the parents (Jokiel 1990; Kyle and Boulding 2000; Colborn et al. 2001; Sponer and Roy 2002; Lester and Ruttenberg 2005; Johnson and Black 2006; Cowen et al. 2007; Lester et al. 2007; Hellberg 2009; Boissin et al. 2015). Hence, dispersal is not the sole, perfect proxy of the distribution range of marine species in general and several other circumstances and particularities can impact distribution ranges such as niche breadth, environmental tolerance, body size, population abundance, latitude, environmental variably at different spatial and temporal scales like substrate type or wave exposure, occurrence of environmental gradients, reproductive strategy, fecundity, lifecycle duration, and physiological constraints (Gaylord and Gaines 2000; Lester and Ruttenberg 2005; Lester et al. 2007; White et al. 2009; Sanford and Kelly 2011). Focusing on meiofauna, some of these factors and concepts have been discussed in the literature, mostly following discoveries of inconsistent and confounding patterns in species' range distribution (Andrade et al. 2011; Tulchinsky et al. 2012). For example, Derycke et al. (2013) suggest that life-history characteristics are important in determining the genetic structure of nematode populations. Similarly, the genetic structure of *Pellioditis marina* might be best explained by its life-history characteristics of a short generation time, high colonization potential and evolutionary potential for local adaptation (Derycke et al. 2005). Furthermore, evidence for rare long-distance dispersal events stems also from the highly similar composition and high diversity of the meiofauna of the Galapagos Islands with other parts of the world (Westheide 1977, 1991). The same may hold truth for colonization of other islands of volcanic origin.

Vicariance has also been proposed not only to explain the establishment of barriers to dispersal as evidenced by the reduced distribution ranges (Table 2), but also as a responsible force underlying the present distributions of meiofaunal species. In specific, the distribution of meiofaunal taxa was suggested to reflect the movement of the tectonic plates and with that the continental landmasses with their coastlines (Sterrer 1973). Arguably, Sterrer overemphasized the importance of this mechanism, and dismissed dispersal (due to the absence of pelagic larval stages) as a viable mechanism, rendering both as mutually exclusive (Sterrer 1973). When considering variation of species distributions through time, the severe differences in geological and climatological events have to be accounted for (Norris and Hull 2012), as these influence population connectivity and distribution ranges in complex ways, both at macro- and microgeological scales. For example, glacial periods resulted in a decrease of the sea level, leading to changes of coastal geography (e.g., increase in island mass) including closure of seaways which were open in interglacial periods. Likewise, temperature, oxygen and salinity gradients were affected by these changes. It is not surprising to assume that the evolutionary history, regardless of the dispersal abilities of ecological communities was severely affected throughout time by such events (Dawson 2001). Hence, considering the registered variance of climatological, sea level and geological changes throughout the last ~ 500 million years, focusing exclusively on vicariance is misleading. Accordingly, none of the studies mentioned above using molecular data supported the hypothesis that the distribution was exclusively the result of plate tectonic events. Indeed, climatic oscillations such as intermittent glacial–interglacial periods resulted in bottlenecks, recent founder-events, and local extinctions in some meiofaunal species (Taylor et al. 1998; Derycke et al. 2005, 2008, 2013; Casu and Curini-Galletti 2006; Tulchinsky et al. 2012). For the gastrotrich *Turbanella cornuta*, Kieneke et al. (2012) found that the most likely colonization to the Baltic Sea was via water connections and corresponding currents about 10,000 years ago rather than by the recent connectivity routes. Moreover, instances of long-distance dispersal within the Northeast Atlantic could be found in other *Turbanella* species. A recent study focusing on the annelid genus *Stygocapitella* demonstrated that considering a strict vicariance hypothesis does not fit meiofauna dispersal (Struck et al. 2017). Applying the vicariance hypothesis strictly would require that the southern species be separated 450 million years ago in the Ordovician with the beginning formation of the Paleo-Tethys Ocean (Fig. 3). Hence, ancient dispersal events (Fig. 3) are more likely, possibly in combination with vicariant events establishing barriers of dispersal (Struck et al. 2017). Furthermore, Derycke et al. (2013) discussed hypotheses considering dispersal and gene flow of free-living meiofaunal nematodes and stressed the importance of historical events in shaping the genetic pattern of marine nematodes, showing that land mass drift, sea level rise and glacial cycles influenced population structuring and distribution of the nematode *Litoditis marina*. From this, they concluded that the evolutionary history of this cryptic species complex is only thoroughly understood when historical events are considered alongside aspects of dispersal. In conclusion, climatological and geological events affect meiofauna distribution and dispersal.

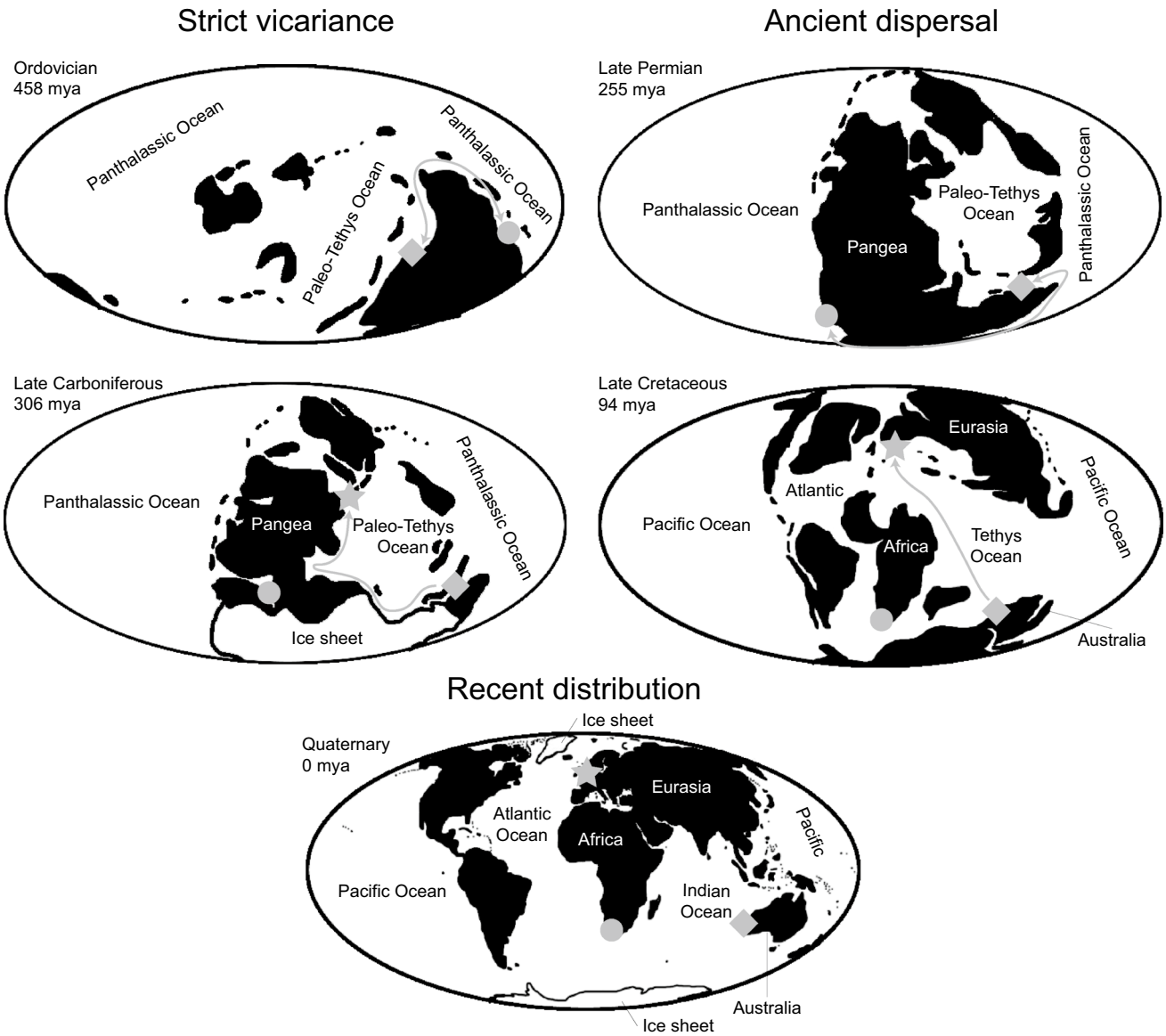


Fig. 3 Two scenarios explaining the distribution of three species of *Stygocapitella* investigated by Struck et al. (2017). The left upper two panels show a strictly vicariant scenario, the right upper two show ancient dispersal and the lowest the recent distribution. Arrows indicate possible dispersal routes either via plate tectonics or long-distance dispersal. Paleomaps modified from Scotese (2002). Star, population of *S. subterranea*; diamond, population of *S australis;* circle, population of *S. minuta*

Conclusions on "How can meiofauna have bridged oceans and occupied distinct shores in the absence of propagative stages?"

A cornerstone of the 'meiofauna paradox' is the expectation of low dispersal of meiofaunal invertebrates. There is an increasing body of experimental and empirical evidence, which clearly contrasts this view. Meiofaunal dispersal, not specifically tied to any restrained evolutionary lineage or taxonomic clade, has been clearly demonstrated. Dispersal abilities seem to account for the distribution of lineages throughout considerably large areas. Nevertheless, this should not be confounded by the ability to maintain cosmopolitan distributions. The expectations of low dispersal seem to emerge from historical views based on the dichotomous presence/absence view on pelagic larval dispersal. The presence of pelagic dispersal plays a role in dispersal and species distribution, but it is not the only considered variable. Meiofaunal biologists should explore the ecological roles and life-history traits of the species to understand the distribution of a species. Ecological and life-history traits effectively affect dispersal and the range distribution of individuals and are seldom considered. Moreover, the inclusion of vicariant events and ancient dispersal routes can explain

the recent distribution of meiofaunal organisms. However, vicariance should not be considered the sole driver of meiofaunal distribution. Only few studies on meiofauna have been conducted so far, which accounted for both (Westheide 2005). Therefore, general conclusions for meiofauna dispersal as not being possible must be regarded as idiosyncratic for the time being.

Future empirical studies on the dispersal of meiofaunal organisms should concentrate on deciphering the contributions of dispersal and vicariance on the recent distribution at several geographic and temporal scales. This will allow more general conclusions regarding dispersal potential, time scales of dispersal and speciation as well as associated processes, especially if these studies are conducted for a broad range of the meiofaunal biodiversity. Empirical data providing indirect evidence of dispersal routes should be complemented by experimental approaches to directly test dispersal capacities, which allow assessing the difference between local recruiters and long-distance dispersers (for further details please see the road map below).

Moreover, analyses of metapopulation dynamics have gained popularity in marine ecological studies (Wares et al. 2001; Kritzer and Sale 2004; Cowen et al. 2007), and have been frequently applied to meiofaunal organisms (Derycke et al. 2006, 2007a, b, 2008; Andrade et al. 2011; Leasi and Norenburg 2016). The complexity and dynamics of meiofauna populations through time and space make them suitable for such analyses. Suitable habitats often consist of relatively small (metre scale) isolated patches of sediment, which are separated from each other by distances of several metres to even hundreds of kilometres of inhospitable habitat (Tulchinsky et al. 2012; Leasi and Norenburg 2016). This often results in mosaic-like population patterns, which are best addressed by taking metapopulation dynamics into account.

Roadmap for meiofauna research: directions of future research

The wide geographical distribution (including cosmopolitanism) of many marine species has puzzled researchers and resulted in the prevalence of several paradoxes. Examples are the extended pelagic stage of some species with restricted distributions (Colborn et al. 2001), the community composition of Rockall (Johannesson 1988) or the cosmopolitan distribution of marine species without free-living larvae (Sponer and Roy 2002). These paradoxes stem from sampling and taxonomic complications and the meiofauna paradox is no exception to these difficulties. Here, we discussed that the meiofauna paradox likely stems from pre-established, historically defined hypotheses, pre-concepts of sea connectivity dynamics and the presence of cryptic species as well as difficulties and biases in meiofauna sampling and collection, identification and characterization. As pointed out in the literature survey, recent evidences indicate that the meiofauna paradox and its underlying assumptions including the wide distribution and low dispersal capability of meiofaunal organisms are not met. First, a considerable amount of studies focusing on cosmopolitan species and applying molecular methods uncovered underlying diversity (cryptic species) often with limited distribution ranges. Hence, the assumption of wide distribution is not given. Second, the limited dispersal capacity seems questionable and a remnant of historical literature. Nonetheless, even though the meiofauna paradox in its strictest sense does most likely not exist, certain aspects of the paradox pose interesting research venues. As such, facing the future we suggest that Giere´s (2009) questions concerning the meiofauna paradox should be considered in terms of morphological similarity and marine connectivity.

To understand phenotypic conservatism, both evolutionary and taxonomic approaches are needed. Future studies should focus on unveiling the selective pressures resulting in phenotypic similarity of meiofaunal species. Overall similarity in meiofauna and its underlying processes warrants potentially interesting evolutionary phenomena (i.e., morphological stasis, recent speciation, parallel or convergent evolution). In the age of 'high-throughput sequencing', genomic scans such as RADseq, anchored hydrid enrichment (AHE), ultra-conserved elements (UCE) or genome re-sequencing in combination with de novo genome assemblies of meiofaunal species will open unprecedented gates to understand the evolutionary history, connectivity, adaptation and selective regimes affecting meiofaunal organisms. Surprisingly though, the provided literature survey captured only seven studies focusing on evolutionary biology (Schmidt and Westheide 1999; Rocha-Olivares et al. 2001; Denis et al. 2009; Yamasaki et al. 2014; Scarpa et al. 2015; Smythe 2015; Randsø et al. 2018) out of a total of 751 studies. Meiofaunal species represent ideal systems to understand selective pressures on cryptic species complexes and deceleration of phenotypic evolution (as generally suggested by Struck et al. 2018a, b). Even though meiofaunal organisms are of small size, recent advantages in whole genome amplification techniques allow working with individual specimens (e.g., Golombek et al. 2013, 2015). On the other hand, the small size can be a potential advantage when investigating the similarity of the whole phenotype as a more complete assessment of the whole phenotype is possible. If such studies are combined with nested sampling strategies of populations and species of a complex as well as of morphological slightly different sister species, selective regimes at different taxonomical levels such as between populations, cryptic species and non-cryptic species can be revealed.

In addition to this evolutionary approach, discovery and description of meiofaunal species should be prioritized, as only a broad taxonomic basis will allow for solid general conclusions about evolutionary processes, speciation and biogeographic history and selective regimes as well as provide the necessary phylogenetic framework for the evolutionary studies. Taxonomic efforts should include DNA sequences when describing species, as these allow a better detection of distribution ranges as discussed above. Guidelines for DNA taxonomy with a focus on meiofauna have been published (Fontaneto et al. 2015). Additionally, the overall phenotype of the meiofaunal species should be described in as much detail as possible, as this will provide the basis to assess similarities across species boundaries. Indeed, following our discussion, a thorough understanding of meiofaunal species' distributions is inevitable to understand the scale and range that meiofaunal species can maintain connectivity. The unravelling of cryptic species, resulting from the overall phenotypic similarity, will help understanding potential barriers of gene flow including historical barriers. Additionally, the discovery of cryptic species will open further research venues such as physiological variability (de Meester et al. 2011) and distribution along ecological gradients. Hence, investment in classical taxonomic research like species characterization and development of identification keys in understudied areas should be a priority of meiofaunal research, likely yielding the discovery of endemic species or species with a more restricted distribution (Garraffoni and Balsamo 2017).

To tackle marine connectivity, both empirical and experimental approaches should be adopted. Empirical research on dispersal and distribution of meiofaunal organisms can apply the methodology outlined above for evolutionary and taxonomic approaches. If an adequate sampling regime is performed, the produced data will be able to tackle questions concerning connectivity, demography and biogeography. Hence, the sampling strategy should be inclusive to both possible vicariance and dispersal events for the group of interest. The dispersal potential of meiofaunal organisms and the influence of vicariant events can then be addressed more thoroughly and systematically in time, space and taxonomic breadth. This includes assessing dispersal potential empirically at local and regional scales, which are potentially affected by historic events like glaciations, comparing sister species pairs with only very few differing biological properties as well as using metapopulation models to get a better fit of the reality of meiofaunal population structure. A strong focus of research is recently on intertidal to shallow-subtidal habitats. However, to achieve a more thorough understanding, marine connectivity research on supralittoral and deep sea habitats should also be emphasized, also having in mind that these could have been temporal habitats in the past. Moreover, genome-scale data are preferable over few molecular markers if possible, as they allow a more accurate assessment of both recent and historic gene flow and hence dispersal capacity based on fewer specimens due to the increased sampling size.

In contrast, only a minority of the surveyed literature directly tested for meiofaunal dispersal in experimental settings. While challenging historical expectations, these works are vital to understand the means of meiofauna connectivity, dispersal and distribution. Considering recent technological advances, the inclusion of DNA sequences on species detection in such studies using metagenomic and metatranscriptomic approaches will enable future works to test for dispersal of meiofauna more accurately (Fonseca et al. 2014; Carugati et al. 2015; Leray and Knowlton 2015, 2016). For example, collecting environmental DNA samples of sediments at various depths and from the adjacent 'pelagic realm' can provide insights if the present meiofaunal species in the pelagic realm are only locally recruited or if specimens from more distant populations are also present. Additionally, metatranscriptomic approaches have the potential to determine which stages of development are responsible for dispersal. However, to validate such approaches, appropriate databases must be established, including genetic and transcriptomic markers specific for certain developmental stages. Therefore, at the present stage, priority should be given to projects compiling such comprehensive databases.

Acknowledgements The authors are indebted to two anonymous reviewers and Diego Fontaneto whose comments have considerably improved the original manuscript. JC is grateful to Zeca Afonso (among other thinkers) for inspiration ("Em cada esquina um amigo; Em cada rosto igualdade"). This is NHM Evolutionary Genomics Lab contribution No. 10.

Compliance with ethical standards

Ethical approval All authors have approved the submitted manuscript

Conflict of interest The authors declare that they have no conflict of interest.

Human animal rights statement This article does not contain any studies with animals performed by any of the authors.

References

Andrade SCS, Norenburg JL, Solferini VN (2011) Worms without borders: genetic diversity patterns in four Brazilian *Ototyphlonemertes* species (Nemertea, Hoplonemertea). Mar Biol 158:2109–2124. https://doi.org/10.1007/s00227-011-1718-3

Ansari KGMT, Pattnaik AK, Rastogi G, Bhadury P (2015a) An inventory of free-living marine nematodes from Asia's largest coastal lagoon, Chilika, India. Wetl Ecol Manag 23:881–890. https://doi.org/10.1007/s11273-015-9426-2

Ansari KGMT, Lyla PS, Khan SA (2015b) New distributional records of free-living marine nematodes from Indian waters I. Chromadorids. Indian J Geo Mar Sci 44:756–765

Ansari KGMT, Lyla PS, Khan SA (2016) New distributional records of free-living marine nematodes from Indian waters II. Monhysterids. Indian J Geo-Marine Sci 45:342–351

Ansari KGMT, Lyla PS, Ajmal Khan S (2017) New distributional records of free-living marine nematodes from Indian waters III. Microlaimids and Laptolaimids. Indian J Geo Mar Sci 46:155–162

Armonies W (1990) Short-term changes of meiofaunal abundance in intertidal sediments. Helgoländer Meeresuntersuchungen 386:375–386

Armonies W (1994) Drifting meio- and macrobenthic invertebrates on tidal flats in Königshafen: a review. Helgoländer Meeresuntersuchungen 48:299–320. https://doi.org/10.1007/BF02367043

Arroyo NL, Aarnio K, Bonsdorff E (2006) Drifting algae as a means of re-colonizing defaunated sediments in the Baltic Sea. A short-term microcosm study. Hydrobiologia 554:83–95. https://doi.org/10.1007/s10750-005-1008-5

Atherton S, Jondelius U (2018) Microstomum (Platyhelminthes, Macrostomorpha, Microstomidae) from the Swedish west coast: two new species and a population description. Eur J Taxon. https://doi.org/10.5852/ejt.2018.398

Baco AR, Etter RJ, Ribeiro PA, von der Heyden S, Beerli P, Kinlan BP (2016) A synthesis of genetic connectivity in deep-sea fauna and implications for marine reserve design. Mol Ecol 25:3276–3298. https://doi.org/10.1111/mec.13689

Baldrighi E, Aliani S, Conversi A, Lavaleye M, Borghini M, Manini E (2013) From microbes to macrofauna: an integrated study of deep benthic communities and their response to environmental variables along the Malta Escarpment (Ionian Sea). Sci Mar 77:625–639. https://doi.org/10.3989/scimar.03811.03b

Barnes DKA (2002) Invasions by marine life on plastic debris. Nature 416:808–809. https://doi.org/10.1038/416808a

Bhadury P, Austen MC, Bilton DT, Lambshead PJD, Rogers AD, Smerdon GR (2006) Molecular detection of marine nematodes from environmental samples: overcoming eukaryotic interference. Aquat Microb Ecol 44:97–103. https://doi.org/10.3354/ame044097

Bhaud M, Duchêne J (1995) Change from planktonic to benthic development: is life cycle evolution an adaptive answer to the constraints of dispersal? Oceanol Acta 19:335–346

Bik HM, Thomas WK, Lunt DH, Lambshead PJD (2010) Low endemism, continued deep-shallow interchanges, and evidence for cosmopolitan distributions in free-living marine nematodes (order Enoplida). BMC Evol Biol 10:389. https://doi.org/10.1186/1471-2148-10-389

Bik HM, Sung W, De Ley P, Baldwin JG, Sharma J, Rocha-Olivares A, Thomas WK (2012) Metagenetic community analysis of microbial eukaryotes illuminates biogeographic patterns in deep-sea and shallow water sediments. Mol Ecol 21:1048–1059. https://doi.org/10.1111/j.1365-294X.2011.05297.x

Boeckner MJ, Sharma J, Proctor HC (2009) Revisiting the meiofauna paradox: dispersal and colonization of nematodes and other meiofaunal organisms in low- and high-energy environments. Hydrobiologia 624:91–106. https://doi.org/10.1007/s10750-008-9669-5

Boissin E, Egea E, Féral JP, Chenuil A (2015) Contrasting population genetic structures in *Amphipholis squamata*, a complex of brooding, self-reproducing sister species sharing life history traits. Mar Ecol Prog Ser 539:165–177. https://doi.org/10.3354/meps11480

Brenzinger B, Haszprunar G, Schrödl M (2013) At the limits of a successful body plan—3D microanatomy, histology and evolution of *Helminthope* (Mollusca: Heterobranchia: Rhodopemorpha), the most worm-like gastropod. Front Zool 10:37. https://doi.org/10.1186/1742-9994-10-37

Callens M, Gheerardyn H, Ndraro SGM, De Troch M, Vanreusel A (2012) Harpacticoid copepod colonization of coral fragments in a tropical reef lagoon (Zanzibar, Tanzania). J Mar Biol Assoc UK 92:1535–1545. https://doi.org/10.1017/S0025315411001597

Carugati L, Corinaldesi C, Dell A, Danovaro R (2015) Marine genomics metagenetic tools for the census of marine meiofaunal biodiversity: an overview. Mar Genom 24:11–20. https://doi.org/10.1016/j.margen.2015.04.010

Casu M, Curini-Galletti M (2004) Sibling species in interstitial flatworms: a case study using *Monocelis lineata* (Proseriata: Monocelididae). Mar Biol 145:669–679. https://doi.org/10.1007/s00227-004-1367-x

Casu M, Curini-Galletti M (2006) Genetic evidence for the existence of cryptic species in the mesopsammic flatworm *Pseudomonocelis ophiocephala* (Rhabditophora: Proseriata). Biol J Linn Soc 87:553–576. https://doi.org/10.1111/j.1095-8312.2006.00588.x

Casu M, Lai T, Sanna D, Cossu P, Curini-Galletti M (2009) An integrative approach to the taxonomy of the pigmented European *Pseudomonocelis* meixner, 1943 (Platyhelminthes: Proseriata). Biol J Linn Soc 98:907–922. https://doi.org/10.1111/j.1095-8312.2009.01316.x

Chatterjee T, Troch M De (2000) Halacaridae (Acari) from Gazi Bay (Kenya): description and biogeography of three new and two known species. Hydrobiologia 427:177–194. https://doi.org/10.1023/A:1003979629889

Christiansen FB, Fenchel TM (1979) Evolution of marine invertebrate reproductive patterns. Theor Popul Biol 16:267–282. https://doi.org/10.1016/0040-5809(79)90017-0

Clausen C (2000) Gastrotricha macrodasyida from the Tromsø region, northern Norway. Sarsia 85:357–384. https://doi.org/10.1080/00364827.2000.10414588

Colborn J, Crabtree RE, Shaklee JB, Pfeiler E, Bowen BW (2001) The evolutionary enigma of bonefishes (*Albula* spp.): cryptic species and ancient separations in a globally distributed shorefish. Evolution (N Y) 55:807–820. https://doi.org/10.1111/j.0014-3820.2001.tb00816.x

Commito JA, Tita G (2002) Differential dispersal rates in an intertidal meiofauna assemblage. J Exp Mar Bio Ecol 268:237–256. https://doi.org/10.1016/S0022-0981(01)00386-0

Cowen RK, Gawarkiewicz G, Pineda J, Thorrold SR, Werner FE (2007) Population connectivity in marine systems. Oceanography 20:14–21. https://doi.org/10.1126/science.1122039

Cristoni C, Colangelo MA, Ceccherelli VU (2004) Spatial scale and meiobenthic copepod recolonisation: testing the effect of disturbance size in a seagrass habitat. J Exp Mar Bio Ecol 298:49–70. https://doi.org/10.1016/j.jembe.2003.08.005

Curini-Galletti M, Puccinelli I (1998) The *Gyratrix hermaphroditus* species complex (Kalyptorhynchia: Polycystididae) in marine habitats of eastern Australia. Hydrobiologia 383:287–298. https://doi.org/10.1023/A:1003456102035

Cuvelier D, Beesau J, Ivanenko VN, Zeppilli D, Sarradin PM, Sarrazin J (2014) First insights into macro- and meiofaunal colonisation patterns on paired wood/slate substrata at Atlantic deep-sea hydrothermal vents. Deep Res Part I Oceanogr Res Pap 87:70–81. https://doi.org/10.1016/j.dsr.2014.02.008

da Fonsêca-Genevois V, Somerfield PJ, Neves MHB, Coutinho R, Moens T (2006) Colonization and early succession on artificial hard substrata by meiofauna. Mar Biol 148:1039–1050. https://doi.org/10.1007/s00227-005-0145-8

Dal Zotto M (2015) *Antygomonas caeciliae*, a new kinorhynch from the Mediterranean Sea, with report of mitochondrial genetic data for the phylum. Mar Biol Res 11:689–702. https://doi.org/10.1080/17451000.2015.1007872

Dal Zotto M, Todaro MA (2016) Kinorhyncha from Italy, a revision of the current checklist and an account of the recent investigations. Zool Anz 265:90–107. https://doi.org/10.1016/j.jcz.2016.01.004

Danielopol DAN, Wouters K (1992) Evolutionary (Paleo)biology of marine interstitial ostracoda. Geobios 25:207–211

Dawson MN (2001) Phylogeography in coastal marine animals: a solution from California? J Biogeogr 28:723–736

De Ley P, De Ley IT, Morris K, Abebe E, Mundo-Ocampo M, Yoder M, Heras J, Waumann D, Rocha-Olivares A, Jay Burr AH, Baldwin JG, Thomas WK (2005) An integrated approach to fast and informative morphological vouchering of nematodes for applications in molecular barcoding. Philos Trans R Soc B Biol Sci 360:1945–1958. https://doi.org/10.1098/rstb.2005.1726

de Meester N, Derycke S, Bonte D, Moens T (2011) Salinity effects on the coexistence of cryptic species: a case study on marine nematodes. Mar Biol 158:2717–2726. https://doi.org/10.1007/s00227-011-1769-5

De Meester N, Derycke S, Moens T (2012) Differences in time until dispersal between cryptic species of a marine nematode species complex. PLoS One 7:1–8. https://doi.org/10.1371/journal.pone.0042674

De Meester N, Derycke S, Rigaux A, Moens T (2015) Active dispersal is differentially affected by inter- and intraspecific competition in closely related nematode species. Oikos 124:561–570. https://doi.org/10.1111/oik.01779

Delogu V, Casu M, Curini-Galletti M (2008) The genera *Parotoplana* Meixner, 1938 and *Parotoplanella* Ax, 1956 (Platyhelminthes: Proseriata) in southern Spain. J Nat Hist 42:157–176. https://doi.org/10.1080/00222930701840696

Denis F, Ravallec R, Pavillon J-F, Van Wormhoudt A (2009) Genetic differentiation of Atlantic populations of the intertidal copepod *Tigriopus brevicornis*. Sci Mar 73:579–587. https://doi.org/10.3989/scimar.2009.73n3579

Derycke S, Remerie T, Vierstraete A, Backeljau T, Vanfleteren JR, Vincx M, Moens T (2005) Mitochondrial DNA variation and cryptic speciation within the free-living marine nematode *Pellioditis marina*. Mar Ecol Prog Ser 300:91–103. https://doi.org/10.3354/meps300091

Derycke S, Backeljau T, Vlaeminck C, Vierstraete A, Vanfleteren J, Vincx M, Moens T (2006) Seasonal dynamics of population genetic structure in cryptic taxa of the *Pellioditis marina* complex (Nematoda: Rhabditida). Genetica 128:307–321. https://doi.org/10.1007/s10709-006-6944-0

Derycke S, Van Vynckt R, Vanoverbeke J, Vincx M, Moens T (2007a) Colonization patterns of Nematoda on decomposing algae in the estuarine environment: community assembly and genetic structure of the dominant species *Pellioditis marina*. Limnol Oceanogr 52:992–1001. https://doi.org/10.4319/lo.2007.52.3.0992

Derycke S, Backeljau T, Vlaeminck C, Vierstraete A, Vanfleteren J, Vincx M, Moens T (2007b) Spatiotemporal analysis of population genetic structure in *Geomonhystera disjuncta* (Nematoda, Monhysteridae) reveals high levels of molecular diversity. Mar Biol 151:1799–1812. https://doi.org/10.1007/s00227-007-0609-0

Derycke S, Remerie T, Backeljau T, Vierstraete A, Vanfleteren J, Vincx M, Moens T (2008) Phylogeography of the *Rhabditis* (Pellioditis) marina species complex: evidence for long-distance dispersal, and for range expansions and restricted gene flow in the northeast Atlantic. Mol Ecol 17:3306–3322. https://doi.org/10.1111/j.1365-294X.2008.03846.x

Derycke S, Sheibani Tezerji R, Rigaux A, Moens T (2012) Investigating the ecology and evolution of cryptic marine nematode species through quantitative real-time PCR of the ribosomal ITS region. Mol Ecol Resour 12:607–619. https://doi.org/10.1111/j.1755-0998.2012.03128.x

Derycke S, Backeljau T, Moens T (2013) Dispersal and gene flow in free-living marine nematodes. Front Zool 10:1. https://doi.org/10.1186/1742-9994-10-1

Derycke S, De Meester N, Rigaux A, Creer S, Bik H, Thomas W, Moens T (2016) Coexisting cryptic species of the *Litoditis marina* complex (Nematoda) show differential resource use and have distinct microbiomes with high intraspecific variability. Mol Ecol. https://doi.org/10.1111/mec.13597

Di Domenico M, Martínez A, Lana P, Worsaae K (2014) Molecular and morphological phylogeny of Saccocirridae (Annelida) reveals two cosmopolitan clades with specific habitat preferences. Mol Phylogenet Evol 75:202–218. https://doi.org/10.1016/j.ympev.2014.02.003

Dujardin F (1851) Sur un petit animal marin, l'Echinodère, formant un type intermédiaire entre les Crustacés et les Vers. Ann Sci Nat Zool 3:158–160

Dunn CW, Giribet G, Edgecombe GD, Hejnol A (2014) Animal phylogeny and its evolutionary implications. Annu Rev Ecol Syst 45:371–395. https://doi.org/10.1146/annurev-ecolsys-120213-091627

Eder B, Schrödl M, Jörger KM (2011) Systematics and redescription of the European meiofaunal slug Microhedyle glandulifera (Kowalevsky, 1901) (Heterobranchia: Acochlidia): Evidence from molecules and morphology. J Molluscan Stud 77:388–400. https://doi.org/10.1093/mollus/eyr030

Fenchel TM (1978) The ecology of micro- and meiobenthos. Annu Rev Ecol Syst 9:99–121

Fonseca VG, Carvalho GR, Nichols B, Quince C, Johnson HF, Neill SP, Lambshead JD, Thomas WK, Power DM, Creer S (2014) Metagenetic analysis of patterns of distribution and diversity of marine meiobenthic eukaryotes. Glob Ecol Biogeogr 23:1293–1302. https://doi.org/10.1111/geb.12223

Fontaneto D, Kaya M, Herniou EA, Barraclough TG (2009) Extreme levels of hidden diversity in microscopic animals (Rotifera) revealed by DNA taxonomy. Mol Phylogenet Evol 53:182–189. https://doi.org/10.1016/j.ympev.2009.04.011

Fontaneto D, Flot JF, Tang CQ (2015) Guidelines for DNA taxonomy, with a focus on the meiofauna. Mar Biodivers 45:433–451. https://doi.org/10.1007/s12526-015-0319-7

Futuyma DJ (2010) Evolutionary constraint and ecological consequences. Evolution (N Y) 64:1865–1884. https://doi.org/10.1111/j.1558-5646.2010.00960.x

Gallucci F, Moens T, Vanreusel A, Fonseca G (2008) Active colonisation of disturbed sediments by deep-sea nematodes: evidence for the patch mosaic model. Mar Ecol Prog Ser 367:173–183. https://doi.org/10.3354/meps07537

Garlitska L, Neretina T, Schepetov D, Mugue N, De Troch M, Baguley JG, Azovsky A (2012) Cryptic diversity of the "cosmopolitan" harpacticoid copepod *Nannopus palustris*: genetic and morphological evidence. Mol Ecol 21:5336–5347. https://doi.org/10.1111/mec.12016

Garraffoni ARS, Balsamo M (2017) Is the ubiquitous distribution real for marine gastrotrichs? Detection of areas of endemism using Parsimony Analysis of Endemicity (PAE). Proc Biol Soc Wash 130:197–210. https://doi.org/10.2988/17-00011

Gaylord B, Gaines SD (2000) Temperature or transport? Range limits in marine species mediated solely by flow. Am Nat 155:769–789. https://doi.org/10.1086/303357

George KH (2013) Faunistic research on metazoan meiofauna from seamounts—a review. Meiofauna Mar 20:1–32

George KH, Schminke HK (2002) Harpacticoida (Crustacea, Copepoda) of the Great Meteor Seamount, with first conclusions as to the origin of the plateau fauna. Mar Biol 141:887–895. https://doi.org/10.1007/s00227-002-0878-6

Gerlach SA (1977) Means of meiofauna dispersal. In: Sterrer W, Ax P (eds) The meiofauna species in time and space. Mikrofauna Meeresbod, vol 61, pp 89–103

Giard A (1904) Sur une faunule charactéristique des sables à diatomées d'Ambleteuse. C R Séanc Soc Biol Paris 56:107–165

Giere O (2009) Meiobenthology: the microscopic motile fauna of aquatic sediments, 2nd edn. Springler-Verlag, Berlin Heidelberg

Gobin JF, Warwick RM (2006) Geographical variation in species diversity: a comparison of marine polychaetes and nematodes. J Exp Mar Bio Ecol 330:234–244. https://doi.org/10.1016/j.jembe.2005.12.030

Golombek A, Tobergte S, Nesnidal MP, Purschke G, Struck TH (2013) Mitochondrial genomes to the rescue—diurodrilidae in the myzostomid trap. Mol Phylogenet Evol 68:312–326

Golombek A, Tobergte S, Struck TH (2015) Elucidating the phylogenetic position of Gnathostomulida and first mitochondrial genomes of Gnathostomulida, Gastrotricha and Polycladida (Platyhelminthes). Mol Phylogenet Evol 86:49–63. https://doi.org/10.1016/j.ympev.2015.02.013

Gruber-Vodicka HR, Dirks U, Leisch N, Baranyi C, Stoecker K, Bulgheresi S, Heindl NR, Horn M, Lott C, Loy A, Wagner M, Ott J (2011) *Paracatenula*, an ancient symbiosis between thiotrophic Alphaproteobacteria and catenulid flatworms. Proc Natl Acad Sci 108:12078–12083. https://doi.org/10.1073/pnas.1105347108

Guilini K, Soltwedel T, van Oevelen D, Vanreusel A (2011) Deep-sea nematodes actively colonise sediments, irrespective of the presence of a pulse of organic matter: results from an in situ experiment. PLoS One. https://doi.org/10.1371/journal.pone.0018912

Gwyther J, Fairweather PG (2005) Meiofaunal recruitment to mimic pneumatophores in a cool-temperate mangrove forest: spatial context and biofilm effects. J Exp Mar Bio Ecol 317:69–85. https://doi.org/10.1016/j.jembe.2004.11.012

Hagerman GM, Rieger RM (1981) Dispersal of benthic meiofauna by wave and current action in Bogue sound, North Carolina, USA. Mar Ecol 2:245–270. https://doi.org/10.1111/j.1439-0485.1981.tb00099.x

Hansen TF, Houle D (2004) Evolvability, stabilizing selection, and the problem of stasis. In: Pigliucci M, Preston K (eds) Phenotypic integration: studying the ecology and evolution of complex phenotypes. Oxford University Press, New York, pp 130–154

Hellberg ME (2009) Gene flow and isolation among populations of marine animals. Annu Rev Ecol Evol Syst 40:291–310. https://doi.org/10.1146/annurev.ecolsys.110308.120223

Higgins RP, Thiel H (1988) Introduction to the study of meiofauna. Smithsonian Institution Press, Washington

Hooper GJ, Davenport J (2006) Epifaunal composition and fractal dimensions of intertidal marine macroalgae in relation to emersion. J Mar Biol Assoc UK 86:1297–1304. https://doi.org/10.1017/S0025315406014329

Hutchings P, Kupriyanova E (2018) Cosmopolitan polychaetes—fact or fiction? Personal and historical perspectives. Invertebr Syst 32:1–9. https://doi.org/10.1071/IS17035

Johannesson K (1988) The paradox of Rockall: why is a brooding gastropod (*Littorina saxatilis*) more widespread than one having a planktonic larval dispersal stage (*L. littorea*)? Mar Biol 99:507–513. https://doi.org/10.1007/BF00392558

Johnson MS, Black R (2006) Islands increase genetic subdivision and disrupt patterns of connectivity of intertidal snails in a complex archipelago. Evolution (N Y) 60:2498–2506. https://doi.org/10.1111/j.0014-3820.2006.tb01885.x

Jokiel PL (1990) Long-distance dispersal by rafting: reemergence of an old hypothesis. Endeavour 14:66–73

Jörger KM, Schrödl M (2013) How to describe a cryptic species? Practical challenges of molecular taxonomy. Front Zool 10:59. https://doi.org/10.1186/1742-9994-10-59

Jörger KM, Norenburg JL, Wilson NG, Schrödl M (2012) Barcoding against a paradox? Combined molecular species delineations reveal multiple cryptic lineages in elusive meiofaunal sea slugs. BMC Evol Biol 12:245. https://doi.org/10.1186/1471-2148-12-245

Jörger KM, Neusser TP, Brenzinger B, Schrödl M (2014) Exploring the diversity of mesopsammic gastropods: how to collect, identify, and delimitate small and elusive sea slugs? Am Malacol Bull 32:290–307. https://doi.org/10.4003/006.032.0205

Jouin-Toulmond C, Gambi MC (2007) Description of Saccocirrus goodrichi sp. nov. (Annelida: Polychaeta: Saccocirridae), a new Mediterranean species and new data on the chaetae of S. papillocercus and S. major. Cah Biol Mar 48:381–390

Junkins R, Kelaher B, Levinton J (2006) Contributions of adult oligochaete emigration and immigration in a dynamic soft-sediment community. J Exp Mar Bio Ecol 330:208–220. https://doi.org/10.1016/j.jembe.2005.12.028

Kajihara H, Ikoma M, Yamasaki H, Hiruta SF (2015) *Trilobodrilus itoi* sp. nov., with a re-description of *T. nipponicus* (Annelida: Dinophilidae) and a molecular phylogeny of the genus. Zool Sci 32:405–417. https://doi.org/10.2108/zs140251

Kånneby T, Bernvi DC, Jondelius U (2015) Distribution, delimitation and description of species of *Archaphanostoma* (Acoela). Zool Scr 44:218–231. https://doi.org/10.1111/zsc.12092

Karanovic I, Tanaka H, Tsukagoshi A (2016) Congruence between male upper lip morphology and molecular phylogeny in Parapolycope (Ostracoda), with two new species from Korea Congruence between male upper lip morphology and molecular phylogeny in Parapolycope (Ostracoda), with two new species from. Invertebr Syst 30:231–254

Kelly RP, Palumbi SR (2010) Genetic structure among 50 species of the northeastern pacific rocky intertidal community. PLoS One. https://doi.org/10.1371/journal.pone.0008594

Kieneke A, Nikoukar H (2017) Integrative morphological and molecular investigation of *Turbanella hyalina* Schultze, 1853 (Gastrotricha: Macrodasyida), including a redescription of the species. Zool Anz 267:168–186. https://doi.org/10.1016/j.jcz.2017.03.005

Kieneke A, Martínez Arbizu PM, Fontaneto D (2012) Spatially structured populations with a low level of cryptic diversity in European marine Gastrotricha. Mol Ecol 21:1239–1254. https://doi.org/10.1111/j.1365-294X.2011.05421.x

Klautau M, Russo CAM, Lazoski C, Boury-esnault N, Thorpe JP, Solecava AM, Klautau M, Russo CAM, Lazoski C, Boury-esnault N, John P, Solt-cava AM (1999) Does cosmopolitanism result from overconservative systematics? A case study using the marine sponge *Chondrilla nucula*. Evolution (N Y) 53:1414–1422

Knowlton N (1993) Sibling species in the sea. Annu Rev Ecol Syst 24:189–216

Kritzer JP, Sale PF (2004) Metapopulation ecology in the sea: from Levins' model to marine ecology and fisheries science. Fish Fish 5:131–140. https://doi.org/10.1111/J.1467-2979.2004.00131.X

Kyle CJ, Boulding EG (2000) Comparative population genetic structure of marine gastropods (*Littorina* spp.) with and without pelagic larval dispersal. Mar Biol 137:835–845. https://doi.org/10.1007/s002270000412

Leasi F, Norenburg JL (2014) The necessity of DNA taxonomy to reveal cryptic diversity and spatial distribution of meiofauna, with a focus on Nemertea. PLoS One. https://doi.org/10.1371/journal.pone.0104385

Leasi F, Norenburg JL (2016) At least some meiofaunal species are not everywhere. Indication of geographic, ecological and geological barriers affecting the dispersion of species of *Ototyphlonemertes* (Nemertea, Hoplonemertea). Mol Ecol 25:1381–1397. https://doi.org/10.1111/mec.13568

Leasi F, Todaro MA (2007) The Muscular system of *Musellifer delamarei* and other chaetonotidans with implication for the phylogeny and systematisation of the Paucitubulatina (Gastrotricha). Biol J Linn Soc 94:379–398

Leasi F, Todaro MA (2009) Meiofaunal cryptic species revealed by confocal microscopy: the case of *Xenotrichula intermedia* (Gastrotricha). Mar Biol 156:1335–1346. https://doi.org/10.1007/s00227-009-1175-4

Leasi F, Tang CQ, De Smet WH, Fontaneto D (2013) Cryptic diversity with wide salinity tolerance in the putative euryhaline Testudinella clypeata (Rotifera, Monogononta). Zool J Linn Soc 168:17–28. https://doi.org/10.1111/zoj.12020

Leray M, Knowlton N (2015) DNA barcoding and metabarcoding of standardized samples reveal patterns of marine benthic diversity. Proc Natl Acad Sci 2014:201424997. https://doi.org/10.1073/pnas.1424997112

Leray M, Knowlton N (2016) Censusing marine eukaryotic diversity in the twenty-first century. Philos Trans R Soc B Biol Sci 371:20150331. https://doi.org/10.1098/rstb.2015.0331

Lester SE, Ruttenberg BI (2005) The relationship between pelagic larval duration and range size in tropical reef fishes: a synthetic analysis. Proc Biol Sci 272:585–591. https://doi.org/10.1098/rspb.2004.2985

Lester SE, Ruttenberg BI, Gaines SD, Kinlan BP (2007) The relationship between dispersal ability and geographic range size. Ecol Lett 10:745–758. https://doi.org/10.1111/j.1461-0248.2007.01070.x

Lins L, Vanreusel A, van Campenhout J, Ingels J (2013) Selective settlement of deep-sea canyon nematodes after resuspension—an experimental approach. J Exp Mar Bio Ecol 441:110–116. https://doi.org/10.1016/j.jembe.2013.01.021

Lovén S (1844) Chaetoderma, ett nytt maskslägte n.g. Öfvers K Vetenskaps-Akad Förh 1:116 + pl.112

Mcfarlane CBA, Drolet D, Barbeau MA, Hamilton DJ, Ollerhead J (2013) Dispersal of marine benthic invertebrates through ice rafting. Ecology 94:250–256

Méndez N, Linke-Gamenick I, Forbes VE (2000) Variability in reproductive mode and larval development within the *Capitella capitata* species complex. Invertebr Reprod Dev 38:131–142. https://doi.org/10.1080/07924259.2000.9652448

Mevenkamp L, Van Campenhout J, Vanreusel A (2016) Experimental evidence for selective settlement of meiofauna from two distinct environments after sediment suspension. J Exp Mar Bio Ecol 474:195–203. https://doi.org/10.1016/j.jembe.2015.10.005

Meyer-Wachsmuth I, Curini Galletti M, Jondelius U (2014) Hypercryptic marine meiofauna: species complexes in Nemertodermatida. PLoS One. https://doi.org/10.1371/journal.pone.0107688

Muenter L, Kieneke A (2017) Novel myo-anatomical insights to the *Xenotrichula intermedia* species complex (Gastrotricha: Paucitubulatina): Implications for a pan-European species and reconsideration of muscle homology among Paucitubulatina. Proc Biol Soc Wash 130:165–185. https://doi.org/10.2988/17-00013

Neusser TP, Heß M, Schrödl M (2009) Tiny but complex - interactive 3D visualization of the interstitial acochlidian gastropod *Pseudunela cornuta* (Challis, 1970). Front Zool. https://doi.org/10.1186/1742-9994-6-20

Neusser TP, Jörger KM, Schrödl M (2011) Cryptic species in tropic sands—interactive 3D anatomy, molecular phylogeny and evolution of meiofaunal Pseudunelidae (Gastropoda, Acochlidia). PLoS One. https://doi.org/10.1371/journal.pone.0023313

Norris RD, Hull PM (2012) The temporal dimension of marine speciation. Evol Ecol 26:393–415. https://doi.org/10.1007/s10682-011-9488-4

Packmor J, Riedl T (2016) Records of Normanellidae Lang, 1944 (Copepoda, Harpacticoida) from Madeira island support the hypothetical role of seamounts and oceanic islands as "stepping stones" in the dispersal of marine meiofauna. Mar Biodivers 46:861–877. https://doi.org/10.1007/s12526-016-0448-7

Palmer M (1988) Dispersal of marine meiofauna: a review and conceptual model explaining passive transport and active emergence with implications for recruitment. Mar Ecol Prog Ser 48:81–91. https://doi.org/10.3354/meps048081

Palmer MA, Gust G (1985) Dispersal of meiofauna in a turbulent tidal creek. J Mar Res 43:179–210. https://doi.org/10.1357/002224085788437280

Pietsch A, Westheide W (1985) Ultrastructural investigations of presumed photoreceptors as a means of discrimination and identificafion of closely related species of the genus *Microphtbalmus* (Polychaeta, Hesionidae). Zoomorphology 105:265–276

Prasath D, Balasubramaniam J, Marimuthu P, Jayaraj KA (2017) New record of two free-living marine nematode species, *Sphaerolaimus balticus* and *Sphaerolaimus islandicus* (Nematoda: Sphaerolaimaidae) from Sipphighat mangrove region, South Andaman. Indian J Geo Mar Sci 46:1105–1109

Pugh PJA (1996) Using artificial substrata to monitor how cryptofaunal acari colonize littoral algae on sub-antarctic south Georgia. Acarologia 37:188–200

Radziejewska T, Gruszka P, Rokicka-Praxmajer J (2006) A home away from home: a meiobenthic assemblage in a ship's ballast water tank sediment. Oceanologia 48:259–265

Randsø PV, Domenico MD, Herranz M, Lorenzen ED, Sørensen MV (2018) Population genetic structure of the intertidal kinorhynch *Echinoderes marthae* (Kinorhyncha; Cyclorhagida; Echinoderidae) across the São Sebastião Channel, Brazil. Proc Biol Soc Wash 131:36–46. https://doi.org/10.2988/17-00005

Remane A (1933) Verteilung und organisation der benthonischen mikrofauna der Kieler Bucht. Wissenschaftliche Meeresuntersuchungen 21:161–221

Rinaldo A, Garraffoni S, Araújo TQ (2017) Phylogeny of *Pseudostomella* Swedmark, 1956 (Gastrotricha: Macrodasyida) base on morphological data and first insights on the historical biogeography of Thaumastodermatidae. Proc Biol Soc Wash 130:222–238. https://doi.org/10.2988/17-00014

Rocha-Olivares A, Fleeger JW, Foltz DW (2001) Decoupling of molecular and morphological evolution in deep lineages of a meiobenthic Harpacticoid Copepod. Mol Biol Evol 18:1088–1102. https://doi.org/10.1093/oxfordjournals.molbev.a003880

Ronce O (2007) How does it feel to be like a rolling stone? Ten questions about dispersal evolution. Annu Rev Ecol Evol Syst 38:231–253. https://doi.org/10.1146/annurev.ecolsys.38.091206.095611

Rundell RJ, Leander BS (2014) Molecular examination of kalyptorhynch diversity (Platyhelminthes: Rhabdocoela), including descriptions of five meiofaunal species from the north-eastern Pacific Ocean. J Mar Biol Assoc UK 94:499–514. https://doi.org/10.1017/S0025315413001471

Sahraean N, Van Campenhout J, Rigaux A, Mosallanejad H, Leliaert F, Moens T (2017) Lack of population genetic structure in the marine nematodes *Ptycholaimellus pandispiculatus* and *Terschellingia longicaudata* in beaches of the Persian Gulf, Iran. Mar Ecol. https://doi.org/10.1111/maec.12426

Sánchez N, Yamasaki H, Pardos F, Sørensen MV, Martínez A (2016) Morphology disentangles the systematics of a ubiquitous but elusive meiofaunal group (Kinorhyncha: Pycnophyidae). Cladistics 32:479–505. https://doi.org/10.1111/cla.12143

Sands CJ, Convey P, Linse K, McInnes SJ (2008) Assessing meiofaunal variation among individuals utilising morphological and molecular approaches: an example using the Tardigrada. BMC Ecol 8:7. https://doi.org/10.1186/1472-6785-8-7

Sanford E, Kelly MW (2011) Local adaptation in marine invertebrates. Annu Rev Mar Sci 3:509–537. https://doi.org/10.1146/annurev-marine-120709-142756

Scarpa F, Cossu P, Sanna D, Lai T, Norenburg JL, Curini-Galletti M, Casu M (2015) An 18S and 28S-based clock calibration for marine Proseriata (Platyhelminthes). J Exp Mar Bio Ecol 463:22–31. https://doi.org/10.1016/j.jembe.2014.10.020

Schmidt H, Westheide W (1999) Genetic relationships (RAPD-PCR) between geographically separated populations of the

"cosmopolitan" interstitial polychaete Hesionides gohari (Hesionidae) and the evolutionary origin of the freshwater species Hesionides riegerorum. Biol Bull 196:216–226. https://doi.org/10.2307/1542567

Schmidt H, Westheide W (2000) Are the meiofaunal polychaetes Hesionides arenaria and Stygocapitella subterranea true cosmopolitan species?—results of RAPD-PCR investigations. Zool Scr 29:17–27. https://doi.org/10.1046/j.1463-6409.2000.00026.x

Schratzberger M, Rees HL, Boyd SE (2000) Effects of simulated deposition of dredged material on structure of nematode assemblages—the role of burial. Mar Biol 136:519–530

Scotese CR (2002) PALEOMAP project website. http://www.scotese.com. Accessed May 2017

Sedlacek L, Thistle D (2006) Emergence on the continental shelf: differences among species and between microhabitats. Mar Ecol Prog Ser 311:29–36

Shanks AL, Walters K (1997) Holoplankton, meroplankton, and meiofauna associated with marine snow. Mar Ecol Prog Ser 156:75–86

Smythe AB (2015) Evolution of feeding structures in the marine nematode order Enoplida. Integr Comp Biol 55:228–240. https://doi.org/10.1093/icb/icv043

Sponer R, Roy MS (2002) Phylogeographic analysis of the brooding brittle star *Amphipholis squamata* (Echinodermata) along the coast of New Zealand reveals high cryptic genetic variation and cryptic dispersal potential. Evolution (N Y) 56:1954–1967

Sterrer W (1973) Plate tectonics as a mechanism for dispersal and speciation in interstitial sand fauna. Neth J Sea Res 7:200–222

Sterrer W, Sørensen MV (2006) *Chirognathia dracula* gen. et spec. nov. (Gnathostomulida) from the west coast of North America. Mar Biol Res 2:296–302. https://doi.org/10.1080/17451000600895013

Struck TH, Koczula J, Stateczny D, Meyer C, Purschke G (2017) Two new species in the annelid genus *Stygocapitella* (Orbiniida, Parergodrilidae) with comments on their biogeography. Zootaxa 4286:301–332. https://doi.org/10.11646/zootaxa.4286.3.1

Struck TH, Feder JL, Bendiksby M, Birkeland S, Cerca J, Gusarov VI, Kistenich S, Larsson K-H, Liow LH, Nowak MD, Stedje B, Bachmann L, Dimitrov D (2018a) Cryptic species—more than terminological chaos: a reply to heethoff. Trends Ecol Evol 33:310–312. https://doi.org/10.1016/j.tree.2018.02.008

Struck TH, Feder JL, Bendiksby M, Birkeland S, Cerca J, Gusarov VI, Kistenich S, Larsson K-H, Liow LH, Nowak MD, Stedje B, Bachmann L, Dimitrov D (2018b) Finding evolutionary processes hidden in cryptic species. Trends Ecol Evol. https://doi.org/10.1016/j.tree.2017.11.007

Suatoni E, Vicario S, Rice S, Snell T, Caccone A (2006) An analysis of species boundaries and biogeographic patterns in a cryptic species complex: the rotifer—*Brachionus plicatilis*. Mol Phylogenet Evol 41:86–98. https://doi.org/10.1016/j.ympev.2006.04.025

Swift HF, Daglio LG, Dawson MN (2016) Three routes to crypsis: stasis, convergence, and parallelism in the *Mastigias* species complex (Scyphozoa, Rhizostomeae). Mol Phylogenet Evol 99:103–115. https://doi.org/10.1016/j.ympev.2016.02.013

Tanaka H, Ohtsuka S (2016) Historical biogeography of the genus *Polycopissa* (Ostracoda: Myodocopa: Cladocopina), with the description and DNA barcode of the second Indo-Pacific species from the Seto Inland Sea. Mar Biodivers 46:625–640. https://doi.org/10.1007/s12526-015-0412-y

Taylor DJ, Finston TL, Hebert PDN (1998) Biogeography of a widespread freshwater crustacean: pseudocongruence and cryptic endemism in the North American *Daphnia laevis* complex. Evolution (N Y) 52:1648–1670

Teasdale M, Vopel K, Thistle D (2004) The timing of benthic copepod emergence. Limnol Oceanogr 49:884–889

Thistle D (2003) Harpacticoid copepod emergence at a shelf site in summer and winter: implications for hydrodynamic and mating hypotheses. Mar Ecol Prog Ser 248:177–185. https://doi.org/10.3354/meps248177

Thomas MC, Lana PC (2011) A new look into the small-scale dispersal of free-living marine nematodes. Zoologia 28:449–456. https://doi.org/10.1590/S1984-46702011000400006

Todaro MA, Fleeger JW, Hu YP, Hrincevich AW, Foltz DW (1996) Are meiofaunal species cosmopolitan? Morphological and molecular analysis of *Xenotrichula intermedia* (Gastrotricha: Chaetonotida). Mar Biol 125:735–742

Todaro MA, Telford MJ, Lockyer AE, Littlewood DTJ (2006) Interrelationships of the Gastrotricha and their place among the Metazoa inferred from 18S rRNA genes. Zool Scr 35:251–259. https://doi.org/10.1111/j.1463-6409.2006.00228.x

Todaro MA, Leasi F, Hochberg R (2014) A new species, genus and family of marine Gastrotricha from Jamaica, with a phylogenetic analysis of Macrodasyida based on molecular data. Syst Biodivers 12:473–488. https://doi.org/10.1080/14772000.2014.942718

Tulchinsky AY, Norenburg JL, Turbeville JM (2012) Phylogeography of the marine interstitial nemertean *Ototyphlonemertes parmula* (Nemertea, Hoplonemertea) reveals cryptic diversity and high dispersal potential. Mar Biol 159:661–674. https://doi.org/10.1007/s00227-011-1844-y

Ullberg J, Ólafsson E (2003a) Effects of biological disturbance by *Monoporeia affinis* (Amphipoda) on small-scale migration of marine nematodes in low-energy soft sediments. Mar Biol 143:867–874. https://doi.org/10.1007/s00227-003-1139-z

Ullberg J, Ólafsson E (2003b) Free-living marine nematodes actively choose habitat when descending from the water column. Mar Ecol Prog Ser 250:141–149. https://doi.org/10.3354/meps260141

Van Campenhout J, Derycke S, Moens T, Vanreusel A (2014) Differences in life-histories refute ecological equivalence of cryptic species and provide clues to the origin of bathyal *Halomonhystera* (Nematoda). PLoS One. https://doi.org/10.1371/journal.pone.0111889

van Oppen MJH, Klerk H, Olsen JL, Stam WT (1996) Hidden diversity in marine algae: some examples of genetic variation below the species level. J Mar Biol Assoc UK 76:239–242

Van Steenkiste NWL, Herbert ER, Leander BS (2018) Species diversity in the marine microturbellarian Astrotorhynchus bifidus sensu lato (Platyhelminthes: Rhabdocoela) from the Northeast Pacific Ocean. Mol Phylogenet Evol 120:259–273. https://doi.org/10.1016/j.ympev.2017.12.012

Villora-Moreno S, de Grimaldi SZ (1993) Redescription and ecology of *Batillipes phreaticus* Renaud-Debyser, 1959 (Arthrotardigrada, Batillipedidae) in the gulf of Valencia (western mediterranean). Cah Biol Mar 34:387–399

von Soosten C, Schmidt H, Westheide W (1998) Genetic variability and relationships among geographically widely separated populations of *Petitia amphophthalma* (Polychaeta: Syllidae). Results from RAPD-PCR investigations. Mar Biol 131:659–669. https://doi.org/10.1007/s002270050358

Wada S, Kameda Y, Chiba S (2013) Long-term stasis and short-term divergence in the phenotypes of microsnails on oceanic islands. Mol Ecol 22:4801–4810. https://doi.org/10.1111/mec.12427

Wares JP, Gianes SD, Cunningham CW (2001) A comparative study of asymmetric migration events across a marine biogeographic boundary. Evolution (N Y) 55:295–306

Westheide W (1977) The geographical distribution of interstitial polychaetes. Mikrofauna Meeresb 61:287–302

Westheide W (1991) The meiofauna of the galapagos: a review. In: James Mathew J (ed) Galápagos marine invertebrates. Springler, New York, pp 37–69

Westheide W (2005) Meiofauna geographic distribution: vicariance and dispersal. Meiofauna Mar 14:201–207

Westheide W, Hass-Cordes E (2001) Molecular taxonomy: description of a cryptic Petitia species (Polychaeta: Syllidae) from the island of Mahe (Seychelles, Indian Ocean) using RAPD markers and ITS2 sequences. J Zool Syst Evol Res 39:103–111

Westheide W, Rieger RM (1987) Systematics of the amphiatlantic *Microphthalmus listensis* species-group (Polychaeta: Hesionidae): facts and concepts for reconstruction of phylogeny and speciation. Zeitschrift für Zool Syst und Evol 25:12–39

White TA, Stefanni S, Stamford J, Hoelzel AR (2009) Unexpected panmixia in a long-lived, deep-sea fish with well-defined spawning habitat and relatively low fecundity. Mol Ecol 18:2563–2573. https://doi.org/10.1111/j.1365-294X.2009.04218.x

Yamasaki H, Hiruta SF, Kajihara H, Dick MH (2014) Two Kinorhynch species (Cyclorhagida, Echinoderidae, Echinoderes) show different distribution patterns across Tsugaru Strait, Northern Japan. Zool Sci 31:421–429. https://doi.org/10.2108/zs140011

Zawierucha K, Grzelak K, Kotwicki L, Michalczyk Ł, Kaczmarek Ł (2013) *Batillipes pennaki* Marcus, 1946, a new addition to the Thai Tardigrade fauna, with an overview of literature on the species. Pak J Zool 45:801–808

Zeppilli D, Vanreusel A, Danovaro R (2011) Cosmopolitanism and biogeography of the genus *Manganonema* (Nematoda: Monhysterida) in the deep sea. Animals 1:291–305. https://doi.org/10.3390/ani1030291

Zeppilli D, Sarrazin J, Leduc D, Arbizu PM, Fontaneto D, Fontanier C, Gooday AJ, Kristensen RM, Ivanenko VN, Sørensen MV, Vanreusel A, Thébault J, Mea M, Allio N, Andro T, Arvigo A, Castrec J, Danielo M, Foulon V, Fumeron R, Hermabessiere L, Hulot V, James T, Langonne-Augen R, Le Bot T, Long M, Mahabror D, Morel Q, Pantalos M, Pouplard E, Raimondeau L, Rio-Cabello A, Seite S, Traisnel G, Urvoy K, Van Der Stegen T, Weyand M, Fernandes D (2015) Is the meiofauna a good indicator for climate change and anthropogenic impacts? Mar Biodivers 45:505–535. https://doi.org/10.1007/s12526-015-0359-z

Supplementary data for manuscript 2

The supplementary data for this manuscript can be found online in
https://doi.org/10.1007/s00227-018-3383-2

Manuscript 3

Deceleration of morphological evolution in a cryptic species complex and its links to paleontological stasis

Title:

Deceleration of morphological evolution in a cryptic species complex and its link to paleontological stasis

José Cerca*†, Christian Meyer‡, Dave Stateczny‡§, Dominik Siemon§, Jana Wegbrod§, Gunter Purschke‡, Dimitar Dimitrov#, Torsten H. Struck†

* Corresponding author: José Cerca; jose.cerca@gmail.com; ORCID 0000-0001-7788-4367

† Frontiers of Evolutionary Zoology Research Group, Natural History Museum, University of Oslo, 0562 Oslo, Norway

‡ Zoology and Developmental Biology, Department of Biology and Chemistry, University of Osnabrueck, Barbarastr. 11, 49069 Osnabrueck, Germany

§ Zoological Research Museum Alexander Koenig, Adenauerallee 160, 53113 Bonn, Germany

Department of Natural History, University Museum of Bergen, University of Bergen, P.O. Box 7800, 5020 Bergen, Norway

Keywords:

Stasis; Speciation; Sibling species; Morphological acceleration; Morphological evolution; adaptive radiation

Title:

Deceleration of morphological evolution in a cryptic species complex and its link to paleontological stasis

Abstract:

Morphological stasis or the absence of morphological change is a well-known phenomenon in the paleontological record, yet it is poorly integrated with neontological evidence. Recent evidence suggests that cryptic species complexes may remain morphologically identical due to morphological stasis. Here, we describe a case of long-term stasis in the *Stygocapitella* cryptic species complex (Parergodrilidae, Orbiniida, Annelida). Using phylogenetic methods and morphological data, we find that rates of morphological evolution in *Stygocapitella* are significantly slower than in closely related taxa (Nerillidae, Orbiniidae). Assessment of quantitative and qualitative morphology revealed the presence of four morphotypes with only subtle differences, while molecular data supports 10 reproductively isolated clades. Notably, estimates for the time of *Stygocapitella* species divergence range from ~275 million years to ~18 million years, including one case of two morphologically similar species which have diverged about 140 million years ago. These findings provide evidence for morphological deceleration and long-term morphological stasis in *Stygocapitella,* and that speciation is not necessarily accompanied by morphological changes. The deceleration of morphological divergence in *Stygocapitella* can be potentially linked to niche conservatism and tracking, coupled with the fluctuating dynamics of the interstitial environment, or genetic constraints due to progenetic evolution. Finally, we conclude that failing to integrate speciation without morphological evolution in paleontology may bias estimates of rates of speciation and morphological evolution.

Introduction

The occurrence of morphological stasis, defined as little or no morphological evolution over extended periods of time, remains a controversial topic in evolutionary biology (Futuyma 2010). Morphological variation is seen as a desired feature of any biological system and its absence is often interpreted as a potential failure to capture variation (Weiss 2011). Lineages with higher evolvability are expected to occupy broader range of habitats more quickly and efficiently, ultimately replacing less labile groups (Rabosky and Adams 2012) and hence, cases of low-evolvability and morphological stasis are expected to be exceptionally uncommon. Despite this, examples of morphological stasis are commonplace in the fossil record (Cheetham 1986; Futuyma 2005, 2010; Frame et al. 2007; Hunt et al. 2008; Hunt and Rabosky 2014; Voje et al. 2018), where series of invariant morphotypes occur at diverse time-scales in different organismal groups (Cheetham 1986; Hunt 2007).

