University of Cologne

A study on the diversity and ecology of choanoflagellates by integrative taxonomy

Inaugural–Dissertation

zur

Erlangung des Doktorgrades

der Mathematisch-Naturwissenschaftlichen Fakultät

der Universität zu Köln

vorgelegt von

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Köln, 2021

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Tag der mündlichen Prüfung:	01.06.2021

"I am among those who think that science has great beauty."

Marie Curie

Acknowledgements

With these words I would like to express my gratitude to all people who went along with me during my scientific career. First and foremost, I would like to thank the members of the committee, starting with my advisor **Prof. Dr. Hartmut Arndt** who gave me the opportunity to get insights into the field of protistology with unforgettable life experiences like expeditions to the Atacama Desert and the attendance on a research cruise on the Atlantic Ocean. I thank you for the trust in me, my scientific skills and efforts. I would like to thank **PD Dr. Kathrin Lampert** for providing the second expertise on this thesis. I am happy to have got to know you better during one expedition to the Atacama Desert with memorable sampling teamwork sharing beautiful landscape views and mud. I thank **Prof. Dr. Frank Schäbitz** for being the chair of disputation. I would like to express my gratitude in particular to **Dr. Frank Nitsche** who always trusted in my scientific skills and work giving me the opportunity to become a PhD student within his project. I thank you for all journeys, sampling campaigns, attendances on conferences and for sharing your expertise on choanoflagellates.

Additionally, I would like to thank many researchers who crossed my career and became friends. I thank **Prof. Dr. Eric von Elert** who supervised my bachelor thesis and early realized my scientific potential. I thank **Dr. Sonja Rückert** who became a good friend during the first Atacama expedition, I appreciate our yearly summer meetings having lots of fun and good talks. I thank my friend **Dr. Pablo Quintela-Alonso** who shared his expertise on ciliate research with me, I had tremendous fun working with you in the lab. I would like to thank the **German Society of Protozoology** to become a member and rewarding me for a talk. I thank the **choanoflagellate community** to share interesting research areas; especially **Prof. Dr. Barry Leadbeater**, **Prof. Dr. Helge Thomsen**, **Dr. Martin Carr** and **Dr. Daniel Richter** for having great talks, sharing their knowledge and having great field trips to experience the world of choanoflagellates. Additionally, I am very thankful to have met **Prof. Dr. Sebastian Krastel** on a research cruise.

I would like to thank the **whole working group AG Arndt** for best affiliation into the team. Especially I would like to thank **Rosita Bieg**, **Brigitte Gräfe** and **Bärbel Jendral** who were always helpful with technical and bureaucratic support. I thank my friends **Manon Hohlfeld**, **Maria Sachs**, **Dr. Alexandra Schoenle**, **Dr. Alexandra Jeuck** and **Johanna Ahlers** for scientific and mental support. It was a pleasure to share so many good moments with you also besides the work.

Not to forget, I would like to thank the bachelor students **Lennart Gutsche**,

Christiane Spruck, **Sofia Nicolai** and **Caroline Klöcker**; I enjoyed to introduce you to the world of choanoflagellates.

I would like to thank also **all unmentioned people** who contributed in different ways to this thesis, either by scientific or mental assistance. With that I want to say thank you to all my **friends** besides the scientific world for great talks and get-together. Last but not least, I would like to thank my **family** for their mental support. Without your aid and love many things would not have been possible. I thank my **parents** for being patient trying to understand my research topic, my sister **Anna** who always greatly supported me being a scientist and my nephew **Jaro** who always makes me smile with his gorgeous nature. Already with an age of four, he grasps the world of microbiology with "small cut onions in size of bacteria". Thank you.

This work was funded by the German Research Foundation within the priority program SPP 1991 – Taxon-omics.

Contents

Abstract

As a major component and link between trophic levels, protists fulfil a variety of different ecological functions in microbial food webs and are essential components in all ecosystems. In particular, one group of heterotrophic protists, the choanoflagellates play an important role in aquatic habitats, as they are efficient filter feeders, able to handle several food particles at the same time. As choanoflagellates resemble morphologically the choanocytes of sponges, they got already early attention regarding the evolution of multicellularity. The close relation could be proven by phylogenetic analyses, in which choanoflagellates cluster within the Opisthokonta as a sister group to the metazoans, making them the closest unicellular relatives to the animals.

Classical taxonomical studies on this group revealed a high morphological diversity and later on phylogenetic analyses were able to depict the relationship among a variety of species resulting into two major orders, the Craspedida possessing only an organic covering like a theca or a glycocalyx and the Acanthoecida with an inorganic siliceous basket-like covering (lorica). But still, we face a high discrepancy between the number of morphologically described and sequenced species. In addition, investigations on ecological properties proofed to be essential to further extend our knowledge on choanoflagellates concerning evolutionary questions. Moreover, the significance of a comprehensive integrative taxonomy with accurate species descriptions is nowadays undeniable, as modern molecular surveys using high-throughput sequencing methods produce massive amounts of molecular information, which can only be analysed and interpreted with the scaffold of a verified reference database founded on taxonomical data.

Within this study, several diverse habitats from freshwater to hypersaline environments were investigated to extend our current knowledge on the diversity and ecology of choanoflagellates. From freshwater and marine habitats, exemplified by studies from the River Rhine at Cologne and a transect of the Atlantic Ocean respectively, new craspedid choanoflagellate species were described based on detailed morphological (light and scanning electron microscopy) and molecular (SSU and LSU rDNA sequencing as well as transcriptomic) data. In addition, by an empirical survey, insights into salinity tolerances of several craspedid choanoflagellates were gained for a better understanding of their ecological dispersal potential, in particular the potential to cross the marine-freshwater boundary. Furthermore, an unexplored and hostile environment, the Atacama Desert in Northern Chile was investigated. This region is characterized by

hypersaline water bodies, heavy metal contamination and extreme UV radiation. Studying these extreme environments resulted in the description of seven new craspedid choanoflagellates and ecological experiments revealed that these species were able to tolerate a broad range of varying salinities. Molecular data of the SSU and LSU rDNA pointed towards high genetic distances to their closest marine relatives and phylogenetic analyses underlined their isolation-driven speciation derived from biogeographical restrictions. Further multigene analyses based on protein level revealed high mutation rates of aquatic organisms from this area. The biological divergence estimates correlated with geological events, e.g. the formation of the salt flats, and help to understand colonization and radiation processes. The establishment of choanoflagellate cultures and their transcriptomes from this area will be the basis to get further insights into the adaptation strategies also from a molecular perspective. By studying one of the salt flats, a new lorica-bearing species, *Enibas tolerabilis* gen. et sp. nov. could be isolated and described. This species is euryoecious regarding salinity, able to survive freshwater and hypersaline conditions, a characteristic never observed before for acanthoecids. The newly established genus *Enibas* clustered within a separated sister clade to all previously identified nudiform reproducing species in the family of Acanthoecidae, which comprised so far only four genera. Using video microscopy, its nudiform lorica reproduction could be proven by life observation of the cell division. In a targeted resampling approach, a phylogenetic closely related amplicon from molecular surveys was identified and a morphological description to this prior deposited sequence provided. By including other environmental eukaryotic sequences, it became obvious that the species richness within this family was underestimated.

To summarize, several choanoflagellate species belonging to the order of Craspedida and Acanthoecida from diverse aquatic habitats were isolated and described. In particular, the amount of comprehensive transcriptomes of choanoflagellates, which will be the basis for further studies on gene functionality, was doubled. The use and combination of different methodologies gave detailed species descriptions for a comprehensive integrative taxonomy which can elucidate the ecological and evolutionary role of choanoflagellates. The present thesis emphasizes the urgent need of taxonomy to generate reliable reference data for further molecular meta-analyses investigating the biodiversity of protists.

Kurzzusammenfassung

Untersuchungen an einzelligen Mikroorganismen (Protisten) haben einen hohen Stellenwert hinsichtlich ökologischer und evolutionärer Fragestellungen. Als essentielle Komponente des mikrobiellen Nahrungsgewebes sind Protisten in allen Ökosystemen präsent. Insbesondere eine Gruppe von heterotrophen Protisten, die Choanoflagellaten, sind in aquatischen Bereichen sehr häufig vorzufinden. Dies ist hauptsächlich auf ihre Art der Nahrungsaufnahme zurückzuführen; als effiziente Filtrierer sind sie in der Lage, mehrere Nahrungspartikel zur selben Zeit zu verarbeiten. Aus evolutionsbiologischer Perspektive sind diese Organismen besonders interessant, da sie den Choanozyten der Schwämme nicht nur morphologisch ähneln, sondern auch phylogenetisch gesehen innerhalb der Opisthokonta an der Basis aller Metazoen stehen.

Anhand klassischer taxonomischer Studien wurde eine hohe morphologische Vielfalt innerhalb der Choanoflagellaten beschrieben. Molekularbiologische Studien bestätigten die auf morphologischen Daten basierende Einteilung der Organismen in zwei Ordnungen: gehäuse- (Acanthoecida) bzw. nicht gehäusetragend (Craspedida). Es besteht jedoch immer noch eine große Diskrepanz zwischen der Anzahl der morphologisch beschriebenen und sequenzierten Arten. Die bisher wenig erforschten ökologischen Eigenschaften einzelner Arten spielen zudem eine wichtige Rolle, um Einblicke in deren Anpassungsvermögen und Verbreitungspotential zu erhalten. Die Kombination aus morphologischen, molekularbiologischen und ökologischen Daten, auch als integrative Taxonomie bezeichnet, ist insbesondere zur heutigen Zeit unumgänglich, da moderne Untersuchungen mit Hochdurchsatz-Sequenzierungsmethoden enorme molekulare Datensätze produzieren. Diese können allerdings nur auf der Basis einer verifizierten, auf taxonomischen Daten basierenden, Referenzdatenbank analysiert und interpretiert werden.

Im Rahmen der vorliegenden Studie wurden diverse aquatische Lebensräume untersucht, um neue Einblicke in die Vielfalt und Ökologie der Choanoflagellaten zu erhalten. Durch Untersuchungen im limnischen und marinen Bereich konnten neue Arten der Craspedida mithilfe morphologischer (Licht- und Rasterelektronenmikroskopie) und molekularbiologischer Analyse (Sanger Sequenzierung und Transkriptom) beschrieben werden. Darüber hinaus erbrachten empirische Studien an einer Vielzahl von Arten der Craspedida neue Einblicke über das ökologische Verbreitungspotential von limnischen bzw. marinen Arten. Weiterhin wurde ein Extremhabitat, die Atacamawüste in Nordchile, welche durch

hypersaline Gewässer, Schwermetallablagerung und extreme UV-Strahlung gekennzeichnet ist, untersucht. Mittels Studien verschiedenster hypersalinen Gewässern konnten sieben neue Arten der Craspedida mit hohem Potential zur Salztoleranz beschrieben werden. Die biologischen Divergenzschätzungen ergaben hohe Mutationsraten und korrelierten mit geologischen Ereignissen, z. B. der Bildung saliner Gewässern und tragen so zum Verständnis von Kolonisations- und Radiationsprozessen bei. Durch die Etablierung von Zellkulturen und der Auswertung von Transkriptomdaten, wird es in Zukunft möglich sein, weitere Einblicke in die Anpassungsstrategien vor allem aus molekularer Perspektive zu erhalten.

Im Rahmen der Untersuchung eines weiteren salinen Gewässers in der Wüstenregion konnte zudem eine neue gehäusetragende Gattung und Art, *Enibas tolerabilis* gen. et sp. nov., isoliert und beschrieben werden. Diese Art ist in Bezug auf den Salzgehalt euryök und in der Lage, in limnischen bis hypersalinen Bedingungen zu überleben; eine Eigenschaft, die bei den Acanthoeciden noch nie zuvor beobachtet wurde. Die neu etablierte Gattung *Enibas* gruppierte sich innerhalb einer klar definierten Schwesterklade zu allen zuvor identifizierten nudiform reproduzierenden Arten in der Familie der Acanthoecidae, welche bisher nur vier Gattungen umfasste. Mithilfe der Videomikroskopie konnte nun erstmals die nudiforme Reproduktion in Echtzeit durch Beobachtung der Zellteilung nachgewiesen werden. In einer gezielten Probenahme konnte eine phylogenetisch eng verwandte Art, die bisher nur anhand einer Sequenz aus einer früheren Studie bekannt war, morphologisch identifiziert werden. Durch die Einbeziehung anderer eukaryotischer Umweltsequenzen zeichnete sich ab, dass der Artenreichtum innerhalb dieser Familie bei Weitem unterschätzt wurde.

Zusammenfassend konnte in der vorliegenden Studie eine Vielzahl von Choanoflagellatenarten aus verschiedenen aquatischen Lebensräumen isoliert und beschrieben werden. Insbesondere wurde die Anzahl an umfassenden Transkriptomen von Choanoflagellaten, welche die Grundlage für weitere Studien zur Genfunktionalität bilden werden, verdoppelt. Durch die Kombination verschiedener Methoden wurden detaillierte Artbeschreibungen für eine umfassende integrative Taxonomie, welche für die Erforschung der ökologischen und evolutionären Rolle von Choanoflagellaten unumgänglich ist, erstellt. Dies unterstreicht die dringende Notwendigkeit von taxonomischer Arbeit, um verlässliche Referenzdaten für weitere molekulare Metastudien zur Untersuchung der Biodiversität von Protisten zu generieren.

General Introduction

Protist Diversity and Ecology

Protists, unicellular eukaryotic organisms, are probably the most abundant and diverse eukaryotes on Earth. Within all protist lineages, species fulfil a variety of different trophic modes; they are characterized as auto-, mixo-, phagotrophs and even parasites and pathogens exist (Jones, 2000; Morrison, 2009; Stoecker et al., 1989). Heterotrophic (or phagotrophic) protists, feeding on small particles such as bacteria, are major components in the microbial food webs of all aquatic and terrestrial ecosystems (Geisen et al., 2018; Singer et al., 2021; Worden et al., 2015). The food uptake can be differentiated in several predation mechanisms, i.e. filter or suspension feeding, direct interception or raptorial feeding and diffusion feeding (Fenchel, 1987). As a crucial link between trophic levels, they play an essential role in decomposition and remineralisation processes of organic matter (Azam et al., 1983; Sherr and Sherr, 2002).

Until now, only a small fraction of the protistan diversity could be described, but morphological and molecular studies revealed a widespread distribution throughout the eukaryotic tree of life (Adl et al., 2012, 2019; Burki et al., 2020; Cavalier-Smith and Chao, 2003; Patterson, 1999). Though, species concepts for protists are challenged as protists display a high degree of cryptic species complexes (Hausmann et al., 2006; Stoeck et al., 2008; Stoupin et al., 2012). As most of the species lack distinct morphological traits, which allow an identification on species level by light or electron microscopy, the rise of molecular methods was a major benefit to characterize protist species. With this, the relationships between protistan taxa could get untangled by applying phylogenetic analyses based on specific marker genes (Pawlowski et al., 2012). Advances in molecular techniques within the last two decades, in particular high-throughput sequencing methods (HTS), boosted the possibilities to extend the knowledge on biodiversity patterns and species richness of protists on a large scale in all habitats (Bates et al., 2013; de Vargas et al., 2015; Mahé et al., 2017; Moreira and López-García, 2002; Venter et al., 2017). These methods are also applied to explain biogeographical concepts, which have been subject of intensive debates (Dolan, 2005; Fenchel and Finlay, 2004; Foissner, 2007, 2008). In terms of dispersal potentials, salinity is probably the most discussed ecological barrier thought to hinder the dispersal and habitat exchange (Filker et al., 2019; Logares et al., 2007, 2009; Scheckenbach et al., 2006; von der Heyden et al., 2004).

One supergroup within the eukaryotic tree of life, the Amorphea (Burki et al., 2020), is of particular interest as it includes the metazoans. Within this supergroup, heterotrophic choanoflagellates form a sister group to metazoans, sharing a last common ancestor. This fact draws special attention to investigate choanoflagellate species and their ecology in more detail.

The Biology of Choanoflagellates

Choanoflagellates, heterotrophic unicellular eukaryotes, are subject of scientific research areas regarding taxonomical, ecological and evolutionary questions (Leadbeater, 2015). Their unique morphology is defined by a cell (2-15 μm) with a single anterior flagellum surrounded by a collar of microvilli. As suspension feeders, choanoflagellates create a hydrodynamic flow field around their collar by undulation of their flagellum (Fenchel, 1987; Kirkegaard and Goldstein, 2016; Lapage, 1925). Thereby the suspended food particles adhere on the outer surface of the collar, are transported to the protoplast and subsequently ingested by pseudopodia (Dayel and King, 2014; Lighthill, 1976; Pettitt et al., 2002). This way of nutrition, in particular the handling of several food particles at the same time, results in high clearance rates (Boenigk and Arndt, 2000, 2002; Nielsen et al., 2017). For highly efficient filter feeders, a sedentary life cycle stage, in which the cell attaches to a variety of substrata, is essential due to hydrodynamics (Christensen-Dalsgaard and Fenchel, 2003; Sleigh, 1964). In addition to the benthic, sessile stages, also free-living stages, like swarmers or colonies have been described for most species (Dayel et al., 2011; Leadbeater, 1983). Therefore, choanoflagellates are not restricted to benthic habitats but are also frequently found in pelagic communities. Choanoflagellates have a worldwide aquatic distribution with abundance records (up to 10^6 cells l^{-1}) from freshwater and marine habitats (Auer and Arndt, 2001; Buck and Garrison, 1988; Weitere and Arndt, 2003). As filter feeders, they occupy a distinctive niche in aquatic microbial communities and have a great ecological impact on structuring microbial food webs, where they form occasionally up to 40 % of the biomass of heterotrophic flagellates (Arndt et al., 2000; Boenigk and Arndt, 2002; Buck and Garrison, 1988).

Based on morphological and molecular data, the group of Choanoflagellatea Cavalier-Smith, 1997 is separated into two distinct monophyletic orders, both containing about 200 morphologically described species (Carr et al., 2008, 2017; Cavalier-Smith, 1997; Leadbeater, 2015; Nitsche et al., 2011). The order of Craspedida Cavalier-Smith, 1996 contains species with exclusively organic

investments (Figure 1A, left) whereas the order of Acanthoecida Cavalier-Smith, 1996 is comprised of species, which possess a species-specific inorganic siliceous basket-like covering, the lorica (Leadbeater, 2008, 2010; Leadbeater et al., 2008b; Leadbeater and Cheng, 2010) (Figure 1A, right). This morphological trait of a species-specific characteristic allows for species identification by light and electron microscopy. Former morphological classifications of the Craspedida into the

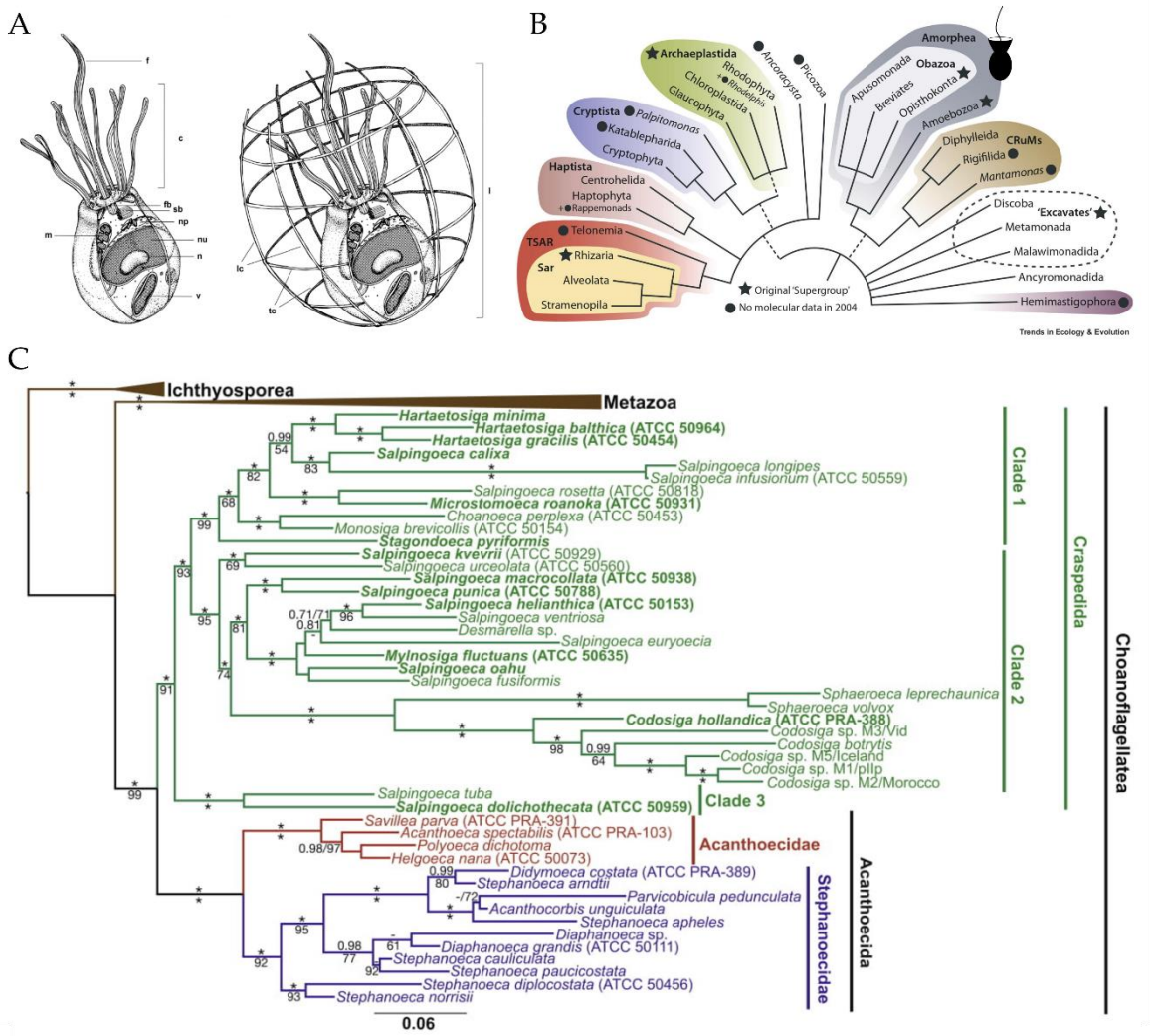

Figure 1. Morphology and systematics of choanoflagellates. (A) General morphology of Craspedida (left) and Acanthoecida (right). Basic features shown for the Craspedida also present in the Acanthoecida. Abbreviations: c – collar of microvilli, f – flagellum, fb – flagellar basal body, l – lorica, lc – longitudinal costae, m – mitochondrion, n – nucleolus, nu – nucleus, np – nuclear pores, sb – second basal body, tc – transverse costae, v – food vacuole. Golgi apparatus not depicted. (Richter and Nitsche, 2017) (B) New tree of eukaryotes based on recent phylogenomic studies. Current 'supergroups' are color-coded. Choanoflagellates cluster within the Opisthokonta at the basis to the Metazoa (modified after Burki et al., 2020). (C) Phylogeny of choanoflagellates using a six-gene analysis (SSU and LSU rDNA, hsp90, tubA, EFL, EF-1A) (Carr et al., 2017).

families Codonosigidae Saville-Kent, 1880-82 and Salpingoecidae Saville-Kent, 1880-82 based on different organic coverings was shown to be misleading as phylogenetic analyses showed no monophyly (Carr et al., 2008, 2017; Nitsche et al., 2011). This observation has led to the amalgamation of both families to only one, the Salpingoecidae Saville-Kent, 1880-82 emend. *sensu* Nitsche et al., 2011. Craspedid species possess two different organic investments. A glycocalyx is a non-restrictive covering (*sensu* Codonosigidae Saville-Kent, 1880-82) with a thin, flexible extracellular organic matrix, which allows for a longitudinal cell division (Bütschli, 1878; de Saedeleer, 1929; Ellis, 1929). On the other hand, restrictive coverings (*sensu* Salpingoecidae Saville-Kent, 1880-82) with a continuous theca result in parental cells that have to partially emerge from the surrounding covering to divide (Boucaud-Camou, 1966; Bourrelly, 1968; de Saedeleer, 1929; Ellis, 1929; von Stein, 1878). Members of the Craspedida have a cosmopolitan distribution in all aquatic environments (Saville-Kent, 1880-82; Zhukov and Karpov, 1985) and were even recorded from soil habitats (Ekelund and Patterson, 1997; Ekelund et al., 2001; Stoupin et al., 2012). In contrast to the Craspedida, the Acanthoecida comprises two families, the Acanthoecidae Norris, 1965 emend. *sensu* Nitsche et al., 2011 with a nudiform (Leadbeater, 2008; Leadbeater et al., 2008b) and the Stephanoecidae Leadbeater, 2011 with a tectiform (Leadbeater, 2010; Leadbeater and Cheng, 2010) lorica reproduction. In the tectiform condition, the mother cell provides a bundle of costal strips prior to cell division to the juvenile cell, whereas in nudiform reproducing species the juvenile cell has to develop the lorica after division independently. This observation could be confirmed by molecular analyses that resulted in distinct phylogenetic clades (Carr et al., 2008, 2017; Leadbeater et al., 2008a; Nitsche et al., 2011). Considering species richness, tectiform choanoflagellates contain a multitude of species compared to nudiforms, where until now only six species have been described (Leadbeater, 2015). Loricates are mainly distributed in brackish to marine habitats with only few freshwater exceptions (Nitsche, 2014; Paul, 2012). Until now, relatively little is known about the functional role of choanoflagellate lorica and ambivalently discussed. One hypothesis regarding a hydrodynamic aspect is that the lorica function to create drag, which counteracts to the flagellar movement (Andersen, 1988/89). By this, the cell does not require to adhere on substratum and can consequently enhance the flagellar activity for feeding. On the other hand, computational modelling could not support such hypothesis and revealed rather a potentially increase of capture efficiency in the presence of a lorica (Asadzadeh et al., 2019).