A theory which aims to explain the occurrence of long periods of stasis is the punctuated equilibrium (Eldredge 1971; Eldredge and Gould 1972). In its essence, punctuated equilibrium suggests that species undergo long periods of morphological stasis, which are disrupted by rapid change during

speciation (Eldredge and Gould 1972; Futuyma 2005). The postulation that morphological evolution occurs exclusively during speciation implies that adaptive and selective processes are insignificant during substantial parts of the evolutionary histories of species (e.g. (Stanley 1975)), and challenged the accumulating evidence of the emerging 'modern synthesis' (Futuyma 2005; Hunt and Rabosky 2014). While the modern synthesis-punctuated equilibrium debate lasted for about 2 decades, over the years paleontological evidence was aligned with the major processes suggested by the modern synthesis: selection, drift, mutation and gene flow (Hunt and Rabosky 2014). However, the conciliation of stasis with these processes was never thoroughly achieved. On one hand, researchers have argued that stasis could result either from biases in the paleontological evidence due to, for example, taphonomical biases (Kidwell and Holland 2002; Pennell et al. 2014), or either be due to the lack of statistical or sampling resolution (Frame et al. 2007; Voje 2016). On the other hand, competing views have focused on developing theoretical frameworks underlying the deceleration of morphological evolution which include scenarios of stabilizing selection (Charlesworth et al. 1982; Hansen and Houle 2004; Futuyma 2010), niche conservatism and tracking (Futuyma 2010, 2015), fluctuating ecological conditions (Futuyma 1987, 2010, 2015; Sheldon 1996; Smith et al. 2011), lack of new ecological interactions (Nordbotten and Stenseth 2016), constraints (Charlesworth et al. 1982; Maynard Smith et al. 1985; Wagner and Schwenk 2000; Hansen and Houle 2004; Futuyma 2010; Smith et al. 2011), recurrent bottlenecks (Futuyma 2010), physiological or behavioural adaptation (Lee and Frost 2002; Futuyma 2010; Lassance et al. 2019), and the influence of particular environments and environmental conditions (Westheide 1977; Futuyma 1987, 2010; Westheide and Rieger 1987; Giere 2009; Gueriau et al. 2016).

One suggested way of integrating components of punctuated equilibrium and modern synthesis can result from variation in rates of anagenetic and cladogenetic change (Futuyma 1987, 2015). If a cladogenetic event (speciation) occurs, and posterior anagenetic changes are slowed or non-existent, daughter species will be similar in morphology. While this idea is plausible, it cannot be tested in the paleontological record because paleontological species are diagnosed based on morphology alone and rely on the evolution of morphological differences (Jackson and Cheetham 1999; Aze et al. 2011; Strotz and Allen 2013). If daughter species are morphologically similar, these estimations will be biased. A solution to this problem is the combination of molecular phylogenetic tools coupled with morphological data in lineages displaying stasis (Bokma 2002, 2008; Mattila and Bokma 2008; Katz 2018). For example, Katz (2019) argues that integrating evidence from palaeontology, paleobiology and molecular phylogenetics is the key to understand the early-burst of the Angiosperms. Applying a holistic approach to the problem of stasis could potentially provide a link between paleontological evidence and evolutionary studies based on neontological evidence. Cryptic species complexes are a potential target for such approach. Morphological stasis has been suggested to occur in extant cryptic species complexes – different species which are similar in morphology (Wada et al. 2013; Swift et al. 2016; Cerca et al. 2018; Struck et al. 2018). Cryptic species have been found widespread across the tree of life (Pfenninger and Schwenk 2007; Pérez-Ponce de León and Poulin 2016), and are being discovered at a faster pace. Matching morphological stasis as seen in

cryptic species and paleontological stasis may provide the possibility to test for phylogenetic signatures of stasis in closely related extant taxa (Mattila and Bokma 2008) and offer the possibility to quantify gene flow and genetic divergence in these complexes (Sheldon 1996; Futuyma 2010; Hunt and Rabosky 2014). In cryptic species, morphological evolution can be potentially decelerated, if adaptive pressures focus on physiological, behavioural or biochemical traits that have no bearing on morphology (Gómez et al. 2002; Lee and Frost 2002; Novo et al. 2010, 2012; Struck et al. 2018; Lassance et al. 2019). Despite this potential, currently described cryptic species complexes are relatively young, contrasting with fossil record evidence which suggest long-lasting stasis. A promising system to close this gap is the *Stygocapitella* cryptic species complex (Parergodrilidae, Orbiniida, Annelida), which until recently was thought to consist of a single-species with a worldwide distribution (Schmidt and Westheide 2000; Struck et al. 2017). Recent evidence suggests that pronounced periods of stasis are intertwined with slight changes in morphology (Struck et al. 2017). However, this work focused on a limited set of species and populations with slight morphological differences, hence lacking resolution to describe morphological and genetic evolution. Here, we present an extended sampling of this genus, with special emphasis on the Northern hemisphere. Specifically, we determine genetic and morphological divergences in the *Stygocapitella* cryptic species complex and in closely related taxa.

Material and methods

Sample collection, identification and preservation

Stygocapitella is an interstitial annelid, generally found around the high-water line of stable, sheltered gravel or sandy beaches (Purschke 2018) (Fig. 1). In each sampling location, we collected sediment samples by drawing transects from the high-water line to the foot of the dune. Every one meter, we dug a one-meter deep hole and, in each of these, we collected approximately 375 cm^3 of sediment every 15 cm-depth. Afterwards, we extracted *Stygocapitella* specimens under dissecting microscope using the $MgCl_2$ method (Westheide and Purschke 1988). For this study a total of 33 sites across the Northern hemisphere were sampled (Fig. 2, Suppl. Table S1 & S2). Specimens used for molecular biology were preserved in 95% ethanol and for morphological analyses in sucrose-picric acid-paraformaldehyde-glutaraldehyde (SPAFG) following Westheide and Purschke (1988) (Suppl. Table S1,3-4).

Molecular biology

Genomic DNA was extracted using phenol-chloroform or the E.Z.N.A Tissue DNA Kit (Omega Bio-Tek). The nuclear markers 18S and ITS1 and the mitochondrial CO1 were amplified with the QIAGEN® Multiplex PCR Kit (Qiagen, Hilden, Germany) in a 10 µl reaction-mix containing 5 µl of multiplex mix, 1 µl Q-solution, 0.8 µl 10 µM of both forward and reverse primer, 1 µl genomic DNA and 1.4 µl deionized water. For the mitochondrial gene 16S, a 25 µl reaction-mix included 15.2 µl of H_2O, 2.5

μl of 10X PCR Buffer I (with $MgCl_2$ added; Applied Biosystems), 2.5 μl of BSA, 0.5 μl of 10mM dNTPs, 1.6 μl 10 μM of both forward and reverse primer and 0.13 μl of amplitaq gold (Applied Biosystems). The following primers have been used: LCO1490-JJ (CHACWAAYCATAAAGATARYGG) & HCO2198-JJ (AWACTTCVGGRTGVCCAAARAATCA; both (Astrin and Stüben 2008)) for COI, 18e (CTGGTTGATCCTGCCAGT; (Hillis and Dixon 1991)) & 18R1779 (TGTTACCGACTTTTACTTCCTCTA; (Struck et al. 2002)) for 18S, species-specific primers Stygo_ITS1_F (TGTTGATTACGTCCCTGCCC) & Stygo_ITS1_R (GTCAACCGACCCTGAGACAG) for ITS1 and 16SarL (CGCCTGTTTATCAAAAACAT; (Palumbi et al. 1991) & 16S_AN-R (GCTTACGCCGGTCTGAACTCAG; (Zanol et al. 2010)) for 16S. The only exceptions were the American populations for COI, where we used polyLCO (GAYTATWTTCAACAAATCATAAAGATATTGG) & polyHCO (TAMACTTCWGGGTGACCAAARAATC; both (Lobo et al. 2016)). PCR conditions for ITS1 included the following protocol (initial denaturation: 15' 95ºC; 40 cycles: 30" 95ºC, 30" 66ºC, 1' 72ºC; final elongation: 20' 72ºC), for 16S a touchdown one (initial denaturation: 15' 95ºC; 40 cycles: 30" 94ºC, 30" 51ºC (touchdown: -0.2ºC per cycle), 2' 65ºC; final elongation: 7' 65ºC), for 18S a touchdown/touch-up (initial denaturation: 15' 95ºC; 15 cycles: 35" 94ºC, 90" 55ºC (touchdown: -1ºC per cycle), 2.5' 72ºC; 25 cycles: 35" 94ºC, 90" 50ºC, 2.5' 72ºC; final elongation: 10' 72ºC), and for COI the same (initial denaturation: 15' 95ºC; 15 cycles: 35" 94ºC, 90" 55ºC (touchdown: -1ºC per cycle), 1.5' 72ºC; 25 cycles: 35" 94ºC, 90" 50ºC, 1.5' 72ºC; final elongation: 10' 72ºC). PCR fragments were purified using a phosphatase-exonuclease mix and Sanger-sequenced by Macrogen Europe. Given the length of the 18S fragment, four additional sequencing primers were included: 18r (CTCTAATTTTTTCAAAGTAAAC), 18L (AGCTCTCAATCTGTCAATCCT; both (Hillis and Dixon 1991)), 18F997 (TTCGAAGACGATCAGATACCG; (Struck et al. 2002)) & 18SF3_Stygo (CCTCGGGATTGGAATGAGTAC; (Struck et al. 2017). After sequencing, all sequences were assembled and the ends automatically trimmed using Geneious (v6.8.1). The quality of the assembly of each sequence was visually checked and each consensus sequence screened for contamination by doing NCBI megablast searches against the complete non-redundant database.

Phylogenetic and molecular clock analyses

Details about amplified markers as well as outgroups obtained from GenBank are provided in Suppl. Table S1. The sequences of each gene were aligned with mafft v7.310 using a maximum of 1,000 iterations and the accurate localpair algorithm (Katoh and Standley 2013). ITS1 was aligned using the genafpair algorithm, which is optimized for gappy sequences. After alignment, both ends of the sequences were trimmed until the first position without missing data. These datasets were concatenated with FASconCAT v1.1 (Kück and Meusemann 2010). A partitioned Maximum Likelihood (ML) analysis of the concatenated dataset was performed using IQ-tree v1.5.5 (Nguyen et al. 2015) with an automatic determination of the best substitution model for each gene, 300 initial parsimony trees, 15 best trees

157 retained during search and 1,000 bootstrap replicates. Similar settings were used for ML analyses of each
158 gene independently.

159 Molecular clock analyses were conducted using BEAST v2.4.7 (Bouckaert et al. 2014). We used
160 IQ-tree's ModelFinder to determine which substitution models best fit the dataset (Kalyaanamoorthy et al.
161 2017). After performing several runs using combinations of different prior models and genes, we selected
162 CO1 and 18S for the final dating analysis because both genes are commonly used for molecular dating of
163 annelids and showed best chain convergence and effective sampling sizes. BEAST was run with linked
164 trees using a TN93 model with equal frequencies for 18S and HKY with estimated frequencies and a Γ-
165 distribution with four categories for CO1. As no fossil record is known for these taxa, we selected a
166 relaxed, log-normal clock to account for rate heterogeneity across lineages and mean values of 0.0001425
167 and 0.0176 as substitution rates per million year for 18S and COI, respectively (Escalante and Ayala 1995;
168 Pérez-Losada et al. 2004; Struck et al. 2017). We applied a birth-death model and constrained the in-group
169 as monophyletic. A MCMC was run for one billion generations, sampling every 100,000th generation. We
170 confirmed chain convergence using Tracer v1.6 (Rambaut et al. 2007), with a 1% burn-in threshold. A
171 Maximum Credibility Consensus Tree was obtained using TreeAnnotator (Bouckaert et al. 2014).
172 Considering the potential biases and criticism of molecular clock approaches, we limit our interpretation
173 of these results.

174 **Genetic diversity**

175 To validate the established lineages, we performed an "Automatic Barcode Gap Discovery"
176 analysis using the ABGD web interface (https://bioinfo.mnhn.fr/abi/public/abgd/) with the COI data
177 (i.e. the "animal barcode"). ABGD analysis was run using the default settings (0.001 > Prior Intraspecific
178 divergence < 0.1; 10 steps; 1.5 relative gap width; 20 bins for distance distribution; JC69 distance).

179 Polymorphic sites, haplotype diversity, sampling variation and Tajima´s D were calculated using
180 DNAsp v6.10.01 (Rozas et al. 2017) and the 16S marker, as it has the highest coverage of species and
181 populations. For the isolation-by-distance test, we applied a mantel test in R using the "ade4" package
182 (Dray and Dufour 2007). For this test, we selected the sequences for each of the four Atlantic species
183 separately, and realigned each individual gene dataset using mafft as previously described. Pairwise F_{ST}
184 were obtained with DNAsp. The least distance between each pair of sites was calculated using google
185 maps' "measure distance" function. After this, we modified the distance line by adding points until the
186 minimum distance between two sites would not cross any landmass. The obtained F_{ST} values and
187 geographic distances between sites were used to compute a mantel test based on 9,999 replicates using the
188 "ade4" R package (Dray and Dufour 2007). For the EA_C we excluded the two American specimens to
189 ensure that the test is not dominated by the long distance to these two specimens, as evidence shows that
190 these might be a result of accidental human translocation (Radziejewska et al. 2006).

191

192 **Morphological fixation and preservation**

193 Morphological divergence was evaluated by Scanning Electron Microscopy (SEM) and
194 morphometrics. Due to the small size of the specimens, we were unable to genotype and obtain
195 morphological data of the same individual (Westheide and Hass-Cordes 2001). Individuals used in
196 morphological studies were identified indirectly based on the investigated site. For SEM, specimens were
197 transferred to a buffered 1% OsO_4 solution for one hour at ambient temperature and dehydrated in a
198 graded ethanol series from 30% to 100%. Dehydrated specimens were critically-point-dried with CO_2,
199 mounted on aluminium stubs, sputter coated with platinum and examined with a Zeiss Auriga field
200 emission SEM. In total we investigated 73 specimens from 16 sites (~4.5 specimens per from: Langebaan,
201 Sarge Bay, Gnarabup Beach, Roche Harbor, Reuben State Park, 4th of July Beach, Canoe Beach, Lubec,
202 Lødingen, Henningsvær, Schilksee, Île Callot, List, Bristol Channel, Gravesend, and Plymouth; Suppl.
203 Table S3). For morphometrics, quantitative measurements of sexually mature specimens were carried out
204 based on pictures taken under a light microscope at an amplification of 10X. Images were assembled using
205 Adobe Photoshop and morphometric traits measured in ImageJ. These included 6 body size
206 characteristics: body length and width, prostomium length and width, and pygidium length and width; of a
207 total of 123 individuals from 18 sites (~7 specimens per site; Langebaan, Sarge Bay, Gnarabup Beach,
208 Roche Harbor, Reuben State Park, 4th of July Beach, Canoe Beach, Lubec, Lødingen, Henningsvær,
209 Schilksee, Île Callot, Glenancross, Keitum, Bristol Channel, Gravesend, Plymouth, and Ellenbogen; Suppl.
210 Table S4).

211 **Rates of morphological and molecular evolution**

212 First, we analysed which morphological characters are variable and/or fixed within the
213 *Stygocapitella* species complex. We did this to obtain character states for each lineage, and to assess the
214 degree of variability among and across lineages. After this initial assessment, we analysed (i) the number of
215 segments with chaetae; and (ii) the number and pattern of chaetal type in each individual chaetiger (Suppl.
216 Table S3). Only the first and the second chaetiger displayed variance in chaetal pattern, and hence the
217 third and following segments were coded only once. Based on this information, we investigated rates of
218 morphological evolution within *Stygocapitella* by means of an ancestral state reconstruction and by a
219 regression of morphological data and pairwise genetic differences. We obtained ancestral state
220 reconstructions by mapping morphological characters on a tree topology derived from the ML and
221 Bayesian analyses above using Mesquite version 3.51. We applied both ML and parsimony reconstructions.
222 In addition, we determined Multidimensional Morphological Disparity (MMD) indices within and between
223 species as described in Struck et al. (2017). In brief, this method relies on the decomposition of variance
224 through a principal component analyses (PCA). Using both discrete morphological and morphometric
225 data, we performed a PCA using the function "prcomp" included in the basic R package "stats" (R Core
226 Team 2013). In both cases the first four principal components were selected for the MMD calculations as
227 they accounted for >99% of the variance. The MMD indices were plotted against the uncorrelated genetic

distances of the 18S gene within and between *Stygocapitella* species, which we obtained using MEGA X (Kumar et al. 2018), by applying 500 bootstrap replications, the TN93 model, and a Γ-distribution.

After this, we compared the rate of morphological evolution within *Stygocapitella* to other closely related groups within Orbiniida (Struck et al. 2015). To do so, we selected 12 species from Orbiniidae and 11 Nerillidae for which morphological and molecular datasets exist (Worsaae 2005; Bleidorn et al. 2009) (Suppl. Tables S5-7). Importantly, both these taxa and *Stygocapitella* have a similar degree of genetic divergence (Struck et al., 2015). Conveniently, Orbiniidae comprises both in faunal and interstitial species, while Nerillidae consists exclusively of interstitial species such as *Stygocapitella*. The integration of the morphological records obtained herein and the records from the literature (Worsaae 2005; Bleidorn et al. 2009; Zrzavý et al. 2009; Struck et al. 2015) led to a morphological data matrix consisting of 32 species (9 from *Stygocapitella*, 11 from Nerillidae, and 12 from Orbiniidae) and 75 morphological characters (Suppl. Tables S5-6). We then conducted a PCA analysis of this dataset using the PCA option of the "FactoMineR" package (Lê et al. 2008). Based on first 18 principal components, which together explain 99.07% of the variation in the dataset, we determined MMD indices between species within *Stygocapitella*, Orbiniidae and Nerillidae separately, and tested if they were statistically different to each other using Tukey's HSD and pairwise students' T tests.

To contrast morphological and molecular evolution, we compiled a dataset of 18S sequences for each species (Suppl. Table S7). Considering that 18S was the slowest evolving gene in the dataset, this gene is the ideal gene to analyse distantly related lineages. Based on this dataset, we reconstructed a ML tree using IQ-Tree (as described above) and obtained pairwise genetic distances between species. Pairwise MMD indices were plotted against the corresponding pairwise genetic distances using the "ggplot" function of the R package "ggplot2" (Wickham 2016) with a loess smoothed fit regression including confidence regions. Finally, we mapped the morphological characters on the ML tree using Mesquite version 3.51 with the ML reconstruction option. We counted the number of changes occurring in each branch, and plotted these on the ML tree to quantify the number of total changes per branch.

Ecological data

Bioclimatic variables were downloaded from the world-climatic database using the "raster" R package (Hijmans 2014). Nineteen variables were downloaded with a 2.5 minutes of a degree resolution (21.62 m^2 at the equator) for each of the sampling sites. Because of the extensive sampling effort in the Eastern Atlantic, we subsampled sites in which only one species occurred. Ideally, we would include variables such as granularity, pH, moisture content – but this was not possible at this stage given the requirement of specialized equipment which was not available at the sampled sites across the entire globe. Additionally, given the strong short-term fluctuations of these parameters (Giere 2009), a comprehensive dataset may only be obtained after repeated and long-term measurements over several years, accounting for events such as heavy rains and stormy weather. "Species" was treated as the dependent variable and

each of the 19 climatic variables as an independent variable. Because species is a multinomial variable, we fitted a multinominal logistic regression using the R package "nnet" (Venables and Ripley 2002). After fitting each model, we performed a least squares means analysis using the R package "lsmeans" (Lenth 2013). We evaluated species-presence pairwise contrasts using F-ratios and its associated *P-value*.

Results

Number of *Stygocapitella* species

We obtained sequences from four gene markers (i.e., 16S, COI, 18S & ITS1) for 301 *Stygocapitella subterranea*, 12 *S. australis* and 18 *S. minuta* specimens from 32 sites, as well as five orbiniid outgroups (Suppl. Table S1). The concatenated dataset comprised 4,081 nucleotide positions. Both maximum likelihood (Suppl. Fig. S1) and Bayesian analyses (Fig. 3) of the concatenated molecular data resulted in the same topology. Ten well supported species were found with bootstrap values ≥95 and posterior probabilities ≥0.95 except for the Eastern Atlantic species B (EA_B) (bootstrap value = 87, posterior probability = 0.81). All species except *S. subterranea*, *S. australis* and *S. minuta*, are new and formal description is pending. Relationships among species received high support with only two cases of bootstrap values <95. Single-gene phylogenies retrieved the same species as the concatenated datasets (Suppl. Table S8; Suppl. Fig. S1-S5). The highly conserved 18S gene was unable to unambiguously distinguish the most recent divergence between closely related species (Suppl. Fig. S2). In congruence with the phylogenetic analysis, the barcoding analyses (ABGD) found an intraspecific maximal distance of 0.031581for COI and the recursive partitioning found eight species within *S. subterranea*.

The three species occurring in Europe, WA and EA_C in North America, as well as EP_D, EP_C and EP_B in the Pacific Ocean occur in sympatry at a total of seven out of 32 sites (Figs. 1 & 2), often co-occurring within the same handful of sand. For example, three of the four Eastern Pacific species co-occur at the 4th of July beach and all three Eastern Atlantic species are found at Musselburgh.

Ancestral state reconstruction

The eight discrete morphological characters present in *Stygocapitella* (out of a total of 75 assessed characters; Fig. 1) exhibited interindividual and interspecific variation. Ancestral character reconstructions of these discrete morphological characters suggest the occurrence of four distinct morphotypes based on the number of chaetigers and the number of specific chaetae in certain chaetigers (Fig. 4A; Suppl. Fig. S6). Morphotype #1 (red in Fig. 4) is restricted to *S. minuta*. Besides having only eight chaetigers, the first chaetiger of #1 has two whip-like chaetae and three bilimbate chaetae. All following chaetigers have four bilimbate chaetae. While #1 is considerably distinct, the other three morphotypes are very similar to each other. All three (#2, #3, #4) have 10 chaetigers and possess an additional chaetal type, forked chaetae. Morphotype #2, observed in *S. australis*, in specimens from 4th of July beach and in one specimen from Plymouth in EA_C consists of two whip-like, two forked and one bilimbate chaeta in the first chaetiger

297 and two bilimbate and two forked in the rest (green in Fig. 4; Suppl. Figs. S6-7). In comparison to #2, #3
298 has one additional bilimbate chaeta in the first and second chaetigers (Fig. 4; Suppl. Fig. S6). This
299 morphotype is observed in EA_B, EA_C and WA as well as in some specimens of EA_A and the Eastern
300 Pacific. Finally, #4 has one additional bilimbate chaetae in the second chaetiger when compared with #3
301 and is present in the Eastern Pacific, in EA_A and in one specimen from Gravesend in EA_C (yellow in
302 Fig. 4; Suppl. Fig. S6). The ancestral state reconstructions unambiguously reveal that #1 is the ancestral
303 condition for *S. minuta*, #2 for EP_A and *S. australis*, #3 for EP_C, EP_D and EA_A, and #4 for EA_B,
304 EA_C and WA.

305 These results show that the *Stygocapitella* species complex is composed of several cryptic species as
306 the majority of species are presently morphologically indistinguishable. Only *S. minuta* can be distinguished
307 from the remaining species having a particularly distinct morphology. The remaining species display
308 morphologies which are not species-exclusive, and differences between the three morphologies are
309 minimal (Fig. 4; Suppl. Fig. S7 – blue; green; yellow morphotypes). The maximal difference is three
310 additional bilimbate chaetae in a total of 44 chaetae for #4 in comparison to #2.

311 **Morphological disparity within *Stygocapitella***

312 We obtained estimates of morphological disparity within the *Stygocapitella* cryptic complex and
313 compared these to estimates from two closely related taxa. Mapping of the 75 morphological characters
314 on the 18S ML tree shows that morphological evolution in *Stygocapitella* is slower when compared to
315 nerillids and orbiniids (Fig. 4B). Indeed, in a total of 16 branches, only 4 are associated with morphological
316 changes in *Stygocapitella*, while nerillids and orbiniids display changes at 19 out of 20 and 18 out of 22
317 branches, respectively. Within *Stygocapitella* we found one branch showing three, two branches displaying
318 two, and a single branch with one morphological change. This translates to an average of 0.5 changes per
319 branch in *Stygocapitella*. In contrast, when considering branches with changes in nerillids, only six showed
320 three or less changes, while 13 had between four to 12 changes, totaling to an average of 4.1
321 morphological changes per branch. Orbiniids displayed eight branches with one to three morphological
322 changes and 10 branches with four to eight changes (Fig. 4B). The average number of changes per branch
323 in orbiniids is 2.8. Thus, in *Stygocapitella* not only the percentage of branches with no changes is higher, but
324 also the amount of change along a branch is considerably smaller considering both the average and the
325 maximum number of changes.

326 Principal component analysis shows that Nerillidae, Orbiniidae and *Stygocapitella* are clearly
327 separated from each other (PC1 and PC2 together explain 64.4% of the variance). In addition to this,
328 Nerillidae and Orbiniidae occupy a substantially larger morphospace area than *Stygocapitella* (Fig. 5A).
329 MMD analysis shows that this difference is not restricted to the first principal components, but holds up
330 for the first 18 principal components. MMD indices of *Stygocapitella* had a mean value of 1.14 with a
331 standard deviation of 1.28 and a median of 0.41. For nerillids, the mean was 7.21 with a standard deviation

of 2.76 and median of 7.86 and for orbiniids it was 7.46 with 3.29 and 7.96, respectively. Boxplots of the distributions of the MMD indices show that the quartiles of *Stygocapitella* do not overlap with the quartiles belonging to Nerillidae and Orbiniidae, while these two overlap completely (Fig. 5B). Accordingly, Tukey's HSD and students' T-tests show that morphological disparity in *Stygocapitella* is significantly lower in comparison to both Nerillidae and Orbiniidae ($P < 0.000001$ in all cases), while there is no significant difference in morphological disparity between Nerillidae and Orbiniidae (Tukey's HSD: $P = 0.7343163$; students' T: $P= 0.45$).

Plotting of the MMD indices against pairwise genetic distances in *Stygocapitella*, nerillids and orbiniids (Fig. 5C) indicates that lower MMD values in *Stygocapitella* are not an artefact of taxonomical ranks (*Stygocapitella* is a genus; Nerillidae and Orbiniidae are families). In *Stygocapitella* MMD indices remain between 0 and 1 until a pairwise genetic distance of 0.025. These values increase slightly to about 4 only at higher molecular distances. This is in agreement with the mapping of morphological change along the 18S ML tree (Fig. 4B) where two branches (out of four having morphological changes) comprise five of the eight morphological changes that were reconstructed in *Stygocapitella*. In clear contrast, MMD indices in Nerillidae and Orbiniidae vary between 5 and 10 at relatively shallow genetic distances of (<0.01; Fig. 5C). Morphological disparity in these lineages remains at high levels with increasing genetic distances and only a few outliers display disparity values as low as *Stygocapitella*. These outliers are at genetic distances of 0.0375. Hence, independent of the genetic distance, morphological disparity in nerillids and orbiniids is on average 2-8 times higher than in *Stygocapitella* (Fig. 5C). Complementarily, we calculated MMD indices for discrete morphological traits and for the morphometric data in the *Stygocapitella* complex while taking the interindividual variation into account and plotted these against genetic distances in 18S (Suppl. Fig. S7). Considering only MMD indices, morphotype #2, #3, and #4 cannot be separated from each other neither in discrete traits nor in morphometrics. Morphotype #1 is clearly set apart from the remaining three but only when considering discrete characters (Suppl. Fig. 7A). When considering morphometric characters, morphotype #1 partially overlaps with the remaining three (Suppl. Fig. 7B). Hence, as above, the morphological similarity across different *Stygocapitella* species is high. Nonetheless, when considering both genetic distances and MMD together we find three clearly separated clusters. The only exceptions being morphotypes #3 and #4. Despite the very low morphological disparity high genetic divergence can be observed between species with different morphotypes indicating that speciation in the *Stygocapitella* complex is not accompanied by morphological changes given the characters analyzed herein.

Finally, we plotted the results of the ancestral state reconstruction (Suppl. Fig. S6) on a time-calibrated tree using the time estimates obtained from the molecular clock analysis (Fig. 3; Suppl. Fig. S6). These results suggest that morphotype #3 is the ancestral condition of EA_B, EA_C and WA and that these three species diverged about 18 MY (5 – 37 MY) ago (Fig 4A; Suppl. Fig. S6). Morphotype #4 was reconstructed as the ancestral condition for a clade comprising EA_A, EP_C, EP_D, as well as for EA_B, EA_C and WA (Suppl. Fig. S6). The age of divergence for this clade (also including EP_B, for which no

morphological data was obtained) was estimated to be about 64 MY (33 – 104 MY) ago (Fig. 4A). Finally, morphotype #2 was reconstructed as the ancestral state for the whole radiation, except for *S. minuta*, and it was dated at ca. 140 MY (75 – 205 MY) (Fig 4A; Suppl. Fig. S6). The age for *Stygocapitella* spp. was dated at about 275 MY (124 – 438 MY). These dates are congruent with previous estimates on a substantially smaller dataset based only on 18S (Struck et al. 2017) which calculated an age of 270 MY for the whole complex and 83 MY for the split of *S. australis* from *S. subterranea*. Hence, even though different morphotypes can be detected, long-term morphological stasis is evident. When considering the 95% confidence interval, morphotype #2 has been maintained for at least 75 MY, and #3 and #4 for at least 5 and 33 MY, respectively. To put the degree of these morphological variations into perspective, even when considering the absolute lowest estimated minimal divergence time for #2 of about 75 MY, only four morphological differences evolved in *Stygocapitella*, while almost the whole radiation of mammals took place during the same time interval.

Demographic changes and ecological separation

We focused on the Atlantic species for the analyses addressing potential ecological drivers of morphological stasis such as niche conservatism and/or the occurrence of fluctuating ecological dynamics (Sheldon 1996; Futuyma 2010; Lindholm 2014). However, analyses at the macro-climatic scale using 19 variables found no statistical differences in the ecological preferences within and among the species (Suppl. Table S9), with *P*-values being generally >0.70. The lowest observed *P*-value was $P = 0.22$, for the comparison between EA_B and EA_C for annual mean temperature.

To investigate potential causes of stasis including niche tracking and reduction of standing genetic variation we assessed the dispersal capacity of different *Stygocapitella* species. Phylogenetic reconstructions indicate that at least five trans-oceanic dispersal events (two across the Pacific Ocean and three across the Atlantic) are necessary to explain present-day distribution. This includes historical transitions, as well as modern translocations potentially due to human activity (Fig. 3, Suppl. Fig. S1). Using Tajima´s D we found indications for reduction in genetic diversity in several populations ($D < 0$ or populations with no polymorphism; Suppl. Table S10) including Bakka, Henningsvær, Kristineberg, and Lødingen as part of EA_A, Glenancross, Île Callot, Keitum, Morsum, and Nairn as part of EA_B, and Bristol Channel, Ellenbogen, Hörnum, Musselburgh, and St. Efflam as part of EA_C. Except for Henningsvær (EA_A), Keitum (EA_B), Ellenbogen, Hörnum, Musselburgh, and St. Efflam (EA_C), all other populations had significant *P-values* or had only a single haplotype. Finally, signatures of balancing selection or population contraction were detected for Cutty Sark (EA_A), Ardtoe (EA_B), Gravesend, List and Plymouth (EA_C) but none of these were significant ($D > 0$; Suppl. Table S10).

Discussion

New perspective on morphological evolution

To the best of our knowledge, this study describes the longest occurrence of stasis in cryptic species complexes known to date. It substantially exceeds results from other cryptic species complexes which uncovered patterns of morphological stasis over a few millions of years. For instance, independent lineages of *Cavernacmella* snails have remained morphologically similar despite estimates of species divergence of about 3 MY ago (Wada et al. 2013) and *Mastigias* jellyfish have remained morphologically similar for 6 MY (Swift et al. 2016). In about 275 MY, the *Stygocapitella* complex evolved at least 10 reproductively isolated species with only with minor morphological differences. Reproductive isolation of these species can be indirectly inferred by complete congruence of independently evolving genetic markers, even when several species occur in sympatry (Coyne and Orr 2004), by species-specific ITS1 length (Suppl. Fig. S8), and by the ABGD analysis.

Rates of morphological evolution are best described as a continuum with two ends represented by 'acceleration' and 'deceleration' (e.g., Stuck et al 2018). In such framework, cryptic species complexes represent cases of substantially decelerated morphological evolution. This is evident when comparing *Stygocapitella* to closely related taxa which demonstrate substantially faster rates of morphological evolution (Fig. 4b). On the other end of the spectrum, accelerated morphological evolution occurs in, for instance, adaptive radiations (Gillespie 2004; Simões et al. 2016) and in character displacement (Brown and Wilson 1956). Generally, increase in rates of morphological evolution seem to be connected with the availability of ecological niches (Gillespie 2004; Losos 2010). Between these extremes, one finds cases where morphological divergence follows genetic divergence or where changes are not as pronounced as in adaptive radiations or conserved in cryptic species complexes. A continuum view will be most informative to future works in morphological evolution which should seek to understand the causes leading to shifts in the pace of morphological evolution (acceleration and deceleration), but also lineage-level morphological evolvability and constraints.

Variations in the rate of morphological change (i.e. acceleration or deceleration/stasis) are likely due to selective pressures, but also 'drift in morphology'. Selection can lead to the evolution of new morphologies, but it can also act to conserve a morphotype (Lynch 1990; Smith et al. 2011; Lidgard and Love 2018). Similar to molecular evolution, the absence of selection or a weak selective pressure on a particular morphotype or trait would lead to "neutral non-adaptive change" (Lynch 1990; Smith et al. 2011). Neutral non-adaptive change occurs when separate populations or species (reproductively isolated or not) accumulate differences in morphology by chance. This could result, for instance, due to random fluctuations in the mean value of a given trait or morphology or fixation of a different trait.

Punctuated Equilibrium

Stasis in *Stygocapitella* is occasionally interrupted by pulses of quantitative and qualitative change, as predicted by punctuated equilibrium (Eldredge and Gould 1972). It is important to note that due to the

lack of fossil data, and due to the few events of morphological change, we cannot infer the exact tempo and mode of morphological evolution in this group. Morphological changes could have occurred either along with speciation events as predicted by the original formulation of the punctuated equilibrium, or they could have occurred in a series of subsequent changes as predicted by gradualism (Mattila and Bokma 2008; Landis and Schraiber 2017). In any case, this is in line with suggestions of punctuated equilibrium-like patterns described in biology, in molecular evolution (Pagel et al. 2006), molecular phylogenies (Bokma 2008), mammal body mass evolution (Mattila and Bokma 2008), and paleontological studies such as the armor development shifts between adaptive peaks in the three-spined stickleback which fits the punctuated equilibrium model (Hunt et al. 2008). Similar to *Stygocapitella*, the evolutionary patterns of other cryptic species complexes such as the *Cavernacmella* snails (Wada et al. 2013) and the *Mastigias* jelly fish (Swift et al. 2016) are best explained by stasis in combination with occasional morphological change in some lineages. In *Cavernacmella*, several species remain morphologically similar, while five became morphologically distinct. Morphological differences in these five lineages seem to be associated with the colonization of limestone outcrops (Wada et al. 2013). This colonization event led to the accumulation of differences in shell morphology in few thousands years despite the evidence for 3 million years long stasis in *C. minima* lineages. This suggests that there are no constraints preventing the evolution of new shell shapes, and that shifts in the pace of morphological evolution could be linked to the ecology or habitat of *Cavernacmella* species, similar to adaptive radiations (Losos 2010). Similarly, in the *Mastigias* species complex, stasis in oceanic offshore species is repeatedly interrupted following independent colonization of marine coastal lakes. Both examples suggest that some degree of stabilizing selection on morphology occurs and when a group of individuals colonizes a new environment, there is a release of the selective pressure leading to the appearance of morphological differences. In *Stygocapitella*, we could not find macro-ecological differences within three morphologically similar species (EA_B, EA_C and WA), and between them and the morphologically distinct species (EA_A). However, our analyses do not include potentially relevant micro-ecological parameters affecting the interstitial environment such as granularity, salinity or moisture distribution (Giere, 2009).

Cryptic species strongly support the hypothesis that speciation is not necessarily coupled with morphological change (Rabosky and Adams 2012; Wada et al. 2013). In *Stygocapitella* 12 out of 16 branches are not associated with morphological change in the assessed characters. In addition, the occurrence of speciation without morphological change adds a layer of complexity to the relationship between speciation, extinction and morphological evolution (Alizon et al. 2008; Silvestro et al. 2018; Katz 2019). Failure to integrate speciation without morphological evolution in paleontology may bias estimates of rates of speciation and morphological evolution (see (Alizon et al. 2008). Because in paleontology a "species" is defined based on morphological differences (Futuyma 1987) and considering the evidence for the widespread occurrence of cryptic species (Pfenninger and Schwenk 2007; Pérez-Ponce de León and Poulin 2016), paleontological estimates of speciation and extinction could be wrong and simply reflect rates of morphological change through time. To mitigate this, we suggest that, when possible, researchers

474 should obtain ratios of cryptic species in extant taxa when focusing on a given paleontological group. For
475 instance, accounting for the presence of cryptic species in marine microplankton fossils demonstrates that
476 the lumping of several cryptic species overestimates the amount of variation within morphospecies, and
477 leads to an apparent slow-down of the rates of evolution (Alizon et al. 2008).

478 While rates of anagenesis and cladogenesis have been estimated in the paleontological record
479 (Jackson and Cheetham 1999; Aze et al. 2011; Strotz and Allen 2013), variation of these rates has received
480 less attention. *Stygocapitella* has two nodes (in a total of eight) associated with more than one morphological
481 change, a single node with a single morphological change and five nodes without any change. This implies
482 that at least five speciation events (i.e. cladogenesis) have occurred without any change in the characters
483 assessed. Additionally, considering that *S. australis* and EP_A share the same morphotype but branch off
484 consecutively, the two subsequent morphological changes occurring in this clade had to be anagenetic.
485 Futuyma (1987) has argued that if variation occurs in rates of anagenesis and cladogenesis, it could result
486 in a pulsated pattern. This is seen in *Stygocapitella*, where we observe that anagenetic changes are lower than
487 the rate of cladogenesis, which results in a pattern of "pulsated changes" between morphotypes.

488 A recent review has argued that discussions about living fossils would benefit from shifting from
489 pattern-description to the causes underlying consistency in morphology through time (Lidgard and Love
490 2018). This is similar to the recently proposed views for cryptic species (Struck et al. 2018). Considering
491 that morphological deceleration occurs in both cryptic species and living fossils, and that it may be due to
492 the same underlying forces, the integration of cryptic species and living fossils could be accomplished
493 under a synthesis of morphological deceleration and stasis. Indeed, cases of long-term stasis in cryptic
494 species could represent living fossils, given a paleontological record (Lidgard and Love 2018). Our results
495 show that some *Stygocapitella* species have been morphologically identical in the assessed characters for at
496 least 50 million years and if there was a fossil record for these lineages, it is very likely that extant species
497 would have been considered living fossils.

498

499 **Morphological stasis**

500 Selection is, likely, the force that maintains highly similar/identical morphologies in *Stygocapitella*
501 species. This may be due to the special properties of the interstitial realm (the space between sand grains)
502 (Noodt 1974; Westheide 1977, 1987; Giere 2009). The interstitial environment is characterized by limited
503 and three-dimensionally structured space, and by constant changes in chemistry due to tides, wave action,
504 seasonal changes, weather conditions, salinity, temperature and input of organic matter. While these
505 factors vary extensively on short time scales (daily and seasonally), it has been shown that abiotic
506 conditions within the interstitial realm have not changed for millions of years (Noodt 1974; Westheide
507 1977; Westheide and Rieger 1987; Giere 2009). This is in agreement with the 'plus ça change, plus c'est la
508 même chose' model by Sheldon (1996), which states that taxa inhabiting environments with severe short-

term abiotic fluctuations, yet stable in the long-term can display stasis. In this model, species are expected to be efficient niche trackers, ultimately resulting in niche conservatism and stasis. Fluctuating conditions have been shown to occur in areas where the three European *Stygocapitella* species occur (i.e., Sylt, Schilksee and Tromsø) (Schmidt 1968, 1969, 1970, 1972a, b). Furthermore, given their mitochondrial divergence (Suppl. Figs. S4-5), *Stygocapitella* species seem to be efficient niche trackers, capable of finding new areas where their habitat occurs. This is shown by their broad distribution ranges, for example, in several populations all individuals have the same haplotype, despite the long-distance between sites. While this is a potential scenario, our attempt to test for reduction in genetic diversity, as a result of recurrent bottlenecks and founder effects (as suggested for meiofauna) did not reveal any general pattern (Andrade et al. 2011; Derycke et al. 2013). If this was the case, absence of standing genetic variation would diminish the potential for selection to act, and potentially drive morphological stasis (Futuyma 2010), yet this must be confirmed using genome-level data. Importantly, the Nerillidae, which are also interstitial, do not exhibit signs of stasis, and hence not every taxa inhabiting the interstitial environment is under stasis.

Conclusions

The *Stygocapitella* cryptic species complex is characterized by decelerated rates of morphological evolution and by long-periods of morphological stasis. In about 275 MYs the *Stygocapitella* complex evolved at least 10 reproductively isolated species, but only four distinct morphotypes with few differences. This highlights that morphological evolution should be represented as a continuum (from accelerated to decelerated) and suggests that the *Stygocapitella* cryptic species complex is one of the most extreme examples for morphological deceleration. Even though we cannot provide conclusive evidence about the causes of stasis in *Stygocapitella*, stasis is likely maintained by niche-conservatism coupled with the ability to track favourable habitats. On the other hand, the comparison with other interstitial species shows that the interstitial realm *per se* is not causing stasis. The increasing numbers of publications describing the occurrence of cryptic species suggest that speciation without morphological changes might be commonplace, and that the presence of morphologically-similar species can bias paleontological rates of speciation, extinction, anagenesis and cladogenesis.

Acknowledgments

We are grateful to Gustav Paulay, Claudia E. Mills, Bernadette Holthuis and Tim Miller for field site suggestions in the USA, and to Tim Worsfold, Andy Mackie, Henning Reiss, Lis Jørgensen for laboratory space in the UK and Norway. We thank Lisbeth Thorbek for her assistance in sequencing, Nataliya Budaeva for primer suggestions and Inês Modesto for fieldwork support in Northern Norway. We are grateful to Peter Wagner and to three anonymous reviewers whose comments substantially improved the manuscript. We acknowledge the use of Norwegian national e-infrastructure for high-performance computing and storage via the projects NN9408K and NS9408K, respectively. JC is grateful

544 to José Mário Branco ("É um lindo sonho para viver; Quando toda a gente assim quiser"), and to Chris
545 Booth and Jane Lewis for late-night discussions about punctuated equilibrium. Fieldwork was partly
546 funded by the Ragen Award from Friday Harbor Laboratories and a Den Grevelige Hjelmstjerne-
547 Rosencroneske Stiftelse ved UiOslo (JC) and by the EU Assemble program (THS). A Forbio travel-grant
548 led JC to Osnabrück to take Light Microscopy and SEM photographs with GP and CM. We thank Kjetil
549 Voje and Lutz Bachmann for reading and commenting a previous version of this manuscript. This is
550 NHM Evolutionary Genomics lab contribution nr **X**.

551 **References**

552 Alizon S, Kucera M, Jansen V (2008) Competition between cryptic species explains variations in rates of
553 lineage evolution. Proc Natl Acad Sci U S A 105:12382–12386. doi: 10.1073/pnas.0805039105

554 Andrade SCS, Norenburg JL, Solferini VN (2011) Worms without borders: Genetic diversity patterns in
555 four Brazilian *Ototyphlonemertes* species (Nemertea, Hoplonemertea). Mar Biol 158:2109–2124. doi:
556 10.1007/s00227-011-1718-3

557 Astrin JJ, Stüben PE (2008) Phylogeny in cryptic weevils: Molecules, morphology and new genera of
558 western Palaearctic Cryptorhynchinae (Coleoptera: Curculionidae). Invertebr Syst 22:503–522. doi:
559 10.1071/IS07057

560 Aze T, Ezard THG, Purvis A, et al (2011) A phylogeny of Cenozoic macroperforate planktonic
561 foraminifera from fossil data. Biol Rev 86:900–927. doi: 10.1111/j.1469-185X.2011.00178.x

562 Bleidorn C, Hill N, Erséus C, Tiedemann R (2009) On the role of character loss in orbiniid phylogeny
563 (Annelida): Molecules vs. morphology. Mol Phylogenet Evol 52:57–69. doi:
564 10.1016/j.ympev.2009.03.022

565 Bokma F (2002) Detection of punctuated equilibrium from molecular phylogenies. J Evol Biol 15:1048–
566 1056. doi: 10.1046/j.1420-9101.2002.00458.x

567 Bokma F (2008) Detection of "punctuated equilibrium" by Bayesian estimation of speciation and
568 extinction rates, ancestral character states, and rates of anagenetic and cladogenetic evolution on a
569 molecular phylogeny. Evolution (N Y) 62:2718–2726. doi: 10.1111/j.1558-5646.2008.00492.x

570 Bouckaert R, Heled J, Kühnert D, et al (2014) BEAST 2: A software platform for bayesian evolutionary
571 analysis. PLoS Comput Biol 10:1–6. doi: 10.1371/journal.pcbi.1003537

572 Brown WL, Wilson EO (1956) Character Displacement. Syst Zool 5:49. doi: 10.2307/2411924

573 Cerca J, Purschke G, Struck TH (2018) Marine connectivity dynamics: clarifying cosmopolitan
574 distributions of marine interstitial invertebrates and the meiofauna paradox. Mar Biol 165:123. doi:
575 10.1007/s00227-018-3383-2

576 Charlesworth B, Lande R, Slatkin M (1982) A Neo-Darwinian commentary on macroevolution. Evolution
577 (N Y) 36:474–498

578 Cheetham AH (1986) Tempo of evolution in a Neogene Bryozoan: Rates of morphologic change within
579 and across species boundaries. Paleobiology 12:190–202

580 Coyne J, Orr H (2004) Speciation. Sinauer Associates, Sunderland, MA

581 Derycke S, Backeljau T, Moens T (2013) Dispersal and gene flow in free-living marine nematodes. Front
582 Zool 10:1. doi: 10.1186/1742-9994-10-1

583 Dray S, Dufour A-B (2007) The ade4 package: Implementing the duality diagram for ecologists. J Stat
584 Softw 22:. doi: 10.18637/jss.v022.i04

585 Eldredge N (1971) The allopatric model and phylogeny in Paleozoic invertebrates. Evolution (N Y)
586 25:156–167

587 Eldredge N, Gould SJ (1972) Punctuated Equilibria: An alternative to phylogetic gradualism. In: Schopf
588 TJM (ed) Models in paleobiology. Freeman, Cooper and Co., San Francisco., pp 82–115

589 Escalante AA, Ayala FJ (1995) Evolutionary origin of *Plasmodium* and other *Apicomplexa* based on rRNA
590 genes. Proc Natl Acad Sci 92:5793–5797. doi: 10.1073/pnas.92.13.5793

591 Frame K, Hunt G, Roy K (2007) Intertidal meiofaunal biodiversity with respect to different algal habitats:
592 A test using phytal ostracodes from Southern California. Hydrobiologia 586:331–342. doi:
593 10.1007/s10750-007-0707-5

594 Futuyma D (2005) Evolution. Sinauer Associates, Inc, Sunderland, MA

595 Futuyma DJ (2010) Evolutionary constraint and ecological consequences. Evolution (N Y) 64:1865–1884.
596 doi: 10.1111/j.1558-5646.2010.00960.x

597 Futuyma DJ (2015) Can modern evolutionary theory explain macroevolution? In: Macroevolution. pp 29–
598 86

599 Futuyma DJ (1987) On the role of species in anagenesis. Am Nat 130:465–473

600 Giere O (2009) Meiobenthology: the microscopic motile fauna of aquatic sediments, 2nd edn. Springler-
601 Verlag, Berlin Heidelberg

602 Gillespie R (2004) Community assembly through adaptive radiation in Hawaiian spiders. Science (80-)
603 303:356–360

604 Gómez A, Serra M, Carvalho GR, et al (2002) Speciation in ancient cryptic species complexes: evidence

605 from the molecular phylogeny of Brachionus plicatilis (Rotifera). Evolution (N Y) 56:1431–1444. doi:
606 10.1554/0014-3820(2002)056[1431:SIACSC]2.0.CO;2

607 Gueriau P, Rabet N, Clément G, et al (2016) A 365-million-year-old freshwater community reveals
608 morphological and ecological stasis in branchiopod crustaceans. Curr Biol 26:383–390. doi:
609 10.1016/j.cub.2015.12.039

610 Hansen TF, Houle D (2004) Evolvability, stabilizing selection, and the problem of stasis. In: Pigliucci M,
611 Preston K (eds) Phenotypic integration: studying the ecology and evolution of complex phenotypes.
612 Oxford University Press, New York, pp 130–154

613 Hijmans RJ (2014) raster: Geographic data analysis and modeling

614 Hillis DM, Dixon MT (1991) Ribosomal DNA: Molecular evolution and phylogenetic inference. Q Rev
615 Biol 66:411–453

616 Hunt G (2007) The relative importance of directional change, random walks, and stasis in the evolution of
617 fossil lineages. Proc Natl Acad Sci 104:18404–18408

618 Hunt G, Bell MA, Travis MP (2008) Evolution toward a new adaptive optimum: Phenotypic evolution in
619 a fossil stickleback lineage. Evolution (N Y) 62:700–710. doi: 10.1111/j.1558-5646.2007.00310.x

620 Hunt G, Rabosky DL (2014) Phenotypic evolution in fossil species: pattern and process. Annu Rev Earth
621 Planet Sci 42:421–441. doi: 10.1146/annurev-earth-040809-152524

622 Jackson JBC, Cheetham AH (1999) Tempo and mode of speciation in the sea. Trends Ecol. Evol. 14:72–
623 77

624 Kalyaanamoorthy S, Minh BQ, Wong TKF, et al (2017) ModelFinder: Fast model selection for accurate
625 phylogenetic estimates. Nat Methods 14:587–589. doi: 10.1038/nmeth.4285

626 Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: Improvements in
627 performance and usability. Mol Biol Evol 30:772–780. doi: 10.1093/molbev/mst010

628 Katz O (2018) Extending the scope of Darwin's "abominable mystery": Integrative approaches to
629 understanding angiosperm origins and species richness. Ann Bot 121:1–8. doi: 10.1093/aob/mcx109

630 Katz O (2019) Conflict and complementarity of paleontological and molecular chronologies? Paleobiology
631 45:7–20. doi: 10.1017/pab.2018.44

632 Kidwell SM, Holland SM (2002) The quality of the fossil record: Implications for evolutionary analyses.
633 Annu Rev Ecol Syst 33:561–588. doi: 10.1146/annurev.ecolsys.33.030602.152151

634 Kück P, Meusemann K (2010) FASconCAT: Convenient handling of data matrices. Mol Phylogenet Evol

635 56:1115–1118. doi: 10.1016/j.ympev.2010.04.024

636 Kumar S, Stecher G, Li M, et al (2018) MEGA X: Molecular evolutionary genetics analysis across
637 computing platforms. Mol Biol Evol 35:1547–1549. doi: 10.1093/molbev/msy096

638 Landis MJ, Schraiber JG (2017) Pulsed evolution shaped modern vertebrate body sizes. Proc Natl Acad
639 Sci 0:201710920. doi: 10.1073/pnas.1710920114

640 Lassance JM, Svensson GP, Kozlov M V., et al (2019) Pheromones and barcoding delimit boundaries
641 between cryptic species in the primitive moth genus *Eriocrania* (Lepidoptera: Eriocraniidae). J Chem
642 Ecol 45:429–439. doi: 10.1007/s10886-019-01076-2

643 Lê S, Josse J, Husson F (2008) FactoMineR: An R Package for Multivariate Analysis. J Stat Softw 25:253–
644 8. doi: 10.1016/j.envint.2008.06.007

645 Lee CE, Frost BW (2002) Morphological stasis in the Eurytemora affinis species complex (Copepoda:
646 Temoridae). In: Hydrobiologia. pp 111–128

647 Lenth R V. (2013) Lsmeans: Least-squares means. R package version 1.10-4. http://CRAN.R-
648 project.org/package=lsmeans

649 Lidgard S, Love AC (2018) Rethinking living fossils. Bioscience 68:760–770. doi: 10.1093/biosci/biy084

650 Lindholm M (2014) Morphologically conservative but physiologically diverse: The mode of stasis in
651 Anostraca (Crustacea: Branchiopoda). Evol Biol 41:503–507. doi: 10.1007/s11692-014-9283-6

652 Lobo J, Teixeira MAL, Borges LMS, et al (2016) Starting a DNA barcode reference library for shallow
653 water polychaetes from the southern European Atlantic coast. Mol Ecol Resour 16:298–313. doi:
654 10.1111/1755-0998.12441

655 Losos JB (2010) Adaptive radiation, ecological opportunity and evolutionary determinism. Am Nat
656 175:623–639. doi: 10.1086/652433

657 Lynch M (1990) The rate of morphological evolution in Mammals from the standpoint of the neutral
658 expectation. Am Nat 136:727–741

659 Mattila TM, Bokma F (2008) Extant mammal body masses suggest punctuated equilibrium. Proc R Soc B
660 Biol Sci 275:2195–2199. doi: 10.1098/rspb.2008.0354

661 Maynard Smith J, Burian R, Kauffman S, et al (1985) Developmental constraints and evolution. Q Rev
662 Biol 60:265–287

663 Nguyen LT, Schmidt HA, Von Haeseler A, Minh BQ (2015) IQ-TREE: A fast and effective stochastic
664 algorithm for estimating maximum-likelihood phylogenies. Mol Biol Evol 32:268–274. doi:

10.1093/molbev/msu300

Noodt W (1974) Anpassungen an interstielle Bedingungen: ein faktor in der evolution höherer taxa der Crustacea. Faun-ökol Mitt 4:445–452

Nordbotten JM, Stenseth NC (2016) Asymmetric ecological conditions favor Red-Queen type of continued evolution over stasis. Proc Natl Acad Sci U S A 113:1847–52. doi: 10.1073/pnas.1525395113

Novo M, Almodóvar A, Fernández R, et al (2012) Appearances can be deceptive: Different diversification patterns within a group of mediterranean earthworms (Oligochaeta, Hormogastridae). Mol Ecol 21:3776–3793. doi: 10.1111/j.1365-294X.2012.05648.x

Novo M, Almodóvar A, Fernández R, et al (2010) Cryptic speciation of hormogastrid earthworms revealed by mitochondrial and nuclear data. Mol Phylogenet Evol 56:507–512. doi: 10.1016/j.ympev.2010.04.010

Pagel M, Venditti C, Meade A (2006) Large punctuational contribution of speciation to evolutionary divergence at the molecular level. Science (80-) 314:119–121. doi: 10.1029/2005GL023216

Palumbi S, Martin A, Romano S, et al (1991) The simple fool's guide to PCR, version 2.

Pennell MW, Harmon LJ, Uyeda JC (2014) Is there room for punctuated equilibrium in macroevolution? Trends Ecol Evol 29:23–32. doi: 10.1016/j.tree.2013.07.004

Pérez-Losada M, Høeg JT, Crandall KA (2004) Unraveling the evolutionary radiation of the thoracican barnacles using molecular and morphological evidence: A comparison of several divergence time estimation approaches. Syst Biol 53:244–264. doi: 10.1080/10635150490423458

Pérez-Ponce de León G, Poulin R (2016) Taxonomic distribution of cryptic diversity among metazoans: not so homogeneous after all. Biol Lett 12:20160371. doi: 10.1098/rsbl.2016.0371

Pfenninger M, Schwenk K (2007) Cryptic animal species are homogeneously distributed among taxa and biogeographical regions. BMC Evol Biol 7:121. doi: 10.1186/1471-2148-7-121

Purschke G (2018) Parergodrilidae Reisinger, 1925. In: Handbook of Zoology Annelida. Vol. 1 Basal Groups and Pleistoannelida, Sedentaria I. Berlin, pp 223–246

R Core Team (2013) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

Rabosky DL, Adams DC (2012) Rates of morphological evolution are correlated with species richness in salamanders. Evolution (N Y) 66:1807–1818. doi: 10.5061/dryad.vt41c78j

695 Radziejewska T, Gruszka P, Rokicka-Praxmajer J (2006) A home away from home: A meiobenthic
696 assemblage in a ship's ballast water tank sediment. Oceanologia 48:259–265

697 Rambaut A, Drummond AJ, Suchard MA (2007) Tracer v1.6

698 Rozas J, Ferrer-Mata A, Sánchez-DelBarrio JC, et al (2017) DnaSP 6: DNA sequence polymorphism
699 analysis of large data sets. Mol Biol Evol 34:3299–3302. doi: 10.1093/molbev/msx248

700 Schmidt H, Westheide W (2000) Are the meiofaunal polychaetes *Hesionides arenaria* and *Stygocapitella*
701 *subterranea* true cosmopolitan species? - results of RAPD-PCR investigations. Zool Scr 29:17–27. doi:
702 doi:10.1046/j.1463-6409.2000.00026.x

703 Schmidt P (1968) Die quantitative verteilung und populationsdynamik des mesopsammons am gezeiten-
704 sandsstrand der Nordseeinsel Sylt - I. Faktorengefüge und biologische Gliederung des Lebensraumes.
705 Int Rev der gesamten Hydrobiol und Hydrogr 53:723–779

706 Schmidt P (1969) Die quantitative Verteilung und Populationdynamik des Mesopsammons am Gezeiten-
707 Sandstrand der Nordseeinsel Sylt - II. Quantitative verteilung und populationsdynamik einzelner
708 Arten. Int Rev der gesamten Hydrobiol und Hydrogr 54:95–174

709 Schmidt P (1970) Zonation of the interstitial polychaete *Stygocapitella subterranea* (Stygocapitellidae) in
710 European sandy beaches. Mar Biol 7:319–323

711 Schmidt P (1972a) Zonierung und jahreszeitliche Fluktuationen des Mesopsammons im Sandstrand von
712 Schilksee (Kieler Bucht). Mikrofauna des Meeresbodens 10:1–60

713 Schmidt P (1972b) Zonierung und jahreszeitliche Fluktuationen der interstitiellen Fauna in Sandstränden
714 des Gebiets von Tromsø (Norwegen). Mikrofauna des Meeresbodens 10:81–164

715 Sheldon PR (1996) Plus ça change - A model for stasis and evolution in different environments.
716 Palaeogeogr Palaeoclimatol Palaeoecol 127:209–227. doi: 10.1016/S0031-0182(96)00096-X

717 Silvestro D, Warnock RCM, Gavryushkina A, Stadler T (2018) Closing the gap between palaeontological
718 and neontological speciation and extinction rate estimates. Nat Commun 9:5237. doi:
719 10.1038/s41467-018-07622-y

720 Simões M, Breitkreuz L, Alvarado M, et al (2016) The Evolving Theory of Evolutionary Radiations.
721 Trends Ecol Evol 31:27–34. doi: 10.1016/j.tree.2015.10.007

722 Smith KL, Harmon LJ, Shoo LP, Melville J (2011) Evidence of constrained phenotypic evolution in a
723 cryptic species complex of agamid lizards. Evolution (N Y) 65:976–992. doi: 10.1111/j.1558-
724 5646.2010.01211.x

725 Stanley SM (1975) A theory of evolution above the species level. Proc Natl Acad Sci USA 72:646–650. doi:

726 10.1016/j.colsurfa.2013.07.017

727 Strotz LC, Allen AP (2013) Assessing the role of cladogenesis in macroevolution by integrating fossil and
728 molecular evidence. Proc Natl Acad Sci 110:2904–2909. doi: 10.1073/pnas.1208302110

729 Struck TH, Feder JL, Bendiksby M, et al (2018) Finding evolutionary processes hidden in cryptic species.
730 Trends Ecol Evol 1–11. doi: 10.1016/j.tree.2017.11.007

731 Struck TH, Golombek A, Weigert A, et al (2015) The evolution of annelids reveals two adaptive routes to
732 the interstitial realm. Curr Biol 1–7. doi: 10.1016/j.cub.2015.06.007

733 Struck TH, Koczula J, Stateczny D, et al (2017) Two new species in the annelid genus *Stygocapitella*
734 (Orbiniida, Parergodrilidae) with comments on their biogeography. Zootaxa 4286:301–332. doi:
735 10.11646/zootaxa.4286.3.1

736 Struck TH, Westheide W, Purschke G (2002) Progenesis in Eunicida ("Polychaeta," Annelida) - Separate
737 evolutionary events? Evidence from molecular data. Mol Phylogenet Evol 25:190–199. doi:
738 10.1016/S1055-7903(02)00231-2

739 Swift HF, Daglio LG, Dawson MN (2016) Three routes to crypsis: stasis, convergence, and parallelism in
740 the *Mastigias* species complex (Scyphozoa, Rhizostomeae). Mol Phylogenet Evol 99:103–115. doi:
741 10.1016/j.ympev.2016.02.013

742 Venables WN, Ripley B d. (2002) Modern applied Statistics with S, Fourth. Springer, New York

743 Voje KL (2016) Tempo does not correlate with mode in the fossil record. Evolution (N Y) 70:2678–2689.
744 doi: 10.1111/evo.13090

745 Voje KL, Starrfelt J, Liow LH (2018) Model adequacy and microevolutionary explanations for stasis in the
746 fossil record. Am Nat 191:000–000. doi: 10.1086/696265

747 Wada S, Kameda Y, Chiba S (2013) Long-term stasis and short-term divergence in the phenotypes of
748 microsnails on oceanic islands. Mol Ecol 22:4801–10. doi: 10.1111/mec.12427

749 Wagner GP, Schwenk K (2000) Evolutionarily stable configurations: Functional integration and the
750 evolution of phenotypic stability. In: Hecht MK, Macintyre RJ, Clegg MT (eds) Evolutionary Biology.
751 Springer US, Boston, MA, pp 155–217

752 Weiss AM (2011) The evolution of evolution: reconciling the problem of stability. Evol Biol 38:42–51. doi:
753 10.1007/s11692-010-9099-y

754 Westheide W (1977) The geographical distribution of interstitial polychaetes. Mikrofauna Meeresb
755 61:287–302

Westheide W (1987) Progenesis as a principle in meiofauna evolution. J Nat Hist 21:843–854. doi: 10.1080/00222938700770501

Westheide W, Hass-Cordes E (2001) Molecular taxonomy: description of a cryptic *Petitia species* (Polychaeta : Syllidae) from the island of Mahe (Seychelles, Indian Ocean) using RAPD markers and ITS2 sequences. J Zool Syst Evol Res 39:103–111

Westheide W, Purschke G (1988) Organism processing. In: Higgins RP, Thiel H (eds) Introduction to the study of meiofauna. Smithsonian Institution Press, Washington, pp 146–160

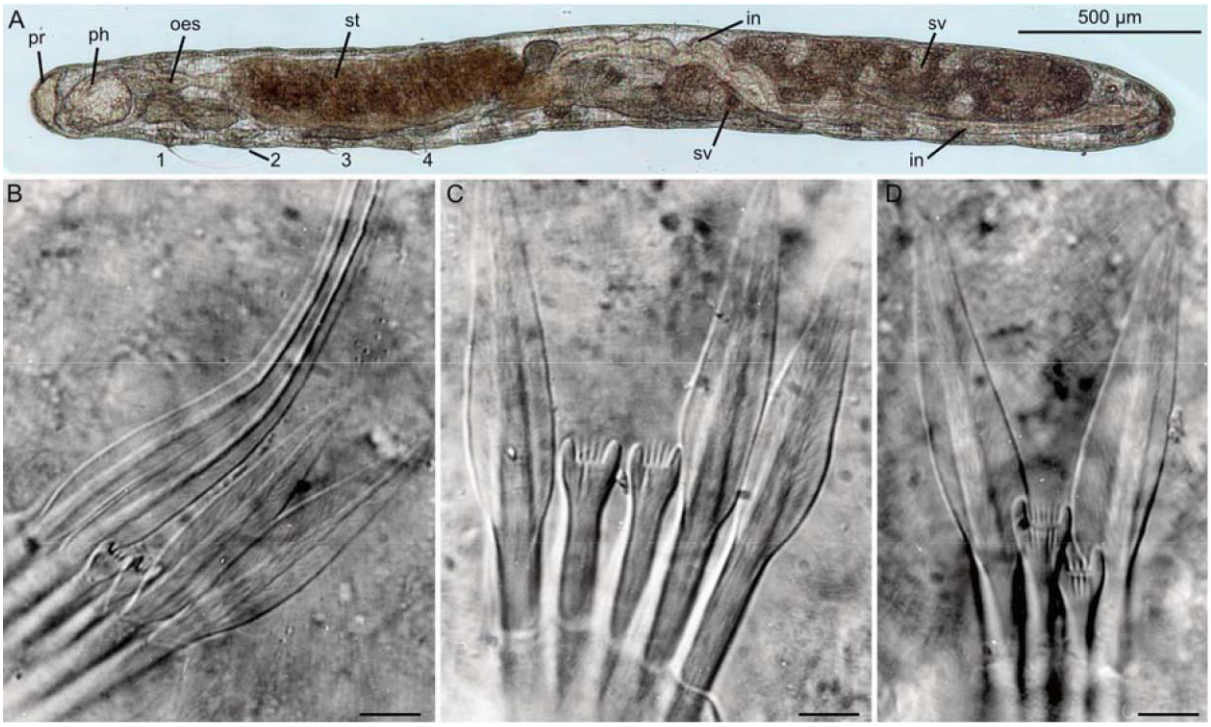
Westheide W, Rieger RM (1987) Systematics of the amphiatlantic *Microphthalmus listensis* species-group (Polychaeta: Hesionidae): facts and concepts for reconstruction of phylogeny and speciation. Zeitschrift für Zool Syst und Evol 25:12–39

Wickham H (2016) ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag, New York

Worsaae K (2005) Phylogeny of Nerillidae (Polychaeta, Annelida) as inferred from combined 18S rDNA and morphological data. Cladistics 21:143–162. doi: 10.1111/j.1096-0031.2005.00058.x

Zanol J, Halanych KM, Struck TH, Fauchald K (2010) Phylogeny of the bristle worm family Eunicidae (Eunicida, Annelida) and the phylogenetic utility of noncongruent 16S, COI and 18S in combined analyses. Mol Phylogenet Evol 55:660–676. doi: 10.1016/j.ympev.2009.12.024

Zrzavý J, Říha P, Piálek L, Janouškovec J (2009) Phylogeny of Annelida (Lophotrochozoa): Total-evidence analysis of morphology and six genes. BMC Evol Biol 9:1–14. doi: 10.1186/1471-2148-9-189



777

778 **Figure 1:** *Stygocapitella subterranea* light microscopy images. A bright field, B-D Normasky interference
779 contrast images. **A:** Whole-mount of living fully mature male individual with prostomium (pr), pharynx
780 (ph), oesophagus (oes), stomach (st), intestine (in) and seminal vesicle (sv). Numbers one to four indicate
781 the chaetae of the 1st, 2nd, 3rd, and 4th chaetiger, respectively. The remaining chaetae are not visible.
782 Individual from Weser estuary, Dedesdort, Germany. **B:** Detail of the left group of chaetae of the 1st
783 chaetiger (indicated as 1 in A) comprising 2 whip-like, 2forked and 2 bilimbate chaetae. **C:** 2nd group of
784 chaetae from chaetiger 2 (left side, indicated as 2 in A) comprising 2 forked and 3 bilimbate chaetae **D:**
785 Detailed view of one chaetal bundle from chaetigers three to ten (such as 3rd and 4th pair of chaetae
786 indicated as 3 and 4 in A) always comprising 2 forked and 2 bilimbate chaetae – Individuals B-D from
787 North Sea Island of Sylt (List-Hausstrand). Scale bars in B-D 10 µm.

788

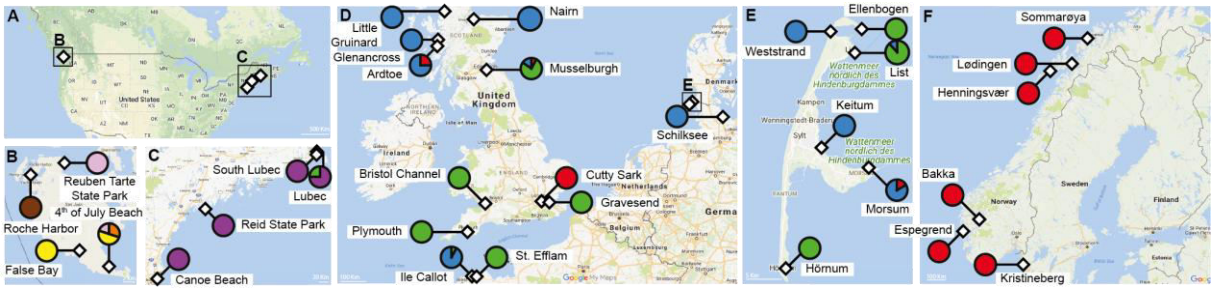
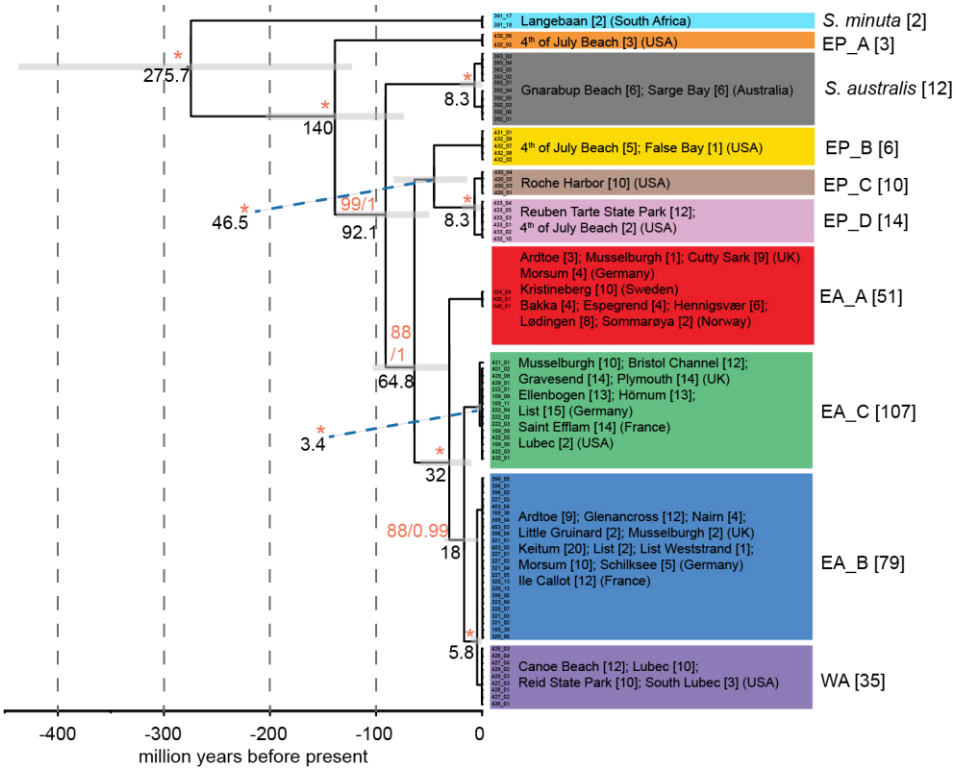


Figure 2: Sampling locations included in this study. A) USA; B) San Juan island; C) US Atlantic coastline; D) UK, France and Germany; E) Island of Sylt; F) Norway. Circles denote species given the phylogeny (Fig. 2): Orange (Eastern Pacific species A; EP_A); Yellow (Eastern Pacific species B; EP_B); Brown Pacific Species (Eastern Pacific species C; EP_C); Pink (Eastern Pacific species D; EP_D); Purple (Western Atlantic species; WA); Red (Eastern Atlantic species A; EA_A); Blue (Eastern Atlantic species B; EA_B); Green (Eastern Atlantic species C; EA_C). Circles with several colours identify sympatry.



797

798 **Figure 3:** Reconstruction of the Bayesian analysis using 18S and COI. Results are congruent with the ML
799 phylogeny of the concatenated data of all four markers (Suppl. Fig. S1). Outgroup is not shown. Above
800 basal nodes bootstrap/posterior support (in red) are shown and below the average divergence date; a grey
801 bar shows the 95% confidence interval. Asterisks (*) indicate a bootstrap value of 100 and a posterior
802 probability of 1. Numbers in square brackets represent the number of specimens for the site included in
803 the ML analysis. For abbreviations see Fig. 1.

804

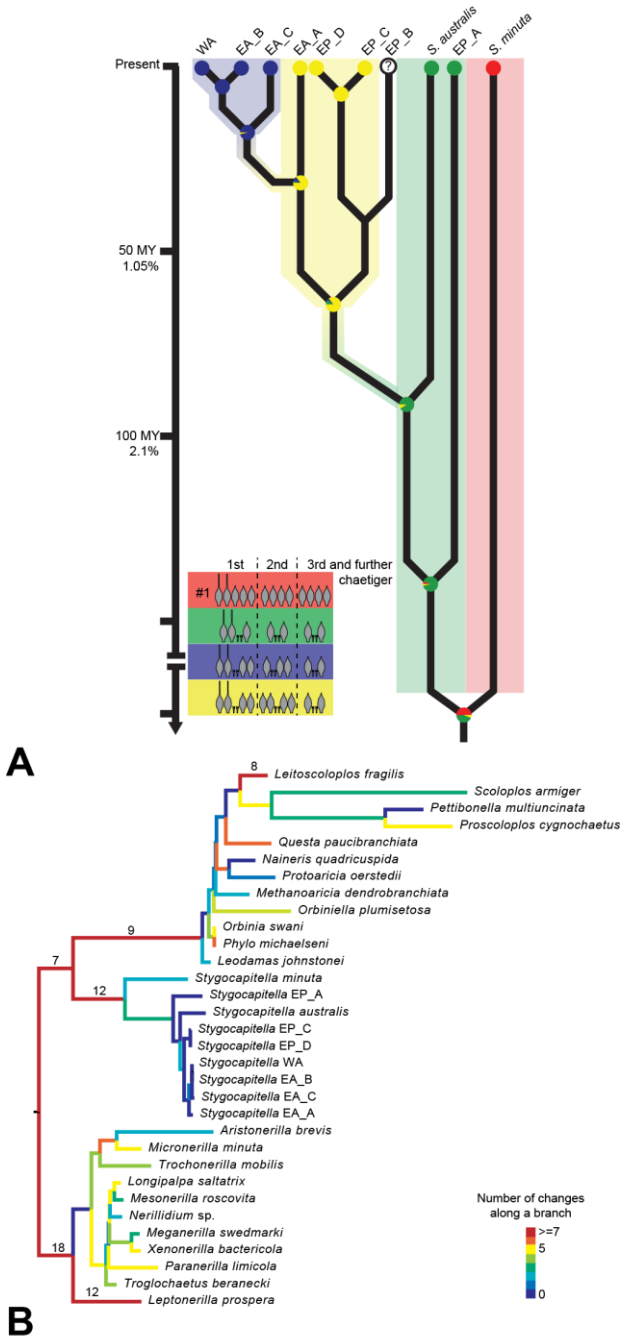
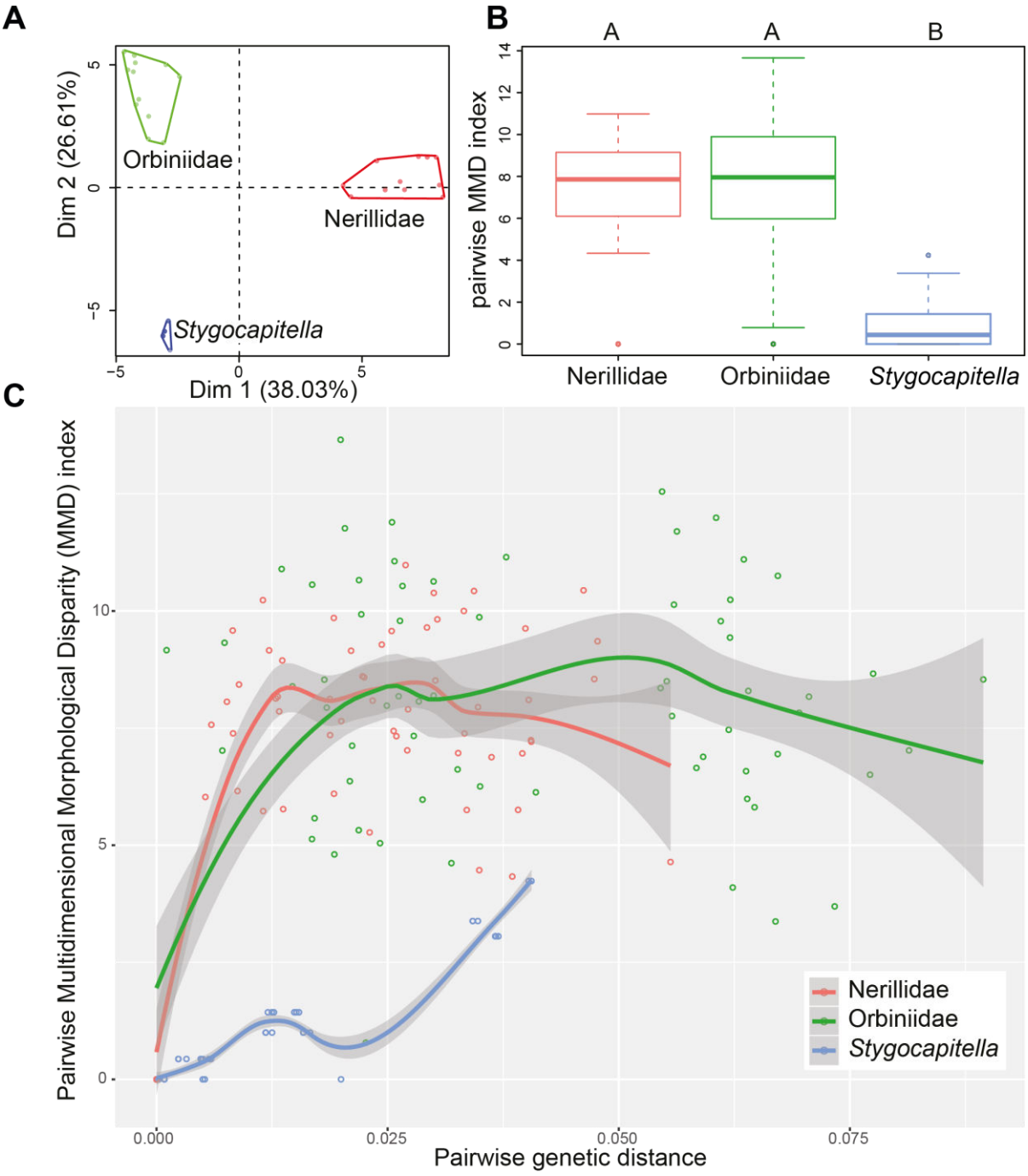


Figure 4: Morphologic and genetic divergence in *Stygocapitella*. A) Tree-like representation of the evolution of the four *Stygocapitella* phenotypes. Morphological assignments follow differences at the chaetigers. Time at the y axis is based on the molecular clock analyses. Morphological transitions between phenotypes are shown by transitioning colours. Tree topology is based on a ML phylogeny (Fig. 2). Pie charts at nodes denote ancestral state reconstructions using a ML approach. Percentages refer to genetic divergence in 18S (0.0002127/MY). For abbreviations see Fig. 1. B) Mapping of character evolution in *Stygocapitella* (Parergodrilidae), Orbiniidae and Nerillidae. Tree topology is based on a 18S ML phylogeny. Number of changes is portrayed in different colours. Zero corresponds to no change occurring along the branch. The number of changes along a branch ≥ 7 is shown on top of the branch.



815

816 **Figure 5**: Principal Component (PC) analysis and Multidimensional Morphological Disparity (MMD)
817 index results among *Stygocapitella*, Orbiniidae and Nerillidae. A) PC analysis of the 75 morphological
818 characters. The first PC explains 38.03% of the variation and the second explains 26.61%. B) Pairwise
819 differences in the MMD index. Outliers are represented by single dots above or below the confidence
820 intervals. Groups which are not significantly different are signed by same letter. C) Plotting of the pairwise
821 MMD indices against the pairwise genetic distance in 18S.

822

823

Supplementary data for manuscript 3

Supplementary Table 1. Accession numbers of sequences used for phylogenetic analyses. Sequences obtained for this study are in bold. For information on sampling sites see Supplementary Table 2.

Taxon	Species	Site	Sampling Code	COI	16S	18S	ITS1
Orbiniidae	*Scoloplos acmeceps*			FJ612519	FJ612470	FJ612488	
	Leitoscoloplos bifurcatus			KR781456	KR349351	KR778793	
	Leitoscoloplos fragilis			FJ612498	AY532341	AY532360	
	Leitoscoloplos robustus				FJ612457	FJ612480	
	Leitoscoloplos pugettensis			HM473442	FJ612454	AY532365	
Parergodrilidae	*Stygocapitella minuta*	Langebaan	327_01	KY503054			
		Langebaan	327_02	KY503055			
		Langebaan	327_03	KY503056			
		Langebaan	327_04	KY503057			
		Langebaan	327_05	KY503058			
		Langebaan	327_06	KY503059			
		Langebaan	327_07	KY503060			
		Langebaan	327_08	KY503061			
		Langebaan	327_10	KY503062			
		Langebaan	327_11	KY503063			
		Langebaan	327_16				
		Langebaan	327_17				
		Langebaan	327_18				
		Langebaan	327_19				
		Langebaan	391_16	KY503064			
		Langebaan	391_17	KY503065		KY503075	
		Langebaan	391_18	KY503066		KY503076	
		Langebaan	391_19	KY503067			
	Stygocapitella australis	Gnarabup Beach	392_01	KY503042			
		Gnarabup Beach	392_03	KY503043			
		Gnarabup Beach	392_04	KY503044			
		Gnarabup Beach	392_05	KY503045		KY503077	
		Gnarabup Beach	392_06	KY503046			
		Gnarabup Beach	392_07	KY503047			
		Sarge Bay	393_01	KY503048		KY503078	
		Sarge Bay	393_02	KY503049			
		Sarge Bay	393_03	KY503050			
		Sarge Bay	393_04	KY503051			
		Sarge Bay	393_05	KY503052			
		Sarge Bay	393_06	KY503053			
	Stygocapitella subterranea	4th July Beach	432_01	MN158589	MN164067		MN162714
		4th July Beach	432_02	MN158382	MN164061	MN162897	MN162736
		4th July Beach	432_03	MN158612	MN164343	MN162996	MN162886
		4th July Beach	432_04		MN164062		MN162738
		4th July Beach	432_05	MN158613	MN164345	MN162997	MN162887
		4th July Beach	432_06	MN158614	MN164344	MN162998	MN162888
		4th July Beach	432_07	MN158385	MN164063	MN162909	MN162739
		4th July Beach	432_08	MN158383	MN164065	MN162895	MN162737
		4th July Beach	432_09	MN158384	MN164064	MN162911	MN162741
		4th July Beach	432_10	MN158597	MN164068	MN162914	MN162724
		Ardtoe	320_01	MN158583	MN164132		
		Ardtoe	320_02	MN158516	MN164320		
		Ardtoe	320_03		MN164133		
		Ardtoe	320_04	MN158525	MN164270		
		Ardtoe	320_05	MN158584	MN164134		
		Ardtoe	320_06	MN158526	MN164298	MN162926	
		Ardtoe	320_07	MN158540	MN164315	MN162927	
		Ardtoe	320_08	MN158551	MN164311		
		Ardtoe	320_12	MN158537	MN164318	MN162958	
		Ardtoe	320_13	MN158541	MN164319	MN162933	
		Ardtoe	320_14	MN158536	MN164312		
		Ardtoe	320_15	MN158523	MN164299		
		Bakka	439_01		MN164090	MN162985	
		Bakka	439_03	MN158582	MN164093	MN162989	
		Bakka	439_07		MN164091		
		Bakka	439_08		MN164094		
		Bristol Channel	422_01	MN158387	MN164135	MN162970	MN162799
		Bristol Channel	422_02	MN158399	MN164136	MN162971	MN162803
		Bristol Channel	422_03	MN158400	MN164144	MN162972	MN162805
		Bristol Channel	422_04	MN158388	MN164148	MN162978	MN162808

Bristol Channel	422_05	MN158413	MN164176		
Bristol Channel	422_06	MN158401	MN164183		MN162802
Bristol Channel	422_07	MN158389	MN164177		MN162800
Bristol Channel	422_08	MN158435			MN162804
Bristol Channel	422_09	MN158415	MN164184		MN162807
Bristol Channel	422_10	MN158480	MN164200		MN162801
Bristol Channel	422_11	MN158390	MN164178		MN162806
Bristol Channel	422_12		MN164149		
Canoe Beach	426_01	MN158481	MN164233	MN162928	MN162768
Canoe Beach	426_02	MN158503		MN162960	MN162770
Canoe Beach	426_03	MN158504	MN164234	MN162939	MN162794
Canoe Beach	426_04	MN158502	MN164235	MN162948	MN162784
Canoe Beach	426_05	MN158507	MN164236		
Canoe Beach	426_06	MN158486	MN164237		MN162795
Canoe Beach	426_07	MN158501	MN164254		MN162771
Canoe Beach	426_08	MN158505	MN164238		MN162785
Canoe Beach	426_09	MN158506	MN164264		MN162796
Canoe Beach	426_10		MN164239		MN162774
Canoe Beach	426_11		MN164249		MN162788
Canoe Beach	426_12		MN164250		MN162797
Cutty Sark	423_01				MN162869
Cutty Sark	423_02		MN164109		MN162870
Cutty Sark	423_03	MN158578	MN164127		
Cutty Sark	423_04		MN164095		
Cutty Sark	423_06		MN164128		
Cutty Sark	423_07	MN158579	MN164110		
Cutty Sark	423_08	MN158580	MN164111		
Cutty Sark	423_09		MN164096		
Cutty Sark	423_10	MN158581	MN164112		MN162871
Ellenbogen	222_01	MN158440	MN164199	MN162982	MN162809
Ellenbogen	222_02	MN158418	MN164153	MN162975	MN162810
Ellenbogen	222_03	MN158396	MN164171	MN162979	MN162832
Ellenbogen	222_04	MN158416	MN164142	MN162984	MN162811
Ellenbogen	222_05	MN158428	MN164195		MN162812
Ellenbogen	222_06	MN158419			MN162820
Ellenbogen	222_07	MN158412			MN162816
Ellenbogen	222_08	MN158421	MN164154		MN162821
Ellenbogen	222_09	MN158422	MN164155		
Ellenbogen	222_10	MN158420	MN164172		
Ellenbogen	222_11	MN158397	MN164173		
Ellenbogen	222_12	MN158423	MN164175		
Ellenbogen	222_13		MN164143		
Espegrend	440_01	MN158588	MN164092	MN162986	MN162872
Espegrend	440_02		MN164097	MN162993	MN162878
Espegrend	440_03		MN164098		
Espegrend	440_04		MN164113		MN162875
False Bay	431_01	MN158386	MN164066	MN162910	MN162740
Glenancross	321_01	MN158538	MN164300	MN162929	
Glenancross	321_02	MN158539	MN164301	MN162952	
Glenancross	321_03	MN158542	MN164303	MN162940	
Glenancross	321_04	MN158534	MN164304	MN162941	
Glenancross	321_05	MN158530	MN164305		
Glenancross	321_06	MN158535	MN164306		
Glenancross	321_07		MN164338		
Glenancross	321_08		MN164302		
Glenancross	321_09	MN158528	MN164307		
Glenancross	321_10	MN158529	MN164310		
Glenancross	321_11	MN158517	MN164308		
Glenancross	321_12	MN158524	MN164309		
Gravesend	424_01	MN158447	MN164201		MN162842
Gravesend	424_02	MN158436	MN164151		
Gravesend	424_03	MN158463	MN164211		
Gravesend	424_04	MN158448	MN164202		
Gravesend	424_05	MN158449	MN164213		MN162843
Gravesend	424_06	MN158450	MN164203		MN162846
Gravesend	424_07	MN158451	MN164212		MN162841
Gravesend	424_08	MN158452	MN164209		MN162848
Gravesend	424_09	MN158461	MN164204		MN162849
Gravesend	424_10	MN158479	MN164205		MN162844
Gravesend	424_11	MN158453	MN164206		MN162838
Gravesend	424_12	MN158431	MN164179		MN162847
Gravesend	424_13	MN158432	MN164191		MN162840
Gravesend	424_14	MN158462	MN164207		MN162845
Henningsvær	437_01				MN162885
Henningsvær	437_02		MN164099		
Henningsvær	437_03		MN164114		MN162877
Henningsvær	437_05		MN164105		

Henningsvær	437_06		MN164100		
Henningsvær	437_07		MN164103		
Hörnum	169_06	MN158391	MN164180		MN162828
Hörnum	169_07	MN158404	MN164192		MN162822
Hörnum	169_08	MN158439	MN164159		
Hörnum	169_09	MN158424	MN164165	MN162973	MN162839
Hörnum	169_10	MN158392	MN164174	MN162974	MN162825
Hörnum	169_11	MN158441		MN162981	MN162835
Hörnum	169_12	MN158433	MN164190	MN162977	
Hörnum	169_13	MN158393	MN164198		MN162836
Hörnum	169_14		MN164157		MN162829
Hörnum	169_15	MN158430	MN164181		MN162826
Hörnum	169_16	MN158405	MN164147		MN162823
Hörnum	169_17	MN158394			MN162830
Île Callot	210_10	MN158556			
Île Callot	210_11	MN158557	MN164321		
Île Callot	210_12	MN158558	MN164284		MN162766
Île Callot	210_13	MN158553	MN164325		
Île Callot	210_14	MN158566	MN164291		
Île Callot	403_03	MN158508	MN164265	MN162950	MN162762
Île Callot	403_04	MN158567	MN164266	MN162942	
Île Callot	403_05	MN158509	MN164267	MN162930	
Île Callot	403_06		MN164268		
Île Callot	403_07	MN158545	MN164322		
Île Callot	403_08	MN158554	MN164273		
Île Callot	403_09		MN164274		
Keitum	169_28		MN164282		
Keitum	169_29	MN158561	MN164283		
Keitum	169_30	MN158569	MN164287		
Keitum	169_31		MN164288		
Keitum	169_32		MN164323		
Keitum	169_33	MN158562	MN164293		
Keitum	169_34	MN158518	MN164326		
Keitum	169_35	MN158563	MN164289		
Keitum	169_36	MN158560	MN164324		
Keitum	169_37		MN164295	MN162953	
Keitum	169_38	MN158568	MN164281	MN162949	
Keitum	169_39	MN158564	MN164290	MN162954	
Keitum	398_04	MN158510	MN164275	MN162956	
Keitum	398_05	MN158546	MN164276		
Keitum	398_06	MN158550	MN164277		
Keitum	398_07	MN158511	MN164278		
Keitum	398_08	MN158512	MN164297		
Keitum	398_09	MN158544	MN164279		
Keitum	398_10	MN158513	MN164269		MN162763
Keitum	398_11	MN158547	MN164280		
Kristineberg	420_01	MN158585	MN164115	MN162987	
Kristineberg	420_02		MN164116	MN162988	
Kristineberg	420_03		MN164124	MN162990	MN162873
Kristineberg	420_04		MN164117		MN162880
Kristineberg	420_05	MN158586	MN164118		MN162882
Kristineberg	420_06		MN164119		MN162881
Kristineberg	420_07		MN164120		MN162874
Kristineberg	420_09		MN164125		
Kristineberg	420_10		MN164122		
Kristineberg	420_12		MN164123		MN162883
List	169_54	MN158446	MN164156		MN162837
List	169_55	MN158548			
List	169_56	MN158406	MN164160	MN162980	MN162817
List	169_57	MN158571	MN164337		
List	169_58	MN158417	MN164164	MN162976	MN162813
List	219_02		MN164161	MN162983	
List	219_03	MN158408	MN164193		MN162824
List	219_04		MN164168		MN162819
List	219_05	MN158442	MN164170		MN162818
List	219_06	MN158443	MN164163		MN162814
List	219_07		MN164158		MN162827
List	219_08	MN158410	MN164150		MN162831
List	219_09		MN164166		MN162833
List	219_10	MN158425	MN164169		
List	219_11	MN158427			
List	219_12		MN164162		
List	219_13		MN164182		
Little Gruinard	322_01	MN158576	MN164314		
Little Gruinard	322_02	MN158577	MN164335		
Lødingen	436_01		MN164106		
Lødingen	436_02		MN164107		

Lødingen	436_03		MN164101		MN162884
Lødingen	436_04		MN164102		
Lødingen	436_05		MN164104		
Lødingen	436_07		MN164108		MN162879
Lubec	429_01	MN158444	MN164138	MN162963	MN162850
Lubec	429_02	MN158482		MN162934	MN162775
Lubec	429_03		MN164255		MN162776
Lubec	429_04		MN164240		
Lubec	429_05		MN164256		MN162777
Lubec	429_06		MN164251		MN162778
Lubec	429_07				MN162793
Lubec	429_08	MN158429	MN164185	MN162967	MN162851
Lubec	429_09	MN158490	MN164257		MN162779
Lubec	429_10	MN158489	MN164241		MN162783
Lubec	429_11	MN158483	MN164242		MN162780
Lubec	429_12	MN158497	MN164258		
Morsum	227_01	MN158519	MN164285	MN162935	
Morsum	227_02	MN158520	MN164286	MN162951	
Morsum	227_03	MN158573	MN164271	MN162955	
Morsum	227_04	MN158521	MN164272	MN162936	
Morsum	227_05	MN158522	MN164316	MN162961	
Morsum	227_06	MN158527	MN164294		
Morsum	227_07	MN158543	MN164292		
Morsum	227_08	MN158555	MN164340		MN162767
Morsum	227_09		MN164296		
Morsum	227_10	MN158559	MN164317		
Morsum	227_14		MN164129		
Morsum	227_15		MN164130		
Musselburgh	324_01	MN158455	MN164223		
Musselburgh	324_02	MN158457	MN164215		
Musselburgh	324_03	MN158565	MN164331		
Musselburgh	324_04	MN158587	MN164131	MN162992	MN162876
Musselburgh	324_05	MN158549	MN164332		MN162764
Musselburgh	324_06	MN158466	MN164216		MN162834
Musselburgh	324_07	MN158458	MN164217		
Musselburgh	324_08	MN158464	MN164222		
Musselburgh	324_09	MN158460	MN164219		
Musselburgh	324_10	MN158459	MN164220		
Musselburgh	324_11	MN158465	MN164218		
Musselburgh	324_12	MN158456	MN164221		
Musselburgh	324_52	MN158468	MN164208		
Nairn	323_01	MN158531	MN164329		
Nairn	323_02	MN158533	MN164333		
Nairn	323_03	MN158552	MN164330		
Nairn	323_04	MN158532	MN164334	MN162957	MN162765
Plymouth	421_01	MN158471	MN164224	MN162964	MN162852
Plymouth	421_02	MN158474	MN164225		
Plymouth	421_03	MN158473	MN164228		MN162865
Plymouth	421_04	MN158438	MN164186		
Plymouth	421_05	MN158475	MN164229		MN162853
Plymouth	421_06	MN158403	MN164187		MN162867
Plymouth	421_07		MN164226		
Plymouth	421_08	MN158472	MN164232		
Plymouth	421_09	MN158476	MN164227		
Plymouth	421_10	MN158478	MN164230		MN162862
Plymouth	421_11		MN164188		
Plymouth	421_13	MN158414	MN164189		MN162855
Plymouth	421_14	MN158426	MN164167		MN162858
Plymouth	421_15	MN158477	MN164231		
Reid State Park	427_01	MN158484	MN164259	MN162943	MN162781
Reid State Park	427_02	MN158495	MN164260	MN162931	MN162772
Reid State Park	427_03	MN158487	MN164243	MN162937	MN162773
Reid State Park	427_04	MN158485	MN164252	MN162944	MN162786
Reid State Park	427_05	MN158499	MN164244		
Reid State Park	427_06	MN158500	MN164245		MN162787
Reid State Park	427_07	MN158492	MN164261		MN162789
Reid State Park	427_08	MN158493	MN164262		MN162792
Reid State Park	427_09	MN158494	MN164246		MN162798
Reid State Park	427_10	MN158496	MN164253		MN162782
Reuben Tarte	433_01	MN158590	MN164069	MN162917	MN162720
Reuben Tarte	433_02	MN158591	MN164075	MN162918	MN162722
Reuben Tarte	433_03	MN158599	MN164070	MN162915	MN162715
Reuben Tarte	433_04	MN158600	MN164076	MN162920	MN162716
Reuben Tarte	433_05	MN158601	MN164071	MN162919	MN162721
Reuben Tarte	433_06	MN158592	MN164077		MN162717
Reuben Tarte	433_07	MN158593	MN164078		
Reuben Tarte	433_08	MN158594	MN164072		MN162719

Reuben Tarte	433_09	MN158595	MN164079		MN162723
Reuben Tarte	433_10	MN158596	MN164080	MN162916	
Reuben Tarte	433_11		MN164073		MN162725
Reuben Tarte	433_12	MN158598	MN164074		MN162718
Roche Harbor	430_01	MN158602	MN164081	MN162921	MN162726
Roche Harbor	430_02			MN162925	MN162727
Roche Harbor	430_03	MN158603	MN164082	MN162922	MN162731
Roche Harbor	430_04	MN158604	MN164083	MN162923	MN162728
Roche Harbor	430_05	MN158605	MN164084	MN162924	MN162729
Roche Harbor	430_06	MN158609	MN164089		MN162735
Roche Harbor	430_07	MN158606	MN164085		MN162733
Roche Harbor	430_08	MN158608	MN164086		MN162730
Roche Harbor	430_09	MN158607	MN164087		MN162734
Roche Harbor	430_10	MN158610	MN164088		MN162732
Schilksee	396_01	KY503068	MN164336	KY503073	
Schilksee	396_02	KY503069	MN164339	KY503074	
Schilksee	396_04	KY503070	MN164327	MN162938	MN162761
Schilksee	396_05	KY503071	MN164313	MN162962	MN162760
Schilksee	396_06	KY503072	MN164328	MN162959	
Sommarøya	438_01		MN164121		
Sommarøya	438_02		MN164126	MN162991	
South Lubec	428_01	MN158491	MN164263	MN162946	MN162791
South Lubec	428_02	MN158498	MN164247	MN162969	MN162790
South Lubec	428_03	MN158488	MN164248	MN162947	MN162769
St. Efflam	210_01	MN158470			MN162864
St. Efflam	210_02	MN158409	MN164197		MN162815
St. Efflam	210_03	MN158395			MN162860
St. Efflam	210_04	MN158467	MN164152		MN162863
St. Efflam	210_05	MN158407	MN164196		
St. Efflam	210_06	MN158411			MN162856
St. Efflam	210_07		MN164141		MN162868
St. Efflam	401_01		MN164139	MN162968	MN162861
St. Efflam	401_02	MN158398	MN164140	MN162966	MN162854
St. Efflam	401_03	MN158454	MN164214	MN162965	
St. Efflam	401_04	MN158437	MN164194		MN162857
St. Efflam	401_05	MN158434	MN164146		
St. Efflam	401_06	MN158445	MN164145		MN162859
St. Efflam	401_07	MN158469	MN164210		MN162866
Weststrand	169_01	MN158574			

Supplementary Table 2. Sampling locations including GPS coordinates for this work.

Site	Coastline (Country)	Latitude	Longitude
4th July Beach	Eastern Pacific (USA)	4846822	-123.00298
False bay	Eastern Pacific (USA)	48.49026	-123.06598
Roche Harbor	Eastern Pacific (USA)	48.59612	-123.16999
Reuben Tarte State Park	Eastern Pacific (USA)	48.61281	-123.09838
Canoe Beach	Western Atlantic (USA)	42.41962	-70.90684
Reid State Park	Western Atlantic (USA)	43.77628	-69.73121
Lubec	Western Atlantic (USA)	44.85482	-66.98179
South Lubec	Western Atlantic (USA)	44.82476	-66.98917
Île Callot	Eastern Atlantic (France)	48.68713	-3.92439
Saint Efflam	Eastern Atlantic (France)	48.684609	-3.62247
Hörnum	Eastern Atlantic (Germany)	54.75619	8.29466
Morsum	Eastern Atlantic (Germany)	54.87822	8.46527
Ellenbogen	Eastern Atlantic (Germany)	55.04397	8.45172
Keitum	Eastern Atlantic (Germany)	54.902	8.36766
List	Eastern Atlantic (Germany)	55.01556	8.43736
Westland	Eastern Atlantic (Germany)	55.040667	8.386944
Schilksee	Eastern Atlantic (Germany)	54.42386	10.17473
Kristineberg	Eastern Atlantic (Sweden)	58.24774	11.44598
Henningsvær	Eastern Atlantic (Norway)	68.26079	14.26836
Lødingen	Eastern Atlantic (Norway)	68.56414	16.49406
Sommarøya	Eastern Atlantic (Norway)	69.63179	18.02713
Espegrend	Eastern Atlantic (Norway)	60.26637	5.22234
Bristol Channel	Eastern Atlantic (Wales)	51.39973	-3.19606
Plymouth	Eastern Atlantic (England)	50.34861	-4.20071
Cutty Sark	Eastern Atlantic (England)	51.48294	-0.0137
Gravesend	Eastern Atlantic (England)	51.44443	0.37764
Ardtoe	Eastern Atlantic (Scotland)	56.76923	-5.88361
Glenancross	Eastern Atlantic (Scotland)	56.94472	-5.85347
Nairn	Eastern Atlantic (Scotland)	57.59653	-3.84176
Musselburgh	Eastern Atlantic (Scotland)	55.94645	-3.07624
Little Gruinard	Eastern Atlantic (Scotland)	57.85223	-5.4533

Supplementary Table 3. Discrete morphological characters. Segments = Number of segments with chaetae (=chaetigers); 1Bilimbate = Number of bilimbate chaetae in chaetiger 1; 2Bilimbate = Number of bilimbate chaetae in chaetiger 2; 3Bilimbate = Number of bilimbate chaetae in chaetiger 3; 4Bilimbate = Number of bilimbate chaetae in chaetiger 4 and the following ones; 1Whip = Number of whipped chaetae in chaetiger 1; 1Forked = Number of forked chaetae in chaetiger 1; 2Forked = Number of forked chaetae in chaetiger 2; 3Forked = Number of forked chaetae in chaetiger 3; 4Forked = Number of forked chaetae in chaetiger 4 and the following ones. If the numbers were different in the left and right parapodium the average values was taken.

Site	Clade	Individual	ID	Segments	1Bilimbate	1Whip	1Forked	2Bilimbate	2Forked	3Bilimbate	3Forked	4Bilimbate	4Forked
Langebaan	*S. minuta*	Holotype	SA_LGB_Holo	8	3	2	0	4	0	4	0	4	0
Langebaan	*S. minuta*	Lost Male	SA_LGB_LostM1	8	3	2	0	4	0	4	0	4	0
Langebaan	*S. minuta*	Paratype ♀ 1	SA_LGB_ParaF1	8	3	2	0	4	0	4	0	4	0
Langebaan	*S. minuta*	Paratype ♀2	SA_LGB_ParaF2	8	3	2	0	4	0	4	0	4	0
Langebaan	*S. minuta*	Paratype ♀3	SA_LGB_ParaF3	8	3	2	0	4	0	4	0	4	0
Langebaan	*S. minuta*	Paratype ♀4	SA_LGB_ParaF4	8	3	2	0	4	0	4	0	4	0
Langebaan	*S. minuta*	Paratype ♀5	SA_LGB_ParaF5	8	3	2	0	4	0	4	0	4	0
Langebaan	*S. minuta*	Paratype ♀6	SA_LGB_ParaF6	8	3	2	0	4	0	4	0	4	0
Langebaan	*S. minuta*	Paratype ♂1	SA_LGB_ParaM1	8	3	2	0	4	0	4	0	4	0
Langebaan	*S. minuta*	Paratype ♂2	SA_LGB_ParaM2	8	3	2	0	4	0	4	0	4	0
Langebaan	*S. minuta*	Paratype ♂3	SA_LGB_ParaM3	8	3	2	0	4	0	4	0	4	0
Langebaan	*S. minuta*	Paratype ♂4	SA_LGB_ParaM4	8	3	2	0	4	0	4	0	4	0
Gnarabup Beach	*S. australis*	Holotype	AUS_GNB_HoloM	10	1	2	2	2	2	2	2	2	2
Gnarabup Beach	*S. australis*	Paratype ♀1	AUS_GNB_ParaF1	10	1	2	2	2	2	2	2	2	2
Gnarabup Beach	*S. australis*	Paratype ♂1	AUS_GNB_ParaM1	10	1	2	2	2	2	2	2	2	2
Gnarabup Beach	*S. australis*	Paratype ♂2	AUS_GNB_ParaM2	10	1	2	2	2	2	2	2	2	2
Sarge Bay	*S. australis*	Paratype ♀2	AUS_SAB_ParaF2	10	1	2	2	2	2	2	2	2	2
Sarge Bay	*S. australis*	Paratype ♀3	AUS_SAB_ParaF3	10	1	2	2	2	2	2	2	2	2
Sarge Bay	*S. australis*	Paratype ♀4	AUS_SAB_ParaF4	10	1	2	2	2	2	2	2	2	2
Sarge Bay	*S. australis*	Paratype ♀5	AUS_SAB_ParaF5	10	1	2	2	2	2	2	2	2	2
Sarge Bay	*S. australis*	Paratype ♂3	AUS_SAB_ParaM3	10	1	2	2	2	2	2	2	2	2
Sarge Bay	*S. australis*	Paratype ♂4	AUS_SAB_ParaM4	10	1	2	2	2	2	2	2	2	2
4th July Beach	EP_A	Individual 1	USA_4JB_Ind1	10	1	2	2	2	2	2	2	2	2
4th July Beach	EP_A	Individual 3	USA_4JB_Ind3	10	1	2	2	2	2	2	2	2	2
Roche Harbor	EP_C	Individual 1	USA_ROH_Ind1	10	2	2	2	3	2	2	2	2	2
Roche Harbor	EP_C	Individual 2	USA_ROH_Ind2	10	2	2	2	4	2	2	2	2	2
Roche Harbor	EP_C	Individual 3	USA_ROH_Ind3	10	2	2.5	2	4	2	2	2	2	2
4th July Beach	EP_D	Individual 4	USA_4JB_Ind4	10	2	2	2	3	2	2	2	2	2
Reuben Tarte	EP_D	Individual 1	USA_RSP_Ind1	10	2	2	2	4	2	2	2	2	2
Reuben Tarte	EP_D	Individual 2	USA_RSP_Ind2	10	2	2	2	4	2	2	2	2	2
Reuben Tarte	EP_D	Individual 4	USA_RSP_Ind3	10	2	2	2	4	2	2	2	2	2
Canoe Beach	WA	Individual 1	USA_CAB_Ind1	10	2	2	2	3	2	2	2	2	2
Canoe Beach	WA	Individual 2	USA_CAB_Ind2	10	2	2	2	3	2	2	2	2	2
Lubec	WA	Individual 1	USA_LUB_Ind1	10	2	2	2	3	2	2	2	2	2
Lubec	WA	Individual 2	USA_LUB_Ind2	10	2	2	2	3	2	2	2	2	2
Lubec	WA	Individual 3	USA_LUB_Ind3	10	2	2	2	3	2	2	2	2	2
Lødingen	EA_A	436.30	NOR_LOE_A436.30	10	2	2	2	3	2	2	2	2	2
Lødingen	EA_A	436.32	NOR_LOE_A436.32	10	2	2	2	3	2	2	2	2	2
Lødingen	EA_A	436.33	NOR_LOE_A436.33	10	2	2	2	4	1.5	2.5	1.5	2	2
Lødingen	EA_A	436.34	NOR_LOE_A436.34	10	2	2	2	3	2	2	2	2	2
Henningsvær	EA_A	437.21	NOR_HEN_A437.21	10	2	2	2	4	2	2	2	2	2
Henningsvær	EA_A	437.22	NOR_HEN_A437.22	10	2	2	2	4	2	2	2	2	2
Henningsvær	EA_A	437.23	NOR_HEN_A437.23	10	2	2	0	4	2	3	0	2	2
Henningsvær	EA_A	437.25	NOR_HEN_A437.25	10	2	2	2	4	2	2	2	2	2
Henningsvær	EA_A	437.28	NOR_HEN_A437.28	10	2	2	2	4	2	2	2	2	2
Henningsvær	EA_A	437.29	NOR_HEN_A437.29	10	2	2	2	4	2	2	2	2	2
Île Callot	EA_B	Individual 2	FRA_ILE_Ind2	10	2	2	2	3	2	2	2	2	2
Île Callot	EA_B	Individual 3	FRA_ILE_Ind3	10	2	2	2	3	2	2	2	2	2
Île Callot	EA_B	Individual 4	FRA_ILE_Ind4	10	2	2	2	3	2	2	2	2	2
Schilksee	EA_B	Neotype	GER_SCS_NeoF	10	2	2	2	3	2	2	2	2	2
Schilksee	EA_B	Paratype ♀1	GER_SCS_ParaF1	10	2	2	2	3	2	2	2	2	2
Schilksee	EA_B	Paratype ♀2	GER_SCS_ParaF2	10	2	2	2	3	2	2	2	2	2
Schilksee	EA_B	Paratype ♂3	GER_SCS_ParaM3	10	2	2	2	3	2	2	2	2	2
Schilksee	EA_B	Paratype ♂4	GER_SCS_ParaM4	10	2	2	2	3	2	2	2	2	2
List	EA_C	Individual 1	GER_HAU_Ind1	10	2	2	2	3	2	2	2	2	2
List	EA_C	Individual 2	GER_HAU_Ind2	10	2	2	2	3	2	2	2	2	2
List	EA_C	Individual 3	GER_HAU_Ind3	10	2	2	2	3	2	2	2	2	2
List	EA_C	Individual 4	GER_HAU_Ind4	10	2	2	2	3	2	2	2	2	2

List	EA_C	Individual 5	GER_HAU_Ind5	10	2	2	2	3	2	2	2	2	2
Gravesend	EA_C	Sample 2 Ind. 1	UK_GRA_Ind2_1	10	2	2	2	3	2	2	2	2	2
Gravesend	EA_C	Sample 2 Ind. 2	UK_GRA_Ind2_2	10	2	3	2	4	2	2	2	2	2
Gravesend	EA_C	Sample 2 Ind. 3	UK_GRA_Ind2_3	10	2	2	2	3	2	2	2	2	2
Gravesend	EA_C	Sample 2 Ind. 4	UK_GRA_Ind2_4	10	2	2	2	3	2	2	2	2	2
Gravesend	EA_C	Sample 3 Ind. 1	UK_GRA_Ind3_1	10	2	2	2	3	2	2	2	2	2
Gravesend	EA_C	Sample 3 Ind. 2	UK_GRA_Ind3_2	10	2	2	2	3	2	2	2	2	2
Gravesend	EA_C	Sample 3 Ind. 3	UK_GRA_Ind3_3	10	2	2	2	3	2	2	2	2	2
Gravesend	EA_C	Sample 3 Ind. 4	UK_GRA_Ind3_4	10	2	2	2	3	2	2	2	2	2
Plymouth	EA_C	Individual 1	UK_PLY_Ind1	10	1	2	2	2	2	2	2	2	2
Plymouth	EA_C	Individual 2	UK_PLY_Ind2	10	2	2	2	3	2	2	2	2	2
Plymouth	EA_C	Individual 3	UK_PLY_Ind3	10	2	2	2	3	2	2	2	2	2
Bristol Channel	EA_C	Individual 1	UK_BCH_Ind1	10	2	2	2	3	2	2	2	2	2
Bristol Channel	EA_C	Individual 2	UK_BCH_Ind2	10	2	2	2	3	2	2	2	2	2
Bristol Channel	EA_C	Individual 3	UK_BCH_Ind3	10	2	2	2	3	2	2	2	2	2

Supplementary Table 4. Morphometric measurements. BodyLength = Length of entire body; BodyWidth = Width of entire body; ProstomiumLength = Length of prostomium; ProstomiumWidth = Width of prostomium; PeristomiumLength = Length of peristomium; PeristomiumWidth = Width of peristomium. All measurements are in μm.

Site	Clade	Individual	ID	BodyLength	BodyWidth	ProstomiumLength	ProstomiumWidth	PygidiumLength	PygidiumWidth
Langebaan	*S. minuta*	Holotype	SA_LGB_Holo	1010.74	104.54	46.6	78.89	30.95	35.21
Langebaan	*S. minuta*	Lost Male	SA_LGB_LostM1	992.34	91.11	39.61	75.01	33.51	37.7
Langebaan	*S. minuta*	Paratype ♀ 1	SA_LGB_ParaF1	977.69	87.79	46.46	75.88	33.13	39.6
Langebaan	*S. minuta*	Paratype ♀3	SA_LGB_ParaF3	999.66	97.2	33.13	66.02	47.41	59.72
Langebaan	*S. minuta*	Paratype ♀4	SA_LGB_ParaF4	912.97	81.1	35.07	72.96	37.35	47.75
Langebaan	*S. minuta*	Paratype ♀5	SA_LGB_ParaF5	1143.29	117.07	46.6	85.51	37.51	55.87
Langebaan	*S. minuta*	Paratype ♀6	SA_LGB_ParaF6	1059.66	89.71	39.8	52.27	30.9	38.53
Langebaan	*S. minuta*	Paratype ♂1	SA_LGB_ParaM1	1155.68	102.67	38.44	69.19	46.86	54.27
Langebaan	*S. minuta*	Paratype ♂2	SA_LGB_ParaM2	1099	110.27	43.24	85.35	44.31	63.48
Langebaan	*S. minuta*	Paratype ♂3	SA_LGB_ParaM3	1117.34	110.65	47.34	90.44	40.46	62.76
Langebaan	*S. minuta*	Paratype ♂4	SA_LGB_ParaM4	1047.34	99.21	38.6	76.46	47.71	57.39
Gnarabup Beach	*S. australis*	Holotype	AUS_GNB_HoloM	2019.02	209.21	73.84	115.29	67.47	123.95
Gnarabup Beach	*S. australis*	Paratype ♀1	AUS_GNB_ParaF1	1900.44	196.75	89.21	138.95	47.87	114.86
Gnarabup Beach	*S. australis*	Paratype ♂1	AUS_GNB_ParaM1	1808	224.39	81.89	130.51	67.19	115.27
Gnarabup Beach	*S. australis*	Paratype ♂2	AUS_GNB_ParaM2	2312.6	235.11	80.59	131.72	37.62	84.99
Sarge Bay	*S. australis*	Paratype ♀2	AUS_SAB_ParaF2	2008.77	202.73	76.18	104.79	53.32	89.06
Sarge Bay	*S. australis*	Paratype ♀3	AUS_SAB_ParaF3	1650.14	172.52	64.76	104.79	49.6	84.56
Sarge Bay	*S. australis*	Paratype ♀4	AUS_SAB_ParaF4	2130.8	185.77	54.33	113.22	61.04	106.34
Sarge Bay	*S. australis*	Paratype ♀5	AUS_SAB_ParaF5	2079.81	204.69	69.79	114.52	57.38	117.42
Sarge Bay	*S. australis*	Paratype ♂3	AUS_SAB_ParaM3	1804.37	145.06	59.7	98.88	60.3	75.89
Sarge Bay	*S. australis*	Paratype ♂4	AUS_SAB_ParaM4	1622.17	122.31	66.23	87.96	59.36	82.77
4th July Beach	EP_A	Individual 2	USA_4JB_Ind2	2075.61	283.45	143.15	240.08	71.07	126.86
4th July Beach	EP_A	Individual 3	USA_4JB_Ind3	1669.4	200.72	87.72	111.82	37.54	82.19
4th July Beach	EP_A	Individual 4	USA_4JB_Ind4	1837.43	214.65	79.28	137.85	68.51	84.66
4th July Beach	EP_A	Individual 5	USA_4JB_Ind5	1718.05	218.64	78.05	130.88	55.85	86.03
4th July Beach	EP_A	Individual 6	USA_4JB_Ind6	2535.21	308.11	104.79	177.73	77.18	123.73
4th July Beach	EP_A	Individual 7	USA_4JB_Ind7	1217.05	154.59	84.29	108.99	27.46	89.47
Roche Harbor	EP_C	Individual 1	USA_ROH_Ind1	1342.86	166.72	60.45	106.08	39.35	74.9
Roche Harbor	EP_C	Individual 2	USA_ROH_Ind2	1874.94	203.63	42.85	86.68	47.96	101.62
Roche Harbor	EP_C	Individual 3	USA_ROH_Ind3	1680.1	216.92	58.03	125.91	50.54	95.9
Roche Harbor	EP_C	Individual 4	USA_ROH_Ind4	2406.77	273.1	81.79	162.75	70.9	143.44
Roche Harbor	EP_C	Individual 5	USA_ROH_Ind5	2326.9	226.89	95.54	182.19	54.18	145.75
Roche Harbor	EP_C	Individual 6	USA_ROH_Ind6	2552.82	273.68	91.58	148.79	63.5	138.1
Roche Harbor	EP_C	Individual 7	USA_ROH_Ind7	1864.44	232.47	58.96	153.64	47.97	116.03
Reuben Tarte	EP_D	Individual 1	USA_RSP_Ind1	2409.03	256.93	71.27	147.03	45.67	137.85
Reuben Tarte	EP_D	Individual 2	USA_RSP_Ind2	2787.72	261.71	78.35	165.59	64.01	131.26
Reuben Tarte	EP_D	Individual 3	USA_RSP_Ind3	2733.84	283.71	103.84	189.07	76.45	174.35
Reuben Tarte	EP_D	Individual 4	USA_RSP_Ind4	2181.42	224.14	114.39	184.93	50.12	129.56
Reuben Tarte	EP_D	Individual 5	USA_RSP_Ind5	2787.91	284.27	95.39	197.67	65.89	200.81
Reuben Tarte	EP_D	Individual 6	USA_RSP_Ind6	2516.54	275.45	109.05	193.42	44.71	171.26
Reuben Tarte	EP_D	Individual 7	USA_RSP_Ind7	2436.34	231.51	86.84	164.01	62.08	138.87
Canoe Beach	WA	Individual 1	USA_CAB_Ind1	1570.1	168.64	73.11	107.38	51.41	86.24
Canoe Beach	WA	Individual 2	USA_CAB_Ind2	1632.26	182.2	66.42	105.87	59.28	72.17
Lubec	WA	Individual 1	USA_LUB_Ind1	2013.09	222.07	68.53	136.84	56.89	121.91
Lubec	WA	Individual 2	USA_LUB_Ind2	1851.4	184.65	63.18	136.1	43.68	106.07
Lubec	WA	Individual 3	USA_LUB_Ind3	2227.13	198.78	71.97	168.34	43.75	98.99
Lubec	WA	Individual 4	USA_LUB_Ind4	1620.44	171.11	49.22	118.02	34.88	72.28
Lubec	WA	Individual 5	USA_LUB_Ind5	1647.19	147.38	73.39	113.84	48.14	77.73
Lubec	WA	Individual 6	USA_LUB_Ind6	2298.89	237.15	56.89	139.88	51.96	116.73
Lubec	WA	Individual 7	USA_LUB_Ind7	1521.28	184.64	51.68	122.04	47.11	98.58
Lødingen	EA_A	436.29	NOR_LOE_A436.29	2365.12	245.9	82.79	127.87	59.84	79.51
Lødingen	EA_A	436.30	NOR_LOE_A436.30	1766.87	198.36	49.18	105.74	52.46	69.67
Lødingen	EA_A	436.31	NOR_LOE_A436.31	2005.23	202.46	45.08	96.72	54.92	82.79
Lødingen	EA_A	436.32	NOR_LOE_A436.32	2388.31	195.87	51.65	123.14	50	80.58
Lødingen	EA_A	436.33	NOR_LOE_A436.33	2277.61	250	61.16	147.93	100.83	116.94
Lødingen	EA_A	436.34	NOR_LOE_A436.34	2456.69	241.32	73.55	112.81	83.47	123.55
Henningsvær	EA_A	437.21	NOR_HEN_A437.21	2293.93	221.9	57.85	118.18	50	83.47
Henningsvær	EA_A	437.23	NOR_HEN_A437.23	2500.06	295.46	84.71	123.55	56.41	126.03
Henningsvær	EA_A	437.24	NOR_HEN_A437.24	2572.16	280.99	54.55	102.07	63.64	123.97
Henningsvær	EA_A	437.25	NOR_HEN_A437.25	1876.3	198.36	85.25	121.31	65.57	114.75

Henningsvær	EA_A	437.26	NOR_HEN_A437.26	2573.28	255.37	57.85	118.18	49.59	113.22
Henningsvær	EA_A	437.28	NOR_HEN_A437.28	1514.25	170.49	50.82	108.2	45.9	81.15
Henningsvær	EA_A	437.29	NOR_HEN_A437.29	2392.82	262.3	44.26	107.38	61.48	104.1
Henningsvær	EA_A	437.30	NOR_HEN_A437.30	2352.62	293.44	84.43	136.07	63.12	119.67
Schilksee	EA_B	Neotype	GER_SCS_NeoF	1692.39	140.99	54.12	82.56	46.55	77.55
Schilksee	EA_B	Paratype ♀1	GER_SCS_ParaF1	1801.36	218.75	65.31	121.51	55.29	76.76
Schilksee	EA_B	Paratype ♀2	GER_SCS_ParaF2	1407.97	159.51	48.13	99.11	45.94	81.02
Schilksee	EA_B	Paratype ♂3	GER_SCS_ParaM3	1841.37	171.13	33.07	93.32	45.23	58.33
Schilksee	EA_B	Paratype ♂4	GER_SCS_ParaM4	1608.5	185.78	61.52	109.71	33.85	80.41
Île Callot	EA_B	Individual 1	FRA_ILE_Ind1	1772.15	186.94	51.36	104.66	38.91	92.79
Île Callot	EA_B	Individual 2	FRA_ILE_Ind2	2074.69	233.75	66.39	109.72	50.68	98.79
Île Callot	EA_B	Individual 3	FRA_ILE_Ind3	2329.07	277.84	79.73	152.68	64.65	160.32
Île Callot	EA_B	Individual 4	FRA_ILE_Ind4	2507.08	251.73	70.58	153.04	36.65	95.99
Île Callot	EA_B	Individual 5	FRA_ILE_Ind5	2441.76	285.81	89.96	167.84	47.14	125.71
Île Callot	EA_B	Individual 6	FRA_ILE_Ind6	1958.52	233.13	72.9	140.66	45.31	118.62
Glenancross	EA_B	321.51	UK_GLE_A321.51	1352.02	204.92	88.53	126.23	43.44	78.69
Glenancross	EA_B	321.52	UK_GLE_A321.52	1277.24	217.36	54.96	114.05	45.87	63.22
Glenancross	EA_B	321.53	UK_GLE_A321.53	1617.29	183.88	66.53	121.49	45.46	82.23
Glenancross	EA_B	321.55	UK_GLE_A321.55	1219.04	143.8	54.13	91.32	39.26	54.13
Glenancross	EA_B	321.56	UK_GLE_A321.56	1201.2	142.88	54.96	102.07	42.15	61.57
Glenancross	EA_B	321.57	UK_GLE_A321.57	1545.18	176.03	51.24	104.13	39.26	60.33
Glenancross	EA_B	321.59	UK_GLE_A321.59	1358.37	185.73	52.89	104.55	42.98	76.86
Glenancross	EA_B	321.60	UK_GLE_A321.60	1099.18	121.31	45.9	105.74	36.89	63.93
Keitum	EA_B	398.1_1	GER_KEI_A398.1_1	1944.72	188.84	71.49	126.45	49.17	104.55
Keitum	EA_B	398.1_2	GER_KEI_A398.1_2	1427.03	146.69	66.12	118.18	45.87	86.36
Keitum	EA_B	398.1_3	GER_KEI_A398.1_3	1909.69	231.41	57.03	130.58	47.11	95.46
Keitum	EA_B	398.1_4	GER_KEI_A398.1_4	2050.62	180.58	72.31	127.27	50.83	99.59
Keitum	EA_B	398.2_1	GER_KEI_A398.2_1	2185.57	149.59	61.57	108.26	43.39	78.93
Keitum	EA_B	398.2_2	GER_KEI_A398.2_2	1452	151.65	61.98	111.98	45.04	83.06
Keitum	EA_B	398.2_3	GER_KEI_A398.2_3	1921.13	161.57	64.88	117.36	48.35	84.3
Keitum	EA_B	398.2_4	GER_KEI_A398.2_4	1401.46	135.95	63.22	108.26	44.63	82.23
Keitum	EA_B	398.16	GER_KEI_A398.16	1396.82	145.9	70.49	124.59	43.44	88.53
Keitum	EA_B	398.17	GER_KEI_A398.17	1613.95	174.79	58.68	114.46	57.85	100
Bristol Channel	EA_C	Individual 1	UK_BCH_Ind1	1873.11	207.79	88.07	111.54	54.05	99.27
Bristol Channel	EA_C	Individual 2	UK_BCH_Ind2	2149.73	227.91	67.44	146.56	53.36	113.37
Bristol Channel	EA_C	Individual 3	UK_BCH_Ind3	1998.95	226.25	66.45	139.53	54.81	145.95
Bristol Channel	EA_C	Individual 4	UK_BCH_Ind4	2294.3	248.21	76.08	147.1	76.32	149.98
Bristol Channel	EA_C	Individual 5	UK_BCH_Ind5	1829.39	227.4	71.96	141.17	51.03	114.78
Gravesend	EA_C	Individual 1	UK_GRA_Ind1	2661.38	338.49	84.97	164.24	64.46	105.47
Gravesend	EA_C	Individual 2	UK_GRA_Ind2	2275.83	226.1	67.52	145.55	59.35	114.8
Gravesend	EA_C	Individual 3	UK_GRA_Ind3	2644.38	267.09	81.52	139.92	52.82	142.71
Gravesend	EA_C	Individual 4	UK_GRA_Ind4	2637.59	291.42	90.99	161.94	59.33	144.71
Gravesend	EA_C	Individual 5	UK_GRA_Ind5	2493.57	264.58	71.32	144.94	50.62	118.22
Gravesend	EA_C	Individual 6	UK_GRA_Ind6	3751.87	423.71	153.28	206.36	51.2	115.59
Gravesend	EA_C	Individual 7	UK_GRA_Ind7	2549.49	189.77	63.13	115.96	56.95	97.98
Gravesend	EA_C	Individual 8	UK_GRA_Ind8	2625.56	234.9	85.71	110.32	61.09	119.39
Gravesend	EA_C	Individual 9	UK_GRA_Ind9	2499.99	297.56	84.72	164.46	57.75	131.38
Plymouth	EA_C	Individual 1	UK_PLY_Ind1	1595.9	169.31	47.96	106.38	41.61	97.98
Plymouth	EA_C	Individual 2	UK_PLY_Ind2	1317	159.65	56.13	104.45	48.81	105.2
Plymouth	EA_C	Individual 3	UK_PLY_Ind3	2903.19	284.06	89.94	161.34	77.43	153.36
Plymouth	EA_C	Individual 4	UK_PLY_Ind4	1713.76	225.3	62.55	130.77	56.28	120.45
Plymouth	EA_C	Individual 5	UK_PLY_Ind5	3021.08	367.8	105.95	200.45	60.57	132.59
Plymouth	EA_C	Individual 6	UK_PLY_Ind6	2093.62	229.65	77.32	133.1	39.05	123.48
Plymouth	EA_C	Individual 7	UK_PLY_Ind7	2689.28	312.04	93.96	171.86	56.55	156.02
Ellenbogen	EA_C	Individual 1	GER_ELL_Ind1	2386.98	255.15	70.92	160.32	160.32	126.17
Ellenbogen	EA_C	Individual 2	GER_ELL_Ind2	2524.11	247.34	75.37	175.99	64.66	97.37
Ellenbogen	EA_C	Individual 3	GER_ELL_Ind3	2780.02	277.53	126.13	222.07	59.78	145.03
Ellenbogen	EA_C	Individual 5	GER_ELL_Ind5	2324.45	213.18	81.59	172.38	53.95	163.36
Ellenbogen	EA_C	Individual 6	GER_ELL_Ind6	2372.22	251.88	87.12	180.05	50.92	137.65
Ellenbogen	EA_C	Individual 7	GER_ELL_Ind7	2283.45	280.01	100.28	190.78	52.69	133
Ellenbogen	EA_C	Individual 8	GER_ELL_Ind8	2674.36	270.7	92.9	183.6	73.28	127.54
Ellenbogen	EA_C	Individual 9	GER_ELL_Ind9	2732.56	264.3	73.54	178.41	65.86	140.89
Ellenbogen	EA_C	Individual 10	GER_ELL_Ind10	2593.88	256.32	140.17	219.23	78.67	155.39

Supplementary Table 5. List of morphological characters used in the analyses comparing *Stygocapitella* with Orbiniidae and Nerillidae.

#	Character	Character states
1	Segmentation	0=absent 1=present
2	Size	0=macrofaunal 1=meiofaunal
3	Numbers of segments	0=more than ten 1=ten 2=nine 3=eight 4=seven
4	Head structure	0=prostomium and ring-like peristomium 1=prostomium and two or more ring-like peristomium 2=prostomium and peristomium limited to lips
5	Shape of prostomium	0=unmodified 1=prostomium narrowly pointed or sharply conical 2=prostomium broadly rounded or truncate on anterior margin 3=prostomium elongate, narrow, rounded anteriorly
6	First body segment	0=similar to following segments, or more or less reduced 1=surrounding head
7	Chaetae on first body segment	0=present 1=absent
8	Second body segment	0=short 1=elongated to form trunk
9	Palps	0=absent 1=present
10	Palp origin	0=absent 1=prostomial 2=peristomial
11	Number of palps	0=absent 1=one pair of palps
12	Median prostomial antenna	0=absent 1=present
13	Relative length median antenna	0=absent 1=longer than prostomial width 2=shorter than prostomial width
14	Shape of antennae	0=absent 1=straight 2=wrinkled
15	Lateral prostomial antennae	0=absent 1=present
16	Relative length paired antennae	0=absent 1=longer than palps and prostomial width 2=shorter than palps and prostomial width
17	Palp structure	0=absent 1=solid
18	Parapodia	0=absent 1=present
19	Shape of notopodium	0=absent 1=tori or small lobes 2=prominent lobes
20	Shape of neuropodium	0=absent 1=tori or small lobes 2=promiment lobes
21	Presence of tori	0=absent 1=present
22	Dorsal cirri	0=absent 1=present
23	Ventral cirri	0=absent 1=present
24	Interramal cirri	0=absent 1=present
25	IC number per segment	0=absent 1=double pair 2=single pair 3=single
26	IC first body segment	0=absent 1=present
27	IC other segments length	0=absent 1=longer than segment 2=shorter than segment 3=rudimentary
28	IC other segments relative length	0=absent 1=increasing towards posterior end

		2=similar throughout
		3=increasing towards posterior end
29	IC other segments shape	0=absent
		1=cirriform
		2=leaf-shaped
		3=bottle-shaped
30	IC last segment	0=absent
		1=present
31	Neuropodial lobes first body segment	0=absent
		1=present
32	Neuropodial lobes following segments	0=absent
		1=present
33	Posterior parapodia elevated	0=absent
		1=present
34	Some parapodia fringed lobes	0=absent
		1=present
35	Subpodial lobes	0=absent
		1=present
36	Crotchets with ligament	0=absent
		1=present
37	Dorsal fold males	0=absent
		1=present
38	Parapodial branchiae	0=absent
		1=present
39	Dorsal branchiae	0=absent
		1=present
40	Lateral organs dorsal cirrus organs	0=absent
		1=present
41	Dorsal organs	0=absent
		1=present
42	Pygidial cirri	0=absent
		1=present
43	Pygidial cirri shape	0=absent
		1=cirriform
		2=wrinkled
		3=leaf-shaped
44	Number of pygidial cirri	0=absent
		1=one pair
		2=two or more pairs
45	Ventral ciliary field	0=absent
		1=present
46	Prostomial ciliation lateral groups between lateral antennae and palps	0=absent
		1=present
47	Dorsal transverse body ciliation	0=absent
		1=present
48	Arrangement of dorsal transverse body ciliation	0=absent
		1=two groups of cilia
		2=a maximum of more than two groups
		3=continuous rows of cilia
		4=dorso-lateral bands between parapodia
49	Ventral transverse body ciliation	0=absent
		1=present
50	Arrangement of ventral transverse body ciliation	0=absent
		1=two groups of cilia
		2=a maximum of more than two groups
		3=continuous rows of cilia
		4=ventro-lateral bands between parapodia
51	Nuchal organs	0=absent
		1=present
		2=internalized
52	Nuchal organ structure	0=pits or grooves
		1=other form
53	Branchial ocelli	0=absent
		1=present
54	Eyes	0=absent
		1=present
55	Number of eyes	0=absent
		1=single pair
		2=double pair
56	Multiciliary eye	0=absent
		1=present
57	Number of multiciliary eyes	0=absent
		1=single pair
		2=double pairs
58	Chaetae	0=absent
		1=present

59	Internatized supporting chaetae	0=absent 1=present
60	Compound chaetae	0=absent 1=present
61	Compound notochaetae	0=absent 1=present
62	Uncini hooks	0=absent 1=present
63	Shape hooks	0=absent 1=swan-shaped
64	Forked chaetae	0=absent 1=present
65	Whipped chaetae	0=absent 1=present
66	Bilimbate chaetae first body segment	0=absent 1=one 2=two 3=three
67	Bilimbate chaetae second body segment	0=absent 1=two 2=three 3=four
68	Bilimbate chaetae other body segment	0=absent 1=two 2=four
69	Crenulated capillary chaetae	0=absent 1=present
70	Anterior parapodia thick modified spines	0=absent 1=present
71	Thoracic neuropodia special chaetae	0=absent 1=present
72	Stomodaeum	0=absent 1=ventral buccal organ
73	Dorsolateral folds	0=absent 1=present
74	Gut	0=straight 1=lateral folds
75	Body distinct thorax-abdomen	0=absent 1=present

Supplementary Table 6. Data matrix of morphological characters used in the analyses comparing *Stygocapitella* with Orbiniidae and Nerillidae.