Molecular studies on choanoflagellates show the highest sampling depth for the small and large subunit of the ribosomal DNA (SSU and LSU rDNA) (Jeuck et

al., 2014; Nitsche and Arndt, 2015; Nitsche et al., 2017). The SSU rDNA is still used as marker gene for species identification and delimitation as choanoflagellates show low intraspecific variability (<0.5 % pairwise distance) within this region (Nitsche and Arndt, 2015; Pawlowski et al., 2012). The molecular analyses got extended by further protein-coding genes and mitochondrial genomes (Burger et al., 2003; Carr et al., 2008). At present, the systematics is based on a six-gene analyses (SSU and LSU rDNA, hsp90, tubA, EFL and EF-1A) which provides a reliable scaffold for phylogenetic analyses (Carr et al., 2017) (Figure 1C). Yet, species delimitation within choanoflagellates is also challenged as cryptic species complexes, e.g. the genus *Codosiga* (Stoupin et al., 2012), highlight the importance of an integrative approach combining different methodologies using molecular, morphological and ecological background.

From an evolutionary perspective, already in 1867, James-Clark first stated the close morphological resemblance of choanoflagellates to the choanocytes of sponges which was much later supported by molecular analyses (Kumar and Rzhetsky, 1996; Lang et al., 2002; Wainright et al., 1993). Within the super group of Amorphea, choanoflagellates cluster within the Obazoa in the Opisthokonta as closest living relatives of the Metazoa (Figure 1B) (Burki et al., 2020). Due to this close relationship, choanoflagellates are in focus of evolutionary research to reconstruct the origin of multicellularity (Hoffmeyer and Burkhardt, 2016; López-Escardó et al., 2019; Maldonado, 2004; Richter and King, 2013). The interest in animal evolution has promoted an increase in the amount of genomic and transcriptomic data of choanoflagellates (Fairclough et al., 2013; King et al., 2008; Richter et al., 2018). In particular, sequencing the genome of *Monosiga brevicollis* gave first insights into the origin of metazoans (King et al., 2008). The annotated genome contains 78 protein domains that are shared exclusively with metazoans, e.g. genes for cell adhesion and signalling processes. A further genome of the species *Salpingoeca rosetta*, which forms rosette-shaped colonies, expands the catalogue of genes, prior exclusively thought to be restricted to the Metazoa (Fairclough et al., 2013). In addition, *S. rosetta* became model organism to investigate the life cycle of choanoflagellates. A specific sulfonolipid, called RIF-1 (rosette inducing factor), of the bacterium *Algoriphagus machipongonensis* induces the formation of colonies (Alegado et al., 2012; Beemelmanns et al., 2014), whereas a chondroitin sulfate, called EroS, produced by the bacterium *Vibrio fischeri*, was shown to induce a sexual life cycle stage (Rossiter and Wuest, 2017; Woznica et al., 2017). These examples underline the linkage of bacteria and heterotroph choanoflagellates and the importance to investigate the microbial food web from several perspectives. The transition from unicellular to multicellular life was mainly driven by biotic factors

(i.e. bacteria as food and their metabolites) which underlie abiotic parameters like temperature and salinity, influencing the prey community and protist behaviour. Understanding the ecology and behaviour of choanoflagellates plays, along with a well resolved systematic, an essential role for the understanding of the origin of multicellularity.

Microbial Life in Hypersaline Water Bodies Exemplified by the Atacama Desert, Northern Chile

The emphasis of Part 2 of this thesis lies on choanoflagellate species from hypersaline water bodies of the Atacama Desert in Northern Chile. In contrast to marine environments, hypersaline water bodies exceeding marine salt concentrations, harbour a distinct biota which requires physiological adaption mechanisms to cope with these extreme saline conditions. In particular, two major strategies for osmoregulation in microorganisms have been identified. The "salt-in" strategy is based on an accumulation of high levels of potassium ions within the cells (Oren, 2013), whereas the "salt-out" strategy is defined by the incooperation of compatible solutes, organic compounds that counteract the water efflux from the cytoplasm (Kempf and Bremer, 1998). These mechanisms (Gunde-Cimerman et al., 2018) were identified in halophilic prokaryotes, a group of well-studied organisms in this research area (Empadinhas and da Costa, 2008; Paul et al., 2008; Roeßler and Müller, 2001; Sleator and Hill, 2002). For a long time, protists were understudied in this field; only the green alga *Dunaliella salina* (Borowitzka and Brown, 1974; Chen and Jiang, 2009; Oren, 2014; Zhao et al., 2011) and some halophilic yeasts (Butinar et al., 2005; Hohmann, 2015; Plemenitaš et al., 2014) were thouroughly studied from the eukaryotic perspective. Recent studies on different halophilic heterotrophic protists like *Halocafeteria seosinensis*, *Pharyngomonas kirbyi* (Harding et al., 2016) and different ciliate species (Qu et al., 2020; Weinisch et al., 2018, 2019) indicate the use of compatible solutes, e.g. glycin betaine and ectoines to persist the high salinities and the resulting osmotic stress in their environment. Phylogenetic analyses of these biosynthesis genes suggest that these mechanisms in eukaryotes were aquired via lateral transfer from the surrounding bacterial community (Czech and Bremer, 2018; Harding et al., 2017; Husnik and McCutcheon, 2018).

Molecular and culture-based studies on halophilic protists increased within the last decades and unveiled an unexpected protist species richness within hypersaline environments with raising species descriptions. The majority of until now examined halophilic (or halotolerant) protozoa belongs to several distinct

groups within ciliates (Cho et al., 2008; Foissner et al., 2014a, 2014b; Fotedar et al., 2016), stramenopiles (Park et al., 2006; Park and Simpson, 2010) and heterolobosean (Kirby et al., 2015; Pánek et al., 2014; Park et al., 2007; Park and Simpson, 2011). On a molecular level, they differ significantly to freshwater or marine relatives and mostly cluster in well-defined phylogenetic clades (Park and Simpson, 2010). Sequenced-based analyses of the SSU rDNA of several hypersaline environments support these findings, showing distinct genotypes compared to marine or freshwater surveys (Alexander et al., 2009; Balzano et al., 2015; Stock et al., 2012; Triadó-Margarit and Casamayor, 2013). With regards to choanoflagellates, craspedids from several hypersaline habitats (Heidelberg et al., 2013; Paul Antony et al., 2013; Triadó-Margarit and Casamayor, 2013) share less than 93 % sequence similiarity to their closest relatives of taxonomically descibed species indicating high molecular novelty (Figure 2B).

Studying the Atacama Desert in Northern Chile with its large number of hypersaline water bodies contributes to understand diversity patterns of halophilic or halotolerant protist species. The Atacama Desert is a paradigm for extremobiospheres as the region is characterized by a large number of hypersaline habitats including high variable salinity regimes (Risacher et al., 1999), heavy metal deposition and extreme UV radiation (Bull et al., 2018; Tapia et al., 2018). These extreme and unique environmental conditions offer the opportunity to study the ecology, biodiversity and physiological adaptation of halophile organisms. The extreme low precipitation in this region and high temperatures have led to the formation of hypersaline endorheic basins which exist for millions of years and provide a unique environment with a high degree of possible endemism, also for microbial life (Bull et al., 2016; Jaksic et al., 1997; Rundel and Palma, 2000) (Figure 2A). Though, seasonal fluctuations of chemical and physical water variabilities in the Altiplano, an elevated plain, can alter species assemblages and ecosystems stability (Márquez-García et al., 2009). This hostile environment was long thought to put life at the limit (Bull et al., 2016; Navarro-Gonzalez et al., 2003) but recent studies suggest a high diversity of prokaryotic life in this habitat (Demergasso et al., 2004, 2008; Dorador et al., 2009; Ghai et al., 2011). These findings initiated the study on halotolerant eukaryotes from the water bodies in the Atacama region. Latest studies indicate a high species richness of heterotrophic placidids (Rybarski et al., under review) and *Percolomonas*-like species (Carduck et al., under review), groups which have been already recorded from other saline places on Earth (Park and Simpson, 2010; Tikhonenkov et al., 2019). To unveil the molecular novelty within choanoflagellates (Triadó-Margarit and Casamayor, 2013), we studied several geographical separated endorheic basins within the Atacama Desert (Figure 2C) to

give detailed taxonomical descriptions. Enlarging the knowledge on various hypersaline environments worldwide offer the opportunity to understand biogeographical distribution patterns and adaptation processes. In particular, the combination with geological proxis, e.g. paleohydrological data (Ritter et al., 2018; Sáez et al., 2012), will further aid to understand colonisation and speciation processes within a certain habitat (CRC 1211 – Earth, Evolution at the dry limit).

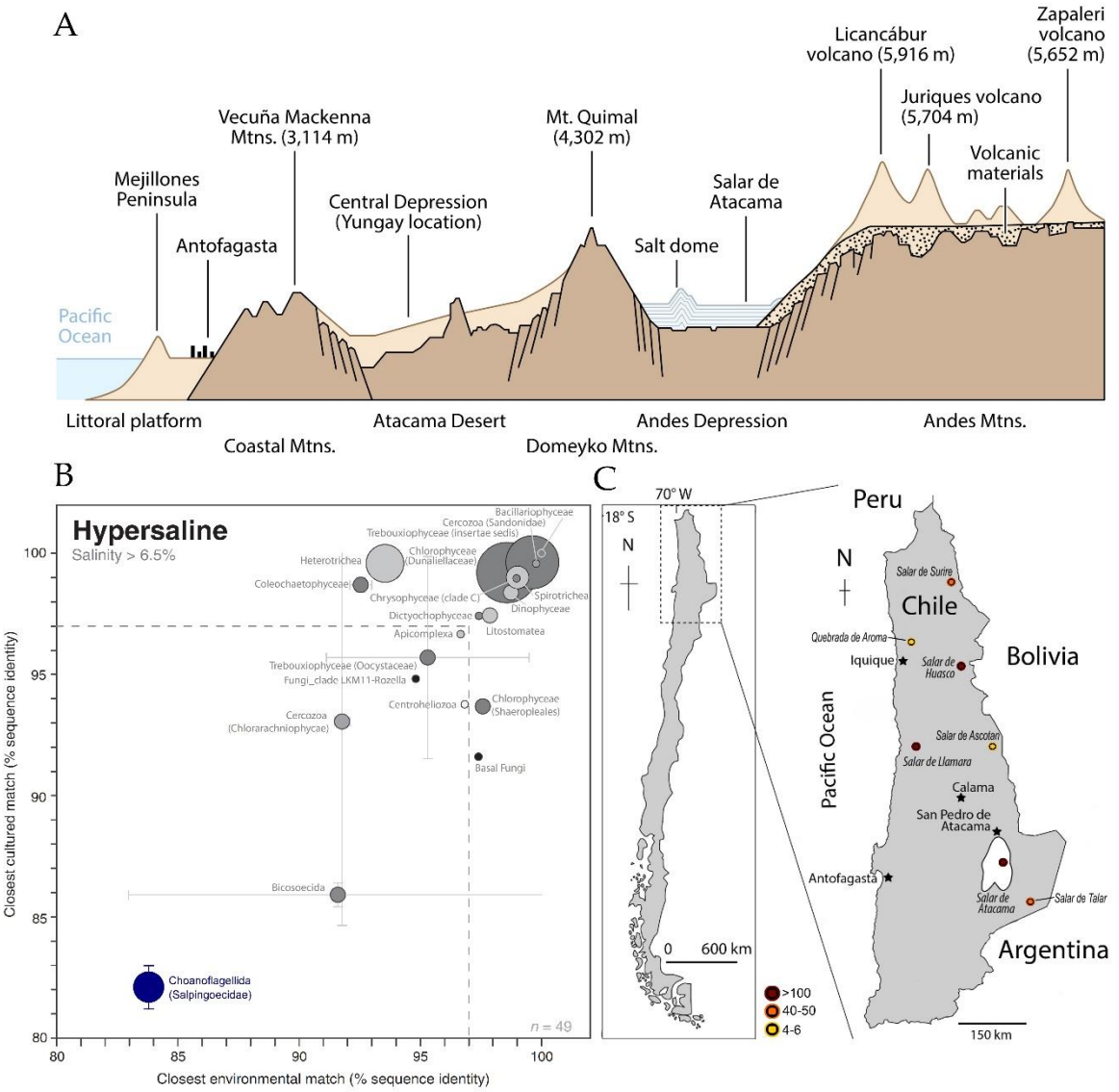

Figure 2. Hypersaline environments exemplified by the Atacama Desert in Northern Chile. (A) Topographical cross section of the Atacama Desert (Bull et al., 2016) (B) Pattern plot of molecular novelty for different eukaryotic groups from hypersaline waters, dot size proportional to sequence number, choanoflagellates are marked in blue (modified after Triadó-Margarit and Casamayor, 2013). (C) Map of Northern Chile with coloured sampling sites, legend indicate salinity values in PSU (map modified after Bull et al., 2018).

Aims

The main objective of the present thesis was to extend our current knowledge on the biogeography and species richness in particular of choanoflagellates, based on verified taxonomical descriptions. By sampling several diverse habitats, detailed morphological, molecular and ecological data were obtained for an integrative taxonomical approach, which is proposed as a standard for species delimitation within protists.