Taxon	Species	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Orbiniidae	*Leitoscoloplos fragilis*	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	2	2	0	0	0	1	3
	Leodamas johnstonei	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	2	2	0	0	0	0	0
	Methanoaricia dendrobranchiata	1	0	0	2	3	0	0	0	0	0	0	0	0	0	0	0	0	1	2	2	0	0	0	0	0
	Naineris quadricuspida	1	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	1	2	2	0	0	0	0	0
	Orbinia swani	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	2	2	0	0	0	0	0
	Orbiniella plumisetosa	1	0	0	1	2	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0
	Pettibonella multiuncinata	1	0	0	1	2	0	0	0	0	0	0	0	0	0	0	0	0	1	2	2	0	0	0	0	0
	Phylo michaelseni	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	2	2	0	0	0	1	3
	Proscoloplos cygnochaetus	1	0	0	1	2	0	0	0	0	0	0	0	0	0	0	0	0	1	2	2	0	0	0	0	0
	Protoaricia oerstedii	1	0	0	1	2	0	0	0	0	0	0	0	0	0	0	0	0	1	2	2	0	0	0	0	0
	Questa paucibranchiata	1	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0
	Scoloplos armiger	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	2	2	0	0	0	0	0
Parergodrilidae	*Stygocapitella minuta*	1	0	3	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Stygocapitella australis	1	0	1	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Stygocapitella EP A	1	0	1	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Stygocapitella EP C	1	0	1	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Stygocapitella EP D	1	0	1	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Stygocapitella WA	1	0	1	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Stygocapitella EA A	1	0	1	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Stygocapitella EA B	1	0	1	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Stygocapitella EA C	1	0	1	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nerillidae	*Aristonerilla brevis*	1	1	4	2	0	0	0	0	1	1	1	1	1	2	1	1	1	1	1	1	0	0	0	1	2
	Leptonerilla prospera	1	1	2	2	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	1	1
	Longipalpa saltatrix	1	1	3	2	0	0	0	0	1	1	1	1	2	1	1	2	1	1	1	1	0	0	0	1	2
	Meganerilla swedmarki	1	1	2	2	0	0	0	0	1	1	1	0	0	0	0	0	1	1	1	1	0	0	0	1	2
	Mesonerilla roscovita	1	1	2	2	0	0	1	0	1	1	1	1	2	1	1	1	1	1	1	1	0	0	0	1	2
	Micronerilla minuta	1	1	3	2	0	0	0	0	1	1	1	1	1	2	1	1	1	1	1	1	0	0	0	1	1
	Nerillidium sp	1	1	3	2	0	0	0	0	1	1	1	0	0	0	1	1	1	1	1	1	0	0	0	1	2
	Paranerilla limicola	1	1	4	2	0	0	0	0	1	1	1	0	0	0	0	0	1	1	1	1	0	0	0	1	2
	Trochonerilla mobilis	1	1	3	2	0	0	0	0	1	1	1	1	2	1	1	2	1	1	1	1	0	0	0	1	2
	Troglochaetus beranecki	1	1	3	2	0	0	1	0	1	1	1	0	0	0	0	0	1	1	1	1	0	0	0	1	2
	Xenonerilla bactericola	1	1	2	2	0	0	1	0	1	1	1	0	0	0	0	0	1	1	1	1	0	0	0	1	2

Supplementary Table 6. Cont.

Taxon	Species	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50
Orbiniidae	*Leitoscoloplos fragilis*	0	2	3	1	0	1	1	1	0	1	0	0	0	1	1	1	1	1	2	0	0	0	0	0	0
	Leodamas johnstonei	0	0	0	0	0	0	1	1	0	0	0	0	0	1	1	1	1	1	2	0	0	0	0	0	0
	Methanoaricia dendrobranchiata	0	0	0	0	0	1	1	0	0	0	0	0	0	1	1	1	1	1	2	0	0	0	0	0	0
	Naineris quadricuspida	0	0	0	0	0	1	1	1	0	0	0	0	0	1	1	1	1	1	2	0	0	0	0	0	0
	Orbinia swani	0	0	0	0	0	1	1	1	1	1	0	0	0	1	1	1	1	1	2	0	0	0	0	0	0
	Orbiniella plumisetosa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	2	0	0	0	0	0	0
	Pettibonella multiuncinata	0	0	0	0	0	1	1	0	0	0	0	0	0	1	1	1	1	1	2	0	0	0	0	0	0
	Phylo michaelseni	0	2	3	1	0	0	1	1	1	1	0	0	0	1	1	1	1	1	2	0	0	0	0	0	0
	Proscoloplos cygnochaetus	0	0	0	0	0	1	1	0	0	0	0	0	0	1	1	1	1	1	2	0	0	0	0	0	0
	Protoaricia oerstedii	0	0	0	0	0	1	1	1	0	0	0	0	0	1	1	1	1	1	2	0	0	0	0	0	0
	Questa paucibranchiata	0	0	0	0	0	1	1	0	0	0	1	1	0	1	1	0	1	1	2	0	0	0	0	0	0
	Scoloplos armiger	0	0	0	0	0	1	1	1	0	1	0	0	0	1	1	1	1	1	2	0	0	0	0	0	0
Parergodrilidae	*Stygocapitella minuta*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Stygocapitella australis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Stygocapitella EP A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Stygocapitella EP C	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Stygocapitella EP D	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Stygocapitella WA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Stygocapitella EA A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Stygocapitella EA B	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Stygocapitella EA C	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nerillidae	*Aristonerilla brevis*	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	1	2	1	1	1	1	2	1	2
	Leptonerilla prospera	1	1	2	1	1	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	3	1	3
	Longipalpa saltatrix	0	2	1	3	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	2	1	2
	Meganerilla swedmarki	1	2	1	2	1	0	0	0	0	0	0	0	0	0	0	0	1	3	1	1	1	1	4	1	4
	Mesonerilla roscovita	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	2	1	2
	Micronerilla minuta	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	1	2	1	1	0	0	0	0	0
	Nerillidium sp	1	2	2	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	2
	Paranerilla limicola	1	3	2	1	1	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	0	1	3	1	3
	Trochonerilla mobilis	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	1	2	1	1	1	1	3	1	3
	Troglochaetus beranecki	0	3	2	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	1	1	1	0	0
	Xenonerilla bactericola	0	2	2	2	1	0	0	0	0	0	0	0	0	0	0	0	1	3	1	1	0	0	0	0	0

Supplementary Table 6. Cont.

Taxon	Species	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75
Orbiniidae	*Leitoscoloplos fragilis*	1	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	1	0	0	1	1	0	1
	Leodamas johnstonei	1	0	0	0	0	0	0	1	1	0	0	0	0	1	0	0	0	0	1	0	1	1	1	0	1
	Methanoaricia dendrobranchiata	1	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	1	0	1	1	1	0	0
	Naineris quadricuspida	1	0	0	1	1	1	1	1	1	0	0	0	0	1	0	0	0	0	1	0	1	1	1	0	1
	Orbinia swani	1	0	0	1	1	1	1	1	1	0	0	0	0	1	0	0	0	0	1	0	1	1	1	0	1
	Orbiniella plumisetosa	1	0	0	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	1	0	1	1	1	0	0
	Pettibonella multiuncinata	1	0	0	1	1	1	1	1	1	0	0	1	1	0	0	0	0	0	1	0	1	1	1	0	1
	Phylo michaelseni	1	0	0	0	0	0	0	1	1	0	0	0	0	1	0	0	0	0	1	1	1	1	1	0	1
	Proscoloplos cygnochaetus	1	0	0	0	0	0	0	1	1	0	0	1	1	0	0	0	0	0	1	0	1	1	1	0	0
	Protoaricia oerstedii	1	0	0	1	1	1	1	1	1	0	0	0	0	1	0	0	0	0	1	0	1	1	1	0	1
	Questa paucibranchiata	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	1	1	0	1
	Scoloplos armiger	1	0	0	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	1	0	1	1	1	0	1
Parergodrilidae	*Stygocapitella minuta*	2	0	0	0	0	0	0	1	0	0	0	0	0	0	1	3	3	2	0	0	0	1	0	0	0
	Stygocapitella australis	2	0	0	0	0	0	0	1	0	0	0	0	0	1	1	1	1	1	0	0	0	1	0	0	0
	Stygocapitella EP A	2	0	0	0	0	0	0	1	0	0	0	0	0	1	1	1	1	1	0	0	0	1	0	0	0
	Stygocapitella EP C	2	0	0	0	0	0	0	1	0	0	0	0	0	1	1	2	3	1	0	0	0	1	0	0	0
	Stygocapitella EP D	2	0	0	0	0	0	0	1	0	0	0	0	0	1	1	2	3	1	0	0	0	1	0	0	0
	Stygocapitella WA	2	0	0	0	0	0	0	1	0	0	0	0	0	1	1	2	2	1	0	0	0	1	0	0	0
	Stygocapitella EA A	2	0	0	0	0	0	0	1	0	0	0	0	0	1	1	2	3	1	0	0	0	1	0	0	0
	Stygocapitella EA B	2	0	0	0	0	0	0	1	0	0	0	0	0	1	1	2	2	1	0	0	0	1	0	0	0
	Stygocapitella EA C	2	0	0	0	0	0	0	1	0	0	0	0	0	1	1	2	2	1	0	0	0	1	0	0	0
Nerillidae	*Aristonerilla brevis*	1	0	0	1	1	1	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0	1	1	0	0
	Leptonerilla prospera	1	0	0	1	1	1	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0	1	1	0	0
	Longipalpa saltatrix	1	0	0	0	0	0	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0	1	1	0	0
	Meganerilla swedmarki	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0
	Mesonerilla roscovita	1	0	0	0	0	0	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0	1	1	0	0
	Micronerilla minuta	1	0	0	1	1	1	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0	1	1	0	0
	Nerillidium sp	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0
	Paranerilla limicola	1	0	0	0	0	0	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0	1	1	0	0
	Trochonerilla mobilis	1	0	0	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0
	Troglochaetus beranecki	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0
	Xenonerilla bactericola	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0

Supplementary Table 7. Accession numbers of 18S sequences used for the phylogenetic analysis in the analyses comparing *Stygocapitella* with Orbiniidae and Nerillidae.

Taxon	Species	Accession number
Orbiniidae	*Leitoscoloplos fragilis*	AY532360
	Leodamas johnstonei	AF508126
	Methanoaricia dendrobranchiata	AY532357
	Naineris quadricuspida	AY532361
	Orbinia swani	AY532363
	Orbiniella plumisetosa	AY532364
	Pettibonella multiuncinata	AY532359
	Phylo michaelseni	AY532362
	Proscoloplos cygnochaetus	AF448162
	Protoaricia oerstedii	AF508123
	Questa paucibranchiata	AF209464
	Scoloplos armiger	FJ612491
Parergodrilidae	*Stygocapitella minuta*	391_17
	Stygocapitella australis	392_01
	Stygocapitella EP_A	432_05
	Stygocapitella EP_C	430_01
	Stygocapitella EP D	433_03
	Stygocapitella WA	427_02
	Stygocapitella EA_A	439_01
	Stygocapitella EA_B	396_06
	Stygocapitella EA_C	422_01
Nerillidae	*Aristonerilla brevis*	AY859530
	Leptonerilla prospera	AY834758
	Longipalpa saltatrix	AY859531
	Meganerilla swedmarki	AY859537
	Mesonerilla roscovita	AY834757
	Micronerilla minuta	AY859533
	Nerillidium sp	AY859536
	Paranerilla limicola	AY859539
	Trochonerilla mobilis	AY834759
	Troglochaetus beranecki	AY859534
	Xenonerilla bactericola	AY859535

Supplementary Table 8. Description of the dataset including phylogenetic placement on the various clades. Partitioned refers to the partitioned phylogenetic model. concatenated to the concatenated dataset. Single genes refer to the single genes dataset. We include the length of ITS1 because samples with 1400+ bp were excluded from the analyses. EP_A=Pacific Clade A; EP_B = Pacific Clade B; PA_C = Pacific Clade C; PA_D=Pacific Clade D; WA= Atlantic American clade; EA_A = Europe Clade A; EA_B = Europe Clade B. Australia refers to Australian samples. South Africa to South African Samples. NA = Not Available; indist. = indistinguishable; ITS1 length refers to the total length of ITS1 in the trimmed dataset.

Site	ID	Combined	COI	16S	18S	ITS1	ITS1 length
4th July Beach	432_01	PC_D	EP_D	EP_D	NA	EP_D	938
4th July Beach	432_02	EP_B	EP_B	EP_B	EP_B	EP_B	759
4th July Beach	432_03	EP_A	EP_A	EP_A	EP_A	EP_A	725
4th July Beach	432_04	EP_B	NA	EP_B	NA	EP_B	759
4th July Beach	432_05	EP_A	EP_A	EP_A	EP_A	EP_A	725
4th July Beach	432_06	EP_A	EP_A	EP_A	EP_A	EP_A	725
4th July Beach	432_07	EP_B	EP_B	EP_B	EP_B	EP_B	759
4th July Beach	432_08	EP_B	EP_B	EP_B	EP_B	NA	759
4th July Beach	432_09	EP_B	EP_B	EP_B	EP_B	EP_B	759
4th July Beach	432_10	EP_D	EP_D	EP_D	EP_D	EP_D	938
Ardtoe	320_01	EA_A	EA_A	EA_A	NA	NA	NA
Ardtoe	320_02	EA_B	EA_B	EA_B	NA	NA	NA
Ardtoe	320_03	EA_A	NA	EA_A	NA	NA	NA
Ardtoe	320_04	EA_B	EA_B	EA_B	NA	NA	NA
Ardtoe	320_05	EA_A	EA_A	EA_A	NA	NA	NA
Ardtoe	320_06	EA_B	EA_B	EA_B	indist.	NA	1461
Ardtoe	320_07	EA_B	EA_B	EA_B	indist.	NA	1462
Ardtoe	320_08	EA_B	EA_B	EA_B	NA	NA	NA
Ardtoe	320_12	EA_B	EA_B	EA_B	indist.	NA	1461
Ardtoe	320_13	EA_B	EA_B	EA_B	indist.	NA	1462
Ardtoe	320_14	EA_B	EA_B	EA_B	NA	NA	1465
Ardtoe	320_15	EA_B	NA	EA_B	NA	NA	1462
Bakka	439_01	EA_A	NA	EA_A	EA_A	NA	NA
Bakka	439_03	EA_A	EA_A	EA_A	EA_A	NA	NA
Bakka	439_07	EA_A	NA	EA_A	NA	NA	NA
Bakka	439_08	EA_A	NA	EA_A	NA	NA	NA
Bristol Channel	422_01	EA_C	EA_C	EA_C	indist.	EA_C	954
Bristol Channel	422_02	EA_C	NA	EA_C	indist.	EA_C	954
Bristol Channel	422_03	EA_C	NA	EA_C	indist.	EA_C	954
Bristol Channel	422_04	EA_C	EA_C	EA_C	indist.	EA_C	952
Bristol Channel	422_05	EA_C	NA	EA_C	NA	NA	NA
Bristol Channel	422_06	EA_C	EA_C	EA_C	NA	EA_C	954
Bristol Channel	422_07	EA_C	EA_C	EA_C	NA	EA_C	954
Bristol Channel	422_08	EA_C	EA_C	EA_C	NA	EA_C	954
Bristol Channel	422_09	EA_C	NA	EA_C	NA	EA_C	953
Bristol Channel	422_10	EA_C	EA_C	EA_C	NA	EA_C	954
Bristol Channel	422_11	EA_C	EA_C	EA_C	NA	EA_C	953
Bristol Channel	422_12	EA_C	NA	EA_C	NA	NA	NA
Canoe Beach	426_01	WA	WA	WA	indist.	WA	944
Canoe Beach	426_02	WA	WA	NA	indist.	WA	943
Canoe Beach	426_03	WA	WA	NA	indist.	WA	943
Canoe Beach	426_04	WA	WA	WA	indist.	WA	943
Canoe Beach	426_05	WA	WA	WA	NA	NA	NA
Canoe Beach	426_06	WA	WA	WA	NA	WA	943
Canoe Beach	426_07	WA	WA	WA	NA	WA	943
Canoe Beach	426_08	WA	WA	WA	NA	WA	943
Canoe Beach	426_09	WA	WA	WA	NA	WA	944
Canoe Beach	426_10	WA	NA	WA	NA	WA	943
Canoe Beach	426_11	WA	NA	WA	NA	WA	943
Canoe Beach	426_12	WA	NA	WA	NA	WA	944
Cutty Sark	423_01	EA_A	NA	NA	NA	EA_A	991
Cutty Sark	423_02	EA_A	NA	EA_A	NA	EA_A	991
Cutty Sark	423_03	EA_A	EA_A	EA_A	NA	NA	NA
Cutty Sark	423_04	EA_A	NA	EA_A	NA	NA	NA
Cutty Sark	423_06	EA_A	NA	EA_A	NA	NA	NA
Cutty Sark	423_07	EA_A	EA_A	EA_A	NA	NA	NA
Cutty Sark	423_08	EA_A	EA_A	EA_A	NA	NA	NA
Cutty Sark	423_09	EA_A	NA	EA_A	NA	NA	NA
Cutty Sark	423_10	EA_A	EA_A	EA_A	NA	EA_A	991
Ellenbogen	222_01	EA_C	EA_C	EA_C	indist.	EA_C	952
Ellenbogen	222_02	EA_C	EA_C	EA_C	indist.	EA_C	952
Ellenbogen	222_03	EA_C	EA_C	EA_C	indist.	EA_C	952

Ellenbogen	222_04	EA_C	EA_C	EA_C	indist.	EA_C	952
Ellenbogen	222_05	EA_C	EA_C	EA_C	NA	EA_C	952
Ellenbogen	222_06	EA_C	EA_C	NA	NA	EA_C	952
Ellenbogen	222_07	EA_C	EA_C	NA	NA	EA_C	952
Ellenbogen	222_08	EA_C	EA_C	EA_C	NA	EA_C	952
Ellenbogen	222_09	EA_C	EA_C	EA_C	NA	NA	NA
Ellenbogen	222_10	EA_C	EA_C	EA_C	NA	NA	NA
Ellenbogen	222_11	EA_C	EA_C	EA_C	NA	NA	NA
Ellenbogen	222_12	EA_C	EA_C	EA_C	NA	NA	NA
Ellenbogen	222_13	EA_C	EA_C	NA	NA	NA	NA
Espegrend	440_01	EA_A	EA_A	EA_A	EA_A	EA_A	991
Espegrend	440_02	EA_A	NA	EA_A	EA_A	EA_A	992
Espegrend	440_03	EA_A	NA	EA_A	NA	NA	NA
Espegrend	440_04	EA_A	NA	EA_A	NA	EA_A	991
False Bay	431_01	EP_B	EP_B	EP_B	EP_B	EP_B	759
Glenancross	321_01	EA_B	EA_B	EA_B	indist.	NA	NA
Glenancross	321_02	EA_B	EA_B	EA_B	indist.	NA	NA
Glenancross	321_03	EA_B	EA_B	EA_B	indist.	NA	NA
Glenancross	321_04	EA_B	EA_B	EA_B	indist.	NA	NA
Glenancross	321_05	EA_B	EA_B	EA_B	NA	NA	1461
Glenancross	321_06	EA_B	EA_B	EA_B	NA	NA	NA
Glenancross	321_07	EA_B	NA	EA_B	NA	NA	1461
Glenancross	321_08	EA_B	NA	EA_B	NA	NA	NA
Glenancross	321_09	EA_B	EA_B	EA_B	NA	NA	NA
Glenancross	321_10	EA_B	EA_B	EA_B	NA	NA	1461
Glenancross	321_11	EA_B	EA_B	EA_B	NA	NA	NA
Glenancross	321_12	EA_B	EA_B	EA_B	NA	NA	NA
Gnarabup Beach	392_01	*S. australis*	*S. australis*	*S. australis*	*S. australis*	NA	NA
Gnarabup Beach	392_03	*S. australis*	*S. australis*	*S. australis*	NA	NA	NA
Gnarabup Beach	392_04	*S. australis*	*S. australis*	*S. australis*	*S. australis*	NA	NA
Gnarabup Beach	392_05	*S. australis*	*S. australis*	*S. australis*	*S. australis*	NA	NA
Gnarabup Beach	392_06	*S. australis*	*S. australis*	*S. australis*	*S. australis*	NA	NA
Gnarabup Beach	392_07	*S. australis*	*S. australis*	*S. australis*	*S. australis*	NA	NA
Gravesend	424_01	EA_C	EA_C	EA_C	NA	EA_C	949
Gravesend	424_02	EA_C	EA_C	EA_C	NA	NA	NA
Gravesend	424_03	EA_C	EA_C	EA_C	NA	NA	NA
Gravesend	424_04	EA_C	EA_C	EA_C	NA	NA	NA
Gravesend	424_05	EA_C	EA_C	EA_C	NA	EA_C	949
Gravesend	424_06	EA_C	EA_C	EA_C	NA	EA_C	949
Gravesend	424_07	EA_C	EA_C	EA_C	NA	EA_C	953
Gravesend	424_08	EA_C	EA_C	EA_C	NA	EA_C	949
Gravesend	424_09	EA_C	EA_C	EA_C	NA	EA_C	949
Gravesend	424_10	EA_C	EA_C	EA_C	NA	EA_C	949
Gravesend	424_11	EA_C	EA_C	EA_C	NA	EA_C	953
Gravesend	424_12	EA_C	EA_C	EA_C	NA	EA_C	949
Gravesend	424_13	EA_C	EA_C	EA_C	NA	EA_C	953
Gravesend	424_14	EA_C	EA_C	EA_C	NA	NA	NA
Henningsvær	437_01	EA_A	NA	NA	NA	EA_A	998
Henningsvær	437_02	EA_A	NA	EA_A	NA	NA	NA
Henningsvær	437_03	EA_A	NA	EA_A	NA	EA_A	992
Henningsvær	437_05	EA_A	NA	EA_A	NA	NA	NA
Henningsvær	437_06	EA_A	NA	EA_A	NA	NA	NA
Henningsvær	437_07	EA_A	NA	EA_A	NA	NA	NA
Hörnum	169_06	EA_C	EA_C	EA_C	NA	EA_C	952
Hörnum	169_07	EA_C	EA_C	EA_C	NA	EA_C	952
Hörnum	169_08	EA_C	EA_C	EA_C	NA	NA	NA
Hörnum	169_09	EA_C	EA_C	EA_C	indist.	EA_C	953
Hörnum	169_10	EA_C	EA_C	EA_C	indist.	EA_C	952
Hörnum	169_11	EA_C	EA_C	NA	indist.	EA_C	953
Hörnum	169_12	EA_C	EA_C	EA_C	indist.	NA	NA
Hörnum	169_13	EA_C	EA_C	EA_C	NA	EA_C	953
Hörnum	169_14	EA_C	NA	EA_C	NA	EA_C	952
Hörnum	169_15	EA_C	EA_C	EA_C	NA	EA_C	952
Hörnum	169_16	EA_C	EA_C	EA_C	NA	EA_C	952
Hörnum	169_17	EA_C	EA_C	NA	NA	EA_C	952
Île Callot	210_10	EA_B	EA_B	NA	NA	NA	1461
Île Callot	210_11	EA_B	EA_B	EA_B	NA	NA	1460
Île Callot	210_12	EA_B	EA_B	EA_B	NA	EA_B	942
Île Callot	210_13	EA_B	EA_B	EA_B	NA	NA	1461
Île Callot	210_14	EA_B	EA_B	EA_B	NA	NA	1461
Île Callot	403_03	EA_B	EA_B	EA_B	indist.	EA_B	942
Île Callot	403_04	EA_B	EA_B	EA_B	indist.	NA	1461
Île Callot	403_05	EA_B	EA_B	EA_B	indist.	NA	1461
Île Callot	403_06	EA_B	NA	EA_B	NA	NA	1461
Île Callot	403_07	EA_B	EA_B	EA_B	NA	NA	1461
Île Callot	403_08	EA_B	EA_B	EA_B	NA	NA	NA
Île Callot	403_09	EA_B	NA	EA_B	NA	NA	NA

Keitum	169_28	EA_B	NA	EA_B	NA	NA	NA
Keitum	169_29	EA_B	EA_B	EA_B	NA	NA	NA
Keitum	169_30	EA_B	EA_B	EA_B	NA	NA	NA
Keitum	169_31	EA_B	NA	EA_B	NA	NA	NA
Keitum	169_32	EA_B	NA	EA_B	NA	NA	NA
Keitum	169_33	EA_B	EA_B	EA_B	NA	NA	NA
Keitum	169_34	EA_B	EA_B	EA_B	NA	NA	NA
Keitum	169_35	EA_B	EA_B	EA_B	NA	NA	NA
Keitum	169_36	EA_B	EA_B	EA_B	NA	NA	NA
Keitum	169_37	EA_B	NA	EA_B	indist.	NA	NA
Keitum	169_38	EA_B	EA_B	EA_B	indist.	NA	1462
Keitum	169_39	EA_B	EA_B	EA_B	indist.	NA	1460
Keitum	398_04	EA_B	EA_B	EA_B	indist.	NA	1460
Keitum	398_05	EA_B	EA_B	EA_B	NA	NA	NA
Keitum	398_06	EA_B	EA_B	EA_B	NA	NA	NA
Keitum	398_07	EA_B	EA_B	EA_B	NA	NA	1460
Keitum	398_08	EA_B	EA_B	EA_B	NA	NA	NA
Keitum	398_09	EA_B	EA_B	EA_B	NA	NA	NA
Keitum	398_10	EA_B	EA_B	EA_B	NA	EA_B	942
Keitum	398_11	EA_B	EA_B	EA_B	NA	NA	1460
Kristineberg	420_01	EA_A	EA_A	EA_A	EA_A	NA	NA
Kristineberg	420_02	EA_A	NA	EA_A	EA_A	NA	NA
Kristineberg	420_03	EA_A	NA	EA_A	EA_A	EA_A	991
Kristineberg	420_04	EA_A	NA	EA_A	NA	EA_A	993
Kristineberg	420_05	EA_A	EA_A	EA_A	NA	EA_A	993
Kristineberg	420_06	EA_A	NA	EA_A	NA	EA_A	993
Kristineberg	420_07	EA_A	NA	EA_A	NA	EA_A	991
Kristineberg	420_09	EA_A	NA	EA_A	NA	NA	NA
Kristineberg	420_10	EA_A	NA	EA_A	NA	NA	NA
Kristineberg	420_12	EA_A	NA	EA_A	NA	EA_A	993
Langebaan	327_01	*S. minuta*	*S. minuta*	*S. minuta*	NA	NA	NA
Langebaan	327_02	*S. minuta*	*S. minuta*	*S. minuta*	NA	NA	NA
Langebaan	327_03	*S. minuta*	*S. minuta*	*S. minuta*	NA	NA	NA
Langebaan	327_04	*S. minuta*	*S. minuta*	*S. minuta*	NA	NA	NA
Langebaan	327_05	*S. minuta*	*S. minuta*	*S. minuta*	NA	NA	NA
Langebaan	327_06	*S. minuta*	*S. minuta*	*S. minuta*	NA	NA	NA
Langebaan	327_07	*S. minuta*	*S. minuta*	*S. minuta*	NA	NA	NA
Langebaan	327_08	*S. minuta*	*S. minuta*	*S. minuta*	NA	NA	NA
Langebaan	327_10	*S. minuta*	*S. minuta*	*S. minuta*	NA	NA	NA
Langebaan	327_11	*S. minuta*	*S. minuta*	*S. minuta*	NA	NA	NA
Langebaan	327_16	*S. minuta*	NA	NA	NA	NA	NA
Langebaan	327_17	*S. minuta*	NA	*S. minuta*	NA	NA	NA
Langebaan	327_18	*S. minuta*	NA	NA	NA	NA	NA
Langebaan	327_19	*S. minuta*	NA	*S. minuta*	NA	NA	NA
Langebaan	391_16	*S. minuta*	*S. minuta*	*S. minuta*	NA	NA	NA
Langebaan	391_17	*S. minuta*	*S. minuta*	*S. minuta*	*S. minuta*	NA	NA
Langebaan	391_18	*S. minuta*	*S. minuta*	*S. minuta*	*S. minuta*	NA	NA
Langebaan	391_19	*S. minuta*	*S. minuta*	NA	NA	NA	NA
List	169_54	EA_C	EA_C	EA_C	NA	EA_C	953
List	169_55	EA_B	EA_B	NA	NA	NA	1460
List	169_56	EA_C	EA_C	EA_C	indist.	EA_C	952
List	169_57	EA_B	EA_B	EA_B	NA	NA	1460
List	169_58	EA_C	EA_C	EA_C	indist.	EA_C	952
List	219_02	EA_C	NA	EA_C	indist.	NA	NA
List	219_03	EA_C	EA_C	EA_C	NA	EA_C	952
List	219_04	EA_C	NA	EA_C	NA	EA_C	952
List	219_05	EA_C	EA_C	EA_C	NA	EA_C	952
List	219_06	EA_C	EA_C	EA_C	NA	EA_C	952
List	219_07	EA_C	NA	EA_C	NA	EA_C	952
List	219_08	EA_C	EA_C	EA_C	NA	EA_C	952
List	219_09	EA_C	NA	EA_C	NA	EA_C	953
List	219_10	EA_C	EA_C	EA_C	NA	NA	NA
List	219_11	EA_C	EA_C	NA	NA	NA	NA
List	219_12	EA_C	NA	EA_C	NA	NA	NA
List	219_13	EA_C	NA	EA_C	NA	NA	NA
Little Gruinard	322_01	EA_B	EA_B	EA_B	NA	NA	NA
Little Gruinard	322_02	EA_B	EA_B	EA_B	NA	NA	NA
Lødingen	436_01	EA_A	NA	EA_A	NA	NA	NA
Lødingen	436_02	EA_A	NA	EA_A	NA	NA	NA
Lødingen	436_03	EA_A	NA	EA_A	NA	EA_A	991
Lødingen	436_04	EA_A	NA	EA_A	NA	NA	NA
Lødingen	436_05	EA_A	NA	EA_A	NA	NA	NA
Lødingen	436_07	EA_A	NA	EA_A	NA	EA_A	991
Lubec	429_01	EA_C	EA_C	EA_C	indist.	EA_C	949
Lubec	429_02	WA	WA	NA	indist.	WA	943
Lubec	429_03	WA	NA	WA	NA	WA	943
Lubec	429_04	WA	NA	WA	NA	NA	NA

Lubec	429_05	WA	NA	WA	NA	WA	943
Lubec	429_06	WA	NA	WA	NA	WA	943
Lubec	429_07	WA	NA	NA	NA	WA	943
Lubec	429_08	EA_C	EA_C	EA_C	indist.	EA_C	949
Lubec	429_09	WA	WA	WA	NA	WA	943
Lubec	429_10	WA	WA	WA	NA	WA	943
Lubec	429_11	WA	WA	WA	NA	WA	943
Lubec	429_12	WA	WA	WA	NA	NA	NA
Morsum	227_01	EA_B	EA_B	EA_B	indist.	NA	1460
Morsum	227_02	EA_B	EA_B	EA_B	indist.	NA	NA
Morsum	227_03	EA_B	EA_B	EA_B	indist.	NA	NA
Morsum	227_04	EA_B	EA_B	EA_B	indist.	NA	1460
Morsum	227_05	EA_B	EA_B	EA_B	indist.	NA	NA
Morsum	227_06	EA_B	EA_B	EA_B	NA	NA	1460
Morsum	227_07	EA_B	EA_B	EA_B	NA	NA	1460
Morsum	227_08	EA_B	EA_B	EA_B	NA	indist.	941
Morsum	227_09	EA_B	NA	EA_B	NA	NA	1460
Morsum	227_10	EA_B	EA_B	EA_B	NA	NA	NA
Morsum	227_14	EA_A	NA	EA_A	NA	NA	NA
Morsum	227_15	EA_A	NA	EA_A	NA	NA	NA
Musselburgh	324_01	EA_C	EA_C	EA_C	NA	NA	NA
Musselburgh	324_02	EA_C	EA_C	EA_C	NA	NA	NA
Musselburgh	324_03	EA_B	EA_B	EA_B	NA	NA	NA
Musselburgh	324_04	EA_A	EA_A	EA_A	EA_A	EA_A	991
Musselburgh	324_05	EA_B	EA_B	EA_B	NA	EA_B	942
Musselburgh	324_06	EA_C	EA_C	EA_C	NA	EA_C	952
Musselburgh	324_07	EA_C	EA_C	EA_C	NA	NA	NA
Musselburgh	324_08	EA_C	EA_C	EA_C	NA	NA	NA
Musselburgh	324_09	EA_C	EA_C	EA_C	NA	NA	NA
Musselburgh	324_10	EA_C	EA_C	EA_C	NA	NA	NA
Musselburgh	324_11	EA_C	EA_C	EA_C	NA	NA	NA
Musselburgh	324_12	EA_C	EA_C	EA_C	NA	NA	NA
Musselburgh	324_52	EA_C	EA_C	EA_C	NA	NA	NA
Nairn	323_01	EA_B	EA_B	EA_B	NA	NA	NA
Nairn	323_02	EA_B	EA_B	EA_B	NA	NA	NA
Nairn	323_03	EA_B	EA_B	EA_B	NA	NA	NA
Nairn	323_04	EA_B	EA_B	EA_B	indist.	EA_B	942
Plymouth	421_01	EA_C	EA_C	EA_C	indist.	EA_C	947
Plymouth	421_02	EA_C	EA_C	EA_C	NA	NA	NA
Plymouth	421_03	EA_C	EA_C	EA_C	NA	EA_C	948
Plymouth	421_04	EA_C	EA_C	EA_C	NA	EA_C	NA
Plymouth	421_05	EA_C	EA_C	EA_C	NA	EA_C	947
Plymouth	421_06	EA_C	EA_C	EA_C	NA	EA_C	953
Plymouth	421_07	EA_C	NA	EA_C	NA	NA	NA
Plymouth	421_08	EA_C	EA_C	EA_C	NA	NA	NA
Plymouth	421_09	EA_C	EA_C	EA_C	NA	NA	NA
Plymouth	421_10	EA_C	EA_C	EA_C	NA	EA_C	947
Plymouth	421_11	EA_C	NA	EA_C	NA	NA	NA
Plymouth	421_13	EA_C	EA_C	EA_C	NA	EA_C	947
Plymouth	421_14	EA_C	EA_C	EA_C	NA	EA_C	947
Plymouth	421_15	EA_C	EA_C	EA_C	NA	NA	NA
Reid State Park	427_01	WA	WA	WA	indist.	WA	943
Reid State Park	427_02	WA	WA	WA	indist.	WA	943
Reid State Park	427_03	WA	WA	WA	indist.	WA	943
Reid State Park	427_04	WA	WA	WA	indist.	WA	943
Reid State Park	427_05	WA	WA	WA	NA	NA	NA
Reid State Park	427_06	WA	WA	WA	NA	WA	943
Reid State Park	427_07	WA	WA	WA	NA	WA	943
Reid State Park	427_08	WA	WA	WA	NA	WA	943
Reid State Park	427_09	WA	WA	WA	NA	WA	944
Reid State Park	427_10	WA	WA	WA	NA	WA	943
Reuben Tarte	433_01	EP_D	EP_D	EP_D	EP_D	EP_D	938
Reuben Tarte	433_02	EP_D	EP_D	EP_D	EP_D	EP_D	938
Reuben Tarte	433_03	EP_D	EP_D	EP_D	EP_D	EP_D	938
Reuben Tarte	433_04	EP_D	EP_D	EP_D	EP_D	EP_D	938
Reuben Tarte	433_05	EP_D	EP_D	EP_D	EP_D	EP_D	938
Reuben Tarte	433_06	EP_D	EP_D	EP_D	NA	EP_D	938
Reuben Tarte	433_07	EP_D	EP_D	EP_D	NA	NA	NA
Reuben Tarte	433_08	EP_D	EP_D	EP_D	NA	EP_D	938
Reuben Tarte	433_09	EP_D	EP_D	EP_D	NA	EP_D	938
Reuben Tarte	433_10	EP_D	EP_D	EP_D	EP_D	NA	NA
Reuben Tarte	433_11	EP_D	NA	EP_D	NA	EP_D	939
Reuben Tarte	433_12	EP_D	EP_D	EP_D	NA	EP_D	938
Roche Harbor	430_01	EP_C	EP_C	EP_C	EP_C	EP_C	936
Roche Harbor	430_02	EP_C	NA	NA	EP_C	EP_C	936
Roche Harbor	430_03	EP_C	EP_C	EP_C	EP_C	EP_C	936
Roche Harbor	430_04	EP_C	EP_C	EP_C	EP_C	EP_C	936

Roche Harbor	430_05	EP_C	EP_C	EP_C	EP_C	EP_C	936
Roche Harbor	430_06	EP_C	EP_C	EP_C	NA	EP_C	942
Roche Harbor	430_07	EP_C	EP_C	EP_C	NA	EP_C	936
Roche Harbor	430_08	EP_C	EP_C	EP_C	NA	EP_C	936
Roche Harbor	430_09	EP_C	EP_C	EP_C	NA	EP_C	936
Roche Harbor	430_10	EP_C	EP_C	EP_C	NA	EP_C	936
Sarge Bay	393_01	*S. australis*	*S. australis*	*S. australis*	*S. australis*	NA	NA
Sarge Bay	393_02	*S. australis*	*S. australis*	*S. australis*	*S. australis*	NA	NA
Sarge Bay	393_03	*S. australis*	*S. australis*	*S. australis*	*S. australis*	NA	NA
Sarge Bay	393_04	*S. australis*	*S. australis*	*S. australis*	*S. australis*	NA	NA
Sarge Bay	393_05	*S. australis*	*S. australis*	*S. australis*	*S. australis*	NA	NA
Sarge Bay	393_06	*S. australis*	*S. australis*	*S. australis*	NA	NA	NA
Schilksee	396_01	EA_B	EA_B	EA_B	indist.	NA	1460
Schilksee	396_02	EA_B	EA_B	EA_B	indist.	NA	1460
Schilksee	396_04	EA_B	EA_B	EA_B	indist.	EA_B	942
Schilksee	396_05	EA_B	EA_B	EA_B	indist.	EA_B	942
Schilksee	396_06	EA_B	EA_B	EA_B	indist.	NA	NA
Sommarøya	438_01	EA_A	NA	EA_A	NA	NA	NA
Sommarøya	438_02	EA_A	NA	EA_A	EA_A	NA	NA
South Lubec	428_01	WA	WA	WA	indist.	WA	943
South Lubec	428_02	WA	WA	WA	indist.	WA	943
South Lubec	428_03	WA	WA	WA	indist.	WA	945
St. Efflam	210_01	EA_C	EA_C	NA	indist.	EA_C	947
St. Efflam	210_02	EA_C	EA_C	EA_C	indist.	EA_C	952
St. Efflam	210_03	EA_C	EA_C	EA_C	indist.	EA_C	947
St. Efflam	210_04	EA_C	EA_C	EA_C	NA	EA_C	947
St. Efflam	210_05	EA_C	EA_C	EA_C	NA	NA	NA
St. Efflam	210_06	EA_C	EA_C	NA	NA	EA_C	947
St. Efflam	210_07	EA_C	NA	EA_C	NA	EA_C	947
St. Efflam	401_01	EA_C	NA	EA_C	NA	EA_C	948
St. Efflam	401_02	EA_C	EA_C	EA_C	NA	EA_C	947
St. Efflam	401_03	EA_C	EA_C	EA_C	NA	NA	NA
St. Efflam	401_04	EA_C	EA_C	EA_C	NA	EA_C	947
St. Efflam	401_05	EA_C	EA_C	EA_C	NA	NA	NA
St. Efflam	401_06	EA_C	EA_C	EA_C	NA	EA_C	947
St. Efflam	401_07	EA_C	EA_C	EA_C	NA	EA_C	948
Weststrand	169_01	EA_B	EA_B	NA	NA	NA	NA

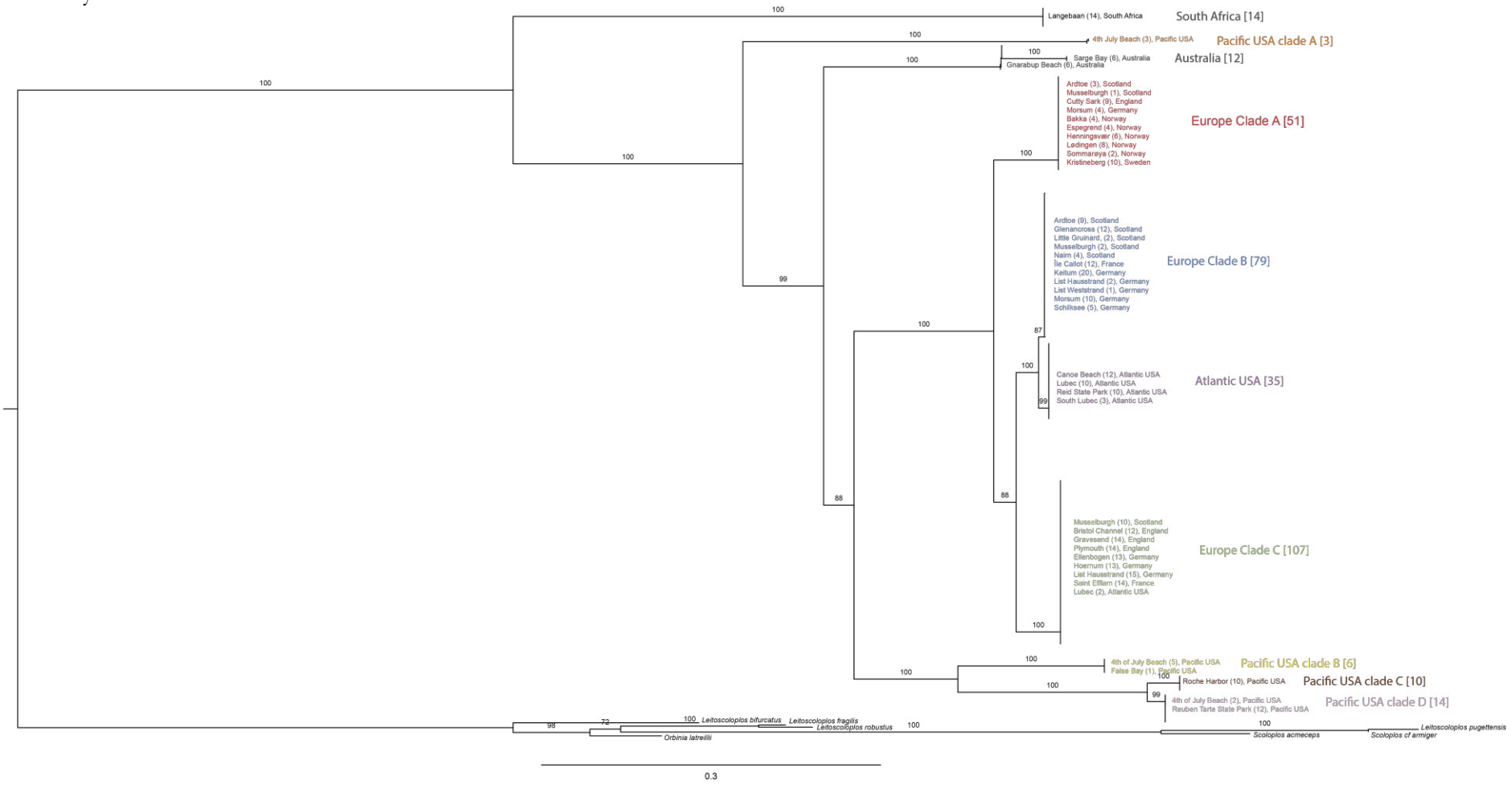
Supplementary Table 9. Pairwise contrasts between European clades (red, blue and green) considering various climatic variables. The results of multinomial logistic regression models are presented. Models included only one fixed variable and as a result, 19 models were fit.. Degrees of freedom 1 and 2 (*Df1-2*), F ratio and *p* significance values are provided. No statistical significances ($p < 0.05$) were obtained.

Pairwise Contrast			**EA_A - EA_B**		**EA_A - EA_C**		**EA_B - EA_C**	
Fixed Variable	***Df1***	***Df2***	**F ratio**	***p***	**F ratio**	***p***	**F ratio**	***p***
Annual Mean Temp.	1	4	0.092	0.78	1.548	0.28	2.109	0.22
Mean Diurnal Range	1	4	0.088	0.78	0.081	0.79	0.000	0.99
Isothermality	1	4	0.036	0.86	0.072	0.80	0.007	0.94
Temp. Seasonality	1	4	0.017	0.90	0.049	0.84	0.009	0.93
Max. Temp. of the Warmest Month	1	4	0.027	0.88	0.260	0.64	0.433	0.55
Min. Temp. of the Coldest Month	1	4	0.177	0.70	0.587	0.49	1.200	0.33
Temp. Annual Range	1	4	0.002	0.96	0.011	0.92	0.003	0.96
Mean Temp. of Wettest Quarter	1	4	0.115	0.75	0.011	0.92	0.199	0.68
Mean Temp. of Driest Quarter	1	4	0.040	0.85	0.160	0.71	0.042	0.85
Mean Temp of Warmest Quarter	1	4	0.041	0.85	0.738	0.44	0.929	0.39
Mean Temp. of Coldest Quarter	1	4	0.183	0.69	0.253	0.64	0.768	0.43
Annual Precipitation	1	4	0.020	0.89	0.155	0.71	0.070	0.80
Precipitation of Wettest Month	1	4	0.006	0.94	0.130	0.74	0.086	0.78
Precipitation of Driest Month	1	4	0.031	0.87	0.123	0.74	0.034	0.86
Precipitation Seasonality	1	4	0.048	0.84	0.057	0.82	0.000	0.99
Precipitation of Wettest Quarter	1	4	0.020	0.89	0.130	0.74	0.053	0.83
Precipitation of Driest Quarter	1	4	0.024	0.88	0.127	0.74	0.045	0.84
Precipitation of Warmest Quarter	1	4	0.043	0.85	0.279	0.63	0.117	0.75
Precipitation of Coldest Quarter	1	4	0.034	0.86	0.073	0.80	0.008	0.93

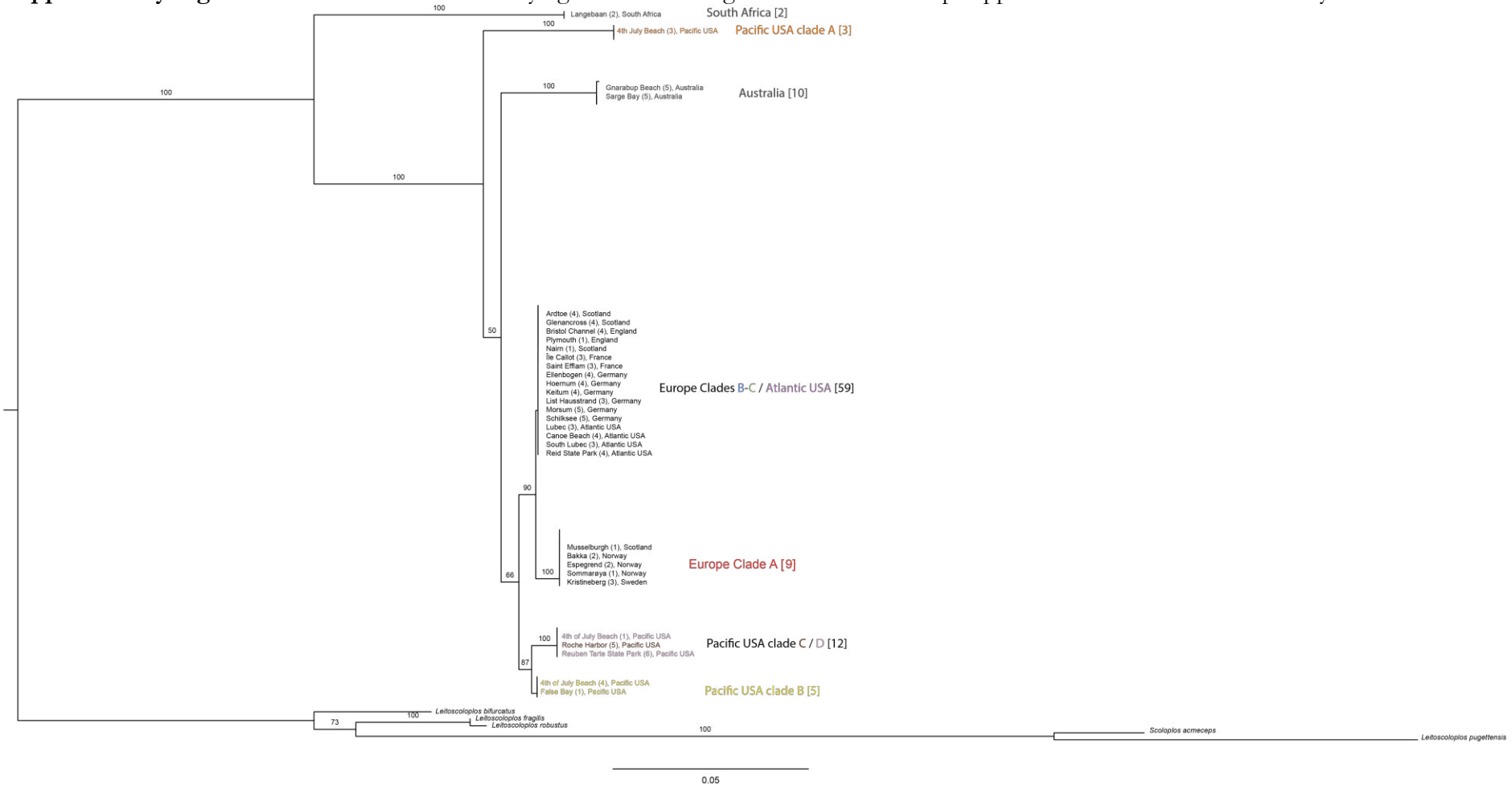
Supplementary Table 10. Calculations of Tajima's D and its correspondent P-value for the three studied European clades. The Atlantic Pacific America is excluded due to the complete absence of polymorphisms in all four populations. Tajima's D was not calculated for populations with less than 4 individuals were ("Not enough samples"), and populations without no data polymorphism (i.e. all individual sequences being exactly identical) are signaled ("all data identical").

	EA_A		**EA_B**		**EA_C**	
	Tajima's D	***p***	**Tajima's D**	***p***	**Tajima's D**	***p***
Ardtoe	Not enough samples		1.26455	>0.1		
Bakka	**All data identical**					
Bristol Channel					-1.79631	***<0.05***
Cutty Sark	0.20364	>0.1				
Ellenbogen					-0.27492	>0.1
Espegrend	Not enough samples					
Glenancross			-1.89423	***<0.05***		
Gravesend					0.51918	>0.1
Henningsvær	-0.81650	>0.1				
Hörnum					-1.23716	>0.1
Île Callot			**All data identical**			
Keitum			-1.76237	0.05 - 0.1		
Kristineberg	**All data identical**					
List			Not enough samples		0.81980	>0.1
Little Gruinard			Not enough samples			
Lødingen	**All data identical**					
Lubec					Not enough samples	
Morsum			**All data identical**			
Musselburgh	Not enough samples		Not enough samples		-1.11173	>0.1
Nairn			**All data identical**			
Plymouth					1.95522	0.05 - 0.1
St. Efflam					-1.19267	>0.1
Schilksee			Not enough samples			
Sommarøya	Not enough samples					

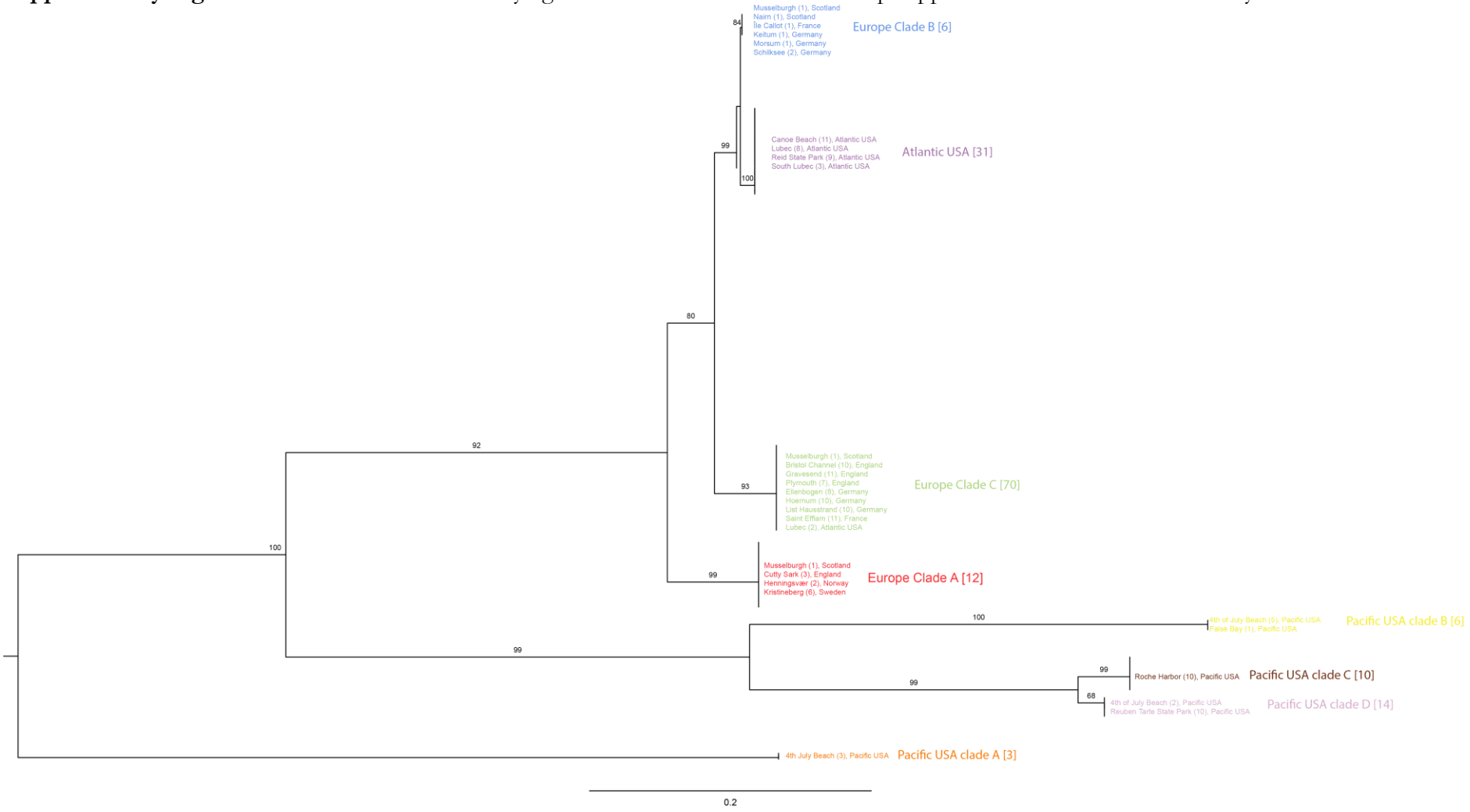
Supplementary Figure 1: Maximum Likelihood Phylogram of the partitioned dataset including the four genes. Bootstrap support values are included for every branch.



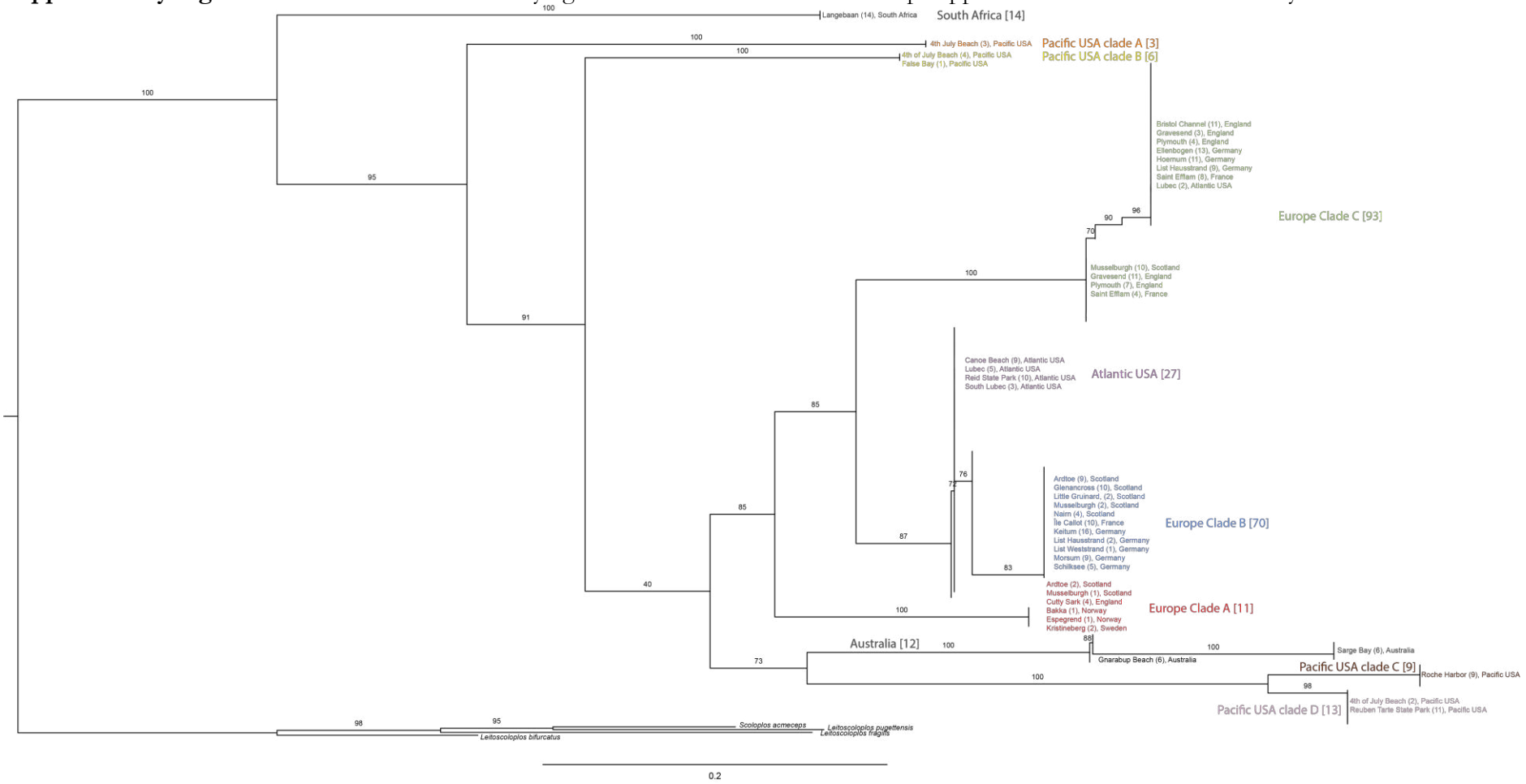
Supplementary Figure 2: Maximum Likelihood Phylogram of the 18S gene dataset. Bootstrap support values are included for every branch.



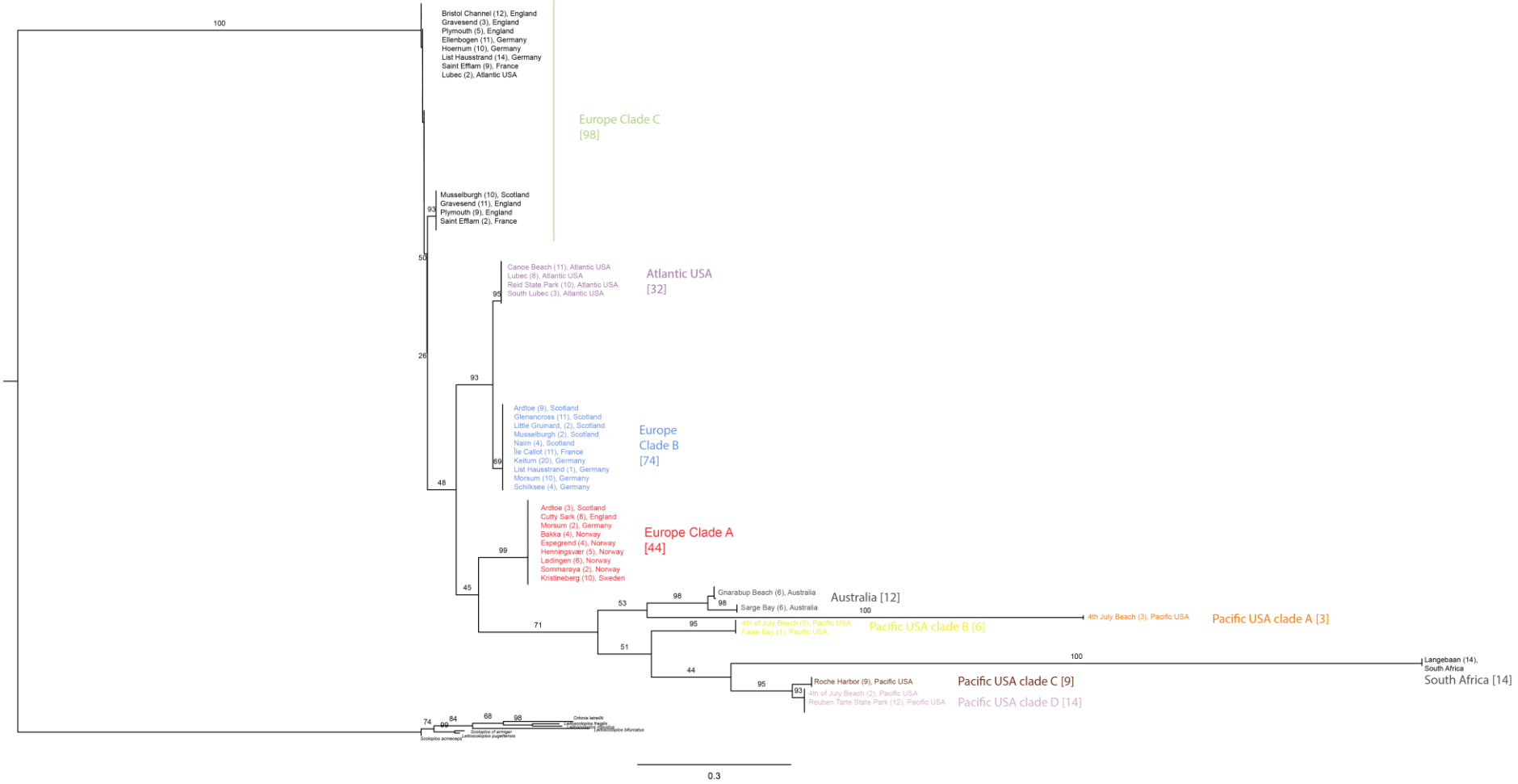
Supplementary Figure 3: Maximum Likelihood Phylogram of the ITS1 dataset. Bootstrap support values are included for every branch.