To gain further knowledge on possible implications of our presented findings for protists in general, additional studies were performed to give insights into a comprehensive ecological context. Thereby following questions were addressed:

1. Are choanoflagellate species able to adapt to different salinities to cross the marine-freshwater boundary?
2. Do extreme habitats harbour a distinct choanoflagellate diversity?
3. Is there a discrepancy between morphological and molecular systematics within loricate choanoflagellates?

Summary of Chapters

The presented findings of the thesis are displayed in three structuring parts, each represented by two or three chapters. In summary, **Part 1** comprises investigations from freshwater and marine environments, where craspedid choanoflagellate species are frequently found. This resulted in new species descriptions and redescriptions of up to now only morphologically described species. **Part 2**, focuses on several hypersaline water bodies of the Atacama Desert in Northern Chile. This allowed for insights into isolation-driven speciation processes of highly adapted choanoflagellates, which also reflect the geological evolution within this area. **Part 3** refers to the family of nudiform loricate choanoflagellates, the Acanthoecidae. Loricate choanoflagellates are, due to their unique lorica, a well-studied group within the choanoflagellates. Despite the high number of studies, in particular the family of nudiforms comprised up to now only five species. With our studies, a new genus within this family could be assigned including a high number of environmental genotypes. In addition, these findings gave first evidence for a specific morphology to be ancestral for all loricates.

Part 1
Choanoflagellates from freshwater to marine environments and the potential to cross the marine-freshwater boundary

Chapter 1: Morphological and molecular investigation on freshwater choanoflagellates (Craspedida, Salpingoecidae) from the River Rhine at Cologne (Germany) To get an overview on the species richness of choanoflagellates in a freshwater river system, a long-term observation and sampling was performed at the Ecological Rhine Station in Cologne. The aim of this study was to analyze the dominant choanoflagellates taxa by morphological and molecular means. By this, six craspedid choanoflagellate species with distinct morphological characteristics, closely related to known freshwater species, were revealed. *Published in European Journal of Protistology, doi: 10.1016/j.ejop.2020.125687.*

Chapter 2: Extending the genus *Hartaetosiga* (Choanoflagellatea, Craspedida, Salpingoecidae) by species from a transect across the Atlantic Ocean During a cruise across the Atlantic Ocean, several strains of the genus *Hartaetosiga* were isolated and analyzed using scanning electron microscopy and transcriptomic data. These data enlarged the recently introduced marine genus by a new species, *H. australis* sp. nov. and extended our knowledge on the biogeography of this genus.

Chapter 3: The potential to cross the freshwater-marine boundary – a case study on craspedid choanoflagellates With further ecological experiments of craspedid choanoflagellates in culture, new insights into salinity tolerances and perspectives on the potential of biogeographical dispersal were gained.

Part 2
Choanoflagellates from extreme hypersaline environments of the Atacama Desert, Northern Chile

Chapter 4: Four new choanoflagellate species from extreme saline environments: Indication for isolation-driven speciation exemplified by highly adapted Craspedida from salt flats in the Atacama Desert (Northern Chile) This study aimed to extend the knowledge on the biogeography and ecology of craspedid choanoflagellates from extreme saline water bodies in the Atacama Desert in Northern Chile. These hostile environments harbor a high degree of molecular novelty with high genetic distances to the closest marine relatives. Within this study, four new craspedid choanoflagellates, which were highly adapted to fluctuating salinities, were described. *Published in European Journal of Protistology, doi: 10.1016/j.ejop.2018.08.001.*

Chapter 5: Mirroring the effect of geological evolution: Protist divergence in the Atacama Desert To combine biological processes with geological evolution, frequently found protists from different isolated environments within the Atacama Desert were compared to geological events to get insights into divergence rates of protists. With the use of transcriptomic data, a molecular clock analysis revealed high mutation rates of choanoflagellates, which correlate with the geological evolution of the isolated salt flats within the desert. *Published in Global and Planetary Change, doi: 10.1016/j.gloplacha.2020.103193.*

Chapter 6: Extended divergence estimates and species descriptions of new craspedid choanoflagellates from the Atacama Desert, Northern Chile This study aimed to extend data on craspedid choanoflagellates from salt flats in the Atacama Desert (Northern Chile). Adding data of four new strains revealed two distinct phylogenetic clades from species of the Atacama. By the use of transcriptomic data, a molecular clock analysis could indicate a correlation of biological divergence estimates and the paleohydrology of the investigated salt flat. *Published in European Journal of Protistology, doi: 10.1016/j.ejop.2021.125798.*

Part 3
Nudiform acanthoecids – an unexpected species richness within loricate choanoflagellates

Chapter 7: First description of an euryoecious acanthoecid choanoflagellate species, *Enibas tolerabilis* gen. et sp. nov. from a salar in the Chilean Andes based on morphological and transcriptomic data Acanthoecid choanoflagellates were mainly described from brackish to marine habitats. This study provides first data of a nudiform acanthoecid choanoflagellate originating from inland waters of the Atacama Desert (Northern Chile). This species is euryoecious regarding salinity, able to survive freshwater to hypersaline conditions. The description of the new genus *Enibas*, promoted the research on this particular acanthoecid family, where until now only five species were described. *Published in European Journal of Protistology, doi: 10.1016/j.ejop.2018.11.004.*

Chapter 8: A needle in the haystack – mapping sequences to morphology exemplified by the loricate choanoflagellate *Enibas thessalia* sp. nov. (Acanthoecida, Acanthoecidae) This study aimed to highlight the potential to resample habitats, which showed interesting amplicons from molecular surveys only. With the use of a single cell isolation approach, a sequence, which clustered closely related to the previous described new genus *Enibas*, was reinvestigated. This resulted in a morphological description to the prior deposited sequence and a verification of the taxonomy of this newly established genus. *Published in Protist, doi: 10.1016/j.protis.2020.125782.*

Part 1

Choanoflagellates from Freshwater to Marine Environments and the Potential to Cross the Marine-Freshwater Boundary

Chapter 1: Morphological and molecular investigation on freshwater choanoflagellates (Craspedida, Salpingoecidae) from the River Rhine at Cologne (Germany)

Published publication

Schiwitza S., Lisson H., Arndt H., Nitsche F., **2020**. Morphological and molecular investigation on freshwater choanoflagellates (Craspedida, Salpingoecidae) from the River Rhine at Cologne (Germany). *European Journal of Protistology 73, 125687. doi: 10.1016/j.ejop.2020.125687*

Chapter 2: Extending the Genus *Hartaetosiga* (Choanoflagellatea, Craspedida, Salpingoecidae) by Species from a Transect Across the Atlantic Ocean

Extending the genus *Hartaetosiga* (Choanoflagellatea, Craspedida, Salpingoecidae) by species from a transect across the Atlantic Ocean

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Abstract

The recently introduced genus *Hartaetosiga* Carr, Richter and Nitsche, 2017 comprised up to now only three species, *H. gracilis* (Saville-Kent) Carr, Richter, Nitsche, 2017, *H. balthica* (Wylezich and Karpov) Carr, Richter and Nitsche, 2017 and *H. minima* (Wylezich and Karpov) Carr, Richter and Nitsche, 2017. Based on distinct molecular data these three species were relocated from the strictly freshwater genus *Codosiga* (Ehrenberg) Bütschli, 1878 to the new genus composed of brackish to marine distributed species. During a cruise across the Atlantic Ocean in 2019, surface water samples of 15 stations along a transect ranging from 35° S to 23° N were taken. We were able to isolate and cultivate 14 strains of the genus *Hartaetosiga* and to analyze transcriptomic data of nine of these strains. Morphometric data showed no distinct morphological traits allowing for a species delineation, indicating a cryptic species complex within the genus. Based on cultivation, morphological data and molecular analyses, we recorded *H. gracilis* for the first time from off-shelf waters of the Atlantic Ocean and could describe a new species, *H. australis* sp. nov., extending the genus *Hartaetoesiga*. This new species was recorded only from sampling stations in the Southern Hemisphere, which may indicate a potential biogeographic distribution likely caused by the Equatorial Counter Current (ECC), dividing the northern and southern surface waters.

Keywords

Atlantic Ocean; Choanoflagellatea; Distribution; *Hartaetosiga*, Surface water, Transcriptome

Introduction

Within marine habitats, unicellular eukaryotes (protists) are important components in ecosystem functioning as they form the major part of primary production and food-web dynamics (Azam et al., 1983; Worden et al., 2015). Heterotrophic protists play an essential role as they remineralize nutrients from bacterial and phytoplankton production and provide them to higher trophic levels (Massana et al., 2009; Pernthaler, 2005). Exemplified by studies from the Atlantic Ocean, only few approaches have been attempted to study the abundance (Mangot et al., 2018; Zubkov et al., 2006), taxonomic composition (Countway et al., 2005, 2007), distribution (de Vargas et al., 1999) and diversity (Patterson et al., 1993) of heterotrophic flagellates in the off-shelf waters. Most studies focused on neritic pelagic zones (Bergesch et al., 2008; Doherty et al., 2007; Gran-Stadniczeñko et al., 2019; Massana et al., 2004), which differ in their community composition compared to oceanic pelagic habitats. The first global metabarcoding approach (Tara Oceans) revealed different protist community patterns in the sunlit ocean (de Vargas et al., 2015). As this study is mainly hindered by the lack of reference sequences for the assignment of reads and the limitation of the used primer set, the species composition of these communities remains uncertain. Still, cultivation-based studies are of great necessity to create reliable data for reference databases and hence increase our knowledge on the ecological functionality of protists in marine ecosystems and refine biogeographical patterns by molecular and morphological characterization.

Heterotrophic choanoflagellates as efficient filter feeders, have great ecological impact on the pelagic zone of marine ecosystems. Several studies revealed positive correlations of choanoflagellates to autotrophic nanoplankton and bacterioplankton abundance respectively making them a key component in marine habitats (Becquevort et al., 1992; Kivi and Kuosa, 1994). Despite their important role, choanoflagellates were mainly studied from polar regions and neritic environments (e.g. Disco Bay, Greenland; Weddell Sea, Antarctica; Kattegat, Denmark; Southampton Bay, UK or Shark Bay, Australia) (Bergesch et al., 2008; Buck and Garrison, 1988; Nitsche et al., 2017; Thomsen et al., 1997, 2016; Thomsen and Østergaard, 2017; Tong, 1997a, 1997b). Only recent morphological studies broadly extended the knowledge on warm water acanthoecid choanoflagellates (Thomsen and Østergaard, 2019a, 2019b, 2019c, 2019d, 2019e; Thomsen et al., 2020a, 2020b). Although molecular data is missing, as these samples were fixed for microscopy, these studies show that the warm water regions harbor along with ubiquitously distributed species a morphological distinct acanthoecid community. Mainly due to their unique morphological characteristic, the species-specific siliceous lorica, most

quantitative and qualitative data are available for acanthoecid choanoflagellates. Craspedid species, although frequently found, are barely assigned to species level, hindered by the lack of distinct morphological traits.

Within a recent revision of the order of Craspedida, the polyphyly of the genus *Codosiga* with its freshwater type species *Codosiga botrytis* (Ehrenberg) Bütschli, 1878 was revealed by molecular data, which separated the species into two distinct phylogenetical and ecological clades (Carr et al., 2017). The marine clade, composed of *C. gracilis* (Saville-Kent) de Saedeleer, 1927, *C. balthica* Wylezich and Karpov, 2012 and *C. minima* Wylezich and Karpov, 2012, was amended by introducing the new genus *Hartaetosiga*. The type species of the genus *Hartaetosiga*, *H. gracilis* (Saville-Kent) Carr, Richter, Nitsche, 2017 was isolated from a tide pool of a salt marsh bordering the Wash at Freiston Shore, England, indicating its marine character. The further findings of *H. balthica* (Wylezich and Karpov) Carr, Richter and Nitsche, 2017 and *H. minima* (Wylezich and Karpov) Carr, Richter and Nitsche, 2017 from the Baltic Sea extended the clade with representatives from hypoxic water bodies (Wylezich et al., 2012). Molecular analyses revealed that the formerly deposited strain *Monosiga gracilis* Saville-Kent, 1880 from North America was identical to *H. balthica*, supporting a marine distribution of this genus.

For further insights into the systematics and biogeography of the genus *Hartaetosiga*, we sampled surface water of a transect across the Atlantic Ocean (35 °S to 23 °N) as the pelagic provinces do affect biological and ecological patterns (Spalding et al., 2012). The Atlantic Ocean is the second largest aquatic ecosystem on Earth and physically structured by varying abiotic conditions, like changes in salinity (influenced by the influx of river systems and high equatorial evaporation) and temperature shifts due to the spatial expansion (e.g. artic waters and tropics) (Fuglister, 1960; Marsh et al., 2007; Montgomery, 1958; Parrilla et al., 1994). The South and North Atlantic are separated by equatorial counter currents at about 8 °N, dividing the surface waters of both regions (Richardson and Walsh, 1986). With a high isolation and cultivation effort, we were able to enlarge the clade of the genus *Hartaetosiga* with several strains, including *H. australis* sp. nov., a species up to now restricted to surface waters of the South Atlantic Ocean only.