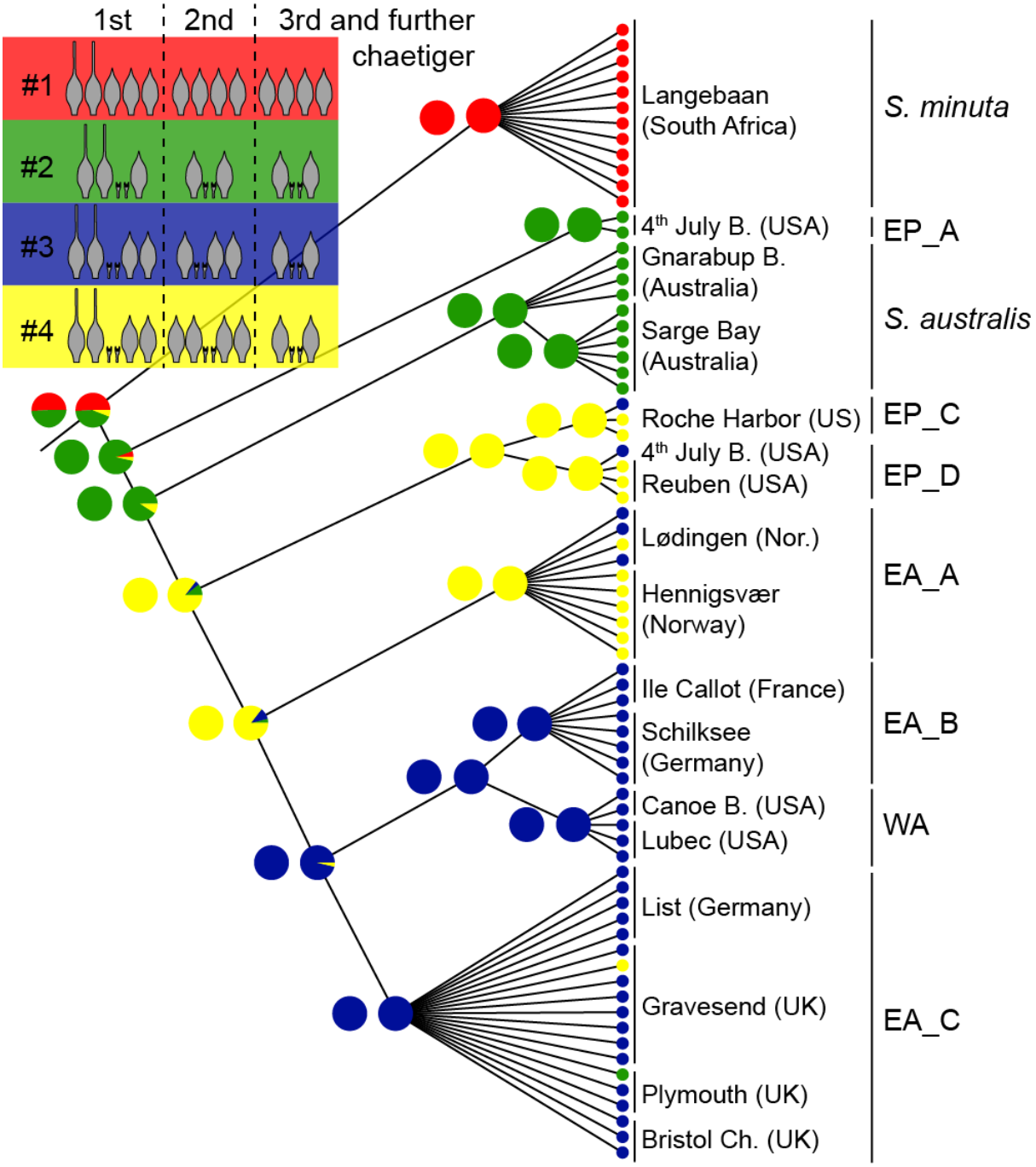
Supplementary Figure 4: Maximum Likelihood Phylogram of the COI dataset. Bootstrap support values are included for every branch.



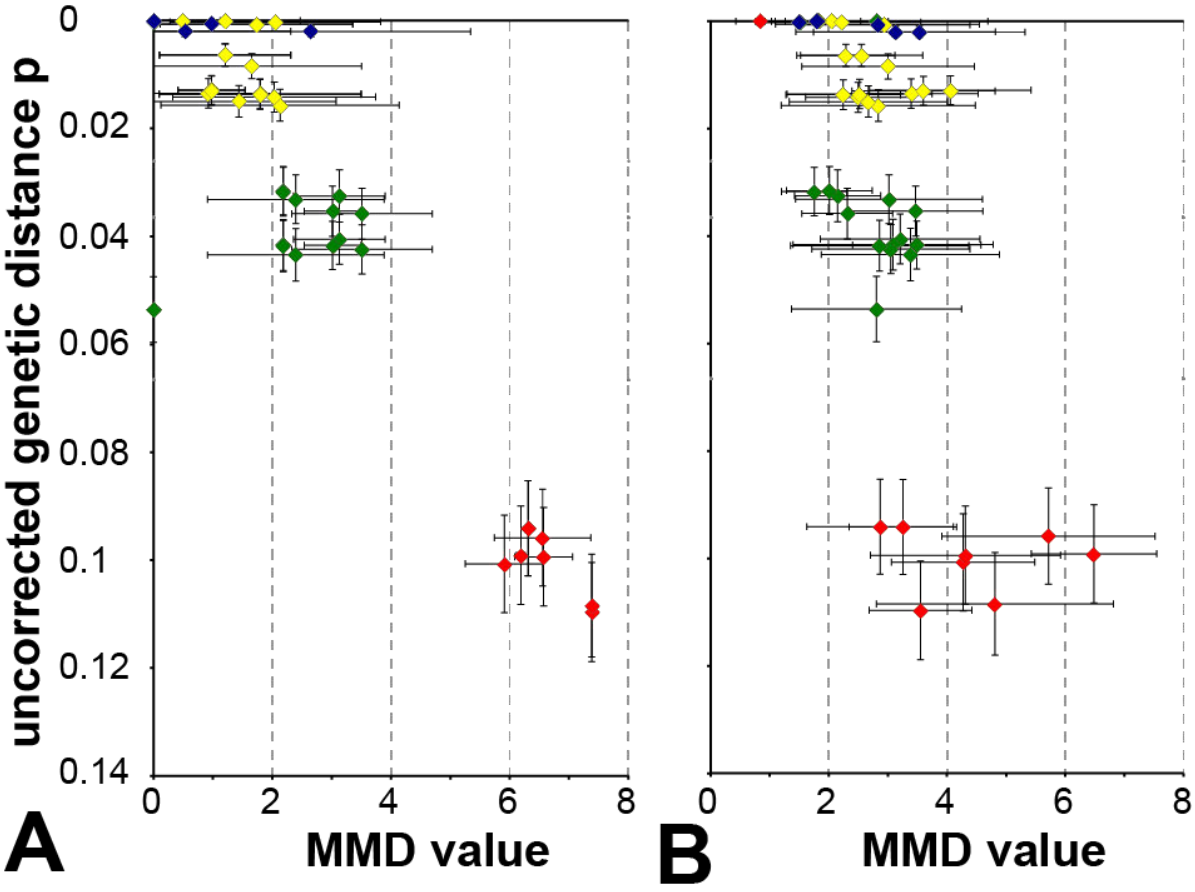
Supplementary Figure 5: Maximum Likelihood Phylogram of the 16S dataset. Bootstrap support values are included for every branch.



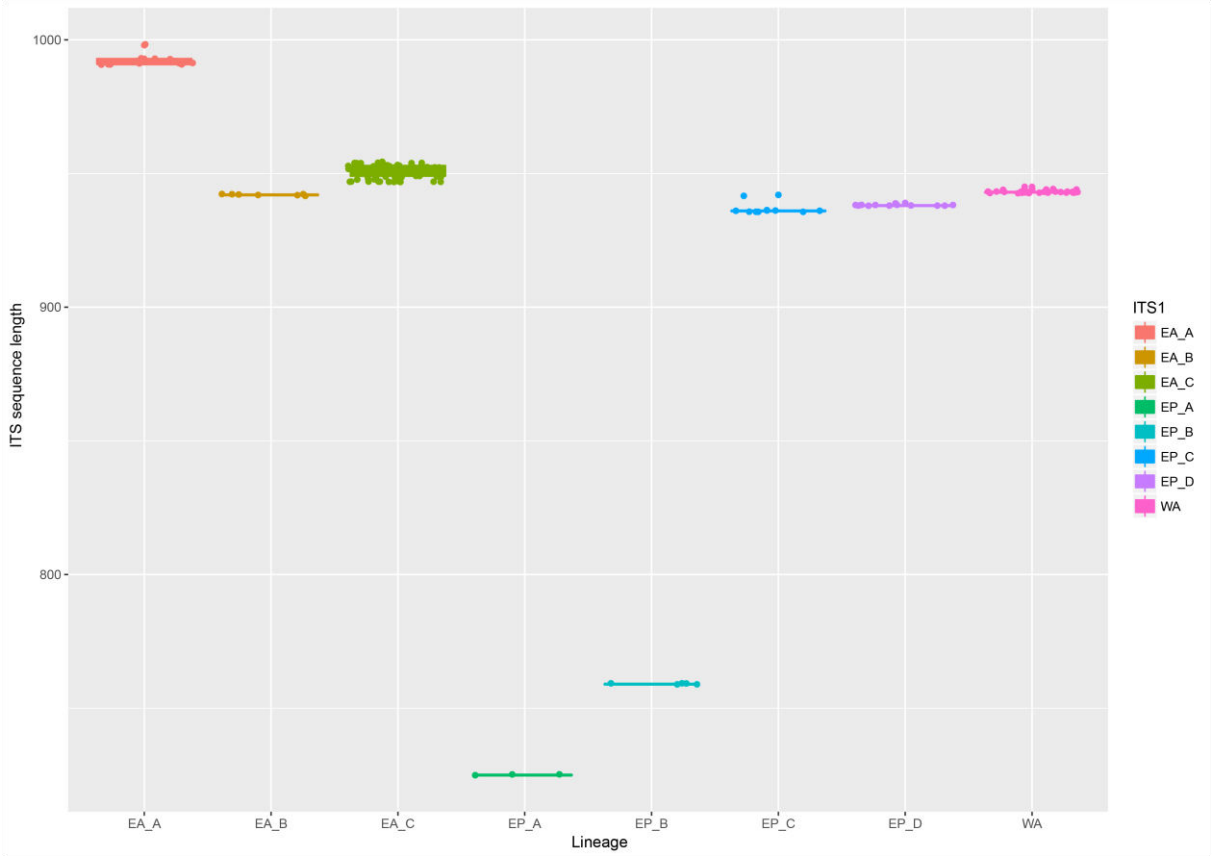
Supplementary Figure 6: Ancestral character reconstruction of morphological characters. Four phenotypes based on the chaetal pattern of the first three chaetae can be distinguished (top left). Red denotes phenotype #1, green denotes phenotype #2, blue denotes phenotype 3, yellow denotes phenotype #4. On the tips we include information of the sampling site, aswell as the country. EC_A = Europe Clade A; EC_B = Europe Clade B; EC_C = Europe Clade C; AA = Atlantic America; PC_A = Pacific Clade A; PC_B = Pacific Clade B; PC_C = Pacific Clade C; PC_D = Pacific clade D



Supplementary Figure 7: Phenotypic and genetic divergence in *Stygocapitella*. A) Morphological disparity for qualitative traits; B) Morphological disparity for quantitative traits. These values result from a Multidimensional morphological disparity (MMD) index plotted against uncorrelated genetic distances of the 18S gene. Red, green, blue and yellow diamonds correspond to different morphortypes (#1, #2, #3, #4), see main text for a through description.



Supplementary Figure 8: ITS length across different clades. EC_A = Europe Clade A; EC_B = Europe Clade B; EC_C = Europe Clade C; AA = Atlantic America; PC_A = Pacific Clade A; PC_B = Pacific Clade B; PC_C = Pacific Clade C; PC_D = Pacific Clade D. In Europe Clade B we display only sequences with 900-1000 basepairs (see main text)



Manuscript 4

Delimitation of cryptic species drastically reduces the geographical of marine interstitial ghost-worms (Stygocapitella; Annelida, Sedentaria)

Molecular phylogenetics and evolution

Delimitation of cryptic species drastically reduces the geographical ranges of marine interstitial ghost-worms (*Stygocapitella*; Annelida, Sedentaria)

José Cerca [a]*, Christian Meyer [b], Günter Purschke [b], Torsten H. Struck [a]

[a] Frontiers of Evolutionary Zoology Research Group, Natural History Museum, University of Oslo, 0562 Oslo, Norway

[b] Zoology and Developmental Biology, Department of Biology and Chemistry, University of Osnabrück, Barbarastr. 11, 49069 Osnabrück, Germany

* Corresponding author: José Cerca; jose.cerca@gmail.com; ORCID 0000-0001-7788-4367

Abstract

The recognition of cryptic species concealed in traditionally established species can reveal new biogeographical patterns and alter the understanding of how biodiversity is geographically distributed. This is particularly relevant for marine ecosystems where the incidence of cryptic species is high and where species distribution data are often challenging to collect and interpret. Here, we studied specimens of the 'cosmopolitan' interstitial meiofaunal annelid *Stygocapitella subterranea* Knöllner, 1934 (Parergodrilidae, Orbiniida), obtaining data from four coastlines in the Northern hemisphere. Using phylogenetic tools and several species-delimitation methods (haplotype networks, GMYC, bPTP, maximum likelihood, posterior probability and morphology) we describe eight new *Stygocapitella* species. With one exception, all species are present along a single coastline, ultimately challenging the idea that *Stygocapitella subterranea* has a cosmopolitan distribution. We found evidence for several oceanic transitions having occurred in the past as well as a recent translocation, potentially due to human activity. No diagnostic characters were found, and qualitative and quantitative morphological data do not allow an unequivocal differentiation of the identified cryptic species. This suggests that (i) neither traditional diagnostic features nor quantitative morphology suffice to recognise species boundaries in cryptic species complexes, such as the *Stygocapitella* species complex; and that (ii) the recognition and description of cryptic species is of seminal importance for biodiversity assessments, biogeography and evolutionary biology.

Keywords

Sibling species; biogeography; marine connectivity; phylogenetics; distribution range; dispersal

1. Introduction

Species distribution data provide a valuable proxy to understand patterns of biodiversity occurrence across the globe, the influence of past geological events on taxa, but also of human impact on biological communities (Holt et al., 2013). However, quantifying biodiversity and species distributions can be challenging (Knowlton, 2000, 1993; Leray and Knowlton, 2016). For instance, in marine environments, sampling of biodiversity often requires expensive equipment and big teams. Boundaries and biogeographic barriers are often hard to determine, and the patchiness of marine populations combined with the wide area occupied by oceans contributes to difficult sampling and collection. Species delimitation and identification are often compromised because organisms can be deformed following their collection and extraction from the water, due to preservation practices, or because they require sound taxonomic expertise, including adult features and unequivocal diagnostic characters (Cerca et al., 2018; Hellberg, 2009; Knowlton, 1993; Sterrer, 1973). As a result, our understanding of evolutionary and ecological processes as well as biogeographic patterns at the sea is severely diminished (Hellberg, 2009; Johannesson, 1988; Knowlton, 1993).

Cryptic species add another layer of complexity to biodiversity assessments (Knowlton, 1993; Pante et al., 2015) and to the determination of species' distributions (Cerca et al., 2018; Knowlton, 2000, 1993). The debate whether cryptic species result from natural phenomena or taxonomic artefacts has recently sparked attention (Fišer et al., 2018; Korshunova et al., 2019, 2017; Pante et al., 2015). A recent view has argued that the uncertainty in this debate essentially derives from placing the focus on the taxonomic history of the species complex, rather than focusing on the accumulation of morphological disparity through time or lack thereof (Struck et al., 2018a, 2018b; Struck and Cerca, 2019). To distinguish between these two aspects, it was suggested that species should be first investigated and delimited using all available data (e.g. morphological, ecological and behavioural data). The assignment of the 'cryptic' status, should then follow, but only after and independent from the species delimitation process. In this step, one should quantify morphological disparity. The status of 'cryptic species' should only be attributed if the species under consideration are morphologically more similar to each other than expected, given the time since species divergence. The comparison with closely related outgroups, displaying clear morphological differences is beneficial to this procedure as it allows gauging the degree of morphological disparity in closely related lineages (for a more detailed discussion of the problems in assigning cryptic species please see (Struck et al., 2018a, 2018b; Struck and Cerca, 2019). These guidelines allow distinguishing species which are morphologically

decelerated (i.e., cryptic species) from cryptic species complexes resulting from taxonomic artefacts such as erroneous descriptions, or from poorly sampled and preserved data. Importantly, distinguishing between both opens the possibility to study processes leading to the deceleration of morphological evolution and its underlying causes.

One particularly interesting group of marine organisms with a high incidence of cryptic species and little-known distribution is the interstitial fauna of coastal sediments (Cerca et al., 2018; Giere, 2009; Westheide, 1977). These habitats harbour a rich biodiversity, with organisms being typically found in the space between sand grains in beaches along continental coastlines (Giere, 2009). The overall convergence of body plan, and the suggested occurrence of high rates of morphological stasis (i.e. retention of the same ancestral character state over an extended period) results in high incidences of cryptic species in this group (Cerca et al., 2018; Jörger and Schrödl, 2013; Westheide, 1987). Taken together, these have been suggested to confound the study of meiofauna species' distribution (Cerca et al., 2018; Leasi and Norenburg, 2016). For instance, *Stygocapitella subterranea* was first described from the Baltic coastline of Germany (Knöllner, 1934). It was later found in numerous places across European coastlines including the Mediterranean and Black Sea, Northern America, as well as New Zealand and Australia (Purschke et al., 2019; Westheide, 2008, 1990), leading to the interpretation that *S. subterranea* is a cosmopolitan-distributed species (Riser, 1984). Recent investigations using a combination of genetic and morphological data from the Southern hemisphere led to the description of two new *Stygocapitella* species, *S. minuta* Struck et al., 2017 and *S. australis* Struck et al., 2017 (Fig. 1). RAPD-PCR data suggested that three different lineages of *S. subterranea* from different coastlines of the Northern hemisphere are genetically distinct (Schmidt and Westheide, 2000), with specimens from Europe and North America (Atlantic) grouping together, and specimens from the Pacific being distantly related to this group (Schmidt and Westheide, 2000). Hence, it is uncertain if *S. subterranea* has a distribution with population structure, or whether these are potentially different species.

The aim of this study is to do a phylogeographic reconstruction of lineages belonging to *Stygocapitella*, including data from *S. subterranea* specimens occurring along the Northern European, East-Northern American, West-Northern American, and Far-Eastern Russian coastlines. We complement this with data from the *S. australis* and *S. minuta* which respectively occur in the Australian and South African coastlines, to determine genetic and morphological differences between lineages, and whether these are different species. We (i) delimitate species using phylogenetic tools, species delimitation algorithms and haplotype networks and follow the

phylogenetic species concept; and (ii) assess morphological similarity between *Stygocapitella* species. We find evidence for several morphologically similar or identical species (i.e. cryptic species), which differ only in their geographic distribution at different coastlines. In addition, we find evidence for the occurrence of several oceanic translocations, including one potentially due to human activity. We discuss the importance of recognising and delimiting cryptic species, as well as the role of morphological-oriented practices in cryptic species complexes.

2. Methods

2.1 Field work and species identification

Stygocapitella spp. are interstitial annelids generally found around and above the high-water line of stable, sheltered gravel or sandy beaches (Purschke et al., 2019). To collect specimens, we selected beaches based on old records or by assessment of the area using google maps (Supplementary Table 1). At each site, we drew a transect roughly perpendicular to the coastline from the high-water line to the foot of the dune. After drawing the transect, we dug a hole every meter and collected sediment samples in plastic bags (volume of 375 cm^3) at intervals of 15 cm (0-15; 15-30; …) until approximately 60-75 cm depth or till the ground water. Interstitial invertebrate communities were then extracted using the $MgCl_2$ method and sorted under a dissecting microscope (Westheide and Purschke, 1988). After identification, we preserved specimens for molecular biology and for morphological analyses, either by transferring these to a solution of ~70% ethanol or to a fixative containing picric acid, formaldehyde and glutaraldehyde adjusted with sucrose to sea water osmolality (Sucrose-picric-acid-parafolmaldehyde-glutaraldehyde; SPAFG) following Westheide and Purschke (1988), respectively. Lists of individuals used for molecular and for quantitative morphological analyses are provided in Supplementary Tables 2 and 3, respectively.

2.2 DNA extraction and amplification

DNA extractions were carried out using either phenol-chloroform or the E.Z.N.A Tissue DNA Kit (Omega Bio-Tek). The nuclear markers 18S (complete) and ITS1 and the mitochondrial CO1 were amplified using the QIAGEN® Multiplex PCR Kit (Qiagen, Hilden, Germany) in a 10 µl reaction-mix containing 5 µl of multiplex mix, 1 µl Q-solution, 0.8 µl 10 µM of both forward and reverse primer, 1 µl genomic DNA and 1.4 µl deionized water. The mitochondrial gene 16S was amplified using a 25 µl reaction-mix, which included 15.2 µl of H_2O, 2.5 µl of 10X PCR Buffer I (with $MgCl_2$ added; Applied Biosystems), 2.5 µl of BSA, 0.5 µl of

10mM dNTPs, 1.6 µl 10 µM of both forward and reverse primer and 0.13 µl of amplitaq gold (Applied Biosystems). For COI, we used the primers LCO1490-JJ (CHACWAAYCATAAAGATARYGG) and HCO2198-JJ (AWACTTCVGGRTGVCCAAARAATCA; both Astrin & Stüben, 2008), for 18S 18e (CTGGTTGATCCTGCCAGT, Hillis & Dixon, 2018) and 18R1779 (TGTTACCGACTTTTACTTCCTCTA; (Struck et al., 2002), and for ITS1 species-specific primers Stygo_ITS1_F (TGTTGATTACGTCCCTGCCC; this study) and Stygo_ITS1_R (GTCAACCGACCCTGAGACAG; this study), and for 16S 16SarL (CGCCTGTTTATCAAAAACAT; Palumbi et al., 1991) and 16S_AN-R (GCTTACGCCGGTCTGAACTCAG; (Zanol et al., 2010). Exceptionally, polyLCO (GAYTATWTTCAACAAATCATAAAGATATTGG) and polyHCO (TAMACTTCWGGGTGACCAAARAATC; both Lobo et al., 2016) were used for individuals from the Atlantic-American sites as they yielded better results. PCR conditions for ITS1 included: an initial denaturation: 15' 95ºC; 40 cycles: 30" 95ºC, 30" 66ºC, 1' 72ºC; and a final elongation: 20' 72ºC; for 16S we included a touchdown procedure: an initial denaturation: 15' 95ºC; 40 cycles: 30" 94ºC, 30" 51ºC (touchdown: -0.2ºC per cycle), 2' 65ºC; final elongation: 7' 65ºC; for 18S a touchdown/touch-up: initial denaturation: 15' 95ºC; 15 cycles: 35" 94ºC, 90" 55ºC (touchdown: -1ºC per cycle), 2.5' 72ºC; 25 cycles: 35" 94ºC, 90" 50ºC, 2.5' 72ºC; final elongation: 10' 72ºC. Finally, for COI: initial denaturation of: 15' 95ºC; 15 cycles: 35" 94ºC, 90" 55ºC (touchdown: -1ºC per cycle), 1.5' 72ºC; 25 cycles: 35" 94ºC, 90" 50ºC, 1.5' 72ºC; final elongation: 10' 72ºC. PCR fragments were purified using a 10 times dilution of a phosphatase-exonuclease mix and Sanger-sequenced by Macrogen-Europe. Considering the length of the 18S fragment, four additional sequencing primers were included as sequencing primers: 18r (CTCTAATTTTTTCAAAGTAAAC), 18L (AGCTCTCAATCTGTCAATCCT; both Hillis & Dixon, 1991), 18F997 (TTCGAAGACGATCAGATACCG; Struck et al., 2002) and 18SF3_Stygo (CCTCGGGATTGGAATGAGTAC; Struck et al., 2017). After sequencing, we assembled sequences using Geneious (v6.8.1). The ends of sequences were automatically trimmed to remove the primers, visually checked, and manually trimmed to account for low quality ends. Finally, consensus sequences were blasted using NCBI database to exclude contamination.

2.3 Phylogenetic and molecular clock analyses

In total, we included sequence information for 353 specimens belonging to 33 sites in the Northern Hemisphere, as well as data from other *Stygocapitella* species of the Southern Hemisphere and species of Orbiniidae, the sister group of Parergodrilidae (Supplementary Tables

1 and 2; Supplementary Figure 1). We aligned COI, 16S and 18S sequences using MAFFT v7.310, with a maximum of 1,000 iterations and using the local pair alignment algorithm (mafft --maxiterate 1000 --localpair –reorder input.fa > output.fa) (Katoh and Standley, 2013). For ITS1 sequences, we adopted a different strategy as these sequences ranged from 750 – 1600 bp, resulting from tandem repeats. As we were not able to align these initially, we removed sequences longer than 1100 bp. This allowed aligning ITS1 using the global pair alignment algorithm (--globalpair), which accounts for gap-rich sequences. After aligning sequences and removing about 5% of the sequences due to long missing-ends, both ends were trimmed until the first position without missing data. To inspect congruence of the datasets (i.e. if separate genes cluster individuals and species similarly), we performed separate maximum likelihood analyses of each gene (Supplementary Figures 2-5). Single gene analyses were conducted using IQ-tree v1.6.7 (Chernomor et al., 2016; Nguyen et al., 2015) with an automatic determination of the best substitution model for each gene, 300 initial parsimony trees, 15 best trees retained during search and 1,000 ultrafrast bootstrap replicates (iqtree -s input.fa -nt AUTO -ninit 300 -nbest 15 -bb 1000 -wbtl) (Hoang et al., 2017). Finally, we concatenated the four genes into a single multi-gene alignment using FASconCAT v1.1 (Kück and Meusemann, 2010), and did a partitioned Maximum Likelihood (ML) analysis using IQ-tree as described for the single-gene analyses (Fig. 2).

Bayesian inference (BI) was applied using BEAST v2.4.7 (Bouckaert et al., 2014). Before running any analyses we determined substitution models that best fit the data using IQ-tree's ModelFinder (Kalyaanamoorthy et al., 2017). After performing several runs using combinations of different prior models and genes, we opted to remove 16S and ITS1 from further analyses because chain convergence could not be achieved. After this, we ran several analyses, some including missing data for either COI or 18S or without missing data (i.e. including only specimens for which both genes were present). Given the substantial differences in chain convergence we opted for the latter strategy. For the final analysis, the trees of COI and 18S were linked and the best fitting-models were TNe+I (Model TN93; Frequencies: All equal) for 18S and TIM+F+I+G4 (F= Empirical base frequencies; I = Invariable sites; G = Gamma model) for COI. To apply the TIM model in BEAST we selected the GTR model, with all frequencies to be estimated, apart for AG and CT, and 4 gamma categories. A relaxed, log-normal clock was applied with a substitution rate of 0.0001425 for 18S (Struck et al., 2017) and 0.0176 for COI (Lehmacher et al., 2016). We selected a birth-death model and a MCMC run for 100,000,000 generations sampling every 100,000 generation. Convergence was confirmed using Tracer v1.6

(Rambaut et al., 2007). A Maximum Credibility Consensus Tree was obtained using TreeAnnotator, with a 10% burn-in (Bouckaert et al., 2014).

2.4 Haplotype networks and species delineation

Haplotype networks of each separate genetic marker were build using TCS (Clement et al., 2000), with a connection limit of 95%. Gaps were considered as a fifth state. Graphical representation was done with tcsBU (Múrias Dos Santos et al., 2015), and then redesigned using Adobe Illustrator. We have adopted several species delineation approaches as suggested as best practice (Carstens et al., 2013). These included a GMYC model at https://species.h-its.org/gmyc/ (Fujisawa and Barraclough, 2013), a bPTP model at https://species.h-its.org/ptp/ (Zhang et al., 2013), 16S- and COI-based 95% connection limits using TCS (Clement et al., 2000), a posterior probability cut-off of 0.9 based on the generated Bayesian tree and a bootstrap cut-off of 95% based on the ML tree. The GMYC analysis was performed based on the obtained Bayesian tree (based on 18S and COI) and the bPTP on the obtained ML tree (concatenated, partitioned dataset including 16S, 18S, COI and ITS1). In addition to the genetic data, we did a 'morphological species delineation' with the aim of obtaining diagnostic features, based on the presence of certain chaetal types on the 1st, 2nd, 3rd and consecutive segments – the only variable morphological features we were able to obtain in our data.

2.5 Morphological data analysis

We investigated morphological disparity by using morphological measurement data obtained via light microscopy. To do so, we photographed single *Stygocapitella* specimens at 10X amplification. Because this resulted in multiple photos, we stitched photos together to form a whole-organism photograph using Photoshop. We then used ImageJ to measure body length and width, prostomium length and width, and pygidium length and width. In total, we obtained measures from 133 *Stygocapitella* specimens (Supplementary Table 3). Measurements were analysed using general linear models (GLM), Least Square Means analyses (Lenth, 2013) and principal component analyses. For each measurement, we fit a GLM model, using "measurement" as the dependent variable and "*Stygocapitella* lineage" as the independent variable. Because GLM models do not allow assessing differences between factorial variables (in this case "*Stygocapitella* lineage"), we fit a Least Square Means analysis to each model. This analysis provides pairwise statistical comparisons between factorial variables (i.e. "*Stygocapitella* lineage"), providing *p*-value evaluations between factors. Significance thresholds were then obtained with a Likelihood ratio test using the function drop1 as part of R's stats-package (R Core Team, 2013). After this, we conducted

principal component analyses using all six measurements and the function prcomp included in R's stats-package (R Core Team, 2013). Plotting of results was done using the ggplot2 package (Wickham, 2016) and the Hmisc package (Harrell Jr and Many Others, 2019).

In addition to morphological measurements, we looked for presence of chaetal differences using scanning electron microscopy (SEM). Specimens selected for SEM were rinsed in phosphate buffer and then treated with a buffered 1% OsO_4 solution for one hour at ambient temperature. This was followed by dehydration in a graded ethanol series starting in 30% ethanol and finishing in 100% ethanol. Dehydrated specimens were then critically-point-dried with CO_2, mounted on aluminium stubs and sputter-coated with platinum. SEM photographs from (i) the whole body, (ii) segments with chaetae (chaetiger), and (iii) the type and number of chaetae in each chaetiger were obtained using a Zeiss Auriga field emission SEM.

3. Results

3.1 Phylogenetic analyses

The dataset of 353 specimens comprised 332 16S, 273 COI, 125 18S and 177 ITS1 sequences. Partitioned ML (Fig. 2) and BI (Fig. 3) of the concatenated data generally resulted in the same topology. Monophyly of both *S. minuta* and *S. australis* is supported by bootstrap support values (BS) of 100 and posterior probabilities (PP) of 1. The species *S. subterranea sensu lato* from the Northern hemisphere was not recovered as monophyletic and is separated into eight lineages (BS = 99-100 & PP = 0.928-1; Fig. 2). Specimens from Volchanets (Russia; new record in Fig. 1) split into two separate lineages (BS = 100 & PP = 0.997; one lineage was only represented by one specimen in the BI). Single-gene ML phylogenies retrieved the same lineages as the concatenated datasets, demonstrating congruence between the genes in the dataset. The only exception was the highly conserved 18S gene that did not unambiguously distinguish the most recent divergences between closely related species (Supplementary Figures 2-5). Eight of the lineages in the tree are formally described as new species below, following species delimitation analyses (see below), and for the sake of clarity we use their new species names (*S. pacifica* sp. nov., *S. furcata* sp. nov., *S. berniei* sp. nov., *S. americae* sp. nov., *S. budaevae* sp. nov., *S. zecai* sp. nov., *S. josemariobrancoi* sp. nov., and *S. westheidei* sp. nov.) in the following sections, as well as in Figs. 2 & 3. *Stygocapitella westheidei* sp. nov. is sister to the amended *S. subterranea sensu stricto*, and *S. josemariobrancoi* sp. nov. is sister to these two (BB = 100 and 96 & PP = 0.991 and 0.998, respectively). These three species are sister to *Stygocapitella zecai* sp. nov., and together they form the North Atlantic clade (BB = 100 & PP = 1).

Stygocapitella budaevae sp. nov. is sister to a species which remains undescribed due to the lack of type material and to which we will refer as undescribed species A (BS = 100 & PP = 1), *Stygocapitella berniei* sp. nov. is sister to *S. americae* sp. nov. (BS = 100 & 1). *Stygocapitella berniei* sp. nov., *S. americae* sp. nov, *S. budaevae* sp. nov. and Spec. A form a clade (BS = 100 & 0.987), which is sister to *S. australis* in the ML analysis (BS = 83, Fig. 2), yet it is sister to the Northern Atlantic clade in the BI analysis (PP = 0.987, Fig. 3). In both analyses all species so far mentioned form a monophyletic group (BS = 100 & PP = 0.948). *Stygocapitella pacifica* sp. nov. is sister to *S. furcata* sp. nov. (BS = 100 & PP = 1), together comprising a clade which is sister to all aforementioned species. All these species form a monophyletic clade, which is sister to *S. minuta* which is the first to branch off in the *Stygocapitella* radiation (BS = 100 & PP = 0.656).

3.2 Network analyses

We retrieved haplotype-networks of COI, 16S and ITS1 focusing on the Northern Atlantic, where we did the majority of the sampling efforts (29 sites; Fig. 4), and for all locations (35 sites; Supplementary Figure 6). Haplotype networks are congruent with phylogenetic results recognizing twelve separate lineages. *S. australis* displays two unconnected haplotypes, separating specimens geographically. For *S. zecai* sp. nov., the 16S haplotype network is divided into two dominant haplotypes which are mostly represented in Scandinavia and Scotland and separated by only one substitution. One of the haplotypes is also present in the North Sea and in Eastern England (Fig. 4). For COI, we were unable to obtain as many sequences as for 16S, yet we observe a network comprising five haplotypes, with six substitution differences between the two most distant haplotypes. For ITS1, specimens in different areas have distinct haplotypes. Individuals from Scandinavian regions are separated by up to 25 substitution differences. In *S. josemariobrancoi* sp. nov. one ITS1, two 16S and three COI haplotype networks are unconnected at 95% thresholds. Differences in COI and 16S are probably due to differences in variability in two genetic markers. For ITS1, the most common haplotype is present in Germany (North Sea), Scotland and France. Specimens from West England have multiple haplotypes, separated between a single substitution up to 31 substitutions. Haplotypes found in East England are subdivided into three closely-related haplotypes, which are 11 substitution away from the dominant haplotype, and by three specimens which are one and three substitutions away from the dominant haplotype. The two specimens from the USA are nested between the two most common haplotypes. For 16S, one of the two haplotype networks displays a major haplotype present in France, Eastern England and Scotland. The second biggest haplotype in this network is 6 substitutions away from the major haplotype and only occurs in Western England. The

second haplotype network occurs in France, Germany (North Sea), the USA and Western England and has two major haplotypes, which are only separated by a single substitution. Rarer haplotypes occur mostly in Germany (North Sea), being up to eight substitutions different from one of the major haplotypes. In COI, one of the three networks is represented by a single specimen from Bristol Channel. Another is comprised by a dominant haplotype which is present in Germany (North Sea), France, USA and Western England, and by several haplotypes separated by only one or two substitutions. The third and remaining COI network is comprised by two dominant haplotypes, one occurring in Western England and the other occurring in Eastern England, France and Scotland. This haplotype network is congruent between COI and 16S. 16S and COI retrieve similar networks for *S. subterranea*, revealing a dominant haplotype occurring in Germany (North Sea), Scotland, and France as well as in COI in Germany (Baltic Sea) and Eastern England (Fig. 4). In both genes, *S. subterranea* has haplotypes separated by 5-19 substitutions from the dominant haplotype, which occurs in Germany (Baltic and North Sea), Scotland and France. For ITS1 in *S. subterranea*, we were unable to amplify this marker in multiple specimens (Supplementary Table 2). However, 17 substitutions separate the two most distant haplotypes, which occur in Germany (North Sea), and another in Germany (Baltic Sea) and France. In *S. westheidei* sp. nov., COI and 16S present a very distinct haplotype structure. In 16S a single haplotype is present at all sites from the USA (circa 400 km) while, on the other hand, COI shows six co-occurring haplotypes without geographic structuring. Four of these haplotypes are present in Canoe Beach, three in Reid State Park and two in Lubec (Supplementary Figure 6). For ITS1, a major haplotype exists, with two minor haplotypes separated by only one substitution from the major haplotype.

3.3 Morphological measurements and morphotypes

A total of four morphotypes can be identified in *Stygocapitella* based on chaetal composition. All these morphotypes can be distinguished by chaetae number and composition of chaetae present in the first two chaetigers (Fig. 5). The first morphotype is specific to *S. minuta* (see Struck et al., 2017). It comprises two whip-like and three bilimbate chaetae in the first and four bilimbates in the second chaetinger. The second morphotype consists of two whip-like, two forked and one bilimbate chaetae in the first, and one bilimbate, two forked and one bilimbate chaetae in the second chaetiger (red morphotype in Fig. 5). This morphotype is seen in *S. pacifica* sp. nov., *S. furcata* sp. nov., and *S. australis*. The third morphotype is distinguished by its two whip-like, two forked and two bilimbate chaetae in the first and two bilimbate, two forked and two bilimbate chaetae in the second chaetiger (blue in Fig. 5). This morphotype is present in *S.*

berniei sp. nov., *S. americae* sp. nov., *S. budaevae* sp. nov., *S. zecai* sp. nov. and presumably in *Stygocapitella* sp. A, for which we lack morphological data. The fourth morphotype is identified by two whip-like, two forked and two bilimbate chaetae in the first and one bilimbate chaetae, two forked and two bilimbate chaetae in the second chaetiger (green in Fig. 5). This morphotype is present in *S. josemariobrancoi sp. nov.*, *S. westheidei sp. nov.*, and *S. subterranea*. All morphotypes have one bilimbate, two forked and one bilimbate chaetae in the third and following chaetigers except for that present in *S. minuta* which only has bilimbate chaetae in these chaetingers.

General Linear Models demonstrate significant differences in measurements: body length (Likelihood Ratio Test scaled dev. = 124.72, p <0.001), body width (LRT scaled dev. = 118.3, p <0.001), prostomium length (LRT scaled dev. = 76.5, p <0.001), prostomium width (LRT scaled dev. = 122.8, p < 0.001), pygidium length (LRT scaled dev. = 45.4, p < 0.001), and pygidium width (LRT scaled dev. = 140.5, p < 0.001). Results from the morphological measurements show that pairwise differences in body length between species are also roughly reflected in the remaining five measurements (Fig. 6, Supplementary Table 4). Considering this, we will concentrate on body length in the following section. Most notably, *S. minuta* is significantly shorter in body length (mean 1046.88; SD 76.29) when compared to the remaining species, with the exception of *S. pacifica* sp. nov. (mean 1261.47; SD 52.21) and *S. budaevae* sp. nov. (mean 1368.49; SD 164.53). For the second morphotype mentioned above we lack light microscopy-based measurement data for *S. furcata* sp. nov.. Within this morphotype, *S. pacifica* sp. nov. is smaller than *S. australis* (mean 1933.61; SD 218.01), but this difference is not statistically significant. For the third morphotype, *S. budaevae* sp. nov. has the shortest body length, followed by *S. berniei* sp. nov. (mean 2006.98; SD 436.99), whereas *S. americae* sp. nov. (mean 2550.4; SD 229.74) has the largest body length, followed by *S. zecai* sp. nov. (mean 2238.23; SD 322.48). In this way, *S. budaevae* sp. nov. is not significantly different in body length from *S. berniei* sp. nov., but it is significantly different from *S. americae* sp. nov. and *S. zecai* sp. nov.. Interestingly, *S. berniei* sp. nov. is not significantly different from all species with the third morphotype in any of the characters except for pygidium width, which is significantly different to *S. budaevae* sp. nov. (Suppl. Table 4). *Stygocapitella americae* sp. nov. and *S. zecai* sp. nov. are not significantly different in body length, but in prostomium length and width and pygidium width. Within the fourth morphotype from above, *S. josemariobrancoi* sp. nov. (mean 2409.7; SD 472.15) is clearly the longest species, while *S. subterranea* (mean 1703.7; SD 380.68) and *S. westheidei* sp. nov. (mean 1820.2; SD 293.68) have overlapping body length values. Accordingly, *S. josemariobrancoi* sp. nov. is significantly different from *S. subterranea* and *S. westheidei* sp. nov., but the latter two are not separated from each other.

Finally, we decomposed the variance in all data using principal component analyses. Considering all species together the first principal component separates only *S. minuta* from the remaining species (PC1 explains 75.4% of the variance; Fig. 7A). The second and third principal components explained 11.9 % and 5.4 % of the variance, respectively, but could not separate any of the species (data for third component not shown). However, when considering the variance within morphotypes, the results are slightly more informative. In the second morphotype, *S. pacifica* sp. nov. is clearly separated from *S. australis* based on the first principal component (Fig. 7B). However, *S. pacifica* sp. nov. is only represented by two specimens. In the third morphotype, the first principal component separates *S. budaevae* sp. nov. from the remaining three species (Fig. 7C). *S. zecai* sp. nov. can also be separated from *S. americae* sp. nov. based on the first two principal components, but both overlap substantially with *S. berniei* sp. nov.. Finally, in the fourth morphotype all three species overlap substantially, so that they cannot be separated based on the PCA analysis (Fig. 7D). This is not due to the lack of data as these are the three species with highest number of sampled specimens.

3.4 Species delimitation

The complementary approaches to species delimitation were generally concordant (Fig. 2). With a total of 16 species, the GMYC algorithm (based on the Bayesian tree) was the method suggesting the most species, while, on the other hand, bPTP (based on the ML tree) proposed the least number of species (13). Every approach suggested that *S. pacifica* sp. nov., *S. furcata* sp. nov., *S. berniei* sp. nov., *S. americae* sp. nov., *S.* spec. A, and *S. zecai* sp. nov. all represent single taxonomic units. *Stygocapitella minuta* and *S. budaevae* sp. nov. are consistently considered as single species in all the approaches with the exception of COI networks which suggest the presence of two species in each of these lineages (Fig. 2). GMYC, bPTP, and network approaches suggest that *S. australis* represent two separate species. For *S. josemariobrancoi* sp. nov.,W COI networks and GMYC suggest the occurrence of three species, which also obtain support by posterior probabilities above 0.90. On the other hand, 16S networks suggest the occurrence of two species, but these were not recovered as monophyletic in the ML tree. Finally, bPTP suggests the occurrence of a single species. In both *S. westheidei* sp. nov. and *S. subterranea*, GMYC suggest two separate species, each of which also obtain posterior probabilities above 0.90, whereas the remaining methods suggest the occurrence of only one species. To avoid over splitting, we have opted for a conservative in species recognition approach which consisted in selecting species based on a most inclusive approach. That is, if one approach found a clade as single species, while another one as more than one the clade, we regarded this clade as a single species.

Additionally, the recognized species had to be retrieved as monophyletic, and strongly supported in all phylogenetic reconstructions of the concatenated datasets (for more details see Taxonomic account).

4. Discussion

Here, we described the genetic divergence and morphological disparity among species in the *Stygocapitella* genus. We find four morphotypes based on chaetal number and composition in a total of 12 species, rendering some species morphological identical. Even considering chaetal pattern (diagnostic feature) and light microscopy measurements (quantitative data), we are unable to distinguish species, which can only be distinguished using molecular tools. The morphological evolution of this species complex is exceptionally slow, as expected for cryptic species under morphological stasis (Cerca et al., 2018; Struck et al., 2018a; Struck and Cerca, 2019). With one exception, all *Stygocapitella* species occur in a single coastline, yet some of them are widely distributed spanning hundreds or thousands of kilometres. The discovery of cryptic species drastically reduced the cosmopolitan distribution of *S. subterranea sensu lato* (e.g. Purschke et al., 2019; Schmidt and Westheide, 2000; Westheide, 1990). We find indirect evidence for a potential oceanic translocation due to human activity in *S. josemariobrancoi* sp. nov. We discuss the relevance of morphology in taxonomy and the impact of cryptic species in marine biogeography.

4.1 Cryptic species: Taxonomic artefacts or evolutionary phenomena?

We describe eight new *Stygocapitella* species, totalling to eleven species in the genus. Additionally, one species is not yet formally described due to lack of type material, as required by the ICZN. While we find evidence for several morphologically similar species to occur, we identified 4 morphotypes based on the number and composition of chaetae in the first three chaetingers. Within each morphotype, clear differences in body measurements were found only between some certain species, but not all. For example, *S. americae* sp. nov. and *S. berniei* sp. nov. co-occur at the Pacific coastline of the US (Friday Harbor, Washington state) share the same morphotype and display no significant differences in quantitative measurements. The same is true for *S. zecai* sp. nov. and *S. berniei* sp. nov.. The only diagnosable difference between these two species is molecular divergence, and potentially their geographic distribution, yet considering the global raise in species introduction by humans (Barnes, 2002; Mack and Lonsdale, 2001; Radziejewska et al., 2006) this cannot be taken as a rigorous diagnostic character. A similar example occurs between *S. subterranea* and *S. westheidei* sp. nov., which cannot be differentiated from each other neither based on morphotype nor morphometrics, but only by molecular data.

Finally, so far *S. pacifica* sp. nov., *S. furcata* sp. nov. and *S. australis* can also only be separated by molecular tools. We have thus provided evidence that in *Stygocapitella* morphology is very similar across different species and that some species are even impossible to be identified based on morphology alone. This calls into question whether these species can be called cryptic species or not.

The most often applied definition of cryptic species requires that a given group of species has been recognized as single species before: "two or more distinct species that are erroneously classified (and hidden) under one species name" (Bickford et al., 2007). This definition places the focus in the taxonomic history of the cryptic species complex (Struck and Cerca, 2019). Strictly under this definition, from the 8 *Stygocapitella* species herein described (and the undescribed species), only seven could be considered cryptic species. These seven represent populations/sites which have been considered to be *S. subterranea* before in the literature (Karling, 1958; Knöllner, 1934; Purschke, 2006, 1999, 1987, 1986; Purschke et al., 2019; Purschke and Fursman, 2005; Purschke and Jördens, 2007; Riser, 1980; Schmidt and Westheide, 2000; Schmidt, 1972a, 1970, 1969; Struck et al., 2017; Westheide, 2008, 1966; Worsfold, 2008). The two only exceptions would be *S. pacifica* sp. nov. and *S. budaevae* sp. nov. which represent new records and have therefore never been identified as *S. subterranea*. This exposes the arbitrary nature of this definition, which has led some workers to argue that cryptic species are not a natural phenomenon, but rather artefacts of taxonomic practices such as, for instance, the lack or inappropriate resolution of morphological data to determine species boundaries (Korshunova et al., 2019, 2017).

It has been suggested that cryptic species should only be considered as a "temporary formalization of the problems with delineation of the species from the same geographic region, when those species demonstrate significant molecular phylogenetic differences, but are hardly distinguished morphologically, ethologically, etc." (Korshunova et al. 2017). Given this definition, which considers cryptic species as a problem, not all *Stygocapitella* species could be considered cryptic species. For instance, *S. westheidei* sp. nov. and *S. subterranea* are morphologically indistinguishable but are present at different coastlines. Following this definition would not solve the "problem" of morphological similarity between these two species because they do not geographically overlap. Importantly, and despite the efforts herein included to determine morphological differences between species, most *Stygocapitella* species lack diagnostic characters and morphological differences which allow an unambiguous identification to the species level. Indeed, only when using molecular data, one is able to distinguish these species. Even when

length measurements are significantly different between species, a substantial overlap between specimens often exists, which does not allow to unambiguously assign an individual to a species and is prone to errors if juveniles get measured. This is evidenced from the PCA, which considers all measurements simultaneously, yet species substantially overlap and are thus unable to be identified. A second critical aspect of this definition is its reliance on sympatry and geography. As we discuss below, we find potential evidence for a recent trans-oceanic translocation by human activity. While we are able to distinguish species based on measurements, if translocated specimens would have been from *S. subterranea* and not *S. josemariobrancoi* sp. nov. it would not be possible to distinguish them from the putatively native species *S. westheidei* sp. nov. without molecular data. Considering its reliance on geography, this definition fails to detect introduced species, when these are morphologically indistinguishable and creates arbitrary challenges on whether species are sympatric or not. Finally, an implicit assumption of this definition is that substantial phenotypic differences will accumulate between species, given enough evolutionary time. This is not the case, as shown by the evidence for long-lasting stasis in palaeontology (Eldredge and Gould, 1972; Futuyma, 2010), as well as in cryptic species complexes (Lee and Frost, 2002; Struck et al., 2017; Swift et al., 2016; Wada et al., 2013). This is evident after contrasting the genetic differences of *Stygocapitella* with its morphological evolution, hence being in line with the hypothesis of cryptic species complexes under stasis, as enough time has passed to allow for the accumulation of phenotypic differences. Despite this, few or no phenotypic differences can be observed. Treating these cases just as a taxonomic problems would overlook the phenomenon of morphological stasis (Lee and Frost, 2002; Struck et al., 2017; Swift et al., 2016; Wada et al., 2013).

An entirely different approach to delimit and understand cryptic species consists in recognising these as the result of evolutionary phenomena resulting in the deceleration of morphological evolution (Struck et al., 2018a, b). Based on this approach, first, a regular species delineation process takes place. This delimitation-step should focus on detecting differences between putative species and include various sorts of data such as ecological, behaviour and morphological data, being as scrupulous as possible. In a second step, an assessment of the species' morphological similarity should be done. This second step allows evaluating whether the species complex is indeed comprised of cryptic species (i.e. species morphologically more similar than expected). To determine if a set of species are more similar than expected, workers should focus on obtaining the time of divergence of the species complex, and, when possible, compare it with a closely related taxon/taxa (i.e. outgroup). By separating the species-delimitation (step 1), from the assignment of cryptic species (step 2), the assignment of cryptic species relies solely on

the degree of morphological disparity, and on the time of divergence. This allows differentiating between taxonomic artefacts and the deceleration of morphological evolution as seen in cryptic species (Struck et al., 2018a). On the negative side, it requires an exhaustive understanding of the species complex as well as meticulous sampling and knowledge of the study system (Korshunova et al., 2019). While this might not be possible in every case, a scrupulous and meticulous approach is essential to define species, be it cryptic species or not. In *Stygocapitella*, as outlined above, only very little morphological differences can be observed despite pronounced genetic divergence. Treating these as mere taxonomic oddities would conceal a true biological phenomenon: morphological stasis (Struck et al., 2018a; Struck and Cerca, 2019; Swift et al., 2016; Wada et al., 2013). Recent estimates suggest that the evolution of the *Stygocapitella* genus has occurred in >200 million years and *S. australis* has been separated from *S. subterranea* for more than 80 million years (Struck et al., 2017). To put this into perspective, the whole radiation of mammals took place in less time. Orbiniidae, which is the sister family to Parergrodiliidae (which includes *Stygocapitella*), comprises 21 genera (Horton and Et. Al., 2019). This family has accumulated much more phenotypic differences in the same time than *Stygocapitella*. Therefore, *Stygocapitella* spp. are a textbook example of cryptic species.

4.2 Delimiting cryptic species and biological diversity

Six of the newly described *Stygocapitella* species were originally considered to be *Stygocapitella subterranea*, highlighting the necessity of proper species description in understanding marine biodiversity. The lack of taxonomic knowledge has consequences for marine conservation (Bernardo, 2011; Bickford et al., 2007; Costa and Carvalho, 2010; Schonrogge et al., 2002), biodiversity assessments (Appeltans et al., 2012; Hawksworth and Lücking, 2017; Meyer-Wachsmuth et al., 2014) and species distribution (Cerca et al., 2018). Ratios of 'crypticness' (i.e. proportion of cryptic species within described species) (Kon et al., 2007) seem to be high in the sea (Cerca et al., 2018; Knowlton, 1993). To name a few examples, 14 cryptic species in *Brachionus plicatilis* (Rotifera) (Suatoni et al., 2006), 10 cryptic species in *Eumida sanguinea* (phyllodocidan polychaetes) (Nygren and Pleijel, 2011), and 25 in 4 described *Terebellides* polychaete species (Nygren et al., 2018). These uncommonly high numbers result from issues related to the obstacles in sampling, re-sampling and identifying marine species (Hellberg, 2009; Knowlton, 2000, 1993) which ultimately jeopardize the understanding of basic biology, such as species distribution and life cycle. Cryptic species should be taken into account when protecting marine biodiversity as these contribute to overlooked species richness (Pante et al., 2015). Yet, we must point that we can only be led to speculate how much biological diversity is missed due to the

occurrence of cryptic species, especially having in mind that we cannot yet determine the proportion of cryptic species which are only taxonomic artefacts (Korshunova et al., 2017; Struck et al., 2018b). Nonetheless, it is worth noticing that morphologically-based practices have failed to report this diversity. Estimations suggest that there might be ca. 9,000 - 36,000 cryptic species in the sea, comprising 3-12% of marine biodiversity (Appeltans et al., 2012). The development of new tools, including recent genomic approaches which have contributed towards closing the gap between population genetics and phylogenetics, is likely to benefit and improve species delimitation, including the delimitation of cryptic species (Singhal et al., 2018; Struck et al., 2018a). For example, new demographic tools allow distinguishing the contribution of gene flow and incomplete lineage sorting. In the brittle star *Ophioderma longicauda* the modelling of relatively complex demographic scenarios led to the delimitation of cryptic species in the face of strong incomplete lineage sorting and past hybridization events (Weber et al., 2019).

4.3 Implications of cryptic species to marine biogeography

The splitting of *Stygocapitella subterranea sensu lato*, originally described as a cosmopolitan species, into nine species with reduced geographical distributions suggests that overlooking cryptic species tends to inflate the distribution of marine organisms (Knowlton, 1993; Struck et al., 2018a). The wide distribution of many marine species (Cerca et al., 2018; Johannesson, 1988), and the paradoxical distribution of species with non-pelagic and pelagic larvae (Hellberg, 2009) remains as one of the most puzzling observations in marine biology. The potential high number and influence of cryptic species with reduced distribution ranges provides a further step to solve these issues. The results herein found are in line with evidence from other meiofaunal taxa, which demonstrate that delimitated cryptic species often have geographically restricted distributions and the range of individual species is smaller than the originally described species (Cerca et al., 2018). For instance, in the rotifer *Brachionus plicatilis*, the discovery of 14 cryptic species led to the reduction of the distribution of the originally described species. While the originally described species was recognised as a cosmopolitan species, cryptic lineages demonstrate a rather localized distribution (Suatoni et al., 2006). Similarly, the gastropod *Pontohedyle milaschewitchii* had been reported in the Indian Ocean, Central Pacific, Western Pacific, Eastern Pacific, Western Atlantic, and Eastern Atlantic including the Mediterranean and Black Sea. The splitting and discovery of 6 cryptic species led to the circumscription of one species to the Western Atlantic, another to the Indian Ocean, Central Pacific, Western Pacific, another to the Central Pacific, one to the Eastern Pacific, another to the Mediterranean and Black Sea, and yet another to the Eastern Atlantic (Jörger et al., 2012).

Despite the observed reduction of geographical distribution, *Stygocapitella* species still maintain wide distributions suggesting wide dispersal capacities. Species for which we obtained multiple specimens show no association between population structure and geography. For example, *S. zecai* sp. nov. is distributed from Northern Norway to Southern England and a 16S haplotype is shared between specimens from Henningsvær and Lødingen (Northern Norway), Ardtoe (Western Scotland) and Cutty Sark (England) suggesting that no population structure occurs for about ~400 km distance. Similarly, the two remaining species occurring in Europe (*S. subterranea* and *S. josemariobrancoi* sp. nov) have wide distributions ranging from Scotland to Germany and France, with haplotypes occurring over long distances. Finally, *S. westheidei* sp. nov. has only a single 16S haplotype along the entire North-western Atlantic coastline in the USA spanning ~450 km (but notice COI). These distributions are coherent with recent evidence from meiofaunal nematodes (Derycke et al., 2008), nemerteans (Leasi and Norenburg, 2016, 2014), xenacoelomorphans (Meyer-Wachsmuth et al., 2014) and molluscs (Jörger et al., 2012), even after the discovery of cryptic species. Generally, the discovery of cryptic species in these groups led to the reduction of the first assigned distribution, but these lineages still maintain wide distribution ranges (Cerca et al., 2018).

The phylogeny of *Stygocapitella* displays a biogeographic signal related to oceanic water bodies. All Northern Atlantic species form a monophyletic group in all analyses. Interestingly, *S. westheidei* sp. nov., which occurs at the North-western Atlantic (American coastline), is nested within the remaining North-eastern Atlantic species (European coastline), suggesting a relatively recent oceanic transition. The Northern Atlantic group is placed among species occurring in the Indo-Pacific Oceans. This indicates that a transition from the Indo-Pacific to the Atlantic has occurred only once. Most prominently, we find evidence for sister species occurring in opposite sides of the Northern Pacific Ocean, with *S. furcata* sp. nov. and *S. pacifica* sp. nov. as well as *Stygocapitella* spec. A and *S. budaevae* sp. nov. occurring in Northern America and Russia, respectively. This potentially reveals that the ancient lineage of each pair could transverse the Pacific Ocean, or speciated allopatrically following vicariance.

Interestingly, the Australian species, *Stygocapitella australis*, nests among the Northern Pacific ones, while *S. minuta*, from South Africa, is the first to branch off in the phylogenetic tree. This suggests that at least two equatorial transitions must have occurred in addition to the oceanic transitions in the Northern hemisphere. Two major hypotheses have been suggested to explain the distribution of meiofaunal groups. These include the strict vicariance hypothesis, which assumes that these organisms are poor dispersers, and the long-distance dispersal

hypothesis. Evidence gathered from this work is congruent with a previous analysis (Struck et al., 2017), which together suggest that a strict vicariance hypothesis does neither fit the observed distribution pattern, neither the phylogeny of these meiofaunal organisms. We find evidence for several events of long-distance dispersal (Westheide, 1991, 1977), which have an important role in establishing new populations across oceans and spreading along coastlines (Derycke et al., 2008; Schmidt and Westheide, 2000).

Two specimens collected in Lubec (Maine, USA) at the North-Western Atlantic coastline were identified as *S. josemariobrancoi* sp. nov. using molecular tools. This species is elsewhere only present along the Northern European coastline (Supplementary Figure 1). The specimens from Lubec share a 16S haplotype with specimens from England, France and Germany. This suggests a very recent dispersal event possibly due to trans-Atlantic trade. Even though we have no evidence to conclude that this translocation was human-based, this result is in line with evidence suggesting that meiofaunal specimens can be dispersed by ballast water or sand translocations (Radziejewska et al., 2006).

5. Taxonomic account

We did not take any formal taxonomic actions for *Stygocapitella* spec. A as we lack material for morphological studies and hence cannot assign type material for this species. We do not take any taxonomic actions on *S. australis* despite some analyses suggesting they are potentially different species. Describing both as separate species could entail that one of them would be non-monophyletic (see Fig. 2). More Western Australian data are needed to solve this issue. Records from New Zealand (Riser, 1984) should also to be taken into account by collection of fresh material for molecular analyses. Interspecific pairwise genetic distances between the species are found in Supplementary Table 5, intraspecific genetic distances are found in Supplementary Table 6. Described species have been registered in in Zoobank.org.

Genus ***Stygocapitella*** **Knöllner, 1934**

Type species ***Stygocapitella subterranea*** **Knöllner, 1934 (*sensu stricto*)**

Stygocapitella subterranea (Karling, 1958; Knöllner, 1934; Purschke, 2006, 1999, 1987, 1986; Purschke et al., 2019; Purschke and Fursman, 2005; Purschke and Jördens, 2007; Schmidt and Westheide, 2000; Schmidt, 1969, 1970; Struck et al., 2017; Westheide, 2008, 1966)

Types and material examined. See Struck et al. (2017).

Diagnosis: See Struck et al. (2017).

Description. See Struck et al. (2017) except for size:bbody length: mean 1703.7 µm (range 2441.8- 1099.2 µm) and width 185.8 µm (285.8 – 121.3 µm); prostomium length 62.4 µm (90 – 33.1 µm) and width 117 µm 167.8 – 82.6 µm); pygidium length 45.6 µm (64.7- 33.9 µm) and width 86.6 µm (160.3 – 54.1 µm) (Fig. 6).

Habitat. See Struck et al. (2017).

Distribution. Restricted to the North-eastern Atlantic comprising the North Sea (British & German coast), the Baltic Sea (German & Southern Swedish coast), and the Eastern Atlantic Ocean (British & French coast; Suppl. Fig 1, Suppl. Table 1).

Remarks. The records from the Mediterranean Sea (French & Tunisian coast), the Black Sea (Romanian coast) and New Zealand in the Southern hemisphere have also been assigned to *S. subterranea* before, but given the results herein it is uncertain whether these records belong to *S. subterranea*, *S. josemariobrancoi* sp. nov., *S. westheidei* sp. nov. or *S. australis* or even constitute new species. Therefore, these records should be considered as *Stygocapitella* sp. for the time being.

***Stygocapitella pacifica* sp. nov.**

Types and material examined. Holotype: Volchanets, Russia, N 42° 54' 37.7" / E 132° 44' 25.0", 5.5 m above high-water line at a depth of 0-15 cm, Coll. Natural History Museum of the University of Oslo (NHMO C6996). Additional material: 1 paratype from Volchanets, Russia, N 42° 54' 37.7" / E 132° 44' 25.0", 5.5 m above high-water line at a depth of 0-15 centimetres, Coll. Natural History Museum of the University of Oslo (NHMO C69967).

Type locality. Volchanets, Russia, N 42° 54' 37.7" / E 132° 44' 25.0"

Diagnosis. Morphology: The first chaetiger possesses two bilimbate chaetae with a whip-like extension, one bilimbate and two forked chaetae, and all following ones two bilimbate and two forked chaetae. For genetic data see Genbank ID MN158611 (COI), MN164341 (16S).

Description. Color: White-transparent with a slightly iridescence surface. Size: body length: mean 1261.5 µm (range 1298.4 – 1224.6 µm) and width 137.5 µm (141.1- 133.9 µm); prostomium length 56 µm (62.9 – 49.2 µm) and width 87.1 µm (92.7 – 81.5 µm); pygidium length 34.3 µm (38.7 – 29.8 µm) and width 58.5 µm (58.9 – 58.1 µm) (Fig. 6). The body comprises a prostomium without appendages, a peristomium bearing the mouth opening, 13 segments and a

round pygidium. 1st to 12th segment biannulated. Chaetae in pairs of ventrolateral bundles are present at segments 2 to 11 in the first ring of each segment. First chaetiger with two bilimbate chaetae with whip-like extensions, two forked chaetae and one bilimbate chaeta in each bundle. All following chaetigers possess two bilimbate and two forked chaetae in each bundle.

Habitat: We found specimens at a beach with medium-sized sand grains at or above the high water level down to a depth of 20 cm.

Distribution: Volchanets (Primorsky Krai region, Russia)

Etymology. The species name derives from presence in the Pacific Ocean.

***Stygocapitella furcata* sp. nov.**

Stygocapitella subterranea partim (Purschke, 2006, 1999; Purschke et al., 2019; Schmidt and Westheide, 2000; Struck et al., 2017; Westheide, 2008), not Köllner 1934.

Types and material examined. Holotype: 4th July Beach, USA (WA), N 48° 28' 05.6" / W 123° 00' 10.7", between 5 m above high-water line and the high-water line, at a depth of 0-15 cm, Coll. Natural History Museum of the University of Oslo (NHMO C7010). Additional material: 1 paratype from Roche Harbor, USA (WA), N 48° 35' 46.0" / W 123° 10' 12.03 m above high-water line, at a depth of 0-20 centimetres. Coll. Natural History Museum of the University of Oslo (NHMO C7009). Besides the holotype and paratype, two specimens for molecular work have been examined.

Type locality. 4th July Beach, USA (WA), N 48° 28' 05.6" / W 123° 00' 10.7"

Diagnosis. For morphology see *S. pacifica* sp. nov.. For genetic data see Genbank ID: MN158612 (COI), MN164343 (16S).

Description. Color: White-transparent with a slightly iridescence surface. The body comprises a prostomium without appendages, a peristomium bearing the mouth opening, 13 segments and a round pygidium. 1st to 12th segment biannulated. Chaetae in pairs of ventrolateral bundles are present at segments 2 to 11 in the first ring of each segment. First chaetiger with two bilimbate chaetae with whip-like extensions, two forked chaetae and one bilimbate chaeta in each bundle. All following chaetigers possess two bilimbate and two forked chaetae in each bundle. No measurements were obtained for this species.

Habitat. Specimens predominantly occur at beaches with medium-sized sand grains at or above the higher water level up.

Distribution. San Juan Island (WA, USA). If the additional records from the North-Eastern Pacific along the US and Canadian Pacific coast (Purschke, 2006, 1999; Purschke et al., 2019; Schmidt and Westheide, 2000; Westheide, 2008) belong to this species, *S. berniei* sp. nov., or *S. americae* sp. nov. or constitute new species altogether is uncertain. Therefore, these records should be considered as *Stygocapitella* sp. for the time being.

Etymology. The species name reflects its having forked chaetae.

***Stygocapitella berniei* sp. nov.**

Stygocapitella subterranea partim (Purschke, 2006, 1999; Purschke et al., 2019; Schmidt and Westheide, 2000; Struck et al., 2017; Westheide, 2008), not Köllner 1934.

Types and material examined. Holotype: Roche Harbor, USA (WA), N 48° 35' 46.0" / W 123° 10' 12.0", between 3 m above high-water line and the high-water line, at a depth of 0-20 centimetres, Coll. Natural History Museum of the University of Oslo (NHMO C6994). Aditional material: 1 paratype Roche Harbor, USA (WA), N 48° 35' 46.0" / W 123° 10' 12.0", between 3 m above high-water line and the high-water line, at a depth of 0-20 centimetres, Coll. Natural History Museum of the University of Oslo (NHMO C6995). Besides the holotype and the paratype, 10 specimens for molecular work and two for SEM have examined.

Type locality. Roche Harbor, USA (WA), N 48° 35' 46.0" / W 123° 10' 12.0"

Diagnosis. Morphology: The first chaetigerous segment possesses two bilimbate chaetae with a whip-like extension, two bilimbate and two forked chaetae. The second segment possesses two bilimbate, followed by two forked, followed by two bilimbate chaetae. The third and remaining segments have two bilimbate and two forked chaetae organized in a bilimbate-forked-forked-bilimbate arrangement. For genetic data please see genbank IDs MN158602 (COI), MN164081 (16S).