Material and methods

Study area and sampling

In April and May 2019 surface water samples (5 L) were collected along a transect in sections of about 5° latitudes in the Atlantic Ocean during the cruise MSM82/2 on the research vessel Maria S. Merian (Figure 1, Table 1). Surface seawater was continuously measured on board with the sensors SBE38, SBE45 and FLNTUS to

obtain the parameters, i.e. surface water temperature [°C], salinity [PSU, practical salinity unit] and chlorophyll *a* [µg/L] respectively. Original data were plotted in Ocean Data View (ODV, Schlitzer, 2021) and displayed with gridded fields using DIVA (data-interpolating variational analysis) gridding for salinity and water temperature with < 0.05 signal-to-noise reduction and quick gridding for chlorophyll *a*.

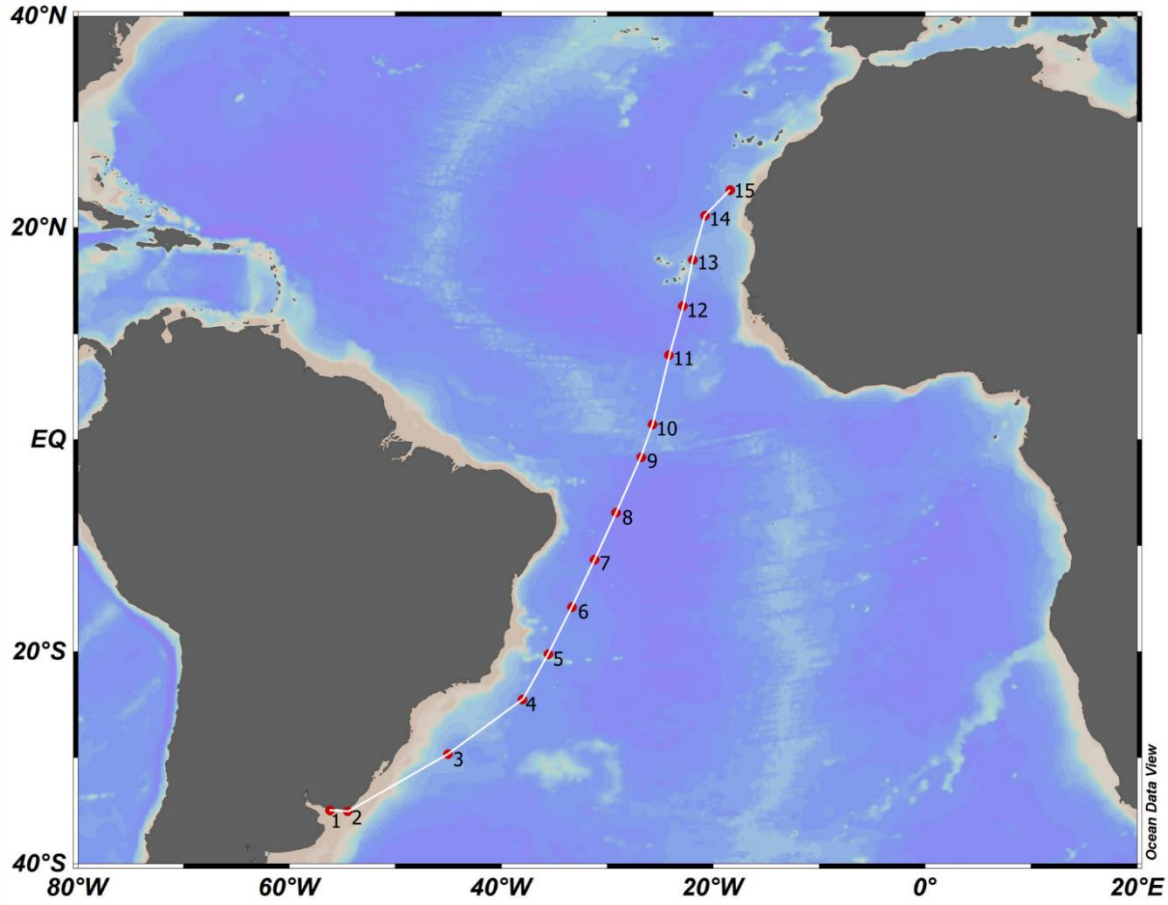

Figure 1. Transect across the Atlantic Ocean, sampling stations marked with a red dot. Map created with ODV (Schlitzer, 2021).

Isolation and cultivation

On board, subsamples were transferred to 50 ml culture flasks (Falcon, Durham, USA) filled with artificial seawater (Instant Ocean, Aquarium Systems, Strasbourg, France) and sterile wheat grains as carbon source for bacterial growth. The culture flasks were regularly monitored with an inverted light microscope (Nikon Diaphot 200). Back in the laboratory, isolation was done by liquid aliquot method (LAM) (Butler and Rogerson, 1995). In brief, samples containing choanoflagellate species, which occurred at several stations, were isolated by transferring diluted subsamples to micro-well plates (TC-Disc 24 Well Standard F, Sarstedt, Nürnbrecht, Germany). Depending on the abundance, 1 to 5 µl were transferred to each 1 ml well. Plates

were regularly monitored for clonal cultures by an inverted light microscope (Axiovert S 100, Zeiss, Oberkochen, Germany). Monoclonal cultures were transferred to culture flasks and further processed for molecular and morphological analyses.

Table 1. Sampling stations, coordinates and corresponding depth of sea ground. Bold line indicates the transition from Southern to Northern Hemisphere. Na= not available.

Station-No.	Latitude	Longitude	Depth (m)
1	35° 00.435' S	056° 08.409' W	14
2	35° 04.657' S	054° 29.982' W	40
3	29° 40.046' S	045° 01.514' W	3601
4	24° 32.409' S	037° 59.203' W	3862
5	20° 14.663' S	035° 33.019' W	3680
6	15° 47.703' S	033° 19.375' W	4604
7	11° 20.632' S	031° 10.961' W	5281
8	06° 54.190' S	029° 09.184' W	5492
9	01° 39.935' S	026° 47.706' W	5129
10	01° 24.991' N	025° 43.545' W	3539
11	07° 58.898' N	024° 10.788' W	na
12	12° 36.477' N	022° 51.152' W	4862
13	16° 57.523' N	021° 53.879' W	3516
14	21° 06.677' N	020° 46.008' W	4124
15	23° 33.222' N	018° 24.305' W	2869

Morphological analyses

For morphological analyses, choanoflagellates cultures were prepared for scanning electron microscopy. Cultures were fixed at 4 °C for 60 min in 2.5 % cacodylate buffered glutaraldehyde (see Nitsche, 2016, for details) in culture flasks, followed by a post fixation with osmium tetroxide (1 %) for 10 min. After a dehydration using an ascending ethanol series using concentrations of 30 %, 50 %, 70 %, 80 %, 90 % and 96 %, samples were treated with a 1:1 hexamethyldisilazane (HMDS)-ethanol solution for 10 min and finally in 100% HMDS for 5 min. Afterwards, the samples were allowed to fall dry for 2 h. The bottom of each culture flask was cut out, mounted on a sample holder and sputter coated with 120 Å layer of gold before

examination. The samples were examined by a FEI Quanta 250 FEG. Cell measurements were performed by ImageJ (Schneider et al., 2012).

Molecular analyses

Total RNA of all choanoflagellates strains was extracted using the ReliaPrepTM RNA Cell Miniprep System (Promega, Madison, USA). PolyA selection during cDNA synthesis was applied to reduce prokaryotic RNA and sequenced at the facility of the Cologne Center of Genomics (CCG) using Illumina NovaSeq (75 nt, paired end). Quality check and assembly was done using Trinity 2.8.5 (Grabherr et al., 2011). Genes of interest (SSU and LSU rDNA, hsp90, tubA, EFL and EF1-A) for a six-gene phylogenetic analyses were searched by blastn based on a choanoflagellate reference database. As quality control, contigs were compared to Sanger Sequencing data of the SSU rDNA. For this, DNA was extracted from clonal cultures using the Quick-DNA Miniprep Plus Kit (Zymo Research Corporation, Ca, USA). Cells were detached from the culture flask using a cell scraper and centrifuged at 4 °C for 20 min. Partial SSU rDNA of choanoflagellates strains was amplified in 50 µl reactions using a PCR Mastermix (2x) (VWR Life Science, Red Taq DNA Polymerase, Hassrode, Belgium) with the primer pair 42F (5′-CTC AAR GAY TAA GCC ATG CA-3′, López-García et al., 2003) and 18S-Rev-1 (5′-ACC TAC GGA AAC CTT GTT ACG-3′, Wylezich et al., 2002) at a primer concentration of 0.1 µM. The PCR was set as follows: a denaturation at 98°C for 2 min, followed by 35 cycles of 96°C for 30 sec, 48°C for 30 sec, 52°C for 30 sec (two-step annealing) and an elongation at 72°C for 2.5 min. Amplification was finalized with a final elongation for 7 min at 72°C.

Phylogenetic analyses

Phylogenetic analyses were performed based on a concatenated five-gene alignment (SSU and LSU rDNA, hsp90, tubA and EFL, EF1-A was not present) (Table 2) and SSU rDNA alone (see supplementary material Figure S1). Phylogenetic trees were calculated using Maximum likelihood (ML) and Bayesian inference (BI) method. Best DNA model was calculated in MrAIC (Nylander, 2004) for ML and BI analyses. ML analyses (concatenated alignment, 11726 nt, unmasked; SSU rDNA, 2824 nt, unmasked) were done by MEGAX using a GTR + I + Γ model, all sites with 1000 replicates for bootstrapping. The Bayesian analysis was performed by MrBayes v.3.2.6 (Ronquist et al., 2012) running the GTR + I + Γ model and a four-category gamma distribution to correct for among site rate variation. The search consisted of two parallel chain sets run at default temperatures with a sample frequency of 10 and run until the average standard deviation of split frequencies dropped below 0.01. The analysis consisted of 500,000 generations with a burnin of 12,500 for the concatenated alignment and 100,000 generations with a burnin of

2,500 for the SSU rDNA alignment before calculating posterior probabilities. The phylogenetic analyses were restricted to only closely related craspedid species and was rooted with six strains of the choanoflagellate genus *Codosiga*. The pairwise distances (based on SSU rDNA) of all strains to their closest relatives were determined by pairwise aligning the uncorrected closest sequences using BioEdit (Hall, 1999). The alignments are available from the author upon request.

Table 2. NCBI accession numbers of all strains used for phylogenetic analyses.

Species/strain	SSU rDNA	LSU rDNA	hsp90	tubA	EFL
***Choanoeca perplexa* Ellis**	KT757437	KT757438	KT757435	KT757439	KT757434
***Codosiga botrytis* (Ehrenberg) Bütschli**	JF706243	KT757422	-	-	-
***Codosiga hollandica* Carr, Richter & Nitsche**	KT757430	KT757431	KT757433	KT757436	-
***Codosiga* sp. Stoupin**	JF706237	KT757440	-	-	-
***Codosiga* sp. Stoupin**	JF706236	KT757441	-	-	-
***Codosiga* sp. Nitsche**	JF706242	KT757442	-	-	-
***Codosiga* sp. Nitsche**	JF706239	KT757443	-	-	-
***Hartaetosiga balthica* (Wylezich & Karpov) Carr, Richter & Nitsche**	KT757421	KT988065	KT757424	KT757425	KT757423
***Hartaetosiga gracilis* (Saville-Kent) Carr, Richter & Nitsche**	KT757426	EU011935	KT757428	KT757429	KT757427
***Hartaetosiga minima* (Wylezich & Karpov) Carr, Richter & Nitsche**	JQ034422	JQ034423	-	-	-
***Monosiga brevicollis* Ruinen**	AF084618	KT757456	AY226081	AY026070	AY026073

ZooBank registration

ZooBank registration of present work (see recommendation 8A of ICZN 2012): urn:lsid:zoobank.org:pub:unpub.

Results

In total, 14 strains of choanoflagellates could be isolated from different sampling stations along the transect across the Atlantic Ocean, ten strains from the Southern Hemisphere and four strains from the Northern Hemisphere. All strains were investigated by scanning electron microscopy (Table 3, Figure 2), whereas molecular data could only be achieved of nine strains (Table 3). The genus-specific colony formation could be monitored in all isolated strains (only shown for strain

HFCC 1306, Figure 3). The described morphological characteristics like cell shape, investment (theca) and stalk correspond to the genus description of *Hartaetosiga*.

Table 3. Data of all isolated strain listed with corresponding sampling station. Abbreviations: SEM - scanning electron microscopy, TR – transcriptome.