Description. Color: White-transparent with a slightly iridescence surface. Size: body length: mean 1926.3 μm (range 2552.8 – 1680.1 μm) and width 217.5 μm (273.7 – 203.63 μm); prostomium length 67.7 μm (95.5 – 42.9 μm) and width 132.7 μm (182.2 -86.7 μm); pygidium length 52.6 μm (70.9 – 48 μm) and width 112.3 μm (145.8 – 95.9 μm) (Fig. 6). The body comprises a prostomium without appendages, a peristomium bearing the mouth opening, 13

segments and a round pygidium. 1st to 12th segment biannulated. Chaetae in pairs of ventrolateral bundles are present at segments 2 to 11 in the first ring of each segment. First chaetiger with two bilimbate chaetae with whip-like extensions, two forked chaetae and two bilimbate chaeta in each bundle. All following chaetigers possess two bilimbate and two forked chaetae in each bundle, except for the second one with four bilimbate chaetae.

Habitat. Specimens predominantly occur at beaches with medium-sized sand grains at or above the high-water line.

Distribution. San Juan Island (WA, USA). If the additional records from the North-Eastern Pacific along the US and Canadian Pacific coast (Purschke, 2006, 1999; Purschke et al., 2019; Schmidt and Westheide, 2000; Westheide, 2008) belong to *S. furcata* sp. nov., or *S. americae* sp. nov. or constitute new species altogether is uncertain. Therefore, these records should be considered as *Stygocapitella* sp. for the time being.

Etymology. The species name reflects upon field collection. The field collection site is in a private property, and while collecting, the caretaker of the property mentioned we could collect sand as long as we would support a progressive such as Bernie Sanders. The species honours Bernie Sanders for his efforts of inclusiveness, diversity and protection of minorities and underrepresented groups.

***Stygocapitella americae* sp. nov.**

Stygocapitella subterranea partim (Purschke, 2006, 1999; Purschke et al., 2019; Schmidt and Westheide, 2000; Struck et al., 2017; Westheide, 2008), not Köllner 1934.

Types and material examined. Holotype: Reuben Tarte State Park, USA (WA), N 48° 28' 05.6" / W 123° 00' 10.7", between 5 m above high-water line and the high-water line, at a depth of 0-15 centimetres Coll. Natural History Museum of the University of Oslo (NHMO C6992). Additional material: 1 paratype from Reuben Tarte State Park, USA (WA), N 48° 28' 05.6" / W 123° 00' 10.7", between 5 m above high-water line and the high-water line, at a depth of 0-15 centimetres. Coll. Natural History Museum of the University of Oslo (NHMO C6993). Besides the holotype and the paratype, 14 specimens for molecular work and two for SEM have examined.

Type Locality. Reuben Tarte State Park, USA (WA), N 48° 28' 05.6" / W 123° 00' 10.7"

Diagnosis. For morphology see *S. berniei* sp. nov.. For genetic data see Genbank ID: MN158590 (COI); MN164069 (16S).

Description. Color: White-transparent with a slightly iridescence surface. Size: body length: mean 2007 µm (range 2552.8 – 1342.9 µm) and width 227.6 µm (273.7 – 166.7 µm); prostomium length 69.9 µm (95.5 – 42.9 µm) and width 138 µm (182.2 – 86.7 µm); pygidium length 53.5 µm (70.9 – 39.4 µm) and width 116.5 µm (145.8 – 74.9 µm) (Fig. 6). The body comprises a prostomium without appendages, a peristomium bearing the mouth opening, 13 segments and a round pygidium. 1st to 12th segment biannulated. Chaetae in pairs of ventrolateral bundles are present at segments 2 to 11 in the first ring of each segment. First chaetiger with two bilimbate chaetae with whip-like extensions, two forked chaetae and two bilimbate chaeta in each bundle. All following chaetigers possess two bilimbate and two forked chaetae in each bundle, except for the second one with four bilimbate chaetae.

Habitat. Specimens predominantly occur at beaches with medium-sized sand grains at or above the high-water line.

Distribution: San Juan Island (WA, USA). If the additional records from the North-Eastern Pacific along the US and Canadian Pacific coast (Purschke, 2006, 1999; Purschke et al., 2019; Schmidt and Westheide, 2000; Westheide, 2008) belong to this species, *S. furcata* sp. nov., or *S. berniei* sp. nov. or constitute new species altogether is uncertain. Therefore, these records should be considered as *Stygocapitella* sp. for the time being.

Etymology. The species name follows the American continent, where it was collected.

***Stygocapitella budaevae* sp. nov.**

Types and material examined. Holotype: Volchanets, Russia, N 42° 54' 37.7" / E 132° 44' 25.0", 4.0 m above high-water line at a depth of 15-30 centimetres, Coll. Natural History Museum of the University of Oslo (NHMO C6990). Additional material: 1 paratype Volchanets, Russia, N 42° 54' 37.7" / E 132° 44' 25.0", 4.0 m above high-water line at a depth of 0-15 centimetres. Coll. Natural History Museum of the University of Oslo (NHMO C6991). Besides the holotype and paratype, 18 specimens for molecular work and two for SEM have examined.

Type locality. Volchanets, Russia, N 42° 54' 37.7" / E 132° 44' 25.0"

Diagnosis: For morphology see *S. berniei* sp. nov. For genetic data check genbank Ids MN158380 (COI), MN164048 (16S)

765 **Description**. Color: White-transparent with a slightly iridescence surface. Size: body
766 length: mean 1368.5 μm (range 1655 - 1141 μm) and width 152.2 μm (167.7 – 131.5 μm);
767 prostomium length 55 μm (62.1 – 46.8 μm) and width 92 μm (97.6 – 87.9 μm); pygidium length
768 42.4 μm (62.9 – 28.2 μm) and width 61.1 μm (76.6 – 45.2 μm) (Fig. 6). The body comprises a
769 prostomium without appendages, a peristomium bearing the mouth opening, 13 segments and a
770 round pygidium. 1st to 12th segment biannulated. Chaetae in pairs of ventrolateral bundles are
771 present at segments 2 to 11 in the first ring of each segment. First chaetiger with two bilimbate
772 chaetae with whip-like extensions, two forked chaetae and two bilimbate chaeta in each bundle.
773 All following chaetigers possess two bilimbate and two forked chaetae in each bundle, except for
774 the second one with four bilimbate chaetae.

775 **Habitat**: Specimens occurred at a beach with medium-sized sand grains at or above the
776 higher water level up to a depth of 20 cm.

777 **Distribution.** Volchanets (Primorsky Krai region, Russia)

778 **Etymology**. The species name honours the Russian polychaete biologist Nataliya
779 Budaeva for her contributions to Annelid systematics.

780 ***Stygocapitella zecai* sp. nov.**

781 *Stygocapitella subterranea* partim (Purschke, 2006, 1999; Purschke et al., 2019; Schmidt,
782 1972b, 1970, 1969; Struck et al., 2017; Westheide, 2008; Worsfold, 2008), not Köllner 1934.

783 **Types and material examined. Holotype**: Henningsvær, Norway, 68°15'38.8"N
784 14°16'06.1"E between high-water line and 6 m above the high-water line, at a depth of 0-15
785 centimetres Coll. Natural History Museum of the University of Oslo (NHMO C6988). Additional
786 material: 1 paratype from Henningsvær 68°15'38.8"N 14°16'06.1"E between 6 m above high-
787 water line and high-water line, at a depth of 0-15 centimetres. Coll. Natural History Museum of
788 the University of Oslo (NHMO C6989). Besides the holotype and paratype, 47 specimens for
789 molecular work and two for SEM have examined.

790 **Type locality.** Henningsvær, Norway, 68°15'38.8"N 14°16'06.1"E

791 **Diagnosis.** For morphology see *S. berniei* sp. nov.. For genetic data please see Genbank
792 ID MN164099 (16S).

Description. Color: White-transparent with a slightly iridescence surface. Size: body length: mean 2238.2 μm (range 2573.3 – 1514.3 μm) and width 236.6 μm (295.5 – 170.5 μm); prostomium length 63.1 μm (85.3 – 44.3 μm) and width 117.8 μm (147.9 – 96.7 μm); pygidium length 61.2 μm (100.8 – 45.9 μm) and width 101.4 μm (126 – 69.7 μm) (Fig. 6). The body comprises a prostomium without appendages, a peristomium bearing the mouth opening, 13 segments and a round pygidium. 1st to 12th segment biannulated. Chaetae in pairs of ventrolateral bundles are present at segments 2 to 11 in the first ring of each segment. First chaetiger with two bilimbate chaetae with whip-like extensions, two forked chaetae and two bilimbate chaeta in each bundle. All following chaetigers possess two bilimbate and two forked chaetae in each bundle, except for the second one with four bilimbate chaetae.

Habitat. Specimens predominantly occur at beaches with medium-sized sand grains at or above the high-water line. Especially at beaches with low tidal exposure.

Distribution. The distribution is predominantly in the Northern Atlantic comprising the Northern and North Seas (Scandinavian, British & German coast) (Supplementary Fig. 1).

Etymology. While collecting in Henningsvær, JC was hearing Zeca Afonso, an important freedom fighter whose songs inspired generations. The name honors him.

***Stygocapitella josemariobrancoi* sp. nov.**

Stygocapitella subterranea partim (Purschke, 2006, 1999; Purschke et al., 2019; Schmidt, 1970, 1969; Struck et al., 2017; Westheide, 2008, 1966; Worsfold, 2008), not Köllner 1934.

Types and material examined. Holotype: Plymouth Bay, 50°20'55.0"N 4°12'02.6"W between high-water line and one meter above, at a depth of 0-15 centimetres. Coll. Natural History Museum of the University of Oslo (NHMO C6986). Additional material: 1 paratype Plymouth Bay, between high-water line and one meter above, at a depth of 0-15 centimetres, 50°20'55.0"N 4°12'02.6"W. Coll. Natural History Museum of the University of Oslo (NHMO C6987). Besides the holotype, paratype and mature additional material 97 specimens for molecular work and two for SEM have examined.

Type locality. Plymouth Bay, 50°20'55.0"N 4°12'02.6"W

Diagnosis. For morphology, see *S. subterranea*. For genetic data, see Genbank ID MN158471 (COI); 16S (MN164224).

Description. Color: White-transparent with a slightly iridescence surface. Size: body length: mean 2409.7 μm (range 3751.9 - 1317 μm) and width 257.8 μm 423.7 – 159.6 μm); prostomium length 84.5 μm (153.3 – 48 μm) and width 157.7 μm (222.1 – 104.5 μm); pygidium length 61.5 μm (160.3 – 39.1 μm) and width 127.6 μm (163.4 – 97.4 μm) (Fig. 6). The body comprises a prostomium without appendages, a peristomium bearing the mouth opening, 13 segments and a round pygidium. 1st to 12th segment biannulated. Chaetae in pairs of ventrolateral bundles are present at segments 2 to 11 in the first ring of each segment. First chaetiger with two bilimbate chaetae with whip-like extensions, two forked chaetae and two bilimbate chaeta in each bundle. The second chaetiger with three bilimbate and two forked chaetae and all following ones with two bilimbate and two forked.

Habitat. Specimens predominantly occur at beaches with medium-sized sand grains at or above the high-water line. Especially at beaches with low tidal exposure.

Distribution. The distribution is predominantly in the North Sea (British & German coast) and the Channel (British & French coast) (Supplementary Fig. 1). Two individuals were also found in Lubec (ME, USA).

Etymology. The name honors José Mário Branco, an important Portuguese singer whose music inspired whole generations. JC was hearing his music in the field.

***Stygocapitella westheidei* sp. nov.**

Stygocapitella subterranea partim, (Purschke, 2006, 1999; Purschke et al., 2019; Riser, 1980; Schmidt and Westheide, 2000; Struck et al., 2017; Westheide, 2008) not Köllner 1934.

Types and material examined. Holotype: Canoe beach, 42°25'10.6"N 70°54'24.6"W between 5 and 7 m above high-water line in a depth of 0-30 cm, Coll. Natural History Museum of the University of Oslo (NHMO C6984). Additional material: 1 paratype Canoe beach, 42°25'10.6"N 70°54'24.6"W between 5 and 7 m above high-water line in a depth of 0-30 cm. Coll. Natural History Museum of the University of Oslo (NHMO C 6985). Besides the holotype, and paratype, 36 specimens for molecular work and two for SEM have examined.

Type locality. Canoe beach, 42°25'10.6"N 70°54'24.6"W

Diagnosis. For morphology see *S. subterranea*. For genetic data see Genbank IDs MN158481 (COI) and MN164233 (16S)

Description. Color: White-transparent with a slightly iridescence surface. Size: body length: mean 1820.2 μm (range 2299- 1521.3 μm) and width 188.5 μm (237.2 – 147.4 μm); prostomium length 63.8 μm (73.4 – 49.2 μm) and width 127.6 μm (168.3 – 105.9 μm); pygidium length 48.6 μm (59.3 – 34.9 μm) and width 94.5 μm (121.9 – 72.2 μm) (Fig. 6). The body comprises a prostomium without appendages, a peristomium bearing the mouth opening, 13 segments and a round pygidium. 1st to 12th segment biannulated. Chaetae in pairs of ventrolateral bundles are present at segments 2 to 11 in the first ring of each segment. First chaetiger with two bilimbate chaetae with whip-like extensions, two forked chaetae and two bilimbate chaeta in each bundle. The second chaetiger with three bilimbate and two forked chaetae and all following ones with two bilimbate and two forked.

Habitat. Specimens predominantly occur at beaches with medium-sized sand grains at or above the high-water line. Especially at beaches with low tidal exposure.

Distribution. The distribution is in the North-western Atlantic (US and Canadian coast) (Supplementary Fig. 1).

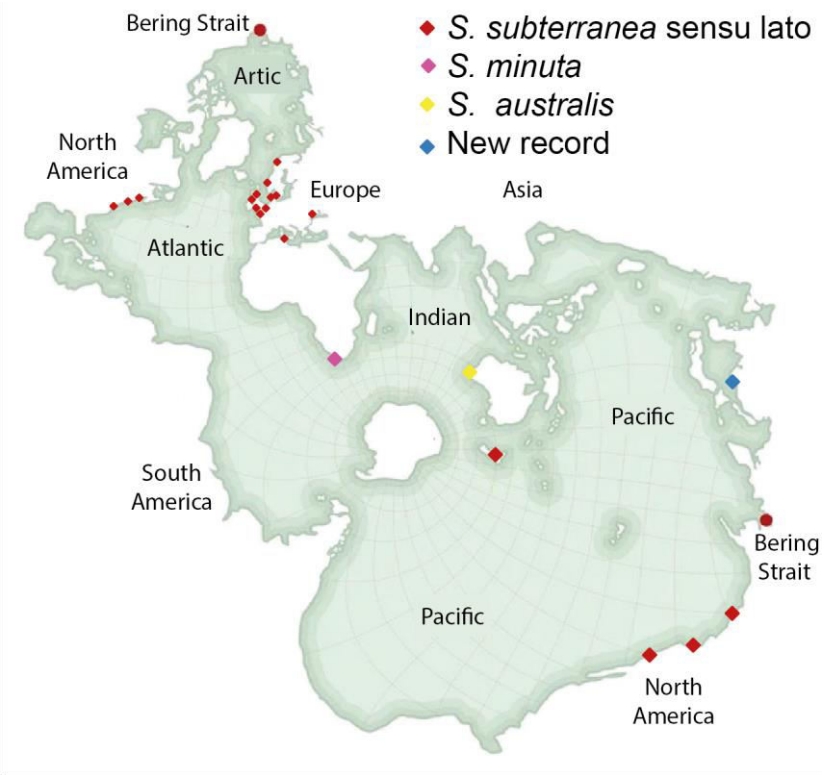
Etymology. The species name honours the polychaete biologist and invertebrate specialist Wilfried Westheide for his numerous contributions to systematics of interstitial polychaetes, the cryptic species problem and invertebrate systematics, and also for his mentorship.

Acknowledgments

JC is grateful to Gustav Paulay, Claudia E. Mills, Bernadette Holthuis and Tim Miller for field site suggestions in the USA. JC is thankful to Tim Worsfold, Andy Mackie, Henning Reiss, Lis Jørgensen for laboratory space in the UK and Norway. JC and THS are grateful to Vasily Radashevsky for hosting us in Russia, and to Nataliya Budaeva for support in obtaining Russian VISAs and funding. JC was partly funded by the Ragen Award from Friday Harbor Laboratories, a Den Grevelige Hjelmstjerne-Rosencroneske Stiftelse ved UiOslo. Funding from FORBIO – Research school in biosystematics (JC) was seminal to obtain photographs as part of a visit to Osnabrück. Funding from the ASSEMBLE project, an EU FP7 research infrastructure initiative, funded the collecting trip to Scotland (THS). JC and THS were partly supported by the SIU-funded MEDUSA project (Multidisciplinary EDUcation and reSearch in mArine biology in Norway and Russia). We acknowledge the use of the Norwegian national e-infrastructure for high-performance computing and storage via the projects NN9408K and NS9408K, respectively.

882 The authors would like to thank two anonymous reviewers for their comments which have
883 substantially improved a previous version of this manuscript. the This is NHM Evolutionary
884 Genomics lab contribution nr **XX**.

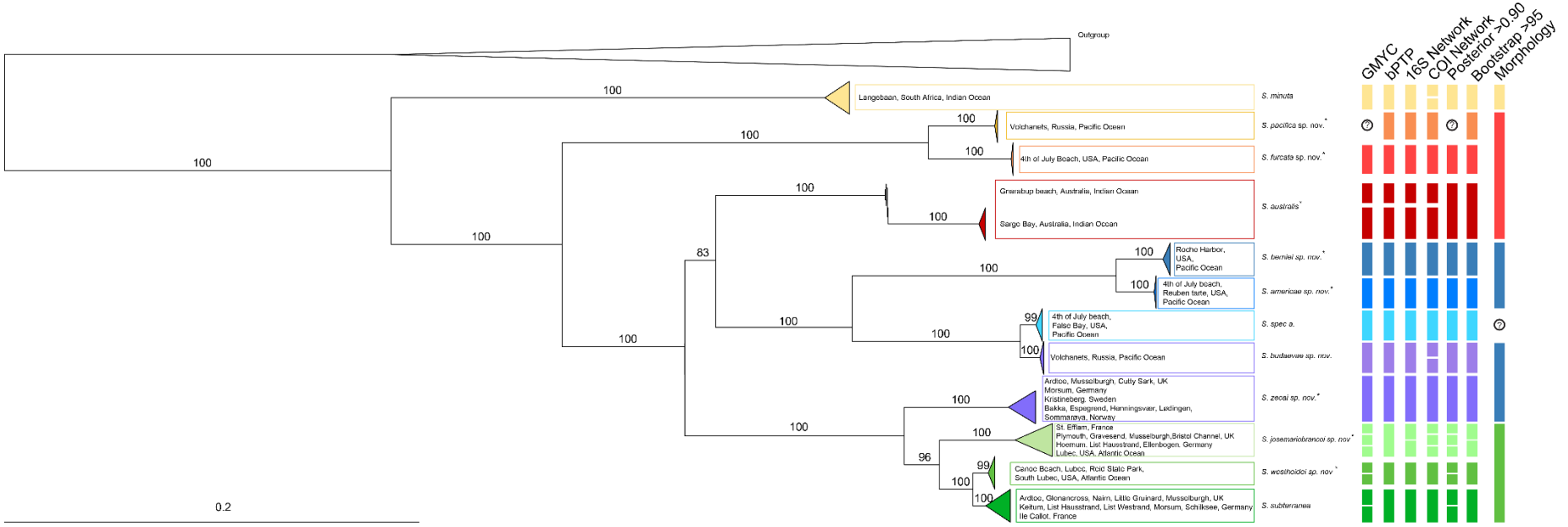
885 **Figure legends**



886

887 **Figure 1:** *Stygocapitella* records considered to date. *Stygocapitella subterranea* (red diamond;
888 *sensu lato*) has been recognised as a cosmopolitan species, *S. minuta* (purple diamond) is found in
889 South Africa and *S. australis* (yellow diamond) in Australia. As part of this work we report
890 *Stygocapitella* occurring in Volchanets (Far-east Russia; blue diamond).

891



892

893 **Figure 2:** Maximum likelihood phylogeny and species delimitation approach. Phylogenetic tree was obtained using a concatenated-partitioned
894 dataset including COI, 16S, 18S and ITS1. Bootstrap support is included above each branch. Sampling locations, as well as species names are included
895 after each edge. Species delimitation analysis include several approaches: Generalized Mixed Yule Coalescent Approach (GMYC; after a Bayesian tree
896 of the concatenated COI and 18S dataset), bPTP (Bayesian Poisson Tree Processes; of the ML tree using the concatenated 18S, COI, 16S and ITS1
897 dataset), 16S network using a 95% cut-off, COI network using a 95% cut-off, posterior probabilities of >0.90, bootstrap support > 95 and
898 morphology. Question marks highlight cases where models did not run. In specific, because we had only one specimen for the final Bayesian analysis,
899 we were unable to obtain posterior probabilities and to run GMYC (question mark in GMYC and posterior columns). The question mark in the
900 morphology column indicates the species in which we were unable to obtain morphological data (SEM photographs). The scale bar shows
901 substitutions per site. Species followed by an asterisk (*) represent species which were previously considered as *Stygocapitella subterranea (sensu lacto)*.

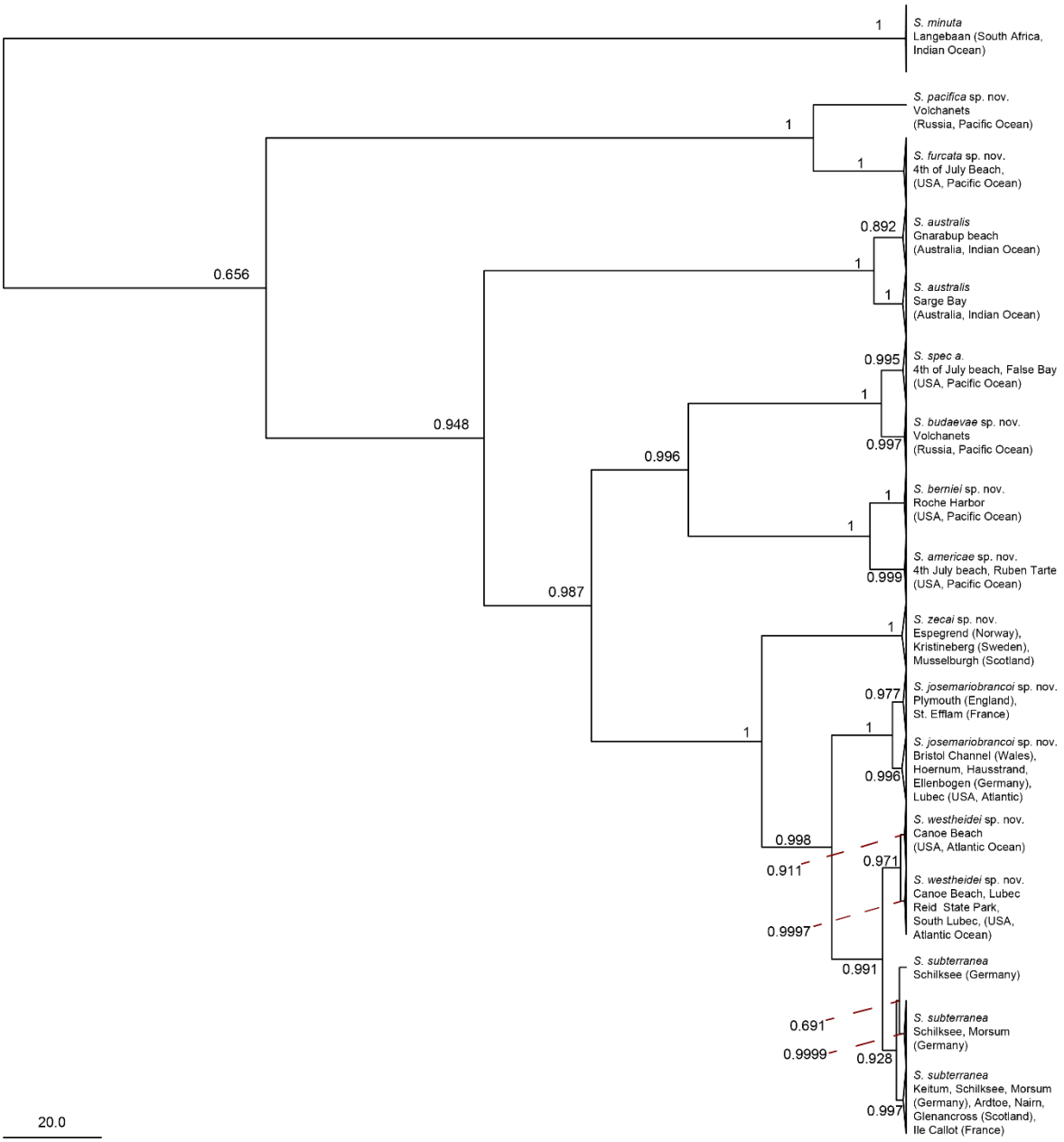
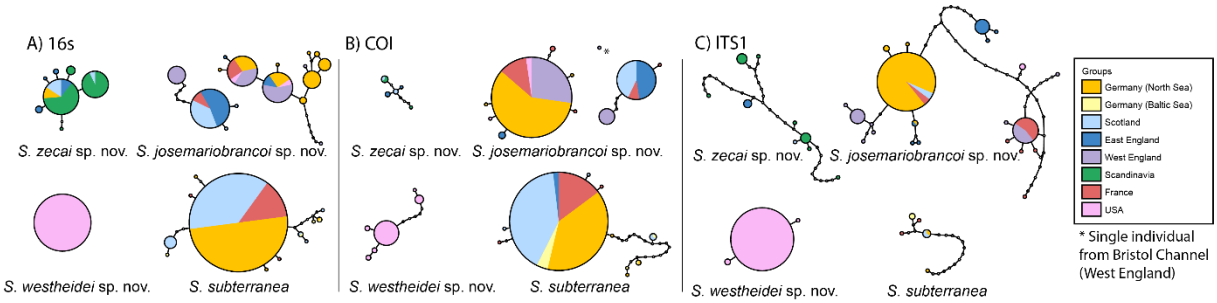
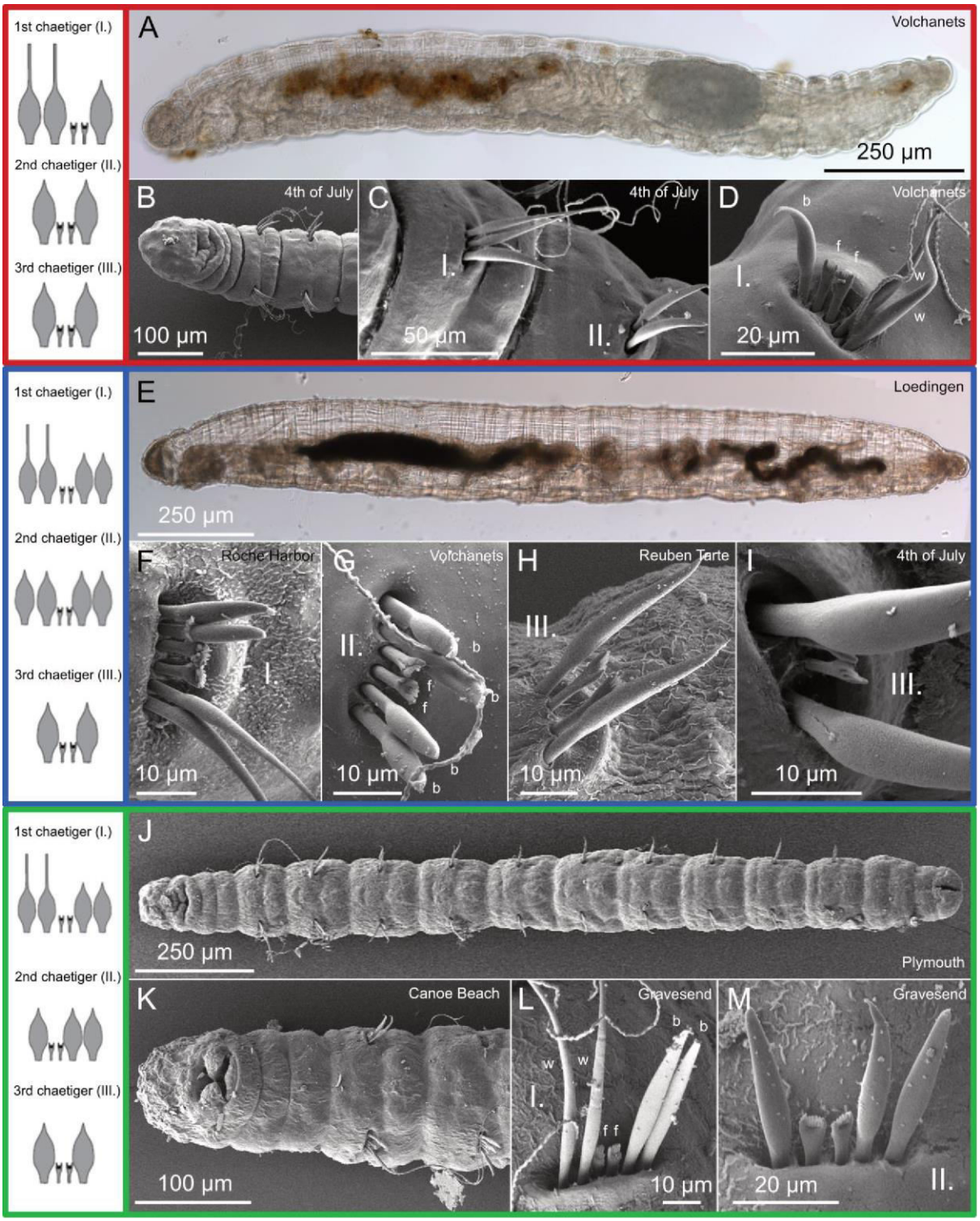


Figure 3: Bayesian phylogenetic tree based on concatenated 18S and COI. Posterior probability is displayed at each branch. Species names and sampling locations are displayed on the species edges.

907 

908 **Figure 4:** Haplotype networks. 16S (A), COI (B), ITS1 (C) based haplotype network of
909 the species present in the Atlantic Ocean (*S. zecai* sp. nov., *S. subterranea*, *S. westheidei* sp. nov., *S.*
910 *josemariobrancoi*). Haplotype sp. nov. networks are colored based on countries and regions.

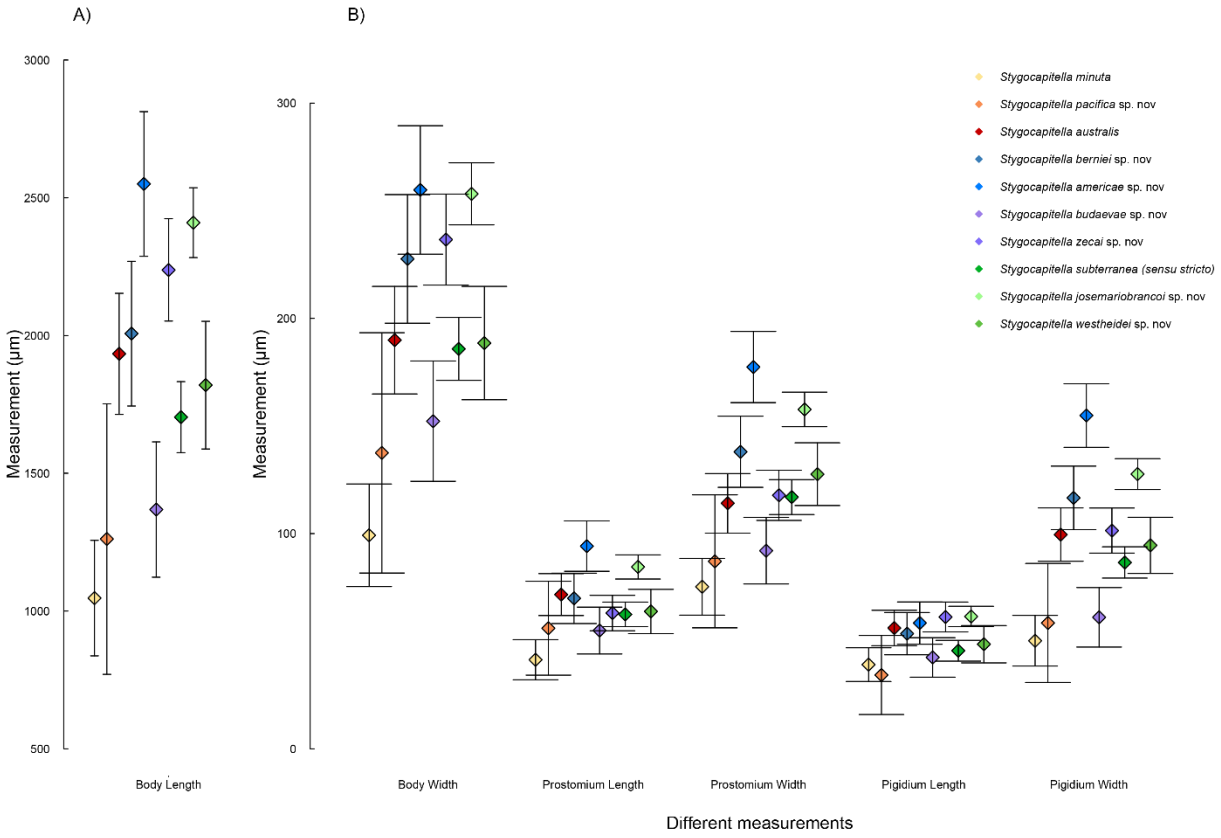
911



912

913 **Figure 5:** Scanning electron microscopy and light microscopy images of *Stygocapitella*.
914 Three morphotypes are represented in red, blue and green boxes (for species delimitation see also
915 Figure 2). A) Light microscopy photograph of *S. pacifica* sp. nov. from Volchanets. B & C) SEM
916 images of *S. furcata* sp. nov. from 4th of July Beach with first (I.) and second (II.) chaetae bearing
917 chaetiger. D) 1st chaetiger of *S. pacifica* sp. nov. with 2 whip-like (w), two forked (f) and 1
918 bilimbate (b) chaetae. E) Light microscopy photograph of *S. zecai* sp. nov. from Lødingen. F) 1st

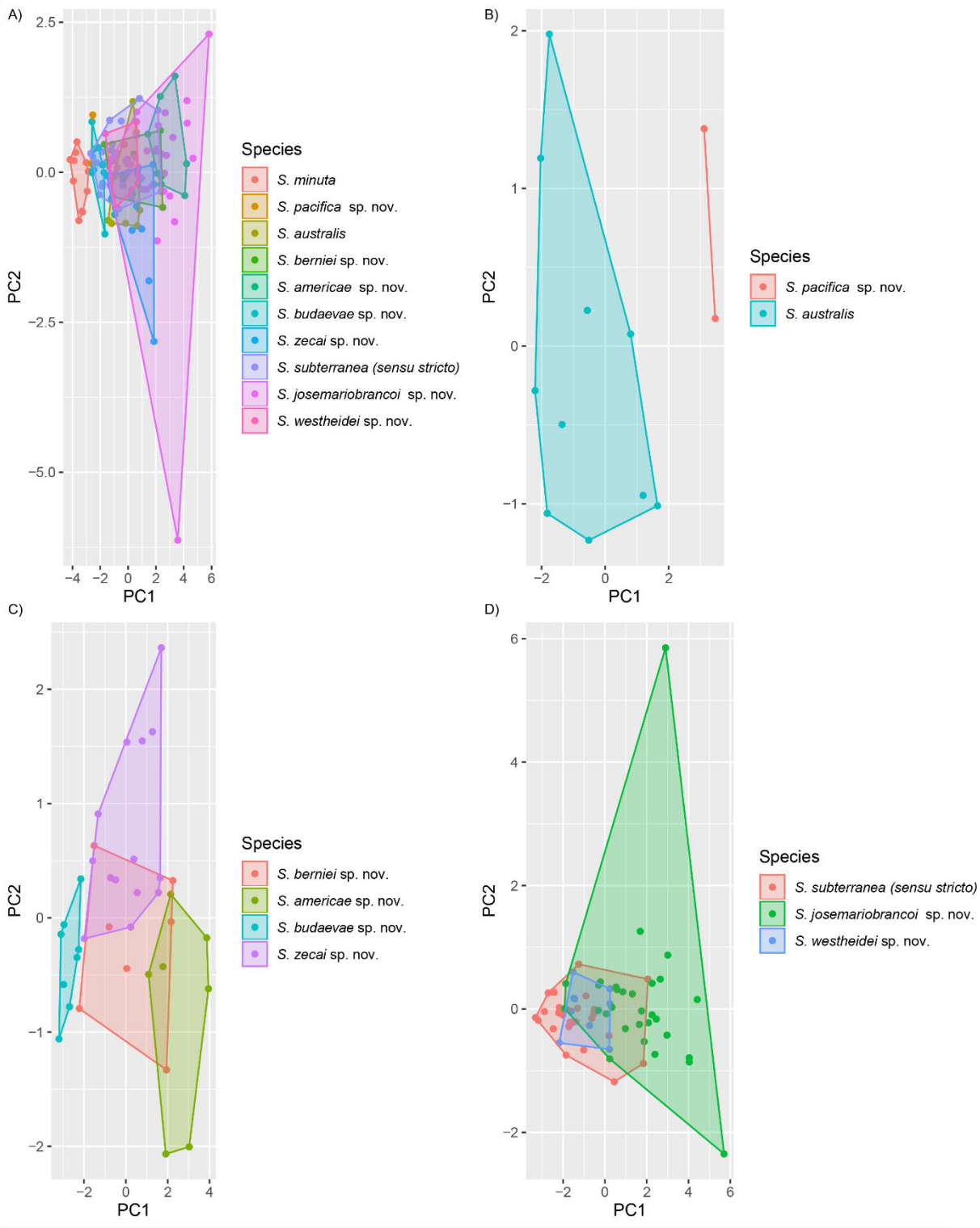
chaetiger of *S. berniei* sp. nov. from Roche Harbor with 2 whip-like, 2 forked and 2 bilimbate chaetae. G) 2nd chaetiger of *S. budaevae* sp. nov. from Volchanets with 2 bilimbate (b), 2 forked (f) and 2 bilimbate (b) chaetae. H) 3rd chaetiger of *S. americae* sp. nov. from Reuben Tarte. I) 3rd chaetiger of *Stygocapitella* from 4th of July Beach. J) SEM images of whole *S. josemariobrancoi* sp. nov. from Plymouth. K) Anterior end of *S. westheidei* sp. nov. from Canoe Beach. L & M) First two chaetigers of *S. josemariobrancoi* sp. nov. from Gravesend. 1st chaetiger with 2 whip-like (w), 2 forked (f) and 2 bilimbate (b) chaetae. 2nd chaetiger with 1 bilimbate (b), two forked (f) and 2 bilimbate (b) chaetae.



928

929 **Figure 6:** Morphometric analysis. Panel A displays body length measurements (µm) and
930 panel B) displays body width, prostomium length and width, and pygidium length and width
931 (µm).

932



933

934 **Figure 7:** Principal component analysis of morphological measurements. Every panel
935 displays the first two principal components (PC1-PC2). Panel A) displays all species. Panel B), C)
936 and D) display only species from separate morphotypes (see figure 5).

937

938 **References**

939 Appeltans, W., Ahyong, S.T., Anderson, G., Angel, M. V., Artois, T., Bailly, N., Bamber, R.,
940 Barber, A., Bartsch, I., Berta, A., Błazewicz-Paszkowycz, M., Bock, P., Boxshall, G., Boyko,
941 C.B., Brandão, S.N., Bray, R.A., Bruce, N.L., Cairns, S.D., Chan, T.Y., Cheng, L., Collins,
942 A.G., Cribb, T., Curini-Galletti, M., Dahdouh-Guebas, F., Davie, P.J.F., Dawson, M.N., De
943 Clerck, O., Decock, W., De Grave, S., De Voogd, N.J., Domning, D.P., Emig, C.C., Erséus,
944 C., Eschmeyer, W., Fauchald, K., Fautin, D.G., Feist, S.W., Fransen, C.H.J.M., Furuya, H.,
945 Garcia-Alvarez, O., Gerken, S., Gibson, D., Gittenberger, A., Gofas, S., Gómez-Daglio, L.,
946 Gordon, D.P., Guiry, M.D., Hernandez, F., Hoeksema, B.W., Hopcroft, R.R., Jaume, D.,
947 Kirk, P., Koedam, N., Koenemann, S., Kolb, J.B., Kristensen, R.M., Kroh, A., Lambert, G.,
948 Lazarus, D.B., Lemaitre, R., Longshaw, M., Lowry, J., MacPherson, E., Madin, L.P., Mah,
949 C., Mapstone, G., McLaughlin, P.A., Mees, J., Meland, K., Messing, C.G., Mills, C.E.,
950 Molodtsova, T.N., Mooi, R., Neuhaus, B., Ng, P.K.L., Nielsen, C., Norenburg, J., Opresko,
951 D.M., Osawa, M., Paulay, G., Perrin, W., Pilger, J.F., Poore, G.C.B., Pugh, P., Read, G.B.,
952 Reimer, J.D., Rius, M., Rocha, R.M., Saiz-Salinas, J.I., Scarabino, V., Schierwater, B.,
953 Schmidt-Rhaesa, A., Schnabel, K.E., Schotte, M., Schuchert, P., Schwabe, E., Segers, H.,
954 Self-Sullivan, C., Shenkar, N., Siegel, V., Sterrer, W., Stöhr, S., Swalla, B., Tasker, M.L.,
955 Thuesen, E. V., Timm, T., Todaro, M.A., Turon, X., Tyler, S., Uetz, P., Van Der Land, J.,
956 Vanhoorne, B., Van Ofwegen, L.P., Van Soest, R.W.M., Vanaverbeke, J., Walker-Smith, G.,
957 Walter, T.C., Warren, A., Williams, G.C., Wilson, S.P., Costello, M.J., 2012. The magnitude
958 of global marine species diversity. Curr. Biol. 22, 2189–2202.
959 https://doi.org/10.1016/j.cub.2012.09.036

960 Astrin, J.J., Stüben, P.E., 2008. Phylogeny in cryptic weevils: Molecules, morphology and new
961 genera of western Palaearctic Cryptorhynchinae (Coleoptera: Curculionidae). Invertebr. Syst.
962 22, 503–522. https://doi.org/10.1071/IS07057

963 Barnes, D.K.A., 2002. Invasions by marine life on plastic debris. Nature 416, 808–809.
964 https://doi.org/10.1038/416808a

965 Bernardo, J., 2011. A critical appraisal of the meaning and diagnosability of cryptic evolutionary
966 diversity, and its implications for conservation in the face of climate change. Clim. Chang.
967 Ecol. Syst. 380–438. https://doi.org/10.1017/CBO9780511974540.019

968 Bickford, D., Lohman, D.J., Sodhi, N.S., Ng, P.K.L., Meier, R., Winker, K., Ingram, K.K., Das,

969 I., 2007. Cryptic species as a window on diversity and conservation. Trends Ecol. Evol. 22,
970 148–155. https://doi.org/10.1016/j.tree.2006.11.004

971 Bouckaert, R., Heled, J., Kühnert, D., Vaughan, T., Wu, C.H., Xie, D., Suchard, M.A., Rambaut,
972 A., Drummond, A.J., 2014. BEAST 2: A software platform for bayesian evolutionary
973 analysis. PLoS Comput. Biol. 10, 1–6. https://doi.org/10.1371/journal.pcbi.1003537

974 Carstens, B.C., Pelletier, T.A., Reid, N.M., Satler, J.D., 2013. How to fail at species delimitation.
975 Mol. Ecol. 22, 4369–4383. https://doi.org/10.1111/mec.12413

976 Cerca, J., Purschke, G., Struck, T.H., 2018. Marine connectivity dynamics: clarifying
977 cosmopolitan distributions of marine interstitial invertebrates and the meiofauna paradox.
978 Mar. Biol. 165, 123. https://doi.org/10.1007/s00227-018-3383-2

979 Chernomor, O., Von Haeseler, A., Minh, B.Q., 2016. Terrace aware data structure for
980 phylogenomic inference from supermatrices. Syst. Biol. 65, 997–1008.
981 https://doi.org/10.1093/sysbio/syw037

982 Clement, M., Posada, D., Crandall, K.A., 2000. TCS: a computer program to estimate gene
983 genealogies. Mol. Ecol. 9, 1657–1660. https://doi.org/10.1046/j.1365-294x.2000.01020.x

984 Costa, F.O., Carvalho, G.R., 2010. New insights into molecular evolution: Prospects from the
985 barcode of life initiative (BOLI). Theory Biosci. 129, 149–157.
986 https://doi.org/10.1007/s12064-010-0091-y

987 Derycke, S., Remerie, T., Backeljau, T., Vierstraete, A., Vanfleteren, J., Vincx, M., Moens, T.,
988 2008. Phylogeography of the *Rhabditis* (Pellioditis) marina species complex: Evidence for
989 long-distance dispersal, and for range expansions and restricted gene flow in the northeast
990 Atlantic. Mol. Ecol. 17, 3306–3322. https://doi.org/10.1111/j.1365-294X.2008.03846.x

991 Eldredge, N., Gould, S.J., 1972. Punctuated Equilibria: An alternative to phylogetic gradualism,
992 in: Schopf, T.J.M. (Ed.), Models in Paleobiology. Freeman, Cooper and Co., San Francisco.,
993 pp. 82–115.

994 Fišer, C., Robinson, C.T., Malard, F., 2018. Cryptic species as a window into the paradigm shift
995 of the species concept. Mol. Ecol. 27, 613–635. https://doi.org/10.1111/mec.14486

996 Fujisawa, T., Barraclough, T.G., 2013. Delimiting species using single-locus data and the

997 generalized mixed yule coalescent approach: A revised method and evaluation on simulated
998 data sets. Syst. Biol. 62, 707–724. https://doi.org/10.1093/sysbio/syt033

999 Futuyma, D.J., 2010. Evolutionary constraint and ecological consequences. Evolution (N. Y). 64,
1000 1865–1884. https://doi.org/10.1111/j.1558-5646.2010.00960.x

1001 Giere, O., 2009. Meiobenthology: the microscopic motile fauna of aquatic sediments, 2nd ed.
1002 Springler-Verlag, Berlin Heidelberg. https://doi.org/10.1016/0022-0981(94)90135-X

1003 Harrell Jr, F.E., Many Others, contributions from, 2019. Hmisc: Harrell Miscellaneous. R
1004 package.

1005 Hawksworth, D.L., Lücking, R., 2017. Fungal diversity revisited : 2.2 to 3.8 million species.
1006 Microbiol. Spetrum 5, 1–17. https://doi.org/10.1128/microbiolspec.FUNK-0052-
1007 2016.Correspondence

1008 Hellberg, M.E., 2009. Gene flow and isolation among populations of marine animals. Annu. Rev.
1009 Ecol. Evol. Syst. 40, 291–310. https://doi.org/10.1146/annurev.ecolsys.110308.120223

1010 Hillis, D.M., Dixon, M.T., 1991. Ribosomal DNA: Molecular evolution and phylogenetic
1011 inference. Q. Rev. Biol. 66, 411–453.

1012 Hoang, D.T., Chernomor, O., von Haeseler, A., Quang Minh, B., Sy Vinh, L., 2017. Ufboot2:
1013 Improving the ultrafast bootstrap approximation 35, 518–522.
1014 https://doi.org/10.5281/zenodo.854445

1015 Holt, B.G., Lessard, J.-P., Borregaard, M.K., Fritz, S.A., Araújo, M.B., Dimitrov, D., Fabre, P.-H.,
1016 Graham, C.H., Graves, G.R., Jønsson, K.A., Nogués-Bravo, D., Wang, Z., Whittaker, R.J.,
1017 Fjeldså, J., Rahbek, C., 2013. An update of Wallace's zoogeographic regions of the world.
1018 Science (80-.). 339, 74–79. https://doi.org/10.1590/S1413-81232011000600020

1019 Horton, T., Et. Al., 2019. World Register of Marine Species, taxon details for Orbiniidae
1020 Hartman, 1942 [WWW Document].
1021 http://www.marinespecies.org/aphia.php?p=taxdetails&id=902&allchildren=1.

1022 Johannesson, K., 1988. The paradox of Rockall: why is a brooding gastropod (*Littorina saxatilis*)
1023 more widespread than one having a planktonic larval dispersal stage (*L. littorea*)? Mar. Biol.
1024 99, 507–513. https://doi.org/10.1007/BF00392558

1025 Jörger, K.M., Norenburg, J.L., Wilson, N.G., Schrödl, M., 2012. Barcoding against a paradox?
1026 Combined molecular species delineations reveal multiple cryptic lineages in elusive
1027 meiofaunal sea slugs. BMC Evol. Biol. 12, 245. https://doi.org/10.1186/1471-2148-12-245

1028 Jörger, K.M., Schrödl, M., 2013. How to describe a cryptic species? Practical challenges of
1029 molecular taxonomy. Front. Zool. 10, 59. https://doi.org/10.1186/1742-9994-10-59

1030 Kalyaanamoorthy, S., Minh, B.Q., Wong, T.K.F., Von Haeseler, A., Jermiin, L.S., 2017.
1031 ModelFinder: Fast model selection for accurate phylogenetic estimates. Nat. Methods 14,
1032 587–589. https://doi.org/10.1038/nmeth.4285

1033 Karling, T.G., 1958. Zur Kenntnis von *Stygocapitella subterranea* Knöllner und *Parergodrilus heideri*
1034 Reisinger. Ark. för Zool. 1, 307–324.

1035 Katoh, K., Standley, D.M., 2013. MAFFT multiple sequence alignment software version 7:
1036 Improvements in performance and usability. Mol. Biol. Evol. 30, 772–780.
1037 https://doi.org/10.1093/molbev/mst010

1038 Knöllner, F., 1934. *Stypocapitella subterranea* nov.gen. nov.spec. Schriften der
1039 Naturwissenschaftlichen Vereins für Schleswig-Holstein 20, 468–472.

1040 Knowlton, N., 2000. Molecular genetic analyses of species boundaries in the sea. Hydrobiologia
1041 420, 73–90. https://doi.org/10.1023/A:1003933603879

1042 Knowlton, N., 1993. Sibling species in the sea. Annu. Rev. Ecol. Syst. 24, 189–216.

1043 Kon, T., Yoshino, T., Mukai, T., Nishida, M., 2007. DNA sequences identify numerous cryptic
1044 species of the vertebrate: A lesson from the gobioid fish *Schindleria*. Mol. Phylogenet. Evol.
1045 44, 53–62. https://doi.org/10.1016/j.ympev.2006.12.007

1046 Korshunova, T., Martynov, A., Bakken, T., Picton, B., 2017. External diversity is restrained by
1047 internal conservatism: New nudibranch mollusc contributes to the cryptic species problem.
1048 Zool. Scr. 46, 683–692. https://doi.org/10.1111/zsc.12253

1049 Korshunova, T., Picton, B., Furfaro, G., Mariottini, P., Pontes, M., Prkić, J., Fletcher, K.,
1050 Malmberg, K., Lundin, K., Martynov, A., 2019. Multilevel fine-scale diversity challenges the
1051 'cryptic species' concept. Sci. Rep. 9, 6732. https://doi.org/10.1038/s41598-019-42297-5

1052 Kück, P., Meusemann, K., 2010. FASconCAT: Convenient handling of data matrices. Mol.

1053 Phylogenet. Evol. 56, 1115–1118. https://doi.org/10.1016/j.ympev.2010.04.024

1054 Leasi, F., Norenburg, J.L., 2016. At least some meiofaunal species are not everywhere. Indication
1055 of geographic, ecological and geological barriers affecting the dispersion of species of
1056 *Ototyphlonemertes* (Nemertea, Hoplonemertea). Mol. Ecol. 25, 1381–1397.
1057 https://doi.org/10.1111/mec.13568

1058 Leasi, F., Norenburg, J.L., 2014. The necessity of DNA taxonomy to reveal cryptic diversity and
1059 spatial distribution of meiofauna, with a focus on Nemertea. PLoS One 9.
1060 https://doi.org/10.1371/journal.pone.0104385

1061 Lee, C.E., Frost, B.W., 2002. Morphological stasis in the *Eurytemora affinis* species complex
1062 (Copepoda: Temoridae). Hydrobiologia 480, 111–128.
1063 https://doi.org/10.1023/A:1021293203512

1064 Lehmacher, C., Ramey-balci, P.A., Wolff, L.I., Fiege, D., 2016. Ultrastructural differences in
1065 presumed photoreceptive organs and molecular data as a means for species discrimination
1066 in Polygordius (Annelida, Protodriliformia , Polygordiidae). Org. Divers. Evol.
1067 https://doi.org/10.1007/s13127-016-0272-8

1068 Lenth, R. V., 2013. Lsmeans: Least-squares means. R package version 1.10-4. http://CRAN.R-
1069 project.org/package=lsmeans [WWW Document].

1070 Leray, M., Knowlton, N., 2016. Censusing marine eukaryotic diversity in the twenty-first century.
1071 Philos. Trans. R. Soc. B Biol. Sci. 371, 20150331.
1072 https://doi.org/http://dx.doi.org/10.1098/rstb.2015.0331

1073 Lobo, J., Teixeira, M.A.L., Borges, L.M.S., Ferreira, M.S.G., Hollatz, C., Gomes, P.T., Sousa, R.,
1074 Ravara, A., Costa, M.H., Costa, F.O., 2016. Starting a DNA barcode reference library for
1075 shallow water polychaetes from the southern European Atlantic coast. Mol. Ecol. Resour.
1076 16, 298–313. https://doi.org/10.1111/1755-0998.12441

1077 Mack, R.N., Lonsdale, W.M., 2001. Humans as global plant dispersers: getting more than we
1078 bargained for. Bioscience 51, 95. https://doi.org/10.1641/0006-
1079 3568(2001)051[0095:hagpdg]2.0.co;2

1080 Meyer-Wachsmuth, I., Curini Galletti, M., Jondelius, U., 2014. Hyper-cryptic marine meiofauna:
1081 Species complexes in Nemertodermatida. PLoS One 9.

https://doi.org/10.1371/journal.pone.0107688

Múrias Dos Santos, A., Cabezas, M.P., Tavares, A.I., Xavier, R., Branco, M., 2015. TcsBU: A tool to extend TCS network layout and visualization. Bioinformatics 32, 627–628. https://doi.org/10.1093/bioinformatics/btv636

Nguyen, L.T., Schmidt, H.A., Von Haeseler, A., Minh, B.Q., 2015. IQ-TREE: A fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Mol. Biol. Evol. 32, 268–274. https://doi.org/10.1093/molbev/msu300

Nygren, A., Parapar, J., Pons, J., Meißner, K., Bakken, T., Kongsrud, J.A., Oug, E., Gaeva, D., Sikorski, A., Johansen, R.A., Hutchings, P.A., Lavesque, N., Capa, M., 2018. A mega-cryptic species complex hidden among one of the most common annelids in the north east Atlantic, PLoS ONE. https://doi.org/10.1371/journal.pone.0198356

Nygren, A., Pleijel, F., 2011. From one to ten in a single stroke - resolving the European Eumida sanguinea (Phyllodocidae, Annelida) species complex. Mol. Phylogenet. Evol. 58, 132–141. https://doi.org/10.1016/j.ympev.2010.10.010

Palumbi, S., Martin, A., Romano, S., McMillan, W.O., Stice, L., Grabowski, G., 1991. The simple fool's guide to PCR, version 2.

Pante, E., Puillandre, N., Viricel, A., Arnaud-Haond, S., Aurelle, D., Castelin, M., Chenuil, A., Destombe, C., Forcioli, D., Valero, M., Viard, F., Samadi, S., 2015. Species are hypotheses: avoid connectivity assessments based on pillars of sand. Mol. Ecol. 24, 525–544. https://doi.org/10.1111/mec.13048

Purschke, G., 2006. Problematic Annelid Groups, in: Rouse, G.W., Pleijel, F. (Eds.), Reproductive Biology and Phylogeny of Annelida. Science Publisher, Enfield, Jersey, Plymouth, pp. 639–667.

Purschke, G., 1999. Terrestrial polychaetes - Models for the evolution of the Clitellata (Annelida)? Hydrobiologia 406, 87–99. https://doi.org/10.1023/A:1003780032497

Purschke, G., 1987. Anatomy and ultrastructure of ventral pharyngeal organs and their phylogenetic importance in Polychaeta (Annelida) - III. The pharynx of the Parergodrilidae. Zool. Jahrbücher Anat. 115, 331–362.

1110 Purschke, G., 1986. Ultrastructure of the nuchal organ in the interstitial polychaete *Stygocapitella*
1111 *subterranea* (Parergodriilidae). Zool. Scr. 15, 13–20.

1112 Purschke, G., Böggemann, M., Westheide, W., 2019. Parergodrilidae Reisinger, 1925, in:
1113 Purschke, G., Böggemann, M., Westheide, W. (Eds.), Annelida, Volume 1: Annelida Basal
1114 Groups and Pleistoannelida, Sedentaria. De Gruyter, Berlin, Boston, pp. 237–250.

1115 Purschke, G., Fursman, M.C., 2005. Spermatogenesis and Spermatozoa in *Stygocapitella subterranea*
1116 (Annelida, Parergodrilidae), an Enigmatic Supralittoral Polychaete. Zoomorphology 124,
1117 137–148. https://doi.org/10.1007/s00435-005-0001-x

1118 Purschke, G., Jördens, J., 2007. Male genital organs in the eulittoral meiofaunal polychaete
1119 stygocapitella subterranea (Annelida, Parergodrilidae): Ultrastructure, functional and
1120 phylogenetic significance. Zoomorphology 126, 283–297. https://doi.org/10.1007/s00435-
1121 007-0047-z

1122 R Core Team, 2013. R: A language and environment for statistical computing. R Foundation for
1123 Statistical Computing, Vienna, Austria. URL http://www.R-project.org/. [WWW
1124 Document].

1125 Radziejewska, T., Gruszka, P., Rokicka-Praxmajer, J., 2006. A home away from home: A
1126 meiobenthic assemblage in a ship's ballast water tank sediment. Oceanologia 48, 259–265.

1127 Rambaut, A., Drummond, A.J., Suchard, M.A., 2007. Tracer v1.6.

1128 Riser, N.W., 1984. General observations on the intertidal interstitial fauna of New Zealand. Tane
1129 30, 239–250.

1130 Riser, N.W., 1980. The aberrant polychaete *Stygocapitella* from some American beaches. Wasmann
1131 J. Biol. 38, 10–17.

1132 Schmidt, H., Westheide, W., 2000. Are the meiofaunal polychaetes *Hesionides arenaria* and
1133 *Stygocapitella subterranea* true cosmopolitan species? - results of RAPD-PCR investigations.
1134 Zool. Scr. 29, 17–27. https://doi.org/doi:10.1046/j.1463-6409.2000.00026.x

1135 Schmidt, P., 1972a. Zonierung und jahreszeitliche Fluktuationen des Mesopsammons im
1136 Sandstrand von Schilksee. Mikrofauna des Meeresbodens 10, 1–60.

1137 Schmidt, P., 1972b. Zonierung und jahreszeitliche Fluktuationen des Mesopsammons im

1138 Sandstrand von Schilksee (Kieler Bucht). Mikrofauna des Meeresbodens 10, 1–60.

1139 Schmidt, P., 1970. Zonation of the interstitial polychaete *Stygocapitella subterranea*
1140 (Stygocapitellidae) in European sandy beaches. Mar. Biol. 7, 319–323.

1141 Schmidt, P., 1969. Die quantitative Verteilung und Populationdynamik des Mesopsammons am
1142 Gezeiten-Sandstrand der Nordseeinsel Sylt - II. Quantitative verteilung und
1143 populationsdynamik einzelner Arten. Int. Rev. der gesamten Hydrobiol. und Hydrogr. 54,
1144 95–174.

1145 Schonrogge, K., Barr, B., Wardlaw, J., Napper, E., Gardner, M., Breen, J., Elmes, G., Thomas,
1146 J.A., 2002. When rare species become endangered: Cryptic speciation in myrmecolphilous
1147 hoverflies. J. Linn. Soc. 75, 291–300. https://doi.org/10.1046/j.1095-8312.2002.00019.x

1148 Singhal, S., Hoskin, C.J., Couper, P., Potter, S., Moritz, C., 2018. A framework for resolving
1149 cryptic species: a case study from the lizards of the Australian Wet Tropics. Syst. Biol.
1150 https://doi.org/10.1093/sysbio/syy026/4960881

1151 Sterrer, W., 1973. Plate tectonics as a mechanism for dispersal and speciation in interstitial sand
1152 fauna. Netherlands J. Sea Res. 7, 200–222.

1153 Struck, T.H., Cerca, J., 2019. Cryptic species and their Evolutionary significance. eLS 1–9.
1154 https://doi.org/10.1002/9780470015902.a0028292

1155 Struck, T.H., Feder, J.L., Bendiksby, M., Birkeland, S., Cerca, J., Gusarov, V.I., Kistenich, S.,
1156 Larsson, K.-H.H., Liow, L.H., Nowak, M.D., Stedje, B., Bachmann, L., Dimitrov, D., 2018a.
1157 Finding evolutionary processes hidden in cryptic species. Trends Ecol. Evol. 1–11.
1158 https://doi.org/10.1016/j.tree.2017.11.007

1159 Struck, T.H., Feder, J.L., Bendiksby, M., Birkeland, S., Cerca, J., Gusarov, V.I., Kistenich, S.,
1160 Larsson, K.-H.H., Liow, L.H., Nowak, M.D., Stedje, B., Bachmann, L., Dimitrov, D.,
1161 2018b. Cryptic Species – More Than Terminological Chaos: A Reply to Heethoff. Trends
1162 Ecol. Evol. 33, 310–312. https://doi.org/10.1016/j.tree.2018.02.008

1163 Struck, T.H., Koczula, J., Stateczny, D., Meyer, C., Purschke, G., 2017. Two new species in the
1164 annelid genus *Stygocapitella* (Orbiniida, Parergodrilidae) with comments on their
1165 biogeography. Zootaxa 4286, 301–332. https://doi.org/10.11646/zootaxa.4286.3.1

1166 Struck, T.H., Westheide, W., Purschke, G., 2002. Progenesis in Eunicida ("Polychaeta,"
1167 Annelida) - Separate evolutionary events? Evidence from molecular data. Mol. Phylogenet.
1168 Evol. 25, 190–199. https://doi.org/10.1016/S1055-7903(02)00231-2

1169 Suatoni, E., Vicario, S., Rice, S., Snell, T., Caccone, A., 2006. An analysis of species boundaries
1170 and biogeographic patterns in a cryptic species complex: The rotifer—*Brachionus plicatilis*.
1171 Mol. Phylogenet. Evol. 41, 86–98. https://doi.org/10.1016/j.ympev.2006.04.025

1172 Swift, H.F., Daglio, L.G., Dawson, M.N., 2016. Three routes to crypsis: stasis, convergence, and
1173 parallelism in the *Mastigias* species complex (Scyphozoa, Rhizostomeae). Mol. Phylogenet.
1174 Evol. 99, 103–115. https://doi.org/10.1016/j.ympev.2016.02.013

1175 Wada, S., Kameda, Y., Chiba, S., 2013. Long-term stasis and short-term divergence in the
1176 phenotypes of microsnails on oceanic islands. Mol. Ecol. 22, 4801–10.
1177 https://doi.org/10.1111/mec.12427

1178 Weber, A.A.-T., Stöhr, S., Chenuil, A., 2019. Species delimitation in the presence of strong
1179 incomplete lineage sorting and hybridization. Mol. Phylogenet. Evol. 131, 240218.
1180 https://doi.org/10.1101/240218

1181 Westheide, W., 2008. Polychaetes: Interstitial families., 2nd Editio. ed. Field Studies Council,
1182 Shrewsbury, 169 pp.

1183 Westheide, W., 1991. The meiofauna of the Galapagos: a review, in: Mathew J. James (Ed.),
1184 Galápagos Marine Invertebrates. Springler, New York, pp. 37–69.

1185 Westheide, W., 1990. Polychaetes: interstitial families. Keys and notes for the identification of the
1186 species., Universal. ed.

1187 Westheide, W., 1987. Progenesis as a principle in meiofauna evolution. J. Nat. Hist. 21, 843–854.
1188 https://doi.org/10.1080/00222938700770501

1189 Westheide, W., 1977. The geographical distribution of interstitial polychaetes. Mikrofauna
1190 Meeresb. 61, 287–302.

1191 Westheide, W., 1966. On the polychaete fauna of the eulitoral of the North Sea island Sylt. Zur
1192 Polychaetenfauna des Eulitorals der Nord. Sylt 13, 203–209.

1193 Westheide, W., Purschke, G., 1988. Organism processing, in: Higgins, R.P., Thiel, H. (Eds.),

Introduction to the Study of Meiofauna. Smithsonian Institution Press, Washington, pp. 146–160.

Wickham, H., 2016. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag, New York.

Worsfold, T.M., 2008. British records of the interstitial polychaete *Stygocapitella subterranea* (Annelida: Parergodrilidae). Mar. Biodivers. Rec. 1, 50–52. https://doi.org/10.1017/S1755267206004295

Zanol, J., Halanych, K.M., Struck, T.H., Fauchald, K., 2010. Phylogeny of the bristle worm family Eunicidae (Eunicida, Annelida) and the phylogenetic utility of noncongruent 16S, COI and 18S in combined analyses. Mol. Phylogenet. Evol. 55, 660–676. https://doi.org/10.1016/j.ympev.2009.12.024

Zhang, J., Kapli, P., Pavlidis, P., Stamatakis, A., 2013. A general species delimitation method with applications to phylogenetic placements. Bioinformatics 29, 2869–2876. https://doi.org/10.1093/bioinformatics/btt499

Supplementary data for manuscript 4

Supplementary Table 1: Sampling locations including GPS coordinates for this work.