Station No.	Number of Heterotrophic Flagellate Collection Cologne (HFCC)	Species	Available data
5	1306	*Hartaetosiga australis* sp. nov.	SEM/ TR
5	1316	*Hartaetosiga australis* sp. nov.	SEM/ TR
6	1318	*Hartaetosiga* sp.	SEM
6	1344	*Hartaetosiga australis* sp. nov.	SEM/ TR
7	1308	*Hartaetosiga australis* sp. nov.	SEM/ TR
7	1319	*Hartaetosiga australis* sp. nov.	SEM/ TR
7	1324	*Hartaetosiga gracilis*	SEM/ TR
9	1322	*Hartaetosiga* sp.	SEM
9	1326	*Hartaetosiga gracilis*	SEM/ TR
9	1358	*Hartaetosiga* sp.	SEM
11	1339	*Hartaetosiga* sp.	SEM
11	1343	*Hartaetosiga gracilis*	SEM/ TR
11	1350	*Hartaetosiga gracilis*	SEM/ TR
13	1314	*Hartaetosiga* sp.	SEM

The concatenated five-gene analyses (SSU and LSU rDNA, hsp90, tubA, EFL) of the nine sequenced strains placed all strains with full molecular support (100 % mlBP; 1.00 biPP) into the genus *Hartaetosiga* (Figure 4). The single gene phylogeny based on SSU rDNA alone revealed the same topology (supplementary material Figure 1), supporting the SSU rDNA as marker gene for species delineation for choanoflagellates. We could recover the whole rDNA sequence of the strains HFCC 1306, 1308, 1316, 1319 and 1344, which were 100 % identical including the variable regions of ITS1, 5.8S and ITS2. The strains showed a pairwise distance of 3.4 % to *H. minima* (based on SSU rDNA) resulting in a species description of *H. australis* sp. nov. with HFCC 1306 as type strain. The other strains clustered together forming a distinct clade of *H. gracilis* with full molecular support (100 % mlBP; 1.00 biPP). The strains HFCC 1324, 1326 and 1350 were 100 % identical based on SSU rDNA and showed a p-distance of 0.1 % to *H. gracilis*. Strain HFCC 1343 had a p-distance of 0.3 % to *H. gracilis* and the other newly isolated strains in this clade.

Morphometric data of the protoplast length and width of all strains resulted in no distinct pattern for a morphological characterisation, reflecting the cryptic species characteristic of this genus, as a species identification of the strains without molecular data is not possible (Figure 5).

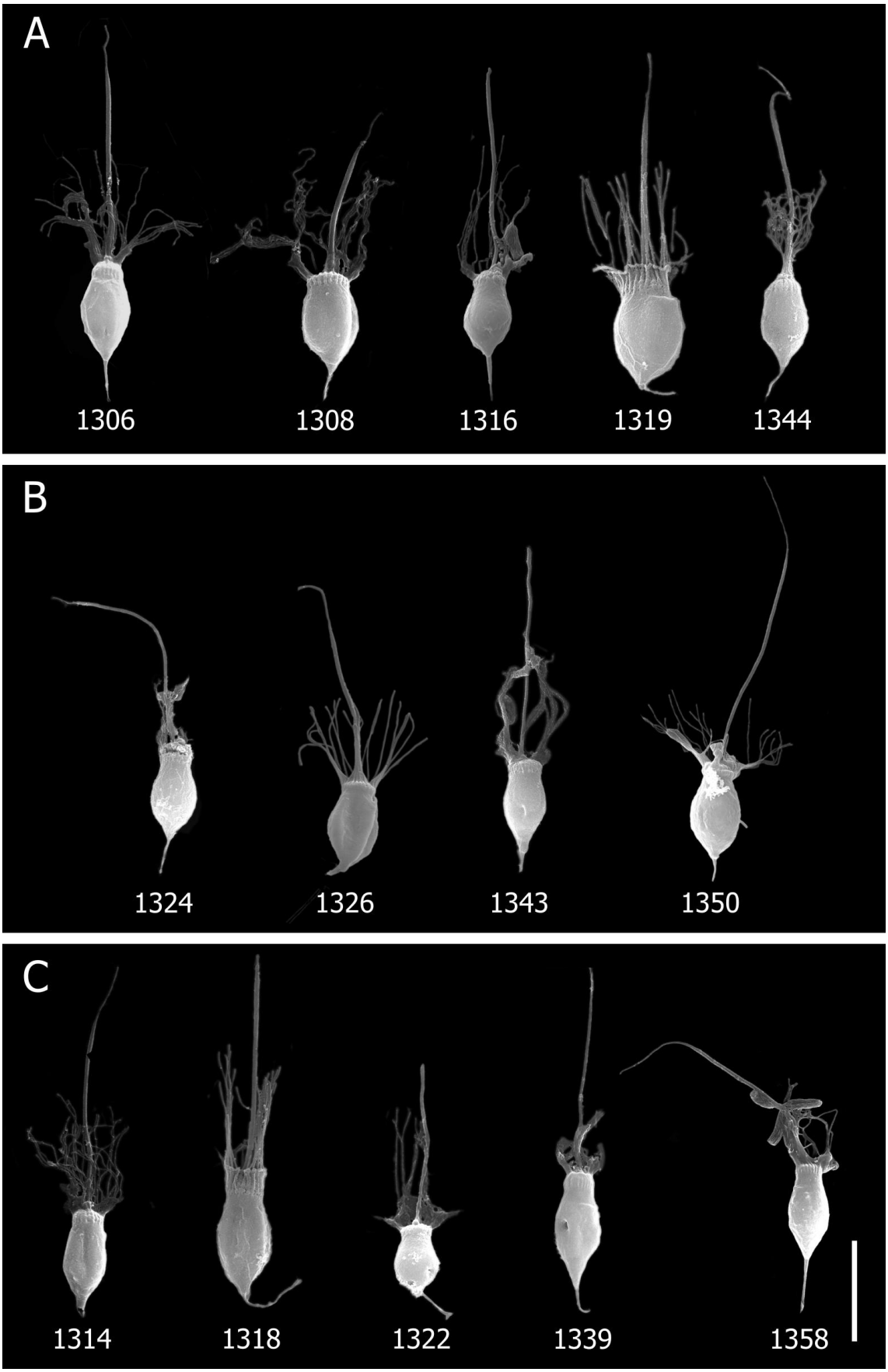

Figure 2. (A-C) Scanning electron micrographs of all *Hartaetosiga* strains isolated during the cruise. (A) *Hartaetosiga australis* sp. nov. (B) *Hartaetosiga gracilis* (C) *Hartaetosiga* sp.; HFCC strain number under each strain. Scale bar: 5 μm.

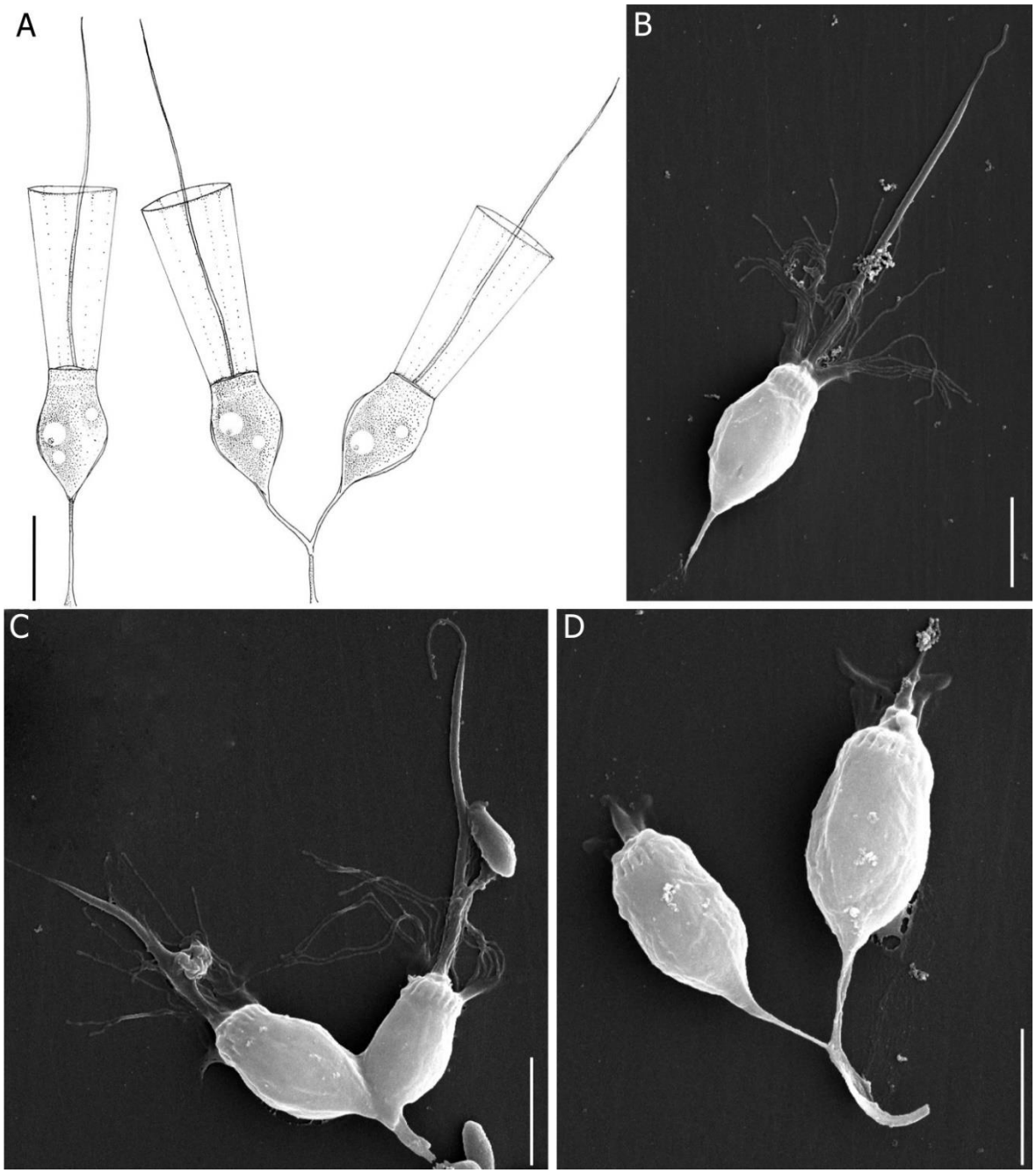

Figure 3. A-D. Morphology of *Hartaetosiga australis* sp. nov. (type strain HFCC 1306) (A, drawings; B-D, SEM). (A) Schematic drawing of a single cell (left) and colony (right). (B) Single cell, stalked. (C) Colony of two cells, stalked (cell-cell attachment due to fixation). (D) Colony of two cells, stalked (missing collar due to fixation). Scalebar: 2 μm.

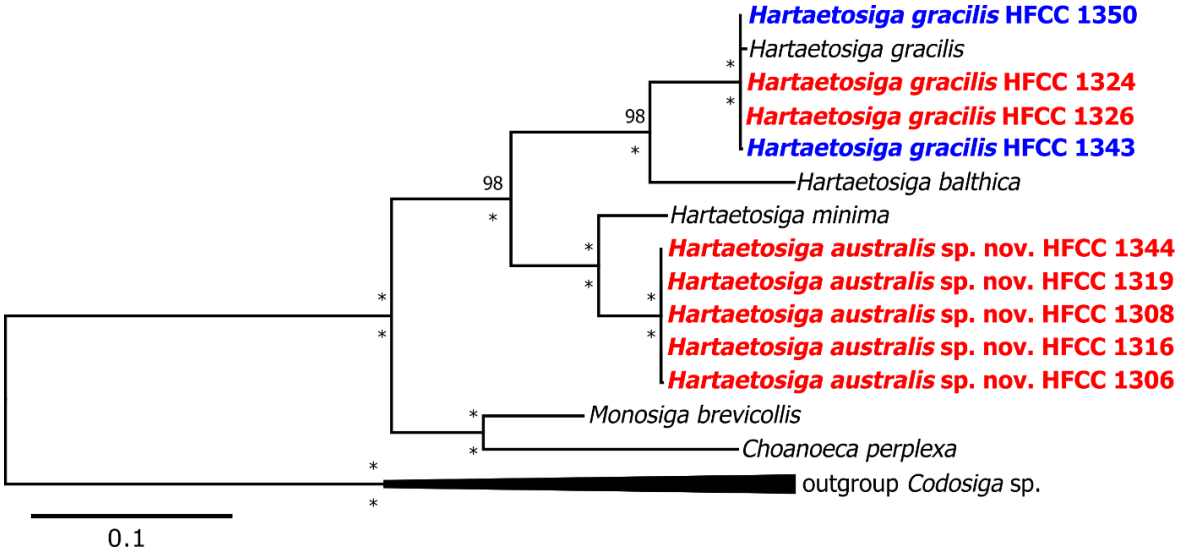

Figure 4. Concatenated five-gene (SSU and LSU rDNA, hsp90, tubA, EFL) maximum likelihood phylogeny of the genus *Hartaetosiga* based on an unmasked alignment (11726 nt). Number of nucleotide substitutions per site defined by scale in the lower left. Support values for ML/BI given at each branch. 100 % ML bootstrap percentage support (mlBP) and 1.00 BI posterior probabilities (biPP) are denoted by a *. Otherwise, mlBP and biPP values are given at each branch respectively. Newly molecular described species/strains are marked by bold letters. Strains isolated from the Northern Hemisphere are displayed in blue, isolates from the Southern Hemisphere in red.

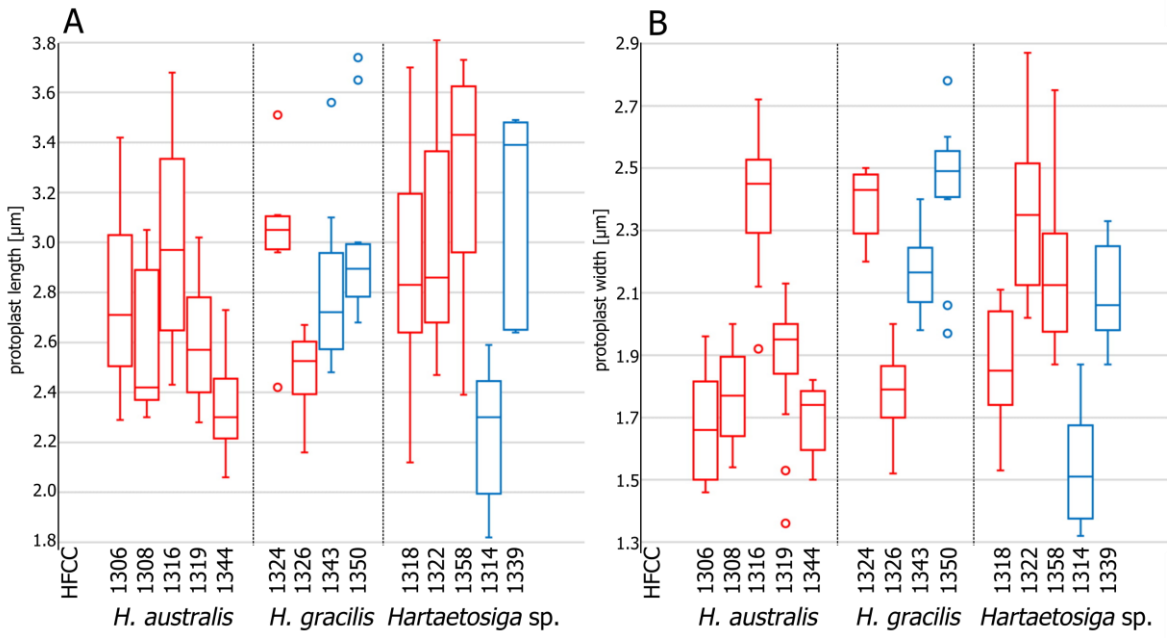

Figure 5. Boxplot analyses of morphometric data of all investigated strain by SEM. (A) protoplast length, (B) protoplast width. Colour code: red – Southern Hemisphere, blue – Northern Hemisphere.

The measured abiotic parameters of the surface water are comparable to standardized global measurements (e.g. NASA Earth Data). In particular, the water temperature was highest in the equatorial region (equivalent to our sampling stations 6 – 9), correlating with the occurrence of *H. australis* sp. nov., and decreased slightly in the subequatorial region (station 4/5, 10/11) (Figure 6B). Colder water at

the NW coast of Africa correlates with the rise of chlorophyll *a* (Figure 6D) indicating an upwelling region. Salinity values ranged from 34.95 to 37.87 PSU (low values in the estuary of Rio de La Plata, Uruguay as outliers were not visualized). Peak of salinity was monitored in the Southern Tropics; equatorial region showed a stable salinity regime (Figure 6C). Correlated with choanoflagellates occurrence and isolation, most strains could be isolated from warmer waters shown by sampling station 5-9 (Table 3, Figure 6B). Chlorophyll *a* concentrations and low differences in salinity had no influence regarding choanoflagellate occurrence.

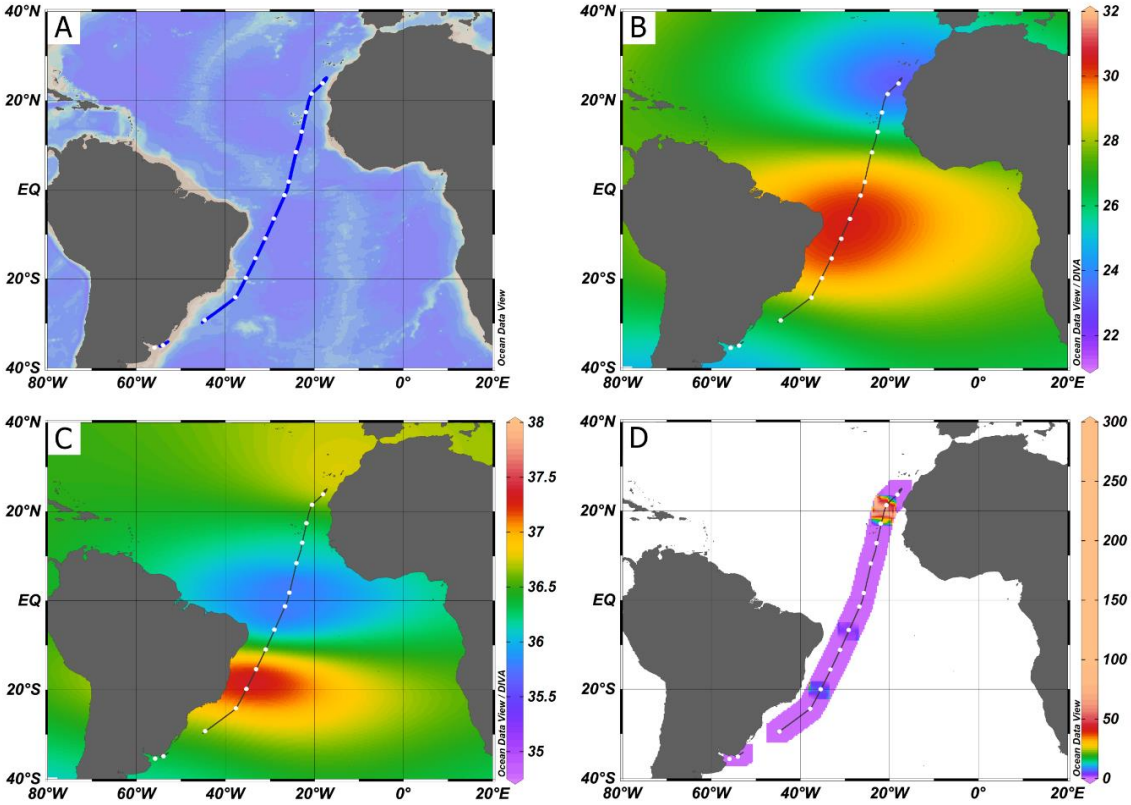

Figure 6. Data of parameters measured during the cruise. (A) Cruise track, (B) surface water temperature [°C], (C) salinity [PSU], (D) chlorophyll *a* [µg/L]. Plots created with ODV (Schlitzer, 2021) using gridded fields.

Taxonomic summary

Order: Craspedida Cavalier-Smith, 1997

Family: Salpingoecidae Saville-Kent, 1880

Genus: *Hartaetosiga* Carr, Richter, Nitsche, 2017

Species: *Hartaetosiga australis* sp. nov. Schiwitza and Nitsche (Figure 3 A-D)

Diagnosis: Thecated choanoflagellates, single celled and colonies (two protoplasts) present, with drop-shaped protoplast about 2.8 x 1.7 µm in size. Collar about 4.2 µm and flagellum about 8.0 µm long. Stalked. Found in warm waters (28.5 – 31°C) of the Southern Hemisphere.

Etymology: *australis* (Latin, adjective) alluding to the only observation and isolation from the South Atlantic.

Type locality: South Atlantic (20°14.663' S 35°33.019' W)

Holotype: Specimen illustrated in Figure 3B (ICZN 1999, Article 73.1.4). The holotype is from the clonal strain HFCC 1306 of the Heterotrophic Flagellate Collection Cologne.

Description: Protoplast drop-shaped, 2.3 – 3.4 μm (2.8 ± 0.4 μm on average) long and 1.5 – 2.0 μm (1.7 ± 0.2 μm on average) wide. Collar length 3.0 – 5.5 μm (4.2 ± 1.0 μm on average). Flagellum length 4.8 – 12.4 μm (8.0 ± 2.8 μm on average). Stalk length 1.0 – 4.2 μm (2.4 ± 1.1 μm on average). Single cells and colonies comprised of two protoplasts observed. Number of microvilli variable.

Type sequence data: The sequences (SSU and LSU rDNA, hsp90, tubA, EFL) of *H. australis* sp. nov. strain HFCC 1306 have been deposited in GenBank with the accession numbers unpub. (SSU rDNA), unpub. (LSU rDNA), unpub. (hsp90), unpub. (tubA) and unpub. (EFL).

Remarks: Species of the genus *Hartaetosiga* display a high degree of cryptic species with similar morphological characteristics. Species delineation based on differences in the SSU rDNA. The five strains of *H. australis* sp. nov. (HFCC 1306, 1308, 1316, 1319, 1344) show high differences regarding the cell size (Figure 5).

Table 4. NCBI accession numbers of all isolated *Hartaetosiga* strains.

Species/strain	SSU rDNA	LSU rDNA	hsp90	tubA	EFL
***Hartaetosiga australis* sp. nov. HFCC 1306**	unpub.	unpub.	unpub.	unpub.	unpub.
***Hartaetosiga australis* sp. nov. HFCC 1308**	unpub.	unpub.	unpub.	unpub.	unpub.
***Hartaetosiga australis* sp. nov. HFCC 1316**	unpub.	unpub.	unpub.	unpub.	unpub.
***Hartaetosiga australis* sp. nov. HFCC 1319**	unpub.	unpub.	unpub.	unpub.	unpub.
***Hartaetosiga australis* sp. nov. HFCC 1344**	unpub.	unpub.	unpub.	unpub.	unpub.
***Hartaetosiga gracilis* HFCC 1324**	unpub.	unpub.	unpub.	unpub.	unpub.
***Hartaetosiga gracilis* HFCC 1326**	unpub.	unpub.	unpub.	unpub.	unpub.
***Hartaetosiga gracilis* HFCC 1343**	unpub.	unpub.	unpub.	unpub.	unpub.
***Hartaetosiga gracilis* HFCC 1350**	unpub.	unpub.	unpub.	unpub.	unpub.

Discussion

The characterisation of protist species diversity in the open ocean is still far from being resolved, also caused by limited access and methodology (e.g. functional high-throughput analysis of protistan assemblages). Global molecular surveys gave first insights into the eukaryotic diversity of plankton communities on a high taxonomical level, lacking morphological and ecological information (de Vargas et al., 2015; Logares et al., 2020). Though, these molecular characterisations unveiled the existence of undiscovered deep-branching lineages, like highly abundant small picoeukaryotes (del Campo et al., 2013; Groisillier et al., 2006; Rodríguez-Martínez et al., 2013). For a comprehensive understanding of species diversity and the functioning of each player within the microbial food web, a characterisation on species level with morphological and autecological data would be important to decipher the species-specific functional role (Sherr et al., 2007). Due to the use of universal eukaryotic primers in global metabarcoding surveys (Amaral-Zettler et al., 2009; de Vargas et al., 2015), the presence of *Hartaetosiga* across the Atlantic Ocean remained undiscovered. Although these primers are applicable for many other choanoflagellate taxa, they do not amplify particularly in this genus, biasing our perspective on the actual diversity of choanoflagellate taxa in marine habitats. Our present data extend the marine genus *Hartaetosiga* by several strains with further implications on their biogeography. As already observed for other craspedid species (Stoupin et al., 2012), morphological surveys cannot accurately distinguish between several species due to morphological plasticity. Only the molecular characterisation revealed the existence of the two species, *H. gracilis* and *H. australis* sp. nov. in the Atlantic Ocean. The distribution of *H. australis* sp. nov. seems to be restricted to surface warm water regions of the Southern Atlantic, whereat the ECC might play a significant role in the distribution. This hypothesis is confirmed by the absence of *H. australis* sp. nov. in CTD samples from deeper, colder water layers (data not shown).

For choanoflagellate species delineation, the SSU rDNA is a suitable marker gene (Nitsche and Arndt, 2015; Pawlowski et al., 2012), also shown in our single gene phylogenetic analysis, which resulted in the same topology as the concatenated analysis. With our transcriptomic data, we could extend the scarce molecular data of the hypervariable regions ITS1 and ITS2 for choanoflagellate taxa. All strains of *H. australis* sp. nov. were 100% identical regarding the SSU rDNA and complete ITS, indicating an intraspecifically conserved region.

The equatorial province of the Atlantic Ocean with its equatorial counter currents was shown to affect population structures for planktonic organisms (Goetze et al., 2017). Investigations on off-shelf waters are frequently aiming to

understand protist distribution. The putative unrestricted distribution of microbiota (based on fast reproduction rates and small sizes) is more and more challenged as several global studies found biogeographical patterns within prokaryotes, archaea and unicellular eukaryotes (Casteleyn et al., 2010; Friedline et al., 2012; Whitaker, 2003). Whether such biogeographies are matter of dispersal limitations or a result of local environmental selection is still under discussion. Our data on the distribution of the choanoflagellate genus *Hartaetosiga* indicate that surface water currents might be considered as an additional abiotic factor among changes in temperature, salinity and light intensity (Okada and Honjo, 1973) that influence the microbial dispersal.