Site	Coastline (Country)	Latitude	Longitude
4th July Beach	Eastern Pacific (USA)	48.46822	-123.00298
False bay	Eastern Pacific (USA)	48.49026	-123.06598
Roche Harbor	Eastern Pacific (USA)	48.59612	-123.16999
Reuben Tarte State Park	Eastern Pacific (USA)	48.61281	-123.09838
Canoe Beach	Western Atlantic (USA)	42.41962	-70.90684
Reid State Park	Western Atlantic (USA)	43.77628	-69.73121
Lubec	Western Atlantic (USA)	44.85482	-66.98179
South Lubec	Western Atlantic (USA)	44.82476	-66.98917
Île Callot	Eastern Atlantic (France)	48.68713	-3.92439
Saint Efflam	Eastern Atlantic (France)	48.684609	-3.62247
Hörnum	Eastern Atlantic (Germany)	54.75619	8.29466
Morsum	Eastern Atlantic (Germany)	54.87822	8.46527
Ellenbogen	Eastern Atlantic (Germany)	55.04397	8.45172
Keitum	Eastern Atlantic (Germany)	54.902	8.36766
List Hausstrand	Eastern Atlantic (Germany)	55.01556	8.43736
List Westland	Eastern Atlantic (Germany)	55.040667	8.386944
Schilksee	Eastern Atlantic (Germany)	54.42386	10.17473
Kristineberg	Eastern Atlantic (Sweden)	58.24774	11.44598
Bakka	Eastern Atlantic (Norway)	60.920192	6.867506
Henningsvær	Eastern Atlantic (Norway)	68.26079	14.26836
Lødingen	Eastern Atlantic (Norway)	68.56414	16.49406
Sommarøya	Eastern Atlantic (Norway)	69.63179	18.02713
Espegrend	Eastern Atlantic (Norway)	60.26637	5.22234
Bristol Channel	Eastern Atlantic (Wales)	51.39973	-3.19606
Plymouth	Eastern Atlantic (England)	50.34861	-4.20071
Cutty Sark	Eastern Atlantic (England)	51.48294	-0.0137
Gravesend	Eastern Atlantic (England)	51.44443	0.37764
Ardtoe	Eastern Atlantic (Scotland)	56.76923	-5.88361
Glenancross	Eastern Atlantic (Scotland)	56.94472	-5.85347
Nairn	Eastern Atlantic (Scotland)	57.59653	-3.84176
Musselburgh	Eastern Atlantic (Scotland)	55.94645	-3.07624
Little Gruinard	Eastern Atlantic (Scotland)	57.85223	-5.4533
Volchanets	Western Pacific (Russia)	42.910472	132.7402

Supplementary Table 2: Accession numbers of sequences used for phylogenetic analyses. Sequences obtained for this study are in bold. For information on sampling sites see Supplementary Table 2.

Taxon	Species	Site	Sampling Code	COI	16S	18S	ITS1
Orbiniidae	*Scoloplos acmeceps*			FJ612519	FJ612470	FJ612488	
	Leitoscoloplos bifurcatus			KR781456	KR349351	KR778793	
	Leitoscoloplos fragilis			FJ612498	AY532341	AY532360	
	Leitoscoloplos robustus				FJ612457	FJ612480	
	Leitoscoloplos pugettensis			HM473442	FJ612454	AY532365	
Parergodrilidae	*Stygocapitella minuta*	Langebaan	327_01	KY503054			
		Langebaan	327_02	KY503055			
		Langebaan	327_03	KY503056			
		Langebaan	327_04	KY503057			
		Langebaan	327_05	KY503058			
		Langebaan	327_06	KY503059			
		Langebaan	327_07	KY503060			
		Langebaan	327_08	KY503061			
		Langebaan	327_10	KY503062			
		Langebaan	327_11	KY503063			
		Langebaan	327_16				
		Langebaan	327_17				
		Langebaan	327_18				
		Langebaan	327_19				
		Langebaan	391_16	KY503064			
		Langebaan	391_17	KY503065		KY503075	
		Langebaan	391_18	KY503066		KY503076	
		Langebaan	391_19	KY503067			
	Stygocapitella australis	Gnarabup Beach	392_01	KY503042			
		Gnarabup Beach	392_03	KY503043			
		Gnarabup Beach	392_04	KY503044			
		Gnarabup Beach	392_05	KY503045		KY503077	
		Gnarabup Beach	392_06	KY503046			
		Gnarabup Beach	392_07	KY503047			
		Sarge Bay	393_01	KY503048		KY503078	
		Sarge Bay	393_02	KY503049			
		Sarge Bay	393_03	KY503050			
		Sarge Bay	393_04	KY503051			
		Sarge Bay	393_05	KY503052			
		Sarge Bay	393_06	KY503053			
	Stygocapitella subterranea	Ardtoe	320_02	MN158516	MN164320		
		Ardtoe	320_04	MN158525	MN164270		
		Ardtoe	320_06	MN158526	MN164298	MN162926	
		Ardtoe	320_07	MN158540	MN164315	MN162927	
		Ardtoe	320_08	MN158551	MN164311		
		Ardtoe	320_12	MN158537	MN164318	MN162958	
		Ardtoe	320_13	MN158541	MN164319	MN162933	
		Ardtoe	320_14	MN158536	MN164312		
		Ardtoe	320_15	MN158523	MN164299		
		Glenancross	321_01	MN158538	MN164300	MN162929	
		Glenancross	321_02	MN158539	MN164301	MN162952	
		Glenancross	321_03	MN158542	MN164303	MN162940	
		Glenancross	321_04	MN158534	MN164304	MN162941	
		Glenancross	321_05	MN158530	MN164305		
		Glenancross	321_06	MN158535	MN164306		
		Glenancross	321_07		MN164338		
		Glenancross	321_08		MN164302		
		Glenancross	321_09	MN158528	MN164307		
		Glenancross	321_10	MN158529	MN164310		
		Glenancross	321_11	MN158517	MN164308		
		Glenancross	321_12	MN158524	MN164309		
		Île Callot	210_10	MN158556			
		Île Callot	210_11	MN158557	MN164321		
		Île Callot	210_12	MN158558	MN164284		MN162766
		Île Callot	210_13	MN158553	MN164325		
		Île Callot	210_14	MN158566	MN164291		
		Île Callot	403_03	MN158508	MN164265	MN162950	MN162762
		Île Callot	403_04	MN158567	MN164266	MN162942	
		Île Callot	403_05	MN158509	MN164267	MN162930	
		Île Callot	403_06		MN164268		
		Île Callot	403_07	MN158545	MN164322		
		Île Callot	403_08	MN158554	MN164273		
		Île Callot	403_09		MN164274		
		Keitum	169_28		MN164282		
		Keitum	169_29	MN158561	MN164283		
		Keitum	169_30	MN158569	MN164287		

	Keitum	169_31		MN164288		
	Keitum	169_32		MN164323		
	Keitum	169_33	MN158562	MN164293		
	Keitum	169_34	MN158518	MN164326		
	Keitum	169_35	MN158563	MN164289		
	Keitum	169_36	MN158560	MN164324		
	Keitum	169_37		MN164295	MN162953	
	Keitum	169_38	MN158568	MN164281	MN162949	
	Keitum	169_39	MN158564	MN164290	MN162954	
	Keitum	398_04	MN158510	MN164275	MN162956	
	Keitum	398_05	MN158546	MN164276		
	Keitum	398_06	MN158550	MN164277		
	Keitum	398_07	MN158511	MN164278		
	Keitum	398_08	MN158512	MN164297		
	Keitum	398_09	MN158544	MN164279		
	Keitum	398_10	MN158513	MN164269		MN162763
	Keitum	398_11	MN158547	MN164280		
	Little Gruinard	322_01	MN158576	MN164314		
	Little Gruinard	322_02	MN158577	MN164335		
	Morsum	227_01	MN158519	MN164285	MN162935	
	Morsum	227_02	MN158520	MN164286	MN162951	
	Morsum	227_03	MN158573	MN164271	MN162955	
	Morsum	227_04	MN158521	MN164272	MN162936	
	Morsum	227_05	MN158522	MN164316	MN162961	
	Morsum	227_06	MN158527	MN164294		
	Morsum	227_07	MN158543	MN164292		
	Morsum	227_08	MN158555	MN164340		MN162767
	Morsum	227_09		MN164296		
	Morsum	227_10	MN158559	MN164317		
	Morsum	227_14		MN164129		
	Morsum	227_15		MN164130		
	Musselburgh	324_03	MN158565	MN164331		
	Musselburgh	324_05	MN158549	MN164332		MN162764
	Nairn	323_01	MN158531	MN164329		
	Nairn	323_02	MN158533	MN164333		
	Nairn	323_03	MN158552	MN164330		
	Nairn	323_04	MN158532	MN164334	MN162957	MN162765
	Schilksee	396_01	KY503068	MN164336	KY503073	
	Schilksee	396_02	KY503069	MN164339	KY503074	
	Schilksee	396_04	KY503070	MN164327	MN162938	MN162761
	Schilksee	396_05	KY503071	MN164313	MN162962	MN162760
	Schilksee	396_06	KY503072	MN164328	MN162959	
	Weststrand	169_01	MN158574			
Stygocapitella zecai	Ardtoe	320_01	MN158583	MN164132		
	Ardtoe	320_03		MN164133		
	Ardtoe	320_05	MN158584	MN164134		
	Bakka	439_01		MN164090	MN162985	
	Bakka	439_03	MN158582	MN164093	MN162989	
	Bakka	439_07		MN164091		
	Bakka	439_08		MN164094		
	Cutty Sark	423_01				MN162869
	Cutty Sark	423_02		MN164109		MN162870
	Cutty Sark	423_03	MN158578	MN164127		
	Cutty Sark	423_04		MN164095		
	Cutty Sark	423_06		MN164128		
	Cutty Sark	423_07	MN158579	MN164110		
	Cutty Sark	423_08	MN158580	MN164111		
	Cutty Sark	423_09		MN164096		
	Cutty Sark	423_10	MN158581	MN164112		MN162871
	Espegrend	440_01	MN158588	MN164092	MN162986	MN162872
	Espegrend	440_02		MN164097	MN162993	MN162878
	Espegrend	440_03		MN164098		
	Espegrend	440_04		MN164113		MN162875
	Henningsvær	437_01				MN162885
	Henningsvær	437_02		MN164099		
	Henningsvær	437_03		MN164114		MN162877
	Henningsvær	437_05		MN164105		
	Henningsvær	437_06		MN164100		
	Henningsvær	437_07		MN164103		
	Kristineberg	420_01	MN158585	MN164115	MN162987	
	Kristineberg	420_02		MN164116	MN162988	
	Kristineberg	420_03		MN164124	MN162990	MN162873
	Kristineberg	420_04		MN164117		MN162880
	Kristineberg	420_05	MN158586	MN164118		MN162882
	Kristineberg	420_06		MN164119		MN162881
	Kristineberg	420_07		MN164120		MN162874
	Kristineberg	420_09		MN164125		

	Kristineberg	420_10		MN164122		
	Kristineberg	420_12		MN164123		MN162883
	Lødingen	436_01		MN164106		
	Lødingen	436_02		MN164107		
	Lødingen	436_03		MN164101		MN162884
	Lødingen	436_04		MN164102		
	Lødingen	436_05		MN164104		
	Lødingen	436_07		MN164108		MN162879
	Musselburgh	324_04	MN158587	MN164131	MN162992	MN162876
	Sommarøya	438_01		MN164121		
	Sommarøya	438_02		MN164126	MN162991	
Stygocapitella josemariobrancoi	Bristol Channel	422_01	MN158387	MN164135	MN162970	MN162799
	Bristol Channel	422_02	MN158399	MN164136	MN162971	MN162803
	Bristol Channel	422_03	MN158400	MN164144	MN162972	MN162805
	Bristol Channel	422_04	MN158388	MN164148	MN162978	MN162808
	Bristol Channel	422_05	MN158413	MN164176		
	Bristol Channel	422_06	MN158401	MN164183		MN162802
	Bristol Channel	422_07	MN158389	MN164177		MN162800
	Bristol Channel	422_08	MN158435			MN162804
	Bristol Channel	422_09	MN158415	MN164184		MN162807
	Bristol Channel	422_10	MN158480	MN164200		MN162801
	Bristol Channel	422_11	MN158390	MN164178		MN162806
	Bristol Channel	422_12		MN164149		
	Ellenbogen	222_01	MN158440	MN164199	MN162982	MN162809
	Ellenbogen	222_02	MN158418	MN164153	MN162975	MN162810
	Ellenbogen	222_03	MN158396	MN164171	MN162979	MN162832
	Ellenbogen	222_04	MN158416	MN164142	MN162984	MN162811
	Ellenbogen	222_05	MN158428	MN164195		MN162812
	Ellenbogen	222_06	MN158419			MN162820
	Ellenbogen	222_07	MN158412			MN162816
	Ellenbogen	222_08	MN158421	MN164154		MN162821
	Ellenbogen	222_09	MN158422	MN164155		
	Ellenbogen	222_10	MN158420	MN164172		
	Ellenbogen	222_11	MN158397	MN164173		
	Ellenbogen	222_12	MN158423	MN164175		
	Ellenbogen	222_13	XX000000	MN164143		
	Gravesend	424_01	MN158447	MN164201		MN162842
	Gravesend	424_02	MN158436	MN164151		
	Gravesend	424_03	MN158463	MN164211		
	Gravesend	424_04	MN158448	MN164202		
	Gravesend	424_05	MN158449	MN164213		MN162843
	Gravesend	424_06	MN158450	MN164203		MN162846
	Gravesend	424_07	MN158451	MN164212		MN162841
	Gravesend	424_08	MN158452	MN164209		MN162848
	Gravesend	424_09	MN158461	MN164204		MN162849
	Gravesend	424_10	MN158479	MN164205		MN162844
	Gravesend	424_11	MN158453	MN164206		MN162838
	Gravesend	424_12	MN158431	MN164179		MN162847
	Gravesend	424_13	MN158432	MN164191		MN162840
	Gravesend	424_14	MN158462	MN164207		MN162845
	Hörnum	169_06	MN158391	MN164180		MN162828
	Hörnum	169_07	MN158404	MN164192		MN162822
	Hörnum	169_08	MN158439	MN164159		
	Hörnum	169_09	MN158424	MN164165	MN162973	MN162839
	Hörnum	169_10	MN158392	MN164174	MN162974	MN162825
	Hörnum	169_11	MN158441		MN162981	MN162835
	Hörnum	169_12	MN158433	MN164190	MN162977	
	Hörnum	169_13	MN158393	MN164198		MN162836
	Hörnum	169_14		MN164157		MN162829
	Hörnum	169_15	MN158430	MN164181		MN162826
	Hörnum	169_16	MN158405	MN164147		MN162823
	Hörnum	169_17	MN158394			MN162830
	List	169_54	MN158446	MN164156		MN162837
	List	169_55	MN158548			
	List	169_56	MN158406	MN164160	MN162980	MN162817
	List	169_57	MN158571	MN164337		XX000000
	List	169_58	MN158417	MN164164	MN162976	MN162813
	List	219_02		MN164161	MN162983	
	List	219_03	MN158408	MN164193		MN162824
	List	219_04		MN164168		MN162819
	List	219_05	MN158442	MN164170		MN162818
	List	219_06	MN158443	MN164163		MN162814
	List	219_07		MN164158		MN162827
	List	219_08	MN158410	MN164150		MN162831
	List	219_09		MN164166		MN162833
	List	219_10	MN158425	MN164169		
	List	219_11	MN158427			

	List	219_12		MN164162		
	List	219_13		MN164182		
	Lubec	429_01	MN158444	MN164138	MN162963	MN162850
	Lubec	429_08	MN158429	MN164185	MN162967	MN162851
	Plymouth	421_01	MN158471	MN164224	MN162964	MN162852
	Plymouth	421_02	MN158474	MN164225		
	Plymouth	421_03	MN158473	MN164228		MN162865
	Plymouth	421_04	MN158438	MN164186		
	Plymouth	421_05	MN158475	MN164229		MN162853
	Plymouth	421_06	MN158403	MN164187		MN162867
	Plymouth	421_07		MN164226		
	Plymouth	421_08	MN158472	MN164232		
	Plymouth	421_09	MN158476	MN164227		
	Plymouth	421_10	MN158478	MN164230		MN162862
	Plymouth	421_11		MN164188		
	Plymouth	421_13	MN158414	MN164189		MN162855
	Plymouth	421_14	MN158426	MN164167		MN162858
	Plymouth	421_15	MN158477	MN164231		
	Musselburgh	324_01	MN158455	MN164223		
	Musselburgh	324_02	MN158457	MN164215		
	Musselburgh	324_06	MN158466	MN164216		MN162834
	Musselburgh	324_07	MN158458	MN164217		
	Musselburgh	324_08	MN158464	MN164222		
	Musselburgh	324_09	MN158460	MN164219		
	Musselburgh	324_10	MN158459	MN164220		
	Musselburgh	324_11	MN158465	MN164218		
	Musselburgh	324_12	MN158456	MN164221		
	Musselburgh	324_52	MN158468	MN164208		
	St. Efflam	210_01	MN158470			MN162864
	St. Efflam	210_02	MN158409	MN164197		MN162815
	St. Efflam	210_03	MN158395			MN162860
	St. Efflam	210_04	MN158467	MN164152		MN162863
	St. Efflam	210_05	MN158407	MN164196		
	St. Efflam	210_06	MN158411			MN162856
	St. Efflam	210_07		MN164141		MN162868
	St. Efflam	401_01		MN164139	MN162968	MN162861
	St. Efflam	401_02	MN158398	MN164140	MN162966	MN162854
	St. Efflam	401_03	MN158454	MN164214	MN162965	
	St. Efflam	401_04	MN158437	MN164194		MN162857
	St. Efflam	401_05	MN158434	MN164146		
	St. Efflam	401_06	MN158445	MN164145		MN162859
	St. Efflam	401_07	MN158469	MN164210		MN162866
Stygocapitella westheidei	Canoe Beach	426_01	MN158481	MN164233	MN162928	MN162768
	Canoe Beach	426_02	MN158503		MN162960	MN162770
	Canoe Beach	426_03	MN158504	MN164234	MN162939	MN162794
	Canoe Beach	426_04	MN158502	MN164235	MN162948	MN162784
	Canoe Beach	426_05	MN158507	MN164236		
	Canoe Beach	426_06	MN158486	MN164237		MN162795
	Canoe Beach	426_07	MN158501	MN164254		MN162771
	Canoe Beach	426_08	MN158505	MN164238		MN162785
	Canoe Beach	426_09	MN158506	MN164264		MN162796
	Canoe Beach	426_10		MN164239		MN162774
	Canoe Beach	426_11		MN164249		MN162788
	Canoe Beach	426_12		MN164250		MN162797
	Lubec	429_02	MN158482		MN162934	MN162775
	Lubec	429_03		MN164255		MN162776
	Lubec	429_04		MN164240		
	Lubec	429_05		MN164256		MN162777
	Lubec	429_06		MN164251		MN162778
	Lubec	429_07				MN162793
	Lubec	429_09	MN158490	MN164257		MN162779
	Lubec	429_10	MN158489	MN164241		MN162783
	Lubec	429_11	MN158483	MN164242		MN162780
	Lubec	429_12	MN158497	MN164258		
	Reid State Park	427_01	MN158484	MN164259	MN162943	MN162781
	Reid State Park	427_02	MN158495	MN164260	MN162931	MN162772
	Reid State Park	427_03	MN158487	MN164243	MN162937	MN162773
	Reid State Park	427_04	MN158485	MN164252	MN162944	MN162786
	Reid State Park	427_05	MN158499	MN164244		
	Reid State Park	427_06	MN158500	MN164245		MN162787
	Reid State Park	427_07	MN158492	MN164261		MN162789
	Reid State Park	427_08	MN158493	MN164262		MN162792
	Reid State Park	427_09	MN158494	MN164246		MN162798
	Reid State Park	427_10	MN158496	MN164253		MN162782
	South Lubec	428_01	MN158491	MN164263	MN162946	MN162791
	South Lubec	428_02	MN158498	MN164247	MN162969	MN162790
	South Lubec	428_03	MN158488	MN164248	MN162947	MN162769

Stygocapitella spec. A.	4th July Beach	432_02	MN158382	MN164061	MN162897	MN162736
	4th July Beach	432_04		MN164062		MN162738
	4th July Beach	432_07	MN158385	MN164063	MN162909	MN162739
	4th July Beach	432_08	MN158383	MN164065	MN162895	MN162737
	4th July Beach	432_09	MN158384	MN164064	MN162911	MN162741
	False Bay	431_01	MN158386	MN164066	MN162910	MN162740
Stygocapitella americae	4th July Beach	432_01	MN158589	MN164067		MN162714
	4th July Beach	432_10	MN158597	MN164068	MN162914	MN162724
	Reuben Tarte	433_01	MN158590	MN164069	MN162917	MN162720
	Reuben Tarte	433_02	MN158591	MN164075	MN162918	MN162722
	Reuben Tarte	433_03	MN158599	MN164070	MN162915	MN162715
	Reuben Tarte	433_04	MN158600	MN164076	MN162920	MN162716
	Reuben Tarte	433_05	MN158601	MN164071	MN162919	MN162721
	Reuben Tarte	433_06	MN158592	MN164077		MN162717
	Reuben Tarte	433_07	MN158593	MN164078		
	Reuben Tarte	433_08	MN158594	MN164072		MN162719
	Reuben Tarte	433_09	MN158595	MN164079		MN162723
	Reuben Tarte	433_10	MN158596	MN164080	MN162916	
	Reuben Tarte	433_11		MN164073		MN162725
	Reuben Tarte	433_12	MN158598	MN164074		MN162718
Stygocapitella berniei	Roche Harbor	430_01	MN158602	MN164081	MN162921	MN162726
	Roche Harbor	430_02			MN162925	MN162727
	Roche Harbor	430_03	MN158603	MN164082	MN162922	MN162731
	Roche Harbor	430_04	MN158604	MN164083	MN162923	MN162728
	Roche Harbor	430_05	MN158605	MN164084	MN162924	MN162729
	Roche Harbor	430_06	MN158609	MN164089		MN162735
	Roche Harbor	430_07	MN158606	MN164085		MN162733
	Roche Harbor	430_08	MN158608	MN164086		MN162730
	Roche Harbor	430_09	MN158607	MN164087		MN162734
	Roche Harbor	430_10	MN158610	MN164088		MN162732
Stygocapitella budaevae	Volchanets	442_1		MN164047	MN162891	MN162742
	Volchanets	442_4	MN158372		MN162898	MN162750
	Volchanets	442_5	MN158380	MN164048	MN162899	MN162745
	Volchanets	442_6	MN158374	MN164060	MN162912	MN162744
	Volchanets	442_7	MN158375		MN162913	MN162751
	Volchanets	442_8	MN158378	MN164049	MN162900	MN162752
	Volchanets	442_9	MN158373	MN164050	MN162901	MN162747
	Volchanets	442_12	MN158379		MN162904	MN162757
	Volchanets	442_15	MN158376	MN164051	MN162892	MN162758
	Volchanets	442_16		MN164052	MN162896	MN162748
	Volchanets	442_18		MN164057	MN162905	MN162753
	Volchanets	442_19		MN164053	MN162902	MN162754
	Volchanets	442_20	MN158381	MN164054	MN162903	MN162746
	Volchanets	442_21		MN164055	MN162893	MN162749
	Volchanets	442_22	MN158377	MN164059	MN162906	MN162743
	Volchanets	442_23		MN164058	MN162907	MN162755
	Volchanets	442_24			MN162894	MN162759
	Volchanets	442_25		MN164056	MN162908	MN162756
Stygocapitella furcata	4th July Beach	432_03	MN158612	MN164343	MN162996	MN162886
	4th July Beach	432_05	MN158613	MN164345	MN162997	MN162887
	4th July Beach	432_06	MN158614	MN164344	MN162998	MN162888
Stygocapitella pacifica	Volchanets	442_10	MN158611	MN164341	MN162994	MN162889
	Volchanets	442_11		MN164342	MN162995	MN162890

Supplementary Table 3. Morphometric measurements. BodyLength = Length of entire body; BodyWidth = Width of entire body; ProstomiumLength = Length of prostomium; ProstomiumWidth = Width of prostomium; PeristomiumLength = Length of peristomium; PeristomiumWidth = Width of peristomium. All measurements are in μm.

Site	Clade	Individual	ID	BodyLength	BodyWidth	ProstomiumLength	ProstomiumWidth	PygidiumLength	PygidiumWidth
Langebaan	*S. minuta*	Holotype	SA_LGB_Holo	1010.74	104.54	46.6	78.89	30.95	35.21
Langebaan	*S. minuta*	Lost Male	SA_LGB_LostM1	992.34	91.11	39.61	75.01	33.51	37.7
Langebaan	*S. minuta*	Paratype ♀ 1	SA_LGB_ParaF1	977.69	87.79	46.46	75.88	33.13	39.6
Langebaan	*S. minuta*	Paratype ♀3	SA_LGB_ParaF3	999.66	97.2	33.13	66.02	47.41	59.72
Langebaan	*S. minuta*	Paratype ♀4	SA_LGB_ParaF4	912.97	81.1	35.07	72.96	37.35	47.75
Langebaan	*S. minuta*	Paratype ♀5	SA_LGB_ParaF5	1143.29	117.07	46.6	85.51	37.51	55.87
Langebaan	*S. minuta*	Paratype ♀6	SA_LGB_ParaF6	1059.66	89.71	39.8	52.27	30.9	38.53
Langebaan	*S. minuta*	Paratype ♂1	SA_LGB_ParaM1	1155.68	102.67	38.44	69.19	46.86	54.27
Langebaan	*S. minuta*	Paratype ♂2	SA_LGB_ParaM2	1099	110.27	43.24	85.35	44.31	63.48
Langebaan	*S. minuta*	Paratype ♂3	SA_LGB_ParaM3	1117.34	110.65	47.34	90.44	40.46	62.76
Langebaan	*S. minuta*	Paratype ♂4	SA_LGB_ParaM4	1047.34	99.21	38.6	76.46	47.71	57.39
Gnarabup Beach	*S. australis*	Holotype	AUS_GNB_HoloM	2019.02	209.21	73.84	115.29	67.47	123.95
Gnarabup Beach	*S. australis*	Paratype ♀1	AUS_GNB_ParaF1	1900.44	196.75	89.21	138.95	47.87	114.86
Gnarabup Beach	*S. australis*	Paratype ♂1	AUS_GNB_ParaM1	1808	224.39	81.89	130.51	67.19	115.27
Gnarabup Beach	*S. australis*	Paratype ♂2	AUS_GNB_ParaM2	2312.6	235.11	80.59	131.72	37.62	84.99
Sarge Bay	*S. australis*	Paratype ♀2	AUS_SAB_ParaF2	2008.77	202.73	76.18	104.79	53.32	89.06
Sarge Bay	*S. australis*	Paratype ♀3	AUS_SAB_ParaF3	1650.14	172.52	64.76	104.79	49.6	84.56
Sarge Bay	*S. australis*	Paratype ♀4	AUS_SAB_ParaF4	2130.8	185.77	54.33	113.22	61.04	106.34
Sarge Bay	*S. australis*	Paratype ♀5	AUS_SAB_ParaF5	2079.81	204.69	69.79	114.52	57.38	117.42
Sarge Bay	*S. australis*	Paratype ♂3	AUS_SAB_ParaM3	1804.37	145.06	59.7	98.88	60.3	75.89
Sarge Bay	*S. australis*	Paratype ♂4	AUS_SAB_ParaM4	1622.17	122.31	66.23	87.96	59.36	82.77
4th July Beach	*S. furcata*	Individual 2	USA_4JB_Ind2	2075.61	283.45	143.15	240.08	71.07	126.86
4th July Beach	*S. furcata*	Individual 3	USA_4JB_Ind3	1669.4	200.72	87.72	111.82	37.54	82.19
4th July Beach	*S. furcata*	Individual 4	USA_4JB_Ind4	1837.43	214.65	79.28	137.85	68.51	84.66
4th July Beach	*S. furcata*	Individual 5	USA_4JB_Ind5	1718.05	218.64	78.05	130.88	55.85	86.03
4th July Beach	*S. furcata*	Individual 6	USA_4JB_Ind6	2535.21	308.11	104.79	177.73	77.18	123.73
4th July Beach	*S. furcata*	Individual 7	USA_4JB_Ind7	1217.05	154.59	84.29	108.99	27.46	89.47
Roche Harbor	*S. berniei*	Individual 1	USA_ROH_Ind1	1342.86	166.72	60.45	106.08	39.35	74.9
Roche Harbor	*S. berniei*	Individual 2	USA_ROH_Ind2	1874.94	203.63	42.85	86.68	47.96	101.62
Roche Harbor	*S. berniei*	Individual 3	USA_ROH_Ind3	1680.1	216.92	58.03	125.91	50.54	95.9
Roche Harbor	*S. berniei*	Individual 4	USA_ROH_Ind4	2406.77	273.1	81.79	162.75	70.9	143.44
Roche Harbor	*S. berniei*	Individual 5	USA_ROH_Ind5	2326.9	226.89	95.54	182.19	54.18	145.75
Roche Harbor	*S. berniei*	Individual 6	USA_ROH_Ind6	2552.82	273.68	91.58	148.79	63.5	138.1
Roche Harbor	*S. berniei*	Individual 7	USA_ROH_Ind7	1864.44	232.47	58.96	153.64	47.97	116.03
Reuben Tarte	*S. americae*	Individual 1	USA_RSP_Ind1	2409.03	256.93	71.27	147.03	45.67	137.85
Reuben Tarte	*S. americae*	Individual 2	USA_RSP_Ind2	2787.72	261.71	78.35	165.59	64.01	131.26
Reuben Tarte	*S. americae*	Individual 3	USA_RSP_Ind3	2733.84	283.71	103.84	189.07	76.45	174.35
Reuben Tarte	*S. americae*	Individual 4	USA_RSP_Ind4	2181.42	224.14	114.39	184.93	50.12	129.56
Reuben Tarte	*S. americae*	Individual 5	USA_RSP_Ind5	2787.91	284.27	95.39	197.67	65.89	200.81
Reuben Tarte	*S. americae*	Individual 6	USA_RSP_Ind6	2516.54	275.45	109.05	193.42	44.71	171.26
Reuben Tarte	*S. americae*	Individual 7	USA_RSP_Ind7	2436.34	231.51	86.84	164.01	62.08	138.87
Canoe Beach	*S. westheidei*	Individual 1	USA_CAB_Ind1	1570.1	168.64	73.11	107.38	51.41	86.24
Canoe Beach	*S. westheidei*	Individual 2	USA_CAB_Ind2	1632.26	182.2	66.42	105.87	59.28	72.17
Lubec	*S. westheidei*	Individual 1	USA_LUB_Ind1	2013.09	222.07	68.53	136.84	56.89	121.91
Lubec	*S. westheidei*	Individual 2	USA_LUB_Ind2	1851.4	184.65	63.18	136.1	43.68	106.07
Lubec	*S. westheidei*	Individual 3	USA_LUB_Ind3	2227.13	198.78	71.97	168.34	43.75	98.99
Lubec	*S. westheidei*	Individual 4	USA_LUB_Ind4	1620.44	171.11	49.22	118.02	34.88	72.28
Lubec	*S. westheidei*	Individual 5	USA_LUB_Ind5	1647.19	147.38	73.39	113.84	48.14	77.73
Lubec	*S. westheidei*	Individual 6	USA_LUB_Ind6	2298.89	237.15	56.89	139.88	51.96	116.73
Lubec	*S. westheidei*	Individual 7	USA_LUB_Ind7	1521.28	184.64	51.68	122.04	47.11	98.58
Lødingen	*S. zecai*	436.29	NOR_LOE_A436.29	2365.12	245.9	82.79	127.87	59.84	79.51
Lødingen	*S. zecai*	436.30	NOR_LOE_A436.30	1766.87	198.36	49.18	105.74	52.46	69.67
Lødingen	*S. zecai*	436.31	NOR_LOE_A436.31	2005.23	202.46	45.08	96.72	54.92	82.79
Lødingen	*S. zecai*	436.32	NOR_LOE_A436.32	2388.31	195.87	51.65	123.14	50	80.58
Lødingen	*S. zecai*	436.33	NOR_LOE_A436.33	2277.61	250	61.16	147.93	100.83	116.94
Lødingen	*S. zecai*	436.34	NOR_LOE_A436.34	2456.69	241.32	73.55	112.81	83.47	123.55
Henningsvær	*S. zecai*	437.21	NOR_HEN_A437.21	2293.93	221.9	57.85	118.18	50	83.47
Henningsvær	*S. zecai*	437.23	NOR_HEN_A437.23	2500.06	295.46	84.71	123.55	56.41	126.03

Henningsvær	*S. zecai*	437.24	NOR_HEN_A437.24	2572.16	280.99	54.55	102.07	63.64	123.97
Henningsvær	*S. zecai*	437.25	NOR_HEN_A437.25	1876.3	198.36	85.25	121.31	65.57	114.75
Henningsvær	*S. zecai*	437.26	NOR_HEN_A437.26	2573.28	255.37	57.85	118.18	49.59	113.22
Henningsvær	*S. zecai*	437.28	NOR_HEN_A437.28	1514.25	170.49	50.82	108.2	45.9	81.15
Henningsvær	*S. zecai*	437.29	NOR_HEN_A437.29	2392.82	262.3	44.26	107.38	61.48	104.1
Henningsvær	*S. zecai*	437.30	NOR_HEN_A437.30	2352.62	293.44	84.43	136.07	63.12	119.67
Schilksee	*S. subterranea*	Neotype	GER_SCS_NeoF	1692.39	140.99	54.12	82.56	46.55	77.55
Schilksee	*S. subterranea*	Paratype ♀1	GER_SCS_ParaF1	1801.36	218.75	65.31	121.51	55.29	76.76
Schilksee	*S. subterranea*	Paratype ♀2	GER_SCS_ParaF2	1407.97	159.51	48.13	99.11	45.94	81.02
Schilksee	*S. subterranea*	Paratype ♂3	GER_SCS_ParaM3	1841.37	171.13	33.07	93.32	45.23	58.33
Schilksee	*S. subterranea*	Paratype ♂4	GER_SCS_ParaM4	1608.5	185.78	61.52	109.71	33.85	80.41
Île Callot	*S. subterranea*	Individual 1	FRA_ILE_Ind1	1772.15	186.94	51.36	104.66	38.91	92.79
Île Callot	*S. subterranea*	Individual 2	FRA_ILE_Ind2	2074.69	233.75	66.39	109.72	50.68	98.79
Île Callot	*S. subterranea*	Individual 3	FRA_ILE_Ind3	2329.07	277.84	79.73	152.68	64.65	160.32
Île Callot	*S. subterranea*	Individual 4	FRA_ILE_Ind4	2507.08	251.73	70.58	153.04	36.65	95.99
Île Callot	*S. subterranea*	Individual 5	FRA_ILE_Ind5	2441.76	285.81	89.96	167.84	47.14	125.71
Île Callot	*S. subterranea*	Individual 6	FRA_ILE_Ind6	1958.52	233.13	72.9	140.66	45.31	118.62
Glenancross	*S. subterranea*	321.51	UK_GLE_A321.51	1352.02	204.92	88.53	126.23	43.44	78.69
Glenancross	*S. subterranea*	321.52	UK_GLE_A321.52	1277.24	217.36	54.96	114.05	45.87	63.22
Glenancross	*S. subterranea*	321.53	UK_GLE_A321.53	1617.29	183.88	66.53	121.49	45.46	82.23
Glenancross	*S. subterranea*	321.55	UK_GLE_A321.55	1219.04	143.8	54.13	91.32	39.26	54.13
Glenancross	*S. subterranea*	321.56	UK_GLE_A321.56	1201.2	142.88	54.96	102.07	42.15	61.57
Glenancross	*S. subterranea*	321.57	UK_GLE_A321.57	1545.18	176.03	51.24	104.13	39.26	60.33
Glenancross	*S. subterranea*	321.59	UK_GLE_A321.59	1358.37	185.73	52.89	104.55	42.98	76.86
Glenancross	*S. subterranea*	321.60	UK_GLE_A321.60	1099.18	121.31	45.9	105.74	36.89	63.93
Keitum	*S. subterranea*	398.1_1	GER_KEI_A398.1_1	1944.72	188.84	71.49	126.45	49.17	104.55
Keitum	*S. subterranea*	398.1_2	GER_KEI_A398.1_2	1427.03	146.69	66.12	118.18	45.87	86.36
Keitum	*S. subterranea*	398.1_3	GER_KEI_A398.1_3	1909.69	231.41	57.03	130.58	47.11	95.46
Keitum	*S. subterranea*	398.1_4	GER_KEI_A398.1_4	2050.62	180.58	72.31	127.27	50.83	99.59
Keitum	*S. subterranea*	398.2_1	GER_KEI_A398.2_1	2185.57	149.59	61.57	108.26	43.39	78.93
Keitum	*S. subterranea*	398.2_2	GER_KEI_A398.2_2	1452	151.65	61.98	111.98	45.04	83.06
Keitum	*S. subterranea*	398.2_3	GER_KEI_A398.2_3	1921.13	161.57	64.88	117.36	48.35	84.3
Keitum	*S. subterranea*	398.2_4	GER_KEI_A398.2_4	1401.46	135.95	63.22	108.26	44.63	82.23
Keitum	*S. subterranea*	398.16	GER_KEI_A398.16	1396.82	145.9	70.49	124.59	43.44	88.53
Keitum	*S. subterranea*	398.17	GER_KEI_A398.17	1613.95	174.79	58.68	114.46	57.85	100
Bristol Channel	*S. josemariobrancoi*	Individual 1	UK_BCH_Ind1	1873.11	207.79	88.07	111.54	54.05	99.27
Bristol Channel	*S. josemariobrancoi*	Individual 2	UK_BCH_Ind2	2149.73	227.91	67.44	146.56	53.36	113.37
Bristol Channel	*S. josemariobrancoi*	Individual 3	UK_BCH_Ind3	1998.95	226.25	66.45	139.53	54.81	145.95
Bristol Channel	*S. josemariobrancoi*	Individual 4	UK_BCH_Ind4	2294.3	248.21	76.08	147.1	76.32	149.98
Bristol Channel	*S. josemariobrancoi*	Individual 5	UK_BCH_Ind5	1829.39	227.4	71.96	141.17	51.03	114.78
Gravesend	*S. josemariobrancoi*	Individual 1	UK_GRA_Ind1	2661.38	338.49	84.97	164.24	64.46	105.47
Gravesend	*S. josemariobrancoi*	Individual 2	UK_GRA_Ind2	2275.83	226.1	67.52	145.55	59.35	114.8
Gravesend	*S. josemariobrancoi*	Individual 3	UK_GRA_Ind3	2644.38	267.09	81.52	139.92	52.82	142.71
Gravesend	*S. josemariobrancoi*	Individual 4	UK_GRA_Ind4	2637.59	291.42	90.99	161.94	59.33	144.71
Gravesend	*S. josemariobrancoi*	Individual 5	UK_GRA_Ind5	2493.57	264.58	71.32	144.94	50.62	118.22
Gravesend	*S. josemariobrancoi*	Individual 6	UK_GRA_Ind6	3751.87	423.71	153.28	206.36	51.2	115.59
Gravesend	*S. josemariobrancoi*	Individual 7	UK_GRA_Ind7	2549.49	189.77	63.13	115.96	56.95	97.98
Gravesend	*S. josemariobrancoi*	Individual 8	UK_GRA_Ind8	2625.56	234.9	85.71	110.32	61.09	119.39
Gravesend	*S. josemariobrancoi*	Individual 9	UK_GRA_Ind9	2499.99	297.56	84.72	164.46	57.75	131.38
Plymouth	*S. josemariobrancoi*	Individual 1	UK_PLY_Ind1	1595.9	169.31	47.96	106.38	41.61	97.98
Plymouth	*S. josemariobrancoi*	Individual 2	UK_PLY_Ind2	1317	159.65	56.13	104.45	48.81	105.2
Plymouth	*S. josemariobrancoi*	Individual 3	UK_PLY_Ind3	2903.19	284.06	89.94	161.34	77.43	153.36
Plymouth	*S. josemariobrancoi*	Individual 4	UK_PLY_Ind4	1713.76	225.3	62.55	130.77	56.28	120.45
Plymouth	*S. josemariobrancoi*	Individual 5	UK_PLY_Ind5	3021.08	367.8	105.95	200.45	60.57	132.59
Plymouth	*S. josemariobrancoi*	Individual 6	UK_PLY_Ind6	2093.62	229.65	77.32	133.1	39.05	123.48
Plymouth	*S. josemariobrancoi*	Individual 7	UK_PLY_Ind7	2689.28	312.04	93.96	171.86	56.55	156.02
Ellenbogen	*S. josemariobrancoi*	Individual 1	GER_ELL_Ind1	2386.98	255.15	70.92	160.32	160.32	126.17
Ellenbogen	*S. josemariobrancoi*	Individual 2	GER_ELL_Ind2	2524.11	247.34	75.37	175.99	64.66	97.37
Ellenbogen	*S. josemariobrancoi*	Individual 3	GER_ELL_Ind3	2780.02	277.53	126.13	222.07	59.78	145.03
Ellenbogen	*S. josemariobrancoi*	Individual 5	GER_ELL_Ind5	2324.45	213.18	81.59	172.38	53.95	163.36
Ellenbogen	*S. josemariobrancoi*	Individual 6	GER_ELL_Ind6	2372.22	251.88	87.12	180.05	50.92	137.65
Ellenbogen	*S. josemariobrancoi*	Individual 7	GER_ELL_Ind7	2283.45	280.01	100.28	190.78	52.69	133
Ellenbogen	*S. josemariobrancoi*	Individual 8	GER_ELL_Ind8	2674.36	270.7	92.9	183.6	73.28	127.54
Ellenbogen	*S. josemariobrancoi*	Individual 9	GER_ELL_Ind9	2732.56	264.3	73.54	178.41	65.86	140.89
Ellenbogen	*S. josemariobrancoi*	Individual 10	GER_ELL_Ind10	2593.88	256.32	140.17	219.23	78.67	155.39

Supplementary Table 4: Least-Square means contrasts in morphological measurements of *Stygocapitella* species for different measurements including body length and width, Prostomium length and width and pygidium length and width. Z-ratio (Z) and p-values (*p*) associated to each contrast are presented. Statistically significant values (<0.001) are marked in bold.

		Body length		**Body width**		**Prostomium length**		**Prostomium width**		**Pygidium length**		**Pygidium width**	
Species 1	**Species 2**	Z	*p*	Z	*p*	Z	*p*	Z	*p*	Z	*p*	Z	*p*
S. minuta	*S. pacifica*	-0.79	1	-1.24	0.97	-1.21	0.97	-0.69	1	0.47	1	-0.54	1
	S. australis	**-5.72**	**<0.001**	**-5.15**	**<0.001**	**-4.40**	**<0.001**	-3.98	0.003	-2.94	0.1	**-5.65**	**<0.001**
	S. berniei	**-5.60**	**<0.001**	**-6.59**	**<0.001**	**-3.75**	**<0.01**	**-5.82**	**<0.001**	-2.24	0.43	**-6.87**	**<0.001**
	S. americae	**-8.77**	**<0.001**	**-8.24**	**<0.001**	**-6.94**	**<0.001**	**-9.47**	**<0.001**	-3.01	0.08	**-10.84**	**<0.001**
	S. budaevae	-1.95	0.63	-2.83	0.13	-1.86	0.70	-1.62	0.84	-0.54	1	-1.17	0.98
	S. zecai	**-8.34**	**<0.001**	**-8.46**	**<0.001**	-3.43	0.02	**-4.73**	**<0.001**	-4.14	0.01	**-6.36**	**<0.001**
	S. subterranea	**-5.23**	**<0.001**	**-6.07**	**<0.001**	**-3.78**	**<0.01**	**-5.28**	**<0.001**	-1.38	0.93	**-5.14**	**<0.001**
	S. josemariobrancoi	**-10.91**	**<0.001**	**-11.17**	**<0.001**	**-7.78**	**<0.001**	**-10.48**	**<0.001**	**-4.78**	**<0.001**	**-11.01**	**<0.001**
	S. westheidei	**-4.85**	**<0.001**	**-4.93**	**<0.001**	-3.18	0.048	**-5.22**	**<0.001**	-1.59	0.85	**-4.94**	**<0.001**
S. pacifica	*S. australis*	-2.45	0.30	-1.68	0.81	-1.28	0.96	-1.56	0.87	-2.13	0.51	-2.66	0.19
	S. berniei	-2.62	0.21	-2.79	0.14	-1.10	0.99	-2.85	0.12	-1.81	0.73	-3.6	0.01
	S. americae	**-4.53**	**<0.001**	**-3.78**	**<0.001**	-3.02	0.08	**-5.05**	**<0.001**	-2.27	0.41	**-6.02**	**<0.001**
	S. budaevae	-0.38	1	-0.46	1	0.09	1	-0.28	1	-0.78	1	-0.16	1
	S. zecai	-3.64	0.01	-3.25	0.04	-0.59	1	-1.82	0.72	-2.69	0.18	-2.84	0.12
	S. subterranea	-1.71	0.79	-1.64	0.83	-0.55	1	-1.83	0.72	-1.16	0.98	-1.93	0.65
	S. josemariobrancoi	**-4.43**	**<0.001**	-4.09	<0.01	-2.47	0.28	**-4.33**	**<0.001**	-2.81	0.13	**-4.75**	**<0.001**
	S. westheidei	-2.02	0.59	-1.62	0.84	-0.63	1	-2.32	0.37	-1.38	0.93	-2.31	0.38
S. australis	*S. berniei*	-0.42	1	-1.90	0.67	0.23	1	-2.18	0.47	0.40	1	-1.73	0.78
	S. americae	-3.53	0.02	-3.52	0.02	-2.90	0.10	**-5.76**	**<0.001**	-0.35	1	**-5.63**	**<0.001**
	S. budaevae	3.36	0.03	1.97	0.62	2.24	0.43	2.08	0.54	2.17	0.47	4.06	<0.01
	S. zecai	-2.08	0.55	-2.80	0.14	1.32	0.95	-0.40	1	-0.93	1	-0.23	1
	S. subterranea	1.77	0.76	0.27	1	1.60	0.85	-0.35	1	2.17	0.48	1.77	0.75
	S. josemariobrancoi	-3.68	<0.01	**-4.62**	**<0.001**	-2.24	0.43	**-5.36**	**<0.001**	-1.10	0.98	-3.86	<0.01
	S. westheidei	0.70	1	0.07	1	1.08	0.99	-1.32	0.95	1.24	0.97	0.54	1
S. berniei	*S. americae*	-2.87	0.11	-1.49	0.90	-2.88	0.11	-3.30	0.03	-0.70	1	-3.59	0.01
	S. budaevae	3.48	0.02	3.62	0.01	1.83	0.71	3.98	< 0.01	1.61	0.84	**5.37**	**<0.001**
	S. zecai	-1.41	0.92	-0.48	1	0.93	1	1.96	0.63	-1.26	0.96	1.64	0.83
	S. subterranea	2.03	0.58	2.47	0.29	1.13	0.99	2.24	0.43	1.42	0.92	3.57	0.01
	S. josemariobrancoi	-2.71	0.17	-1.79	0.74	-2.21	0.45	-2.10	0.53	-1.43	0.92	-1.33	0.95
	S. westheidei	1.05	0.99	1.93	0.65	0.76	1	0.93	1	0.74	1	2.19	0.46
S. americae	*S. budaevae*	**6.44**	**< 0.001**	**5.15**	**< 0.001**	**4.81**	**< 0.001**	**7.39**	**<0.001**	2.33	0.37	**9.08**	**<0.001**
	S. zecai	1.90	0.67	1.24	0.97	**4.27**	**< 0.001**	**5.77**	**<0.001**	-0.46	1	**5.79**	**<0.001**
	S. subterranea	**5.67**	**<0.001**	**4.35**	**<0.001**	**4.79**	**<0.001**	**6.43**	**<0.001**	2.30	0.39	**8.13**	**<0.001**
	S. josemariobrancoi	0.95	0.99	0.11	1	1.46	0.91	2.10	0.53	-0.55	1	3.25	0.04
	S. westheidei	4.09	<0.01	3.51	0.02	3.82	<0.01	**4.43**	**<0.001**	1.47	0.90	**6.00**	**<0.001**
S. budaevae	*S. zecai*	**-5.54**	**<0.001**	**-4.72**	**<0.001**	-1.17	0.98	-2.60	0.22	-3.20	0.045	**-4.56**	**<0.001**
	S. subterranea	-2.37	0.35	-2.09	0.54	-1.19	0.97	-2.80	0.14	-0.59	1	-3.2	0.045
	S. josemariobrancoi	**-7.38**	**<0.001**	**-6.59**	**<0.001**	**-4.702**	**<0.001**	**-7.40**	**<0.001**	-3.60	0.01	**-8.38**	**<0.001**
	S. westheidei	-2.62	0.21	-1.85	0.70	-1.16	0.98	-3.28	0.03	-0.95	0.99	-3.45	0.02

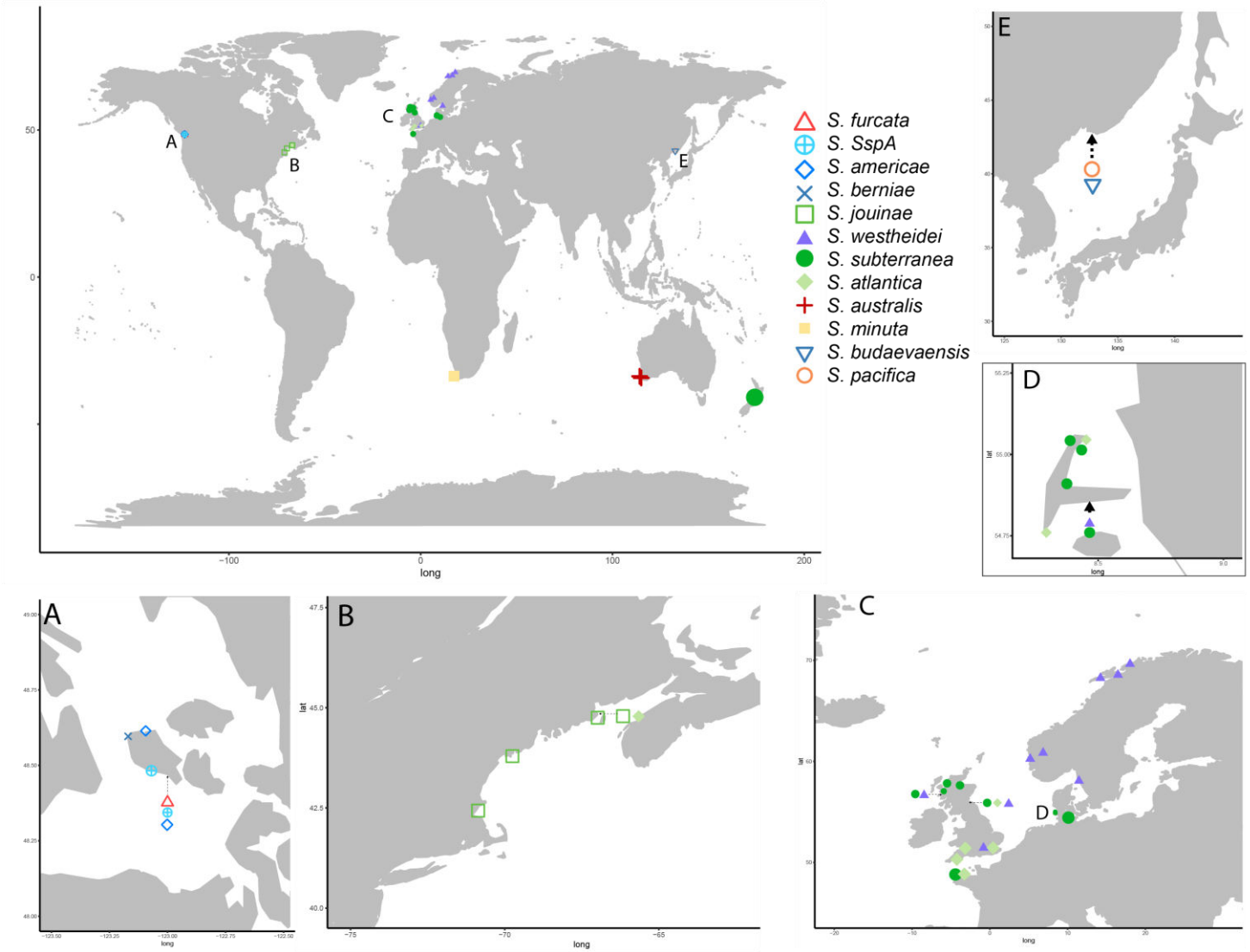
S. zecai	*S. subterranea*	**4.63**	**<0.001**	3.87	<0.01	0.13	1	0.12	1	3.63	0.01	2.29	0.40
	S. josemariobrancoi	-1.49	0.89	-1.63	0.83	**-4.20**	**0.001**	**-5.52**	**<0.001**	-0.05	1	-4.06	<0.01
	S. westheidei	2.79	0.15	2.79	0.14	-0.11	1	-1.03	0.99	2.24	0.43	0.80	1
S. subterrane	*S. josemariobrancoi*	**-7.65**	**<0.001**	**-6.87**	**<0.001**	**-5.39**	**<0.001**	**-7.01**	**<0.001**	**-4.60**	**<0.001**	**-7.90**	**<0.001**
	S. westheidei	-0.86	1	-0.18	1	-0.23	1	-1.25	0.96	-0.59	1	-1.05	0.99
S. westheidei	*S. josemariobrancoi*	**4.38**	**<0.001**	**4.53**	**<0.001**	3.46	0.02	3.55	0.01	2.56	0.24	**4.37**	**<0.001**

Supplementary Table 5: Pairwise interspecific genetic distances using the COI dataset for the studied *Stygocapitella* species. These distances were computed using MEGA X. After defining groups (species), we calculated distances between group mean differences after the bootstrap method (500 replications), model TN-93, Rates among Sites Gamma Distribution, Gamma Parameter 1,00. Values on the lower end of the matrix represent genetic distances, values on the upper end of the matrix represent the standard deviation.

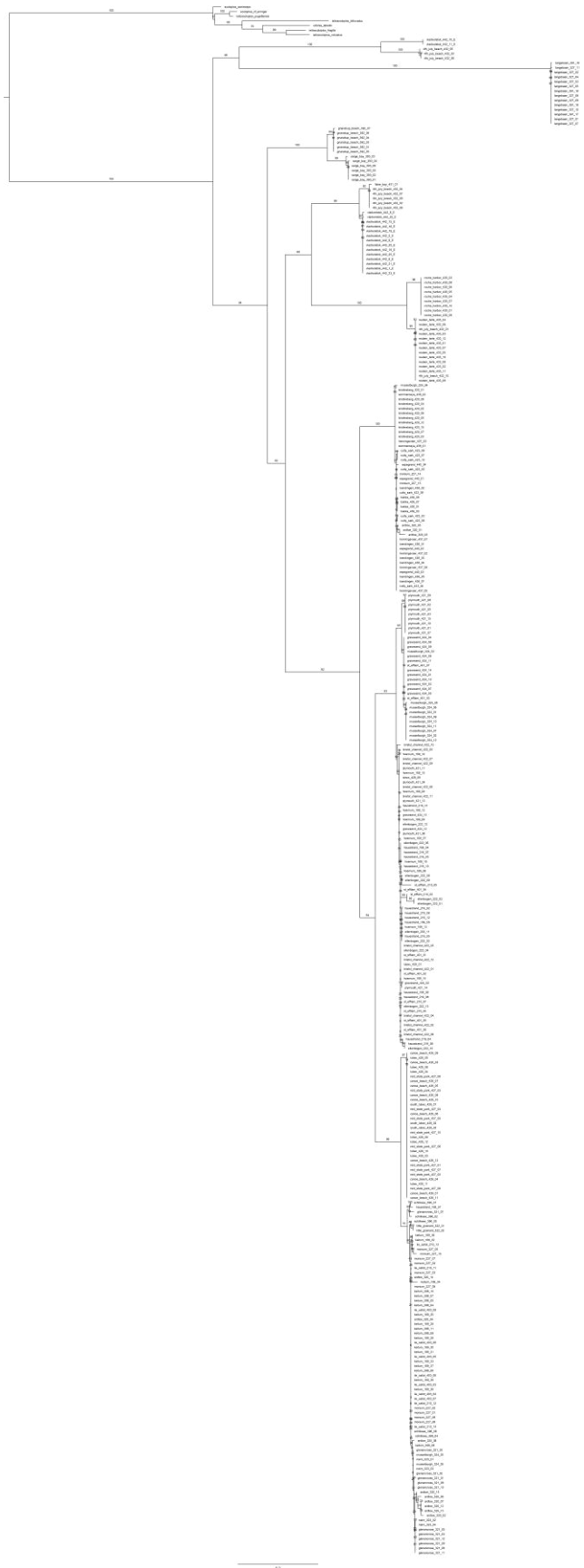
	S. budaevae	*S. pacifica*	*S. josemariobrancoi*	*S. westheidei*	*S. subterranea*	*S. australis*	*S. berniei*	*S. americae*	*S. zecai*	*S. minuta*	*S.spec A*	*S. furcata*
S. budaevae		0.0275	0.0289	0.0253	0.0262	0.0272	0.0308	0.0301	0.0261	0.0408	0.0108	0.0284
S. pacifica	0.2944		0.0305	0.0287	0.0290	0.0290	0.0329	0.0342	0.0303	0.0362	0.0291	0.0227
S. josemariobrancoi	0.3034	0.3405		0.0171	0.0173	0.0246	0.0278	0.0281	0.0231	0.0398	0.0282	0.0288
S. westheidei	0.2545	0.3157	0.1639		0.0095	0.0227	0.0263	0.0263	0.0186	0.0366	0.0248	0.0282
S. subterranea	0.2565	0.3194	0.1634	0.0577		0.0222	0.0269	0.0269	0.0202	0.0374	0.0251	0.0278
S. australis	0.2975	0.3208	0.2690	0.2450	0.2356		0.0302	0.0276	0.0239	0.0379	0.0262	0.0308
S. berniei	0.3342	0.3768	0.2949	0.2816	0.3013	0.3322		0.0144	0.0282	0.0393	0.0287	0.0348
S. americae	0.3202	0.3784	0.3029	0.2692	0.2825	0.2983	0.1180		0.0279	0.0404	0.0299	0.0352
S. zecai	0.2629	0.3291	0.2371	0.1741	0.1846	0.2555	0.3019	0.3044		0.0357	0.0255	0.0307
S. minuta	0.4096	0.4043	0.3961	0.3824	0.3940	0.3870	0.4205	0.4264	0.3814		0.0392	0.0381
S. spec A	0.0694	0.3227	0.2967	0.2449	0.2477	0.2961	0.3005	0.3017	0.2574	0.4012		0.0293
S. furcata	0.2885	0.2303	0.3298	0.2956	0.3093	0.3593	0.3748	0.3851	0.3337	0.4107	0.3132	

Supplementary Table 6: Intraspecific genetic distances using the COI dataset for the studied *Stygocapitella* species. These distances were computed using MEGA X. After defining groups (species), we calculated genetic distances for each group after the bootstrap method (500 replications), model TN-93, Rates among Sites Gamma Distribution, Gamma Parameter 1,00. 'n/c' stands for non-calculated due to a reduced number of individuals.

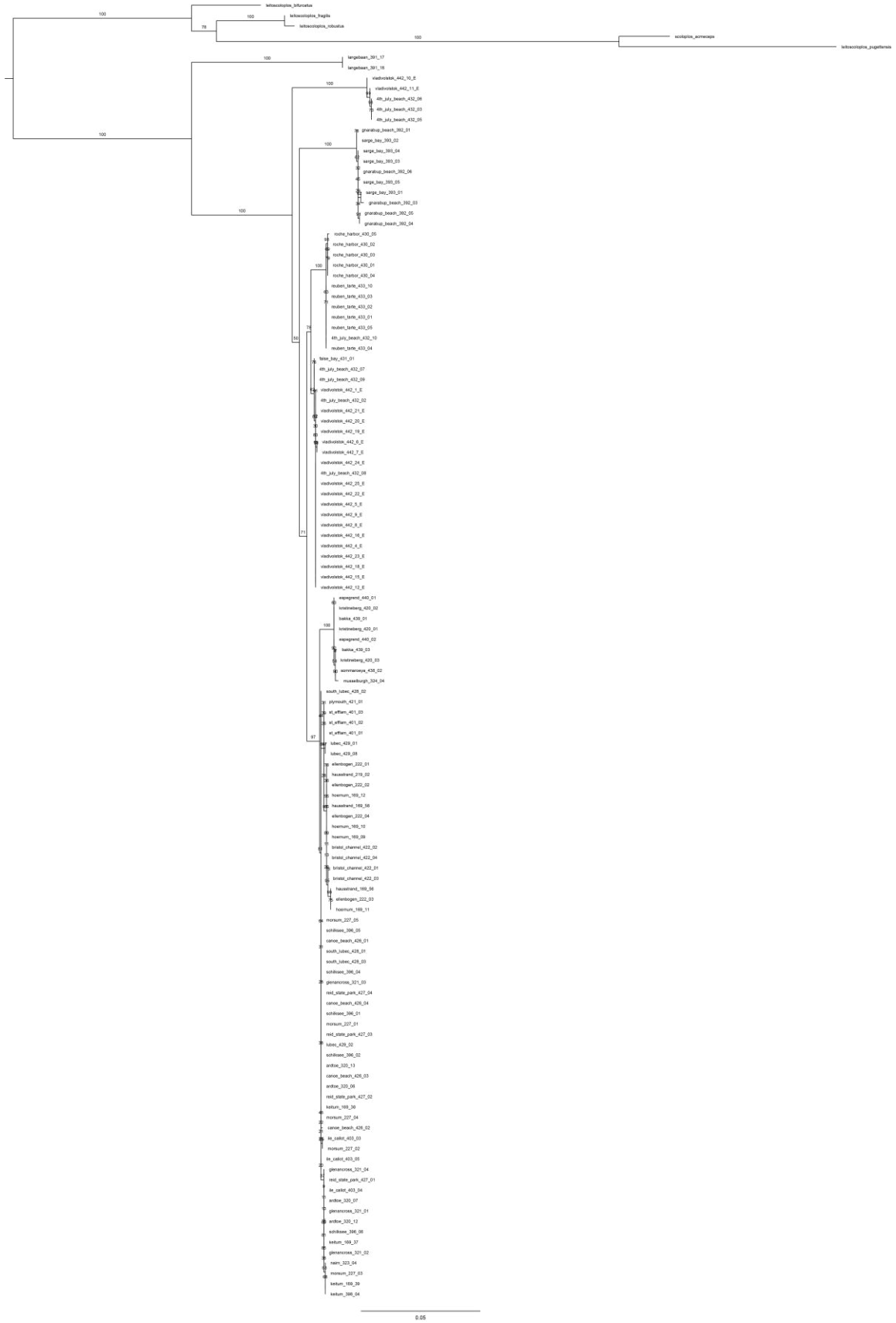
	Distance	*Standard Error*
S. budaevae	0.001961233	0.000949231
S. pacifica	n/c	n/c
S. josemariobrancoi	0.01622295	0.003269977
S. westheidei	0.004264828	0.001335192
S. subterranea	0.005740379	0.001110693
S. australis	0.067016213	0.008159899
S. berniei	0.001071403	0.000580938
S. americae	0	0
S. zecai	0.004776885	0.001455438
S. minuta	0.009434387	0.002140802
S. spec A	0.003217497	0.001383465
S. furcata	0.004283353	0.002097259



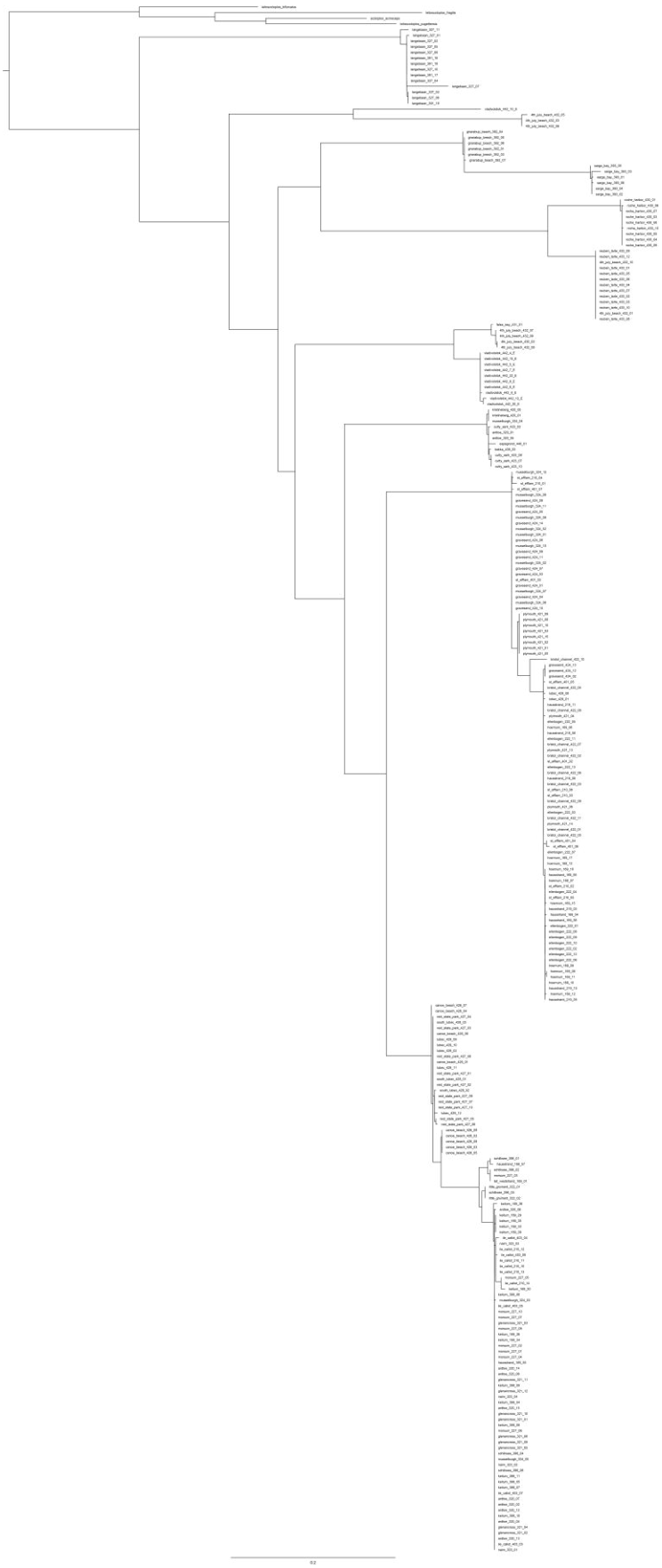
Supplementary Figure 1: World-map with sampling locations included as part of Cerca et al.. The world-view panel displays sampling locations as well as symbols for every species included in this study. As part of this panel, we provide indications of: Panel A) sampling area in the Pacific US; Panel B) sampling locations in the Atlantic coastline of the USA; Panel C) sampling locations in European coastline; Panel D) displays the island of Sylt in Northern Germany; Panel E) displays sampling locations in Far-East-Russia.



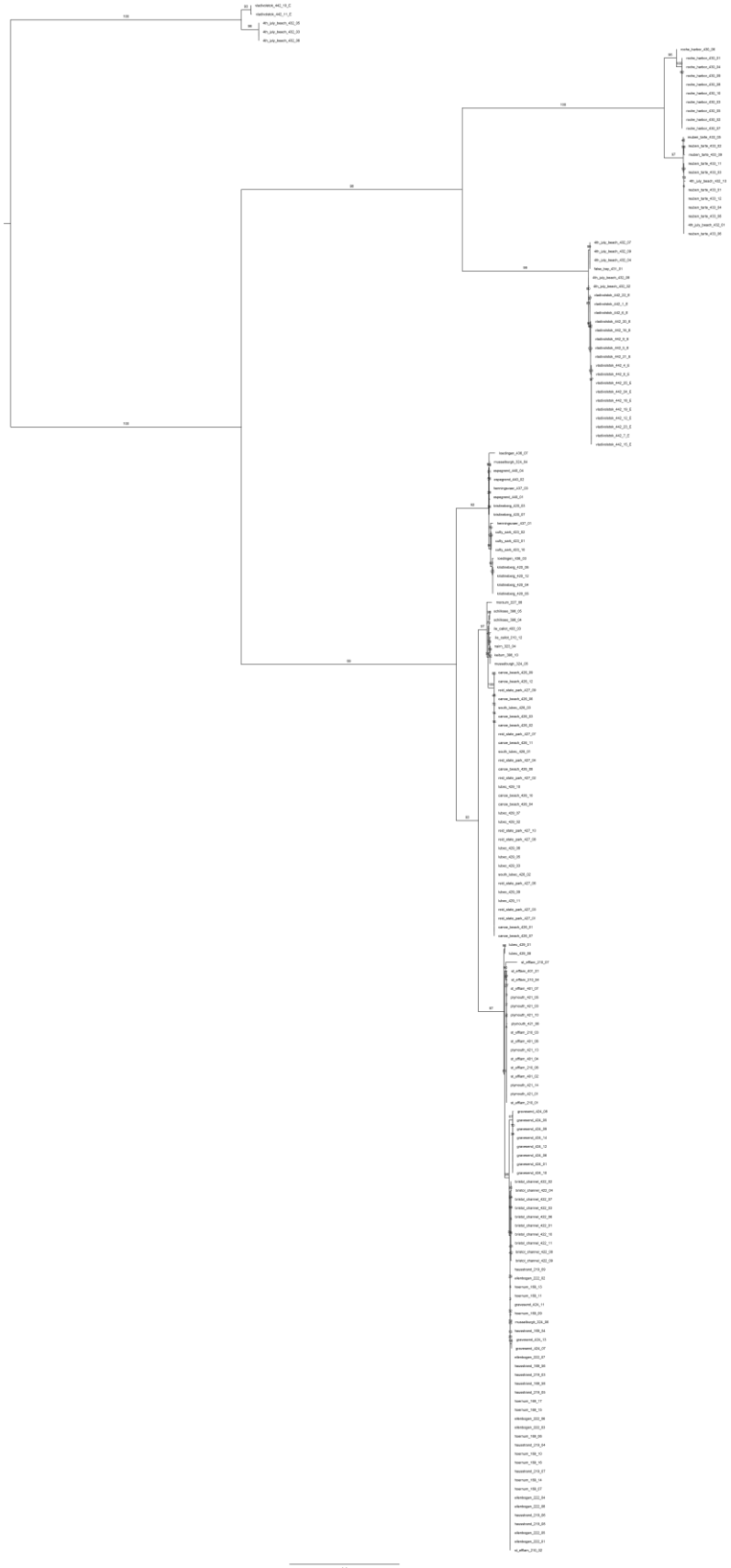
Supplementary Figure 2: Maximum Likelihood Phylogram of the 16S dataset. Bootstrap support values are included for every branch.



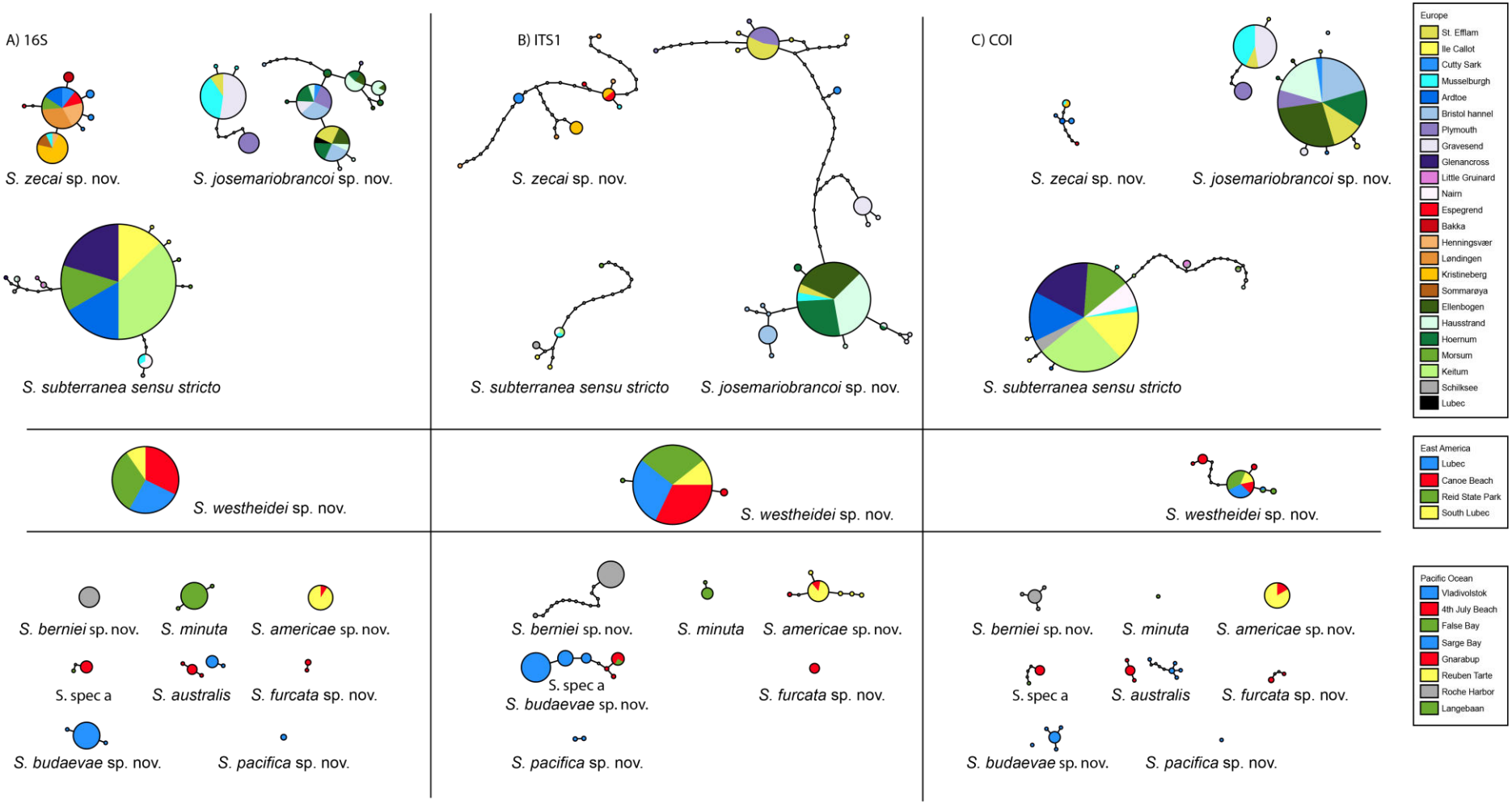
Supplementary Figure 3: Maximum Likelihood Phylogram of the 18S dataset. Bootstrap support values are included for every branch.



Supplementary Figure 4: Maximum Likelihood Phylogram of the COI dataset. Bootstrap support values are included for every branch.



Supplementary Figure 5: Maximum Likelihood Phylogram of the ITS1 dataset. Bootstrap support values are included for every branch.



Supplementary Figure 6: Haplotype networks colored by sampling location. Panel A) displays 16S; Panel B) displays ITS1; Panel C) displays COI. Networks are divided by ocean and coastlines (horizontal divisions) including the North European coastline, the East American Coastline and the Pacific Ocean.

Appendices including non-peer reviewed replies and book chapters 1-2

Appendix 1

Cryptic Species – more than terminological chaos: A reply to Heethoff

References

1. Kuparinen, A. and Uusi-Heikkilä, S. (2018) Sustainability of fishing is about abundance: a response to Bernatchez *et al*. *Trends Ecol. Evol.* 33, 307–308
2. Bernatchez, L. *et al.* (2017) Harnessing the power of genomics to secure seafood future. *Trends Ecol. Evol.* 9, 665–680
3. Willette, D. *et al.* (2014) So, you want to use next-generation sequencing in marine systems? Insight from the Pan-Pacific Advanced Studies Institute. *Bull. Mar. Sci.* 90, 79–122
4. Pearse, D. (2016) Saving the spandrels? Adaptive genomic variation in conservation and fisheries management. *J. Fish Biol.* 89, 2697–2716
5. Kelley, J.L. *et al.* (2016) The life aquatic: advances in marine vertebrate genomics. *Nat. Rev. Genet.* 4, 523–534
6. Waples, R.S. *et al.* (2008) Integrating genetic data into management of marine resources: how can we do it better? *Fish Fish.* 9, 423–449

Letter

Cryptic Species – Conceptual or Terminological Chaos? A Response to Struck *et al*.

Michael Heethoff[1,*]

In a recent article, Struck *et al*. [1] aimed at finding evolutionary processes hidden in cryptic species. They provided a broad overview on the different usage of the term 'cryptic species' and called for a more rigorous definition by comparing phenotypic (morphological) disparity with the degree of genetic differentiation. They conclude 'if biologists cannot even agree on what to consider different species, then how can we reach consensus on what represents cryptic species?' I argue that there is only one solution to both of these issues and that cryptic species represent nothing more than an incompatibility of species 'concepts' in applied taxonomy. Hence, 'cryptic species' can neither be defined nor are they outcomes of an evolutionary process like 'cryptic speciation'.

Species delimitation has been confused with species conceptualization, leading to a controversy on what the species category is and how species can be delineated [2]. The evolutionary species concept [3] represents a general primary concept, however, without much value for applied taxonomy. Applied taxonomy mostly refers to the morphological species concept, although there is no clearly defined workflow for species delineation [4]. In this context, Struck *et al*. suggest that 'morphological variation needs to be explicitly quantified', and I could not agree more. The biological species concept [5] is often used to confirm or reject morphospecies hypotheses, but is only applicable to sexually reproducing organisms. Using genetic differences for species delineation has also been proposed (e.g., [6]), and has recently been applied to split giraffes into four distinct species despite them interbreeding in captivity [7]. Hence, whether a species is cryptic or not depends on nothing else than the underlying species concept. Struck *et al*. implicitly used the morphological species concept and 'tested' it against genetic divergence. Hence, they compared two classes of species concepts (morphological vs. genetical) regarding their compatibility (i.e., supporting the same boundaries of species), and 'define' species to be cryptic when they are morphologically similar but genetically distinct (which is here taken as a proxy for 'reduced gene flow' and 'reproductive isolation' and would thus confirm the biological species hypothesis). This approach prioritizes the 'evolutionary truth' of genetic over morphological species concepts – probably a valid approach in many if not most cases. Ten years ago, Bickford and colleagues defined cryptic species as 'two or more distinct species classified as a single species' [8], rendering 'cryptic' species as nothing more than grouping artifacts. I agree and conclude that cryptic species do not exist as a concept, but that the term 'cryptic' is only used to prioritize one species concept over others. Eventually, it may turn out that cryptic species are not so cryptic at all [9].

Hence, we should not aim at defining what 'cryptic species' are, but what species concept we believe to represent evolutionary entities that we can use as fundamental units in biology – even if such a concept may lack clear instructions for applied taxonomy.

[1]Ecological Networks, Technische Universität Darmstadt, Schnittspahnstraße 3, 64287 Darmstadt, Germany

*Correspondence:
heethoff@bio.tu-darmstadt.de34 (M. Heethoff).

https://doi.org/10.1016/j.tree.2018.02.006

References

1. Struck, T.H. *et al.* (2017) Finding evolutionary processes hidden in cryptic species. *Trends Ecol. Evol.* 33, 153–163
2. De Queiroz, K. (2007) Species concepts and species delimitation. *Syst. Biol.* 56, 879–886
3. Simpson, G.G. (1951) The species concept. *Evolution* V, 285–298
4. Mayden, R.L. *et al.* (1997) A hierarchy of species concepts: the denouement in the saga of the species problem. In *Species: The Units of Biodiversity* (Claridge, M.F., ed.), pp. 381–424, Chapman & Hall
5. Mayr, E. (1940) Speciation phenomena in birds. *Am. Nat.* 74, 249–278
6. Birky, C.W. *et al.* (2010) Using population genetic theory and DNA sequences for species detection and identification in asexual organisms. *PLoS One* 5, e1060951
7. Fennessy, J. *et al.* (2016) Multi-locus analyses reveal four giraffe species instead of one. *Curr. Biol.* 26, 2543–2549
8. Bickford, D. *et al.* (2007) Cryptic species as a window on diversity and conservation. *Trends Ecol. Evol.* 22, 148–155
9. Sykes, B.C. *et al.* (2014) Genetic analysis of hair samples attributed to yeti, bigfoot and other anomalous primates. *Proc. Biol. Sci.* 281, 20140161

Letter

Cryptic Species – More Than Terminological Chaos: A Reply to Heethoff

Torsten H. Struck,[1,*]
Jeffrey L. Feder,[2]
Mika Bendiksby,[1,3]
Siri Birkeland,[1] Jose Cerca,[1]
Vladimir I. Gusarov,[1]
Sonja Kistenich,[1]

CellPress
REVIEWS

Karl-Henrik Larsson,[1]
Lee Hsiang Liow,[1,4]
Michael D. Nowak,[1]
Brita Stedje,[1] Lutz Bachmann,[1]
and Dimitar Dimitrov[1,5]

Recently we discussed problems and challenges associated with inconsistent definitions and methods used to identify cryptic species, and how these hamper studies of their evolutionary significance. We proposed a conceptual framework that is focussed on evolutionary processes and advocated for a shift from pattern- to process-driven research concerning cryptic species, in order to circumvent these issues [1]. In his response, Heethoff [2] argued that cryptic species are merely a reflection of the limitations of applied taxonomy. He stated that cryptic species 'represent nothing more than an incompatibility of species "concepts" in applied taxonomy'. As such, he rejected our proposed framework as an approach that 'prioritizes the "evolutionary truth" of genetic over morphological species concepts'. In our opinion Heethoff's conclusions are based on his misconceptions about the proposed framework.

In essence, Heethoff repeats the often-cited opinion that cryptic species are only a temporary taxonomical formalization problem of species delineation [3,4] and not a natural phenomenon. He supports this view with examples of diverging species in the so-called grey zone of speciation [5,6], like the giraffe, and ignores many examples of good species with unusually high phenotypic similarity despite restricted gene flow, sometimes over long time periods [7–11]. Importantly, our survey of the literature revealed that there are as many old cryptic species reported as young ones [1]. However, the current taxonomic practice makes it difficult to differentiate between these two cases and to understand the evolutionary processes and mechanisms underlying their origins.

Thus, we proposed a framework that in contrast to previous approaches explicitly separated the two steps, that is, species delineation and assignment of the status cryptic. Specifically, we concluded that what is needed is a rigorous quantitative assessment of phenotypic similarity in an evolutionary context [1]. To this end, our framework provides explicit means of differentiating taxonomical errors from true cryptic biodiversity. The first basic requirement of our framework is to show that the lineages in question are clearly separate species [5]. This can be achieved by applying any species concept. We provided examples based on molecular approaches, but we did not advocate for any particular species concept, as presumed by Heethoff. In fact, our proposed call for proof of species being distinct is not different from any other species delineation attempt, and evidence should be based on as many sources of information as possible [5].

The second step of assigning the status cryptic implies that a null expectation (hypothesis) is to be formulated and tested. Specifically, it requires evidence for substantially higher degrees of phenotypic similarity between species in question than expected. This can, for example, be achieved by comparing evolutionary rates to other species pairs within the lineage. If the homogenizing effect of gene flow is shown to be low or absent, high phenotypic similarity becomes less likely with increasing divergence time. Hence, only species exhibiting statistically lower degrees of phenotypic disparity than expected for a given divergence time are accepted as cryptic in the proposed framework. Genetic divergence was proposed as a proxy, when actual divergence times cannot be determined with high confidence. This proposal offers further advantages because genetic divergence estimates can also serve as proxy for the degree of gene flow. However, this does not mean that genetic divergence serves as a proxy for the biological species concept, as implied by Heethoff. Species delineation is done in the first step from the sum whole of information, including genetic data, before cryptic status is assigned.

Finally, it is important to note that we did not advocate that cryptic species are the outcome 'of an evolutionary process like cryptic speciation'. We clearly stated that this term is misleading and should not be used. When it comes to cryptic species, only recent divergence can be directly related to the speciation processes. Other processes such as convergence, parallelism, and stasis are not related to the speciation process itself, but describe macroevolutionary processes that have led to high phenotypic similarity as the outcome of evolution. We explicitly outlined the advantages of cryptic species as models for understanding these processes [1], an end goal of our proposed framework.

Responding to concerns raised by Heethoff [2], we conclude that they are the result of misconceptions about our framework. We reaffirm that our approach to evaluating whether species are cryptic is transparent, repeatable, and independent of taxonomic treatment (e.g., usage of species concepts and history of synonymies). We all make errors and overlook things sometimes, taxonomists included, but cryptic species are more than errors. If we take the time to define and quantify cryptic species with rigor, then they have potentially much to teach us about evolution beyond just learning from our mistakes.

[1]Natural History Museum, University of Oslo, 0318 Oslo, Norway
[2]Department of Biological Sciences, University of Notre Dame, Notre Dame, IN 46556, USA
[3]NTNU University Museum, Norwegian University of Science and Technology, 7491 Trondheim, Norway
[4]Centre for Ecological & Evolutionary Synthesis (CEES), Department of Biosciences, University of Oslo, 0316 Oslo, Norway

[5]Center for Macroecology, Evolution and Climate, Natural History Museum of Denmark, University of Copenhagen, Copenhagen, Denmark

*Correspondence: t.h.struck@nhm.uio.no (T.H. Struck).

https://doi.org/10.1016/j.tree.2018.02.008

References

1. Struck, T.H. *et al.* (2018) Finding evolutionary processes hidden in cryptic species. *Trends Ecol. Evol.* 33, 153–163
2. Heethoff, M. (2018) Cryptic species – conceptual or terminological chaos? A response to Struck *et al*. *Trends Ecol. Evol.* 33, 310
3. Korshunova, T. *et al.* (2017) External diversity is restrained by internal conservatism: new nudibranch mollusc contributes to the cryptic species problem. *Zool. Scripta* 46, 683–692
4. Bickford, D. *et al.* (2007) Cryptic species as a window on diversity and conservation. *Trends Ecol. Evol.* 22, 148–155
5. Pante, E. *et al.* (2015) Species are hypotheses: avoid connectivity assessments based on pillars of sand. *Mol. Ecol.* 24, 525–544
6. Sukumaran, J. and Knowles, L.L. (2017) Multispecies coalescent delimits structure, not species. *Proc. Natl. Acad. Sci. U. S. A.* 114, 1607–1612
7. Smith, K.L. *et al.* (2011) Evidence of constrained phenotypic evolution in a cryptic species complex of agamid lizards. *Evolution* 65, 976–992
8. Struck, T.H. *et al.* (2017) Two new species in the annelid genus *Stygocapitella* (Orbiniida, Parergodrilidae) with comments on their biogeography. *Zootaxa* 4286, 301–332
9. Wada, S. *et al.* (2013) Long-term stasis and short-term divergence in the phenotypes of microsnails on oceanic islands. *Mol. Ecol.* 22, 4801–4810
10. Swift, H.F. *et al.* (2016) Three routes to crypsis: stasis, convergence, and parallelism in the *Mastigias* species complex (Scyphozoa, Rhizostomeae). *Mol. Phylogenet. Evol.* 99, 103–115
11. Cursino, M. *et al.* (2014) The role of chromosome variation in the speciation of the red brocket deer complex: the study of reproductive isolation in females. *BMC Evol. Biol.* 14, 40

Appendix 2

Cryptic species and their evolutionary significance

Cryptic Species and Their Evolutionary Significance

Torsten H Struck, *Natural History Museum, University of Oslo, Oslo, Norway*
José Cerca, *Natural History Museum, University of Oslo, Oslo, Norway*

Advanced article

Article Contents

Online posting date: 16th January 2019

Cryptic species are detected at an ever-increasing rate, mainly due to the application of molecular data. While the impact of this hidden diversity on macro-ecology and conversation biology is widely recognized, its evolutionary significance is rarely. In recent years, it became apparent that definitions of cryptic species are too ambiguous to allow the differentiation between natural phenomena from human-made artefacts. Hence, recently, a unifying conceptual framework has been proposed highlighting the necessity to test the degree of reduced phenotypic disparity in cryptic species. Within this reduced disparity also lies the evolutionary significance, as cryptic species can be regarded as the opposite of adaptive radiations. Specifically, studies on evolutionary stasis can substantially benefit from including these by addressing both patterns of reduced disparity and processes resulting in the lack of phenotypic evolution. In addition, this will allow connecting macro-evolutionary and paleontological studies with micro-evolutionary investigations of genotype-phenotype linkage.

Introduction

Taxonomy, the discipline dealing with the delimitation of biological units, remains one of the most contentious and laborious disciplines in biology, yet it represents a fundamental step before understanding underlying evolutionary processes. Interestingly, taxonomical considerations already had a strong impact on Darwin's line of argument concerning evolution (Darwin, 1859). He had already pointed out the problem of delineating species properly, and that the progress from populations to species is a contiguous scale with respect to the variability that can be observed at different levels, rendering it difficult to delineate species boundaries with certainty. The debate about this problem is still vibrant today and the debate about species concepts and how to apply them is in full fledge (for details **see also**: **Species Concepts**). In the last decades, the discussion about cryptic species has been added to this debate, highlighting the necessity to delimitate independently evolving units to understand evolutionary processes.

The concept of cryptic species has been applied as early as 1718 by the English clergyman William Derham focusing on the avian genus *Phylloscopus* (Winker, 2005) and Mayr (1963) coined the term sibling species for cases, where the species are either sister or very closely related to each other. However, the detection of cryptic species really took off with the advent of employing sequence data in delineating species boundaries on a much broader scale since the early 1990s (Struck *et al*., 2018b; **see also**: **Systematics: Relevance to the Twenty-first Century** on barcoding). Since then, cryptic species have been detected at an ever-increasing pace and across all habitats on Earth and the entire tree of life including fungi, algae, plants, protists, invertebrates, but also primates, amphibia, reptiles and crustaceans (Bickford *et al*., 2007; Pfenninger and Schwenk, 2007; Perez-Ponce de Leon and Poulin, 2016; Hawksworth and Lücking, 2017). Accordingly, cryptic species seem to represent an overlooked, yet substantial part of biodiversity with far-reaching implications for ecological research such as diversity estimates, pest control, fisheries management and conservation efforts as well as research in model systems (Bickford *et al*., 2007; Caputi *et al*., 2007; Bernardo, 2011; Pante *et al*., 2015; Fišer *et al*., 2018). However, like with any new emerging concept in biology, the term cryptic species is often applied with very different meanings and hence has different implications. This, among others, affects the general conclusions, which can be drawn concerning cryptic species. Owing to the different meanings the conducted meta-analyses usually compare apples with oranges (Struck *et al*., 2018a; Struck *et al*., 2018b). Therefore, it became noticeable that a unifying theoretical framework is needed for studying cryptic species, which then would also allow for drawing more general and solid conclusions about the ecological and evolutionary

eLS subject area: Evolution & Diversity of Life

How to cite:
Struck, Torsten H and Cerca, José (January 2019) Cryptic Species and Their Evolutionary Significance. In: eLS. John Wiley & Sons, Ltd: Chichester.
DOI: 10.1002/9780470015902.a0028292

significance of cryptic species. Only such conclusions are then able to provide meaningful contributions to, for example conversation management or health issues stemming from cryptic species.

Towards a Unifying Conceptual Framework for Cryptic Species

The first attempt towards a unifying conceptual framework of cryptic species was accomplished by Bickford *et al*. (2007). They defined cryptic species as 'two or more distinct species that are erroneously classified (and hidden) under one species name'. This broad definition was quickly applied in several studies and became the most commonly used one, as it is easily applicable due to its tight link to the taxonomic history of the species. That is, it strictly requires that the species complex is formally described as a single species before. However, this also entails a caveat associated with different schools of taxonomic practice (**see also**: **History of Taxonomy**). For instance, in taxonomy, some schools favour splitting species even in cases of only little support, while others would rather lump these together as a single species (the splitter vs. lumper debate). Hence, some groups would be more prone to have cryptic species only due to different taxonomic practices. For example, the newly described *Marphysa aegypti* (Annelida) from Egyptian waters was previously recorded as *Marphysa sanguinea* (Elgetany *et al*., 2018). Even though both species are morphologically substantially different from each other, they could be called cryptic species or at least pseudo-cryptic species, while in truth the previous records suggest only sloppy taxonomic practices. On the other hand, molecular data supported the traditional assignment of species within the genus *Polygordius* (Annelida) based on geographic regions (Ramey-Balcı *et al*., 2018), despite the indistinguishable adult morphology of *Polygordius lacteus* from *Polygordius neapolitanus* and *Polygordius jouinae* from *Polygordius triestinus*. Nonetheless, both species pairs could not be considered cryptic species given the provided definition by Bickford *et al*. (2007). Moreover, others pointed out that recent definitions of cryptic species are often linked to and depend on the applied species concept (Pante *et al*., 2015; Sukumaran and Knowles, 2017; Fišer *et al*., 2018; Heethoff, 2018). Given these nonbiological aspects, several studies introduced slightly different concepts such as pseudo-cryptic, hyper- or mega-cryptic species (e.g. Adams *et al*., 2014; Cornils and Held, 2014; Nygren *et al*., 2018).

Hence, in recent years, there has been an increased debate again what constitutes a cryptic species. Ultimately, Korshunova *et al*. (2017) argued 'to avoid the terms "cryptic"/"pseudocryptic" species as a reference to a "natural phenomenon" because it is obscuring multilevel character diversity within a complicated taxonomy-dependent framework' and instead 'to use the term "cryptic species" only for a temporary formalization of the problems with delineation of the species from the same geographic region, when those species demonstrate significant molecular phylogenetic differences, but are hardly distinguished morphologically, ethologically, etc.' A recent literature survey found that only 14% of the studies actually provided or applied an explicit definition of cryptic species adding to the problem of uncertainty in the assignment of cryptic species (Struck *et al*., 2018b). Of these, all were explicitly or implicitly taxonomy-based like the Bickford *et al*. (2007) definition. Additional criteria such as usage of molecular data, sympatric occurrence, reproductive isolation, reduced gene flow, or no morphological differences were included in the definitions. Moreover, another problem of assigning cryptic species based on these definitions is that the species delineation process is intermingled with the assignment of the term 'cryptic'. As a consequence, all problems associated with species delineation (**see also**: **Species Problem – A Philosophical Analysis**) also automatically apply to the assignment. In summary, the assignment of a species as cryptic species depends on many nonbiological factors such as the applied definition and species concept as well as the taxonomic tradition resulting in much uncertainty about what constitutes a cryptic species and if it is a natural phenomenon or only a human-made artefact. This problem has dire consequences for meta-analyses investigating the impact of cryptic species in biodiversity studies (Perez-Ponce de Leon and Poulin, 2016; Poulin and Pérez-Ponce de León, 2017), and for the understanding of biological processes.

Two general assumptions, which were at least implicitly applied in the practical procedures, were evident from the literature (Struck *et al*., 2018b). First, given the species concept, cryptic species were generally thought to be 'true' species and, second, these species were so similar in the taxonomically relevant phenotypic characters that they were not or hardly distinguishable from each other. Therefore, Struck *et al*., 2018b provided a new definition for cryptic species reflecting these two assumptions. The process of assigning cryptic species was separated into two clearly separated steps in contrast to the previous attempts. At the first step, it has to be established that the species are truly species given the applied species concept. In **Figure 1**, the white circles indicate cases, which would not be considered species and accordingly also not cryptic species. Hence, this first step is not different from any other species delineation process, independent if these entities are cryptic or not, but it has the advantage that the pitfalls associated with this process are confined to the proper step and are not carried over to the next step. The second step consists in showing that the species are phenotypically more similar to each other than one would expect given the time that has passed since their last common ancestor (or the level of genetic divergence as a proxy for time). These species should hence be called cryptic species only if this level of phenotypic disparity is significantly lower than expected. The red circles in **Figure 1** represent such cases, while the orange and yellow circles do not. This second step is the crucial step in the framework with respect to cryptic species as here the actual assignment occurs. The definition is property-based and independent of the taxonomic history of the species at hand. Specifically, it does not matter if the species have been described as only one before or not. Studies across taxa, habitats, life strategies and so forth can be based on comparable categories like similar applied species concepts, levels of disparity or genetic divergence instead of taking cryptic species at face value allowing more robust conclusions about the impact of cryptic species.

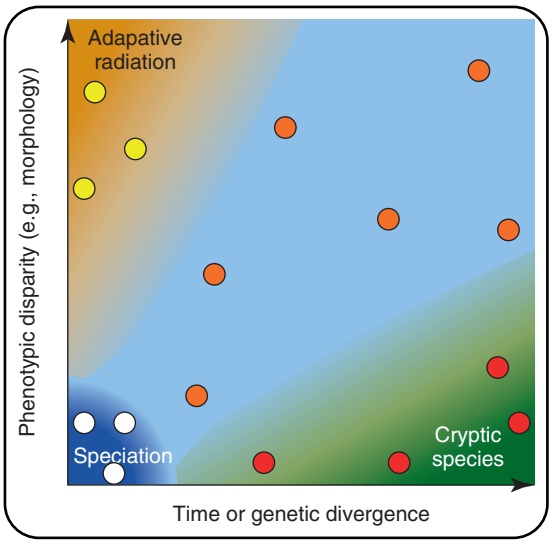


Figure 1 Unifying conceptual framework based on Struck *et al.* (2018b). The *x*-axis represent time since divergence from the last common ancestor. Often genetic divergence is used as proxy for this. The *y*-axis represents the degree of phenotypic disparity. The dark blue area is the area of ongoing speciation and hence no species boundaries have been established yet (white circles). The light blue area indicates evolution between pairs of species (orange circles) as it is intuitively assumed. That is phenotypic disparity more or less increases linear with time. The orange area reflects cases (yellow circles) in which phenotypic evolution occurs at a much higher rate than anticipated such as in adaptive radiations. The green area represents cases (red circles) of significantly reduced phenotypic disparity given time as it is the case in cryptic species.

Evolutionary Significance of Cryptic Species

Understanding the tempo and mode of speciation, and the drivers of phenotypic diversification are major objectives in biology (Rabosky and Adams, 2012). Therefore, identifying and quantifying contrasts between phenotypic disparity and genetic divergence has become popular in recent decades. Cases such adaptive radiations where morphological disparity outpaces genetic divergence received considerable attention (**see also**: **Adaptive Radiation**). Adaptive radiations generally rely on open ecological opportunities like appearance of new resources, evolution of key innovations or colonization of new areas followed by specialization to the open niches and speciation (Losos, 2010). Cryptic species as defined by the aforementioned framework can be regarded as the exact opposite to these radiations, being characterized by a strongly reduced phenotypic or at least morphological disparity in comparison to the observed genetic divergence (yellow vs. red circles in **Figure 1**).

If high rates of phenotypic variation have the potential to result in radiation of organisms and occupation of different evolutionary niches – 'high evolvability' – then low phenotypic disparity as observed in cryptic species can be seen as a paradox as theory predicts that clades with high evolvability supersede clades with low evolvability (Estes and Arnold, 2007; Rabosky and Adams, 2012). Clades of cryptic species should have a lower 'adaptive zone' and occupy less ecological space and, hence, be replaced by clades with a potential to evolve and adapt faster (Rabosky and Adams, 2012). As evolvability is generally considered as a measure of evolutionary success (Rabosky and Adams, 2012), the question arises why so many cryptic species are observed nowadays, which supposedly lack any phenotypic or at least morphological evolution. Several suggestions have been put forth to explain lack of phenotypic evolution in general and especially considering macro-evolutionary patterns (Futuyma, 2010). These include, among others, genetic and developmental constraints, source populations impeding specialization (meta-population dynamics), repeated bottlenecks decreasing standing genetic variation, large populations, stabilizing selection, ephemeral, stressful or fluctuating environments, evolutionary stable configurations or niche conservationism (Maynard Smith, 1983; Eldredge *et al*., 2005; Futuyma, 2010; Haller and Hendry, 2014; Chomicki and Renner, 2017). However, the lack of phenotypic evolution has received considerably less attention in evolutionary biology than its opposite, adaptive radiations, especially at the microevolutionary level, and empirical and experimental evidence for any of the suggestions is low so far. While cryptic species can be ideal systems to inform us on the causes of reduced phenotypic disparity, much needs to be done. First, the evolutionary processes resulting in cryptic species must be identified. Then it can be investigated in how far the different causes mentioned above influenced these processes. Four different processes have been suggested to result in cryptic species: recent divergence, convergence, parallelism and stasis (Swift *et al*., 2016; Struck *et al*., 2018b).

Evolutionary Processes I: Recent Divergence

The most common, but unproven assumption is that cryptic species follow recent speciation and that they did not yet have enough time to accumulate substantial phenotypic, especially morphological differences (Knowlton, 1993; Reidenbach *et al*., 2012). For example (**Figure 2**), in the malaria vector *Anopheles gambiae* (Hexapoda) two forms, the M (now recognized as *Anopheles coluzzii*) and S form, are recognized, which seem to be reproductively isolated (Reidenbach *et al*., 2012). They are at an early stage after speciation differing in an inversion on chromosome-2, which seems to be associated with their ecological differences (Simard *et al*., 2009). The M form mainly exploits stable larval habitats with high level of stressors; the S form exploits unpolluted, predator-free, ephemeral habitats associated with seasonal rainfall (Reidenbach *et al*., 2012). Ecological experiments suggest that these forms seem to outcompete each other in their respective environment and respond to predation differently (Diabaté *et al*., 2008). Hence, in the M and S forms, other traits than morphology are under selection.

Speciation is not necessarily accompanied by morphological change in the early stages as selection acts largely on physiological, immunological, reproductive or behavioural traits (Bensch *et al*., 2004; Damm *et al*., 2010; Derycke *et al*., 2016). Allopatric

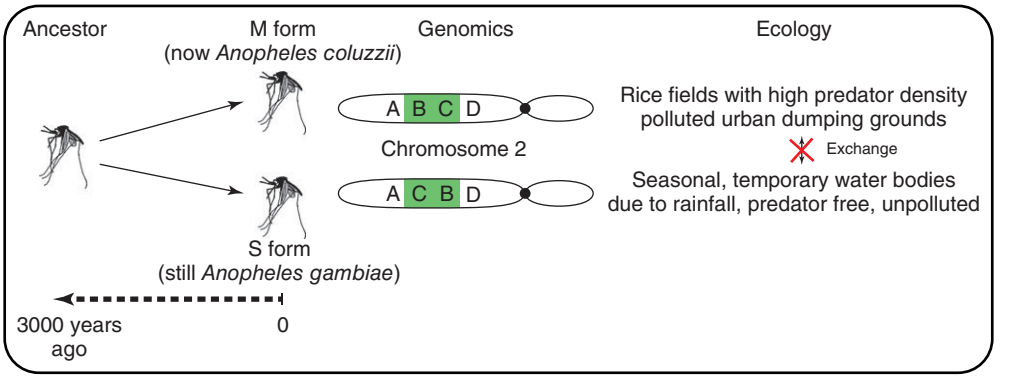


Figure 2 Schematic representation of the *Anopheles* example for recent divergence (based on the results of Reidenbach *et al.,* 2012). The left panel exemplifies the recent divergence of the two cryptic species. In the middle, the genomic inversion at chromosome 2 is shown and the right one lists the ecological differences observed between the two species.

speciation while remaining in one particular ecological niche or habitat might lead to the building-up of nonadaptive divergence without morphological change. As a result, recently diverged species can remain morphologically identical following initial divergence (Korshunova *et al*., 2017). In these cases of recent divergence, cryptic species are nothing special to other species pairs as the speciation event is very recent and generally no or little phenotypic change, particularly in morphology, is expected this shortly after the speciation unless there is a strong selection towards different adaptive optima. Moreover, such cases cannot provide much insight into the supposed lack of phenotypic evolution.

Evolutionary Processes II: Convergence and Parallelism

Phenotypic similarity could also stem from convergence or parallelism (Swift *et al*., 2016; Struck *et al*., 2018b). In both cases, the cryptic species evolved the same phenotypes independently of each other. While for convergence the immediate ancestors of the cryptic species were dissimilar from each other as well as to the extant cryptic species, for parallelism the ancestors were similar to each other, but dissimilar to the extant cryptic species. One example (**Figure 3**) comprising both convergence and parallelism occurs in the *Mastigias* species complex (Scyphozoa) (Swift *et al*., 2016). *Mastigias* species occur in both coastal waters including coves and lagoons ('ocean' phenotypes), and in small-bodies of salt water without an open connection to the ocean ('lake' phenotypes). The 'lake' phenotypes evolved both by parallelism and convergence from the 'ocean' phenotypes. In some cases, the 'lake' phenotypes evolved from the same 'ocean' phenotype (parallelism) and in others from different 'ocean' phenotypes (convergence). Similarly, selective pressures from predators led to parallelism in the Holarctic *Enallagma* species (Hexapoda) (Stoks *et al*., 2005). Cases of convergence of cryptic species were also found in the Deep Sea (Vrijenhoek, 2009).

As for the case of recent divergence, the evolutionary processes of convergence and parallelism cannot contribute to our understanding of the lack of phenotypic evolution, as phenotypic change occurred. Nonetheless, these cases can help us understand how reduced phenotypic disparity evolves. Importantly, it indicates that reduced phenotypic disparity and lack of phenotypic evolution are not necessarily the same. For convergence and parallelism specifically, the question arises what are the driving factors that the cryptic species independently evolved to the same phenotypes? Is it due to intrinsic (e.g. developmental constraints) or extrinsic ones (e.g. extreme environments) confining the available phenotypic landscape to only one solution in the respective situation? Contrary to parallelism, intrinsic factors are expected to be less influential than extrinsic ones in convergence, as evolution starts from more distinct genetic backgrounds. However, maybe even for convergence developmental constraints constrict the available phenotypic landscape more than expected.

Evolutionary Processes III: Stasis

In contrast to the other three processes, under phenotypic stasis, cryptic species retain similar phenotypes for millions of years. The literature survey by Struck *et al*. (2018b) revealed that stasis in cryptic species may occur at least not substantially less than recent divergence and seems to be an important process in the evolution of cryptic species. A prominent example (**Figure 4**) is provided by the *Cavernacmella* complex (Gastropoda) on the Ogasawara Islands (Japan) (Wada *et al*., 2013). The '*Cavernacmella minima*' phenotype occurs in five clades on the different islands of the archipelago (i.e. Mukojima, Chichijima and Hahajima). These represent cryptic species and are unaltered for over 3 million years. In contrast, within the clade of these cryptic species are also five species, which are morphologically distinct from the '*C. minima*' phenotype. This indicates that enough time passed to accumulate morphological differences under certain conditions. This release from morphological arrest might indicate the absence of developmental constraints. Similarly, cichlids have demonstrated the potential for burst of morphological evolution and stasis (Seehausen, 2006). Other examples of stasis comprise *Stygocapitella* (Annelida),

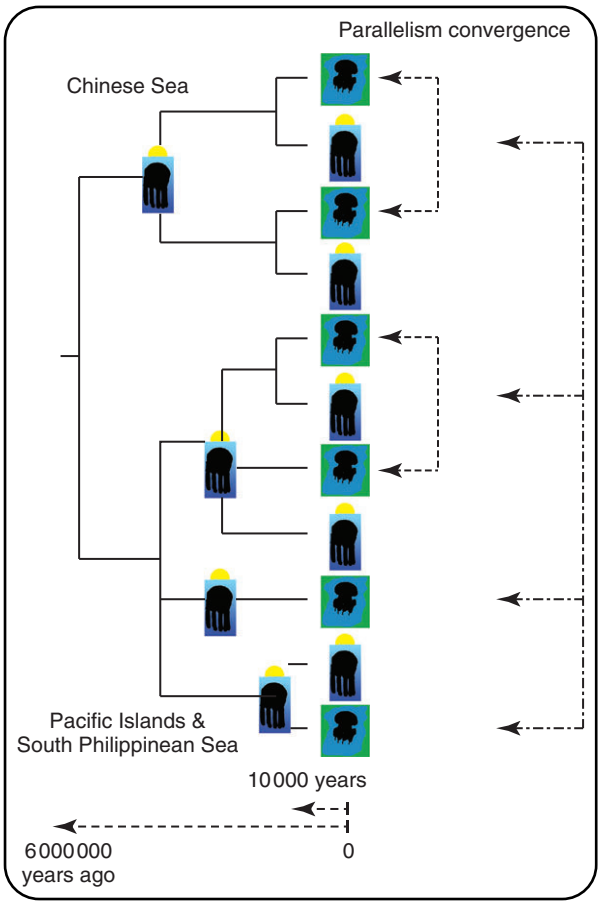


Figure 3 Schematic representation of the *Mastigias* example of parallelism and convergence based on the results by Swift *et al.* (2016). The phylogenetic relationship between the two morphotypes (oceanic and lake; indicated by the two icons) is shown to the left. Swift *et al.* (2016) regarded the origin of the two lake morphotypes within the Chinese Sea as well as two within the Pacific Islands & South Philippinean Seas as examples of parallelism as they originated from the same oceanic species. In contrast, they concluded that the other lake morphotypes to each other as well as to these previous ones evolved by convergence as they originated from different oceanic species.

Diporiphora (Squamata), *Mastigias* (Scyphozoa) or *Cletocamptus* (Crustacea) species complexes (Rocha-Olivares *et al*., 2001; Smith *et al*., 2011; Swift *et al*., 2016; Struck *et al*., 2017). *Stygocapitella* is an example of long-lasting morphological stasis. *Stygocapitella* individuals live between sand grains, by the foot of the dune, at a depth of about 0.5–1 meter and can be found on beaches in all major coastlines with the exception of tropical regions (Westheide, 2008). Molecular data suggest the presence of several cryptic species at the different coastlines and even though rigorous morphological reinvestigations discovered minimal phenotypic differences among the deeply divergent clades, these are estimated to have diverged about 300 to 100 million years ago (Struck *et al*., 2017). As such, the *Stygocapitella* complex seems to be under morphological stasis with slight morphological differentiations having occurred more than 100 million years ago. It is certainly challenging to address why these species did not change morphologically while closely related ones did, and why no phenotypic differences became fixed in the gene pools just by chance (e.g. due to recurrent bottlenecks).

Hence, cryptic species such as the presented examples are ideal systems to investigate stasis using extant taxa. These systems allow addressing both patterns of reduced phenotypic disparity and the process leading to the absence of phenotypic evolution. The term stasis is most often used in macro-evolutionary and paleontological studies and less in micro-evolutionary ones using extant taxa. In macroevolution and palaeontology, it became popularized as an argument for punctuated equilibria (**see also**: **Punctuated Equilibrium and Phyletic Gradualism**), where evidence from fossil timelines lasting for millions of years questioned the power of selection. However, recent efforts have focused on integrating stasis at the microevolutionary level (e.g. Hansen and Houle, 2004; Estes and Arnold, 2007). Futuyma (2010) reviewed many of these models including stabilizing selection, lack of genetic diversity, genetic and developmental constraints, ecological niche tracking and niche conservatism. Other potential, nonexclusive explanations for stasis or arrested evolution have been put forward specifically for more specific scenarios or habitats. For example, for the interstitial realm, that is the space

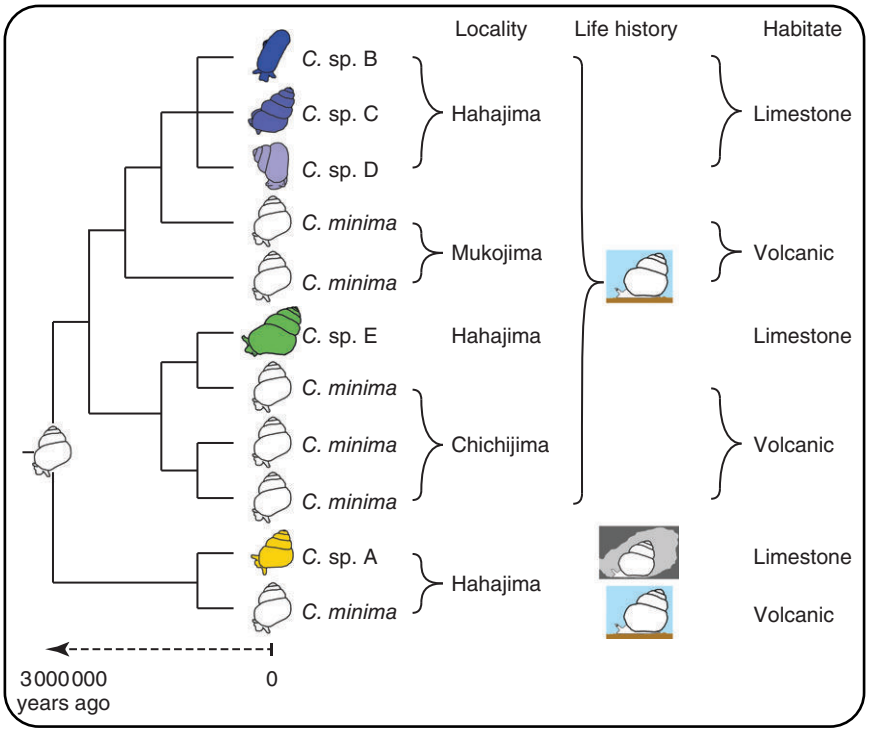


Figure 4 Schematic representation of the *Cavernacmella* example for stasis given the results of Wada *et al.* (2013). Wada *et al.* (2013) recognized a total five cryptic species as well as five morphologically distinct, noncryptic species within them (indicated by the different forms and colours; icons are relative in size to each other). The occurrence of these ten species is indicated as well as their life-history and habitat (epigenean and cave-dwelling; indicated by the two icons).

between the sand grains in marine sediments, one potential explanation stems from the 'plus ça change, plus c'est la même chose' (The more it changes, the more it is the same) model (Sheldon, 1996). This model regards morphological stasis as a response to widely fluctuating physical conditions, which are stable on geological timescales. This fits the interstitial realm, as this is characterized by wide variations in pH, salinity and moisture at short timescales, but this fluctuating characteristic has remained similar for millions of years ago (e.g. Westheide, 1977). It has also been suggested that the phenotypic landspace of species with limited morphological differentiation such as, for example some worms or fungi are too small to allow for change (Bickford *et al*., 2007). In contrast, another suggestion is that intraspecific variation of traits is high and allows for coping with broader ranges of ecological differences, while the traits fluctuate around a stable mean (Voje, 2016).

Conclusions

Studying cryptic species has great potential to further our understanding of evolutionary processes as outlined earlier, but also with respect to research in macroecology and conservation biology (**Figure 5**) (Bickford *et al*., 2007; Bernardo, 2011; Pante *et al*., 2015; Fišer *et al*., 2018). However, to accomplish these goals a pre-requisite is that what is a cryptic species can be determined with certainty. It is important to differentiate cryptic species complexes from those complexes arising from taxonomic biases or malpractice. Only the former will be able to inform us on biological processes in evolution and ecology as they reflect true natural properties. A two-step conceptual framework to accomplish this has been provided and hence the theoretical foundation been laid. However, the literature survey by Struck *et al*. (2018b) also clearly showed that methodological improvement is needed in all aspects to achieve this. Often the phenotype is not studied at all, only a single, uni-parentally inherited genetic marker is used, and/or the results are not set in relation to time or other noncryptic species to assess if they are really exceptionally different from them. Moreover, biology is transforming into a 'big data' science including among others high-throughput sequencing technologies, which allows us to apply population genomic and phylogenomic methods independent of the study object and hence provide much broader data basis for their conclusions (**Figure 5**).

On the other hand, improved delineations of cryptic species will result in and contribute to an improved understanding of the causes of evolutionary processes like convergence, parallelism and stasis (**Figure 5**). Again, the applications of genomic and transcriptomic approaches studies on cryptic species can aid in linking genotypic change to phenotypic alterations or lack thereof. Similarly, improved delineations will result in improved biodiversity assessments allowing better modelling of

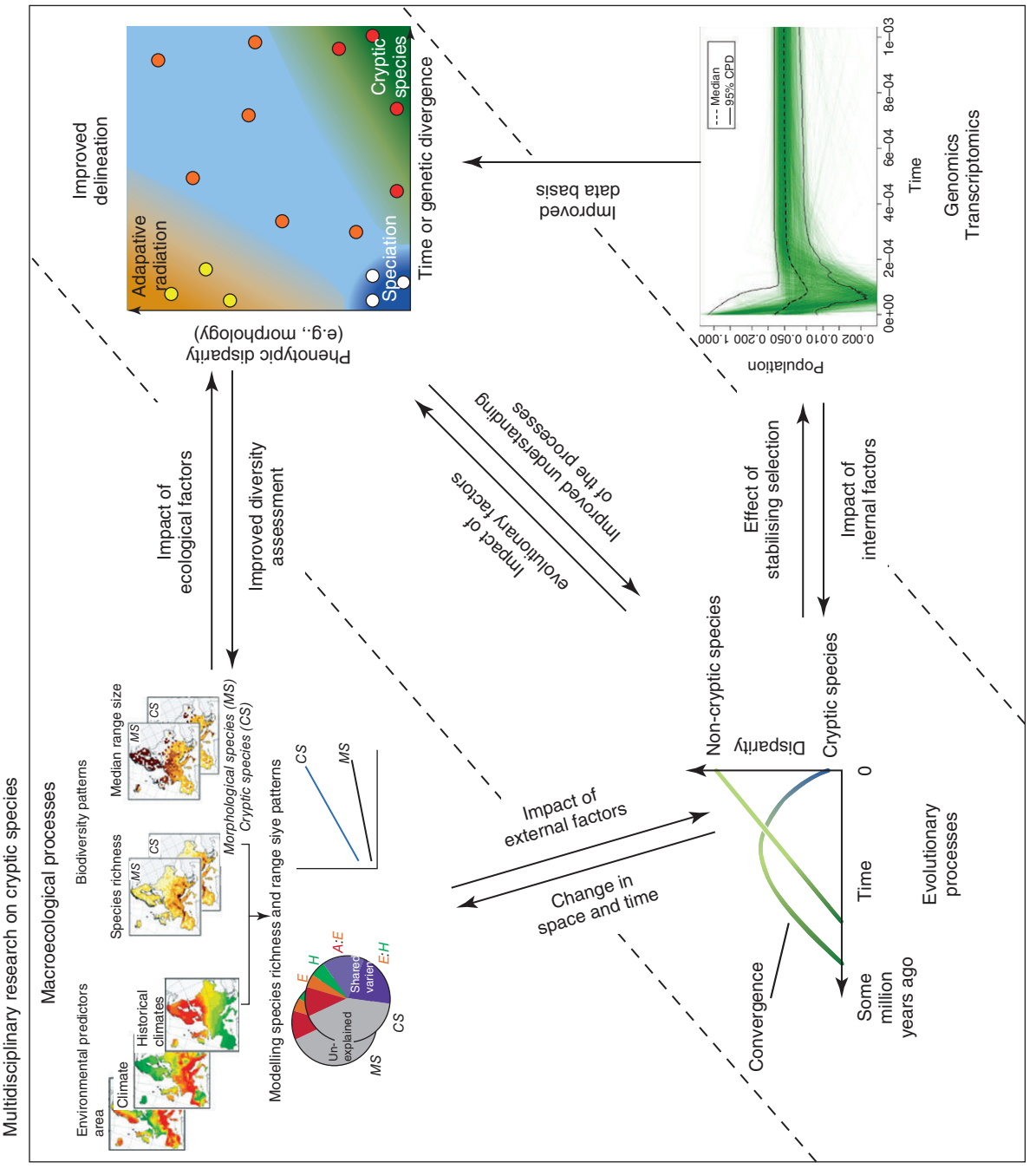


Figure 5 Schematic representation how different biological disciplines can contribute to the research of cryptic species and thereby increase our understanding of macro-ecological, evolutionary and genomic patterns and processes.

macroecological processes. Moreover, an improved understanding of the evolutionary processes shaping cryptic species allows a better assessment of ecological changes and its evolutionary consequences over space and time. Summarizing the research on cryptic species should address the following questions to contribute to our understanding of the evolution of reduced phenotypic disparity and how this affects the macroecological processes in different habitats:

1. Which species complexes are only taxonomic oddities, and which are truly cryptic?
2. Which cryptic species are the results of recent speciation, parallelism, convergence or stasis, and how common are they?
3. What are the relevant intrinsic and extrinsic factors affecting phenotypic evolution of cryptic species and to what degree do they affect their phenotypic landscape?
4. Are there more cryptic species in certain branches of the tree of life, among taxa with certain life histories (e.g. generalists vs. specialists), or in certain habitats?
5. How affect cryptic species the composition and stability of ecosystems or vice versa how vulnerable are cryptic species due to these effects?

Glossary

Convergent evolution Evolution of the same set of morphological traits from different ancestral set of traits.

Cryptic species Different species, which are morphologically very similar or identical.

Morphology The form or structure of the external characters of an organism.

Parallel evolution Evolution of the same set of morphological traits from one ancestral set of traits.

Stasis Maintenance of morphological similarity during long timescales.

References

Adams M, Raadik TA, Burridge CP and Georges A (2014) Global biodiversity assessment and hyper-cryptic species complexes: more than one species of elephant in the room? *Systematic Biology* **63** (4): 518–533.

Bensch S, Pérez-Tris J, Waldenström J and Hellgren O (2004) Linkage between nuclear and mitochondrial DNA sequences in avian malaria parasites: multiple cases of cryptic speciation? *Evolution* **58** (7): 1617–1621.

Bernardo J (2011) A critical appraisal of the meaning and diagnosability of cryptic evolutionary diversity, and its implications for conservation in the face of climate change. In: Hodkinson TR, Jones MB, Waldren S and Parnell JAN (eds) *Climate Change, Ecology and Systematics*, pp. 380–438. Cambridge, UK: Cambridge University Press.

Bickford D, Lohman DJ, Sodhi NS, *et al.* (2007) Cryptic species as a window on diversity and conservation. *Trends in Ecology & Evolution* **22** (3): 148–155.

Caputi L, Andreakis N, Mastrototaro F, *et al.* (2007) Cryptic speciation in a model invertebrate chordate. *Proceedings of the National Academy of Sciences* **104** (22): 9364–9369.

Chomicki G and Renner SS (2017) Partner abundance controls mutualism stability and the pace of morphological change over geologic time. *Proceedings of the National Academy of Sciences* **114** (15): 3951–3956.

Cornils A and Held C (2014) Evidence of cryptic and pseudocryptic speciation in the *Paracalanus parvus* species complex (Crustacea, Copepoda, Calanoida). *Frontiers in Zoology* **11**: 19.

Damm S, Schierwater B and Hadrys H (2010) An integrative approach to species discovery in odonates: from character-based DNA barcoding to ecology. *Molecular Ecology* **19**: 3881–3893.

Darwin C (1859) *On the Origin of Species by Means of Natural Selection: Or the Preservation of Favoured Races in the Struggle of Life*. London: John Murray.

Derycke S, De Meester N, Rigaux A *et al.* (2016) Coexisting cryptic species of the *Litoditis marina* complex (Nematoda) show differential resource use and have distinct microbiomes with high intraspecific variability. *Molecular Ecology* **25**: 2093–2110

Diabaté A, Dabiré RK, Heidenberger K, *et al.* (2008) Evidence for divergent selection between the molecular forms of *Anopheles gambiae*: role of predation. *BMC Evolutionary Biology* **8** (1): 5.

Eldredge N, Thompson JN, Brakefield PM, *et al.* (2005) The dynamics of evolutionary stasis. *Paleobiology* **31** (5): 133–145.

Elgetany AH, El-Ghobashy AE, Ghoneim AM and Struck TH (2018) Description of a new species of the genus *Marphysa* (Eunicidae), *Marphysa aegypti* sp.n., based on molecular and morphological evidence. *Invertebrate Zoology* **15** (1): 71–84.

Estes S and Arnold SJ (2007) Resolving the paradox of stasis: models with stabilizing selection explain evolutionary divergence on all timescales. *The American Naturalist* **169** (2): 227–244.

Fišer C, Robinson CT and Malard F (2018) Cryptic species as a window into the paradigm shift of the species concept. *Molecular Ecology* **27**: 613–635.

Futuyma DJ (2010) Evolutionary constraint and ecological consequences. *Evolution* **64** (7): 1865–1884.

Haller BC and Hendry AP (2014) Solving the paradox of stasis: squashed stabilizing selection and the limits of detection. *Evolution* **68** (2): 483–500.

Hansen TF and Houle D (2004) Evolvability, stabilizing selection, and the problem of stasis. In: Pigliucci M and Preston K (eds) *Phenotypic Integration: Studying the Ecology and Evolution of Complex Phenotypes*, pp. 130–154. Oxford: Oxford University Press.

Hawksworth DL and Lücking R (2017) Fungal diversity revisited: 2.2 to 3.8 million species. *Microbiology Spectrum* **5** (4): FUNK-0052-2016.

Heethoff M (2018) Cryptic species – conceptual or terminological chaos? A response to Struck et al. *Trends in Ecology & Evolution* **33** (5): 310.

Knowlton N (1993) Sibling species in the sea. *Annual Review of Ecology and Systematics* **24** (1): 189–216.

Korshunova T, Martynov A, Bakken T and Picton B (2017) External diversity is restrained by internal conservatism: new nudibranch mollusc contributes to the cryptic species problem. *Zoologica Scripta* **46**: 692–683.

Losos JB (2010) Adaptive radiation, ecological opportunity, and evolutionary determinism. *The American Naturalist* **175** (6): 623–639.

Maynard Smith J (1983) The genetics of stasis and punctuation. *Annual Review of Genetics* **17** (1): 11–25.

Mayr E (1963) *Animal Species and Evolution*. Cambridge: Belknap Press of Harvard University.

Nygren A, Parapar J, Pons J, *et al.* (2018) A mega-cryptic species complex hidden among one of the most common annelids in the North East Atlantic. *PLoS ONE* **13** (6): e0198356.

Pante E, Puillandre N, Viricel A, *et al.* (2015) Species are hypotheses: avoid connectivity assessments based on pillars of sand. *Molecular Ecology* **24** (3): 525–544.

Perez-Ponce de Leon G and Poulin R (2016) Taxonomic distribution of cryptic diversity among metazoans: not so homogeneous after all. *Biology Letters* **12**: 20160371.

Pfenninger M and Schwenk K (2007) Cryptic animal species are homogeneously distributed among taxa and biogeographical regions. *BMC Evolutionary Biology* **7** (1): 121.

Poulin R and Pérez-Ponce de León G (2017) Global analysis reveals that cryptic diversity is linked with habitat but not mode of life. *Journal of Evolutionary Biology* **30** (3): 641–649.

Rabosky DL and Adams DC (2012) Rates of morphological evolution are correlated with species richness in salamanders. *Evolution* **66** (6): 1807–1818.

Ramey-Balcı P, Fiege D and Struck TH (2018) Molecular phylogeny, morphology, and distribution of *Polygordius* (Polychaeta: Polygordiidae) in the Atlantic and Mediterranean. *Molecular Phylogenetics and Evolution* **127**: 919–930.

Reidenbach KR, Neafsey DE, Costantini C, *et al.* (2012) Patterns of genomic differentiation between ecologically differentiated M and S forms of *Anopheles gambiae* in West and Central Africa. *Genome Biology and Evolution* **4** (12): 1202–1212.

Rocha-Olivares A, Fleeger JW and Foltz DW (2001) Decoupling of molecular and morphological evolution in deep lineages of a meiobenthic harpacticoid copepod. *Molecular Biology and Evolution* **18**: 1088–1102.

Seehausen O (2006) African cichlid fish: a model system in adaptive radiation research. *Proceedings of the Royal Society B: Biological Sciences* **273** (1597): 1987–1998.

Sheldon PR (1996) Plus ça change—A model for stasis and evolution in different environments. *Palaeogeography, Palaeoclimatology, Palaeoecology* **127** (1): 209–227.

Simard F, Ayala D, Kamdem GC, *et al.* (2009) Ecological niche partitioning between *Anopheles gambiae* molecular forms in Cameroon: the ecological side of speciation. *BMC Ecology* **9** (1): 17.

Smith KL, Harmon LJ, Shoo LP and Melville J (2011) Evidence of constrained phenotypic evolution in a cryptic species complex of agamid lizards. *Evolution* **65** (4): 976–992.

Stoks R, Nystrom JL, May ML, McPeek MA and Benkman C (2005) Parallel evolution in ecological and reproductive traits to produce cryptic damselfly species across the Holarctic. *Evolution* **59** (9): 1976–1988.

Struck TH, Feder JL, Bendiksby M, *et al.* (2018a) Cryptic species – more than terminological chaos: a reply to Heethoff. *Trends in Ecology & Evolution* **33** (5): 310–312.

Struck TH, Feder JL, Bendiksby M, *et al.* (2018b) Finding evolutionary processes hidden in cryptic species. *Trends in Ecology & Evolution* **33** (3): 153–163.

Struck TH, Koczula J, Stateczny D, Meyer C and Purschke G (2017) Two new species in the annelid genus *Stygocapitella* (Orbiniida, Parergodrilidae) with comments on their biogeography. *Zootaxa* **4286** (3): 301–332.

Sukumaran J and Knowles LL (2017) Multispecies coalescent delimits structure, not species. *Proceedings of the National Academy of Sciences* **114** (7): 1607–1612.

Swift HF, Gómez Daglio L and Dawson MN (2016) Three routes to crypsis: stasis, convergence, and parallelism in the *Mastigias* species complex (Scyphozoa, Rhizostomeae). *Molecular Phylogenetics and Evolution* **99**: 103–115.

Voje KL (2016) Tempo does not correlate with mode in the fossil record. *Evolution* **70** (12): 2678–2689.

Vrijenhoek RC (2009) Cryptic species, phenotypic plasticity, and complex life histories: assessing deep-sea faunal diversity with molecular markers. *Deep Sea Research Part II: Topical Studies in Oceanography* **56** (19): 1713–1723.

Wada S, Kameda Y and Chiba S (2013) Long-term stasis and short-term divergence in the phenotypes of microsnails on oceanic islands. *Molecular Ecology* **22** (18): 4801–4810.

Westheide W (1977) The geographical distribution of interstitial polychaetes. *Mikrofauna Meeresboden* **61**: 287–302.

Westheide W (2008) *Polychaetes: Interstitial Families*. Shrewsbury: Field Studies Council.

Winker K (2005) Sibling species were first recognized by William Derham (1718). *The Auk* **122**: 706–707.

Further Reading

Appeltans W, Ahyong Shane T, Anderson G, *et al.* (2012) The magnitude of global marine species diversity. *Current Biology* **22** (23): 2189–2202.

Cerca J, Purschke G and Struck TH (2018) Marine connectivity dynamics: clarifying cosmopolitan distributions of marine interstitial invertebrates and the meiofauna paradox. *Marine Biology* **165**: 123.

Charlesworth B, Lande R and Slatkin M (1982) A Neo-Darwinian commentary on macroevolution. *Evolution* **36**: 474–498.

Coyne J and Orr H (2004) *Speciation*. Sunderland, MA: Sinauer Associates.

Giere O (2009) *Meiobenthology–The Microscopic Motile Fauna of Aquatic Sediments*. Berlin Heidelberg: Springer-Verlag.

Karanovic T, Djurakic M and Eberhard SM (2016) Cryptic species or inadequate taxonomy? Implementation of 2D geometric morphometrics based on integumental organs as landmarks for delimitation and description of copepod taxa. *Systematic Biology* **65** (2): 304–327.

Meleg IN, Zakšek V, Fišer C, Kelemen BS and Moldovan OT (2013) Can environment predict cryptic diversity? The case of *Niphargus* inhabiting western Carpathian groundwater. *PLoS ONE* **8** (10): e76760.

Nygren A (2013) Cryptic polychaete diversity: a review. *Zoologica Scripta* **43**: 172–183.

Schwenk K and Wagner GP (2001) Function and the evolution of phenotypic stability: connecting pattern to process. *American Zoologist* **41** (3): 552–563.

Winston JE (1999) *Describing Species–Practical Taxonomic Procedure for Biologists*. New York: Columbia University Press.