Author contributions

S.S. – Data curation, formal analysis, investigation, methodology, visualization, original draft, review & editing; C.S. – Investigation, methodology; F.N.– Conceptualization, funding acquisition, project administration, supervision, validation, review & editing

Acknowledgements

We gratefully acknowledge the heartily cooperation with the captain and his crew of the RV Maria S. Merian during the cruise MSM82/2. We appreciate the support by the Leitstelle Deutsche Forschungsschiffe (German Research Fleet Coordination Centre) in Hamburg. In particular, we would like to thank the chief scientist, Sebastian Krastel for cruise organization and incorporating our biological project. Thanks to Alexandra Jeuck and Johanna Ahlers, who helped with the sampling on board. Many thanks to Maria Sachs for the drawings. This work was funded by the grants of the German Research Foundation (DFG) in the frame of the SPP 1991: Taxon-omics − New Approaches for Discovering and Naming Biodiversity to F.N. (NI1097/3-1) and the Leitstelle Deutsche Forschungsschiffe for Taxonomics-Atlantic to F.N. (GPF 18-3_76).

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Supplementary material

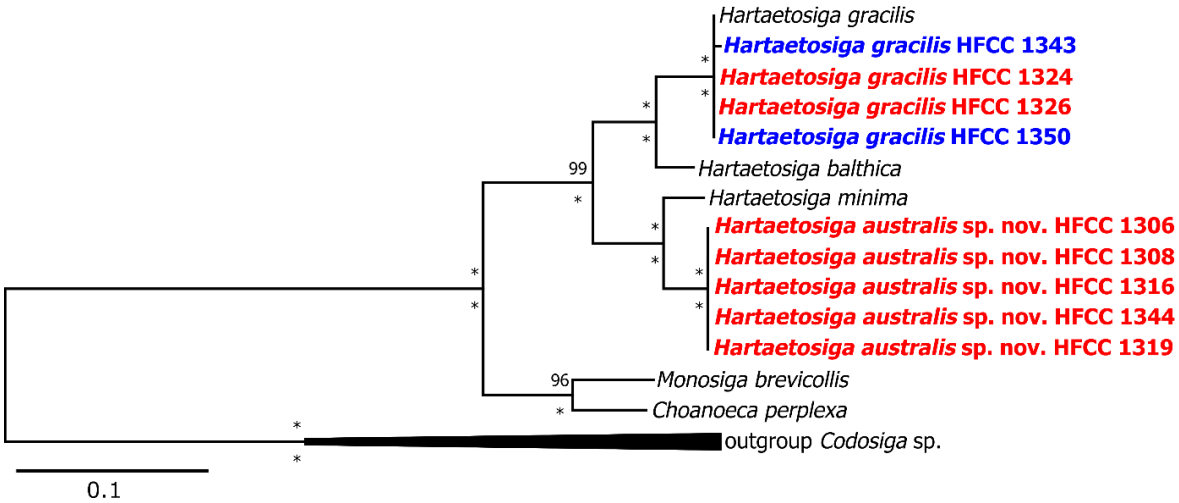

Figure S1. SSU rDNA maximum likelihood phylogeny of the genus *Hartaetosiga* based on an unmasked alignment (2824 nt). Number of nucleotide substitutions per site defined by scale in the lower left. Support values for ML/BI given at each branch. 100 % ML bootstrap percentage support (mlBP) and 1.00 BI posterior probabilities (biPP) are denoted by a *. Otherwise, mlBP and biPP values are given at each branch respectively. Newly molecular described species/strains are marked by bold letters. Strains isolated from the Northern Hemisphere are displayed in blue, isolates from the Southern Hemisphere in red.

Chapter 3: The Potential to Cross the Marine-Freshwater Boundary – a Case Study on Craspedid Choanoflagellates

The potential to cross the marine-freshwater boundary - a case study on craspedid choanoflagellates

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Abstract
Various phylogenetic analyses of microbial taxa revealed distinct phylogenetic clusters of freshwater and marine lineages. This marine-freshwater boundary seems to be a margin in the potential of protist species dispersal. In order to also approach these phylogenetic relationships from an ecological point of view, we performed autecological osmotolerance experiments on several genetically distinct craspedid choanoflagellate species (either marine or freshwater). The results uncovered a great capability of craspedids to colonize freshwater to marine habitats or vice versa. Applying this ecological approach, we show that habitat preferences cannot be inferred unambiguously from common phylogenetic analyses, and hitherto biases our present perspective on dispersal. We suggest to apply a combined, specific pathway-based molecular and autecological approach to elucidate the true nature of the environmental distribution potential for protists.

Keywords
Autecology; Choanoflagellatea; Craspedida; Habitat selection and distribution; Osmotolerance; Marine-freshwater transition

Significance

Protists, unicellular eukaryotes, fulfill a variety of different ecological functions within all ecosystems. Due to their size and high reproduction rates, they have a potential high dispersal capability, but clear patterns of distribution are up to now not apparent. Since the use of molecular techniques, the relationship and environment-specific diversification are considered from a phylogenetical perspective. This image is blurred by the lack of autecological data as the genes commonly used for phylogenetic analyses do not allow deducing the character of osmotolerance. Hitherto, the evolution and dispersal of protists is still far from understood and ecological traits must be taken into account for a thoroughly and comprehensive picture.

Introduction

Unicellular eukaryotes, as well as other microbes (Archaea, Bacteria) are present in all aquatic environments and play an important part in biogeochemical cycling and ecosystem functioning (Azam et al., 1983; Bell et al., 2005; Cotner and Biddanda, 2002; Morin and McGrady-Steed, 2004; Suttle, 2007). They are characterized by fast evolutionary diversification processes resulting from large population sizes (Giovannoni and Stingl, 2005; Snoke et al., 2006), high reproduction and mutation rates (Drake, 1999) and putative high dispersal capabilities (Fenchel and Finlay, 2004; Finlay, 2002). Though, also protists have a certain degree of biogeography as physiochemical and ecological barriers can hinder the dispersal and habitat exchange (Dolan, 2005; Foissner, 2008). Since the use of molecular techniques, the subject of dispersal, biogeographical distribution and ecological restrictions were thought to be decipherable from a phylogenetic perspective. Molecular studies on Archaea and Bacteria give strong evidence for phyloecological clusters of highly environment-specific diversification (Nikolic et al., 2012; Whitaker, 2003), and even viruses show distinct phylobiogeographical clusters (Gaunt et al., 2001). As the major physiochemical barrier, salinity was previously reviewed as the main factor in determining the distribution and evolution of microbial taxa, bacteria and in general microbes (Hahn, 2006; Logares et al., 2009). The marine-freshwater transition was in focus of many independent microbial studies regarding the relationship between freshwater and marine protistan species which revealed distinct ecological clades (Logares et al., 2007; Scheckenbach et al., 2006; von der Heyden et al., 2004). Varying salt concentrations affect different metabolic pathways demanding a physiological adaptation to balance the osmotic pressure, already well-described for prokaryotes and discussed for bacterivorous protists as possible

mechanisms by horizontal gene transfer (Czech and Bremer, 2018; Empadinhas and da Costa, 2008; Oren, 2008; Padan et al., 1989; Steil et al., 2003).

Within this study, we focus on the evolutionary relationship between freshwater and marine craspedid choanoflagellates, as previous studies suggested a phylogenetically supported separation of freshwater and marine species clusters (Carr et al., 2017, 2008, Schiwitza et al., 2020). Based on taxonomy and molecular studies (Nitsche et al., 2011) choanoflagellates are classified into two orders. The Acanthoecida Cavalier-Smith, 1997 comprising two families, the Acanthoecidae Norris, 1965 emend. *sensu* Nitsche et al., 2011 and Stephanoecidae Leadbeater, 2011 which are mainly distributed in marine waters with a few exceptions from freshwater sites (Nitsche, 2014; Paul, 2012). The Craspedida Cavalier-Smith, 1997 with only one family the Salpingoecidae Saville-Kent, 1880-82 emend. *sensu* Nitsche et al., 2011 (Carr et al., 2008; Nitsche et al., 2011) show an extensive dispersal potential as they are ubiquitously distributed from freshwater to marine conditions (Jeuck et al., 2014; Nitsche et al., 2007; Wylezich et al., 2012), with even hypersaline records (Schiwitza et al., 2018; Triadó-Margarit and Casamayor, 2013). Carr et al. (2017) separated the Craspedida into three major clades of which Clade 1 and 3 represent the monophyletic marine lineages, and only Clade 2 the paraphyletic freshwater lineage (with one marine recolonization). To verify the relation of phylogenetic clustering and environment-specific diversification within craspedid choanoflagellates, we aimed to integrate autecological data, in particular osmotolerance, to phylogenetic analyses as previous studies indicated broad salinity tolerances of craspedid choanoflagellates (Nitsche and Arndt, 2015; Schiwitza et al., 2018). Based on the recent revision of the so-called freshwater Clade 2 by several freshwater species from the River Rhine, Cologne (Schiwitza et al., 2020), we tested whether common phylogenetic analyses, based on housekeeping genes, reflect the true nature of potential dispersal within choanoflagellates. For this purpose, we refer here to the osmotolerance data of 21 species evenly spread across the craspedid phylogeny to analyze the potential to cross the marine-freshwater boundary.

Material and methods

Salinity tolerance experiments were performed for several species from freshwater (*Codosiga hollandica*, *C.* sp. M1/pIIp, *Paramonosiga coloniensis, P. thecata*, *Salpingoeca euryoecia, S. fluviatilis, S. fusiformis, S. helianthica* and *S. loutrophoria*) and marine habitats (*Hartaetosiga gracilis, Microstomoeca roanoka, Monosiga brevicollis*, *S. longipes*, *S. macrocollata*, *S. rosetta* and *Stangondoeca pyriformis*) according to a modified protocol of Schiwitza et al. (2018) testing only the salinity range between freshwater

and marine conditions. In brief, species were cultured in artificial seawater (Instant Ocean, Aquarium Systems, Strasbourg, France) or WC medium (Guillard and Lorenzen, 1972). Salinity was increased and decreased respectively every two days by one practical salinity unit (PSU) below ten PSU and by five PSU above ten PSU until no viability (in particular flagellar movement) was observed. Each experiment was done in triplicates and cultures were daily monitored. Salinity tolerance data from *S. crinita*, *S. huasca*, *S. surira and S. tuba* were implemented from previous publications (Nitsche and Arndt, 2015; Schiwitza et al., 2018). Within a cladogram of craspedid species based on the latest published phylogenetic analyses of choanoflagellates (Schiwitza et al., 2020), a generated heat map shows phyloecological patterns regarding the original habitat and empirical tested osmotolerance.

Results

Up to now, phylogenetic analyses of craspedid choanoflagellates are based on 43 species, from freshwater, brackish, marine or hypersaline environments (Figure 1).

Salpingoeca tuba and *S. dolichothecata*, deeply branching at the origin of craspedid species, are from marine and hypersaline origin, respectively. Within this clade, *S. tuba* showed a high degree of osmotolerance (7-121 PSU) (Nitsche and Arndt, 2015). More than half of the craspedids (23 species) have been sampled from freshwater. They are divided into two distinct lineages, freshwater Clade 2, but also at the basis of the marine Clade 1. The largest freshwater clade consists of 21 species with only one revertant to the marine habitat, *S. macrocollata*. Within this paraphyletic freshwater clade, we tested eight species, including the marine species *S. macrocollata*. In our experiment, this marine species survived under freshwater as well as under marine conditions (0-35 PSU). In addition, the freshwater species *S. euryoecia* was also able to survive both conditions (0-35 PSU). The other tested freshwater species had a narrower range of salinity tolerance: *S. fusiformis* (0-20 PSU), *Codosiga hollandica* (0-10 PSU), *S. fluviatilis* (0-3 PSU), *S. loutrophoria* (0-3 PSU). Limited to freshwater conditions were *S. helianthica* and *Codosiga* sp. M1/pIIp with a tolerance of 0-1 PSU. The second freshwater clade is represented by two species of the genus *Paramonosiga*, branching at the basis of a marine and hypersaline clade. The empirically tested osmotolerance of *P. thecata*, ranging from freshwater to marine (0-35 PSU), already indicated the potential of a ubiquitous distribution, whereas the other species, *P. coloniensis* survived only low changes in salinity (0-2 PSU). In addition, the autecological experiments revealed that four marine species (*Microstomoeca roanoka*, *Monosiga brevicollis*, *S. longipes* and *S. rosetta*) show the potential to live in freshwater systems (0-35 PSU). The other tested marine species

were restricted to marine to brackish conditions: *Hartaetosiga gracilis* (5-35 PSU) and *Stagondoeca pyriformis* (5-40 PSU). The craspedid species from hypersaline environments clustered within two clades and showed broad salinity tolerances excluding freshwater conditions: *S. crinita* (12-150 PSU), *S. huasca* (5-150 PSU), *S. prava* (12-90 PSU), *S. surira* (5-150 PSU) (Schiwitza et al., 2018).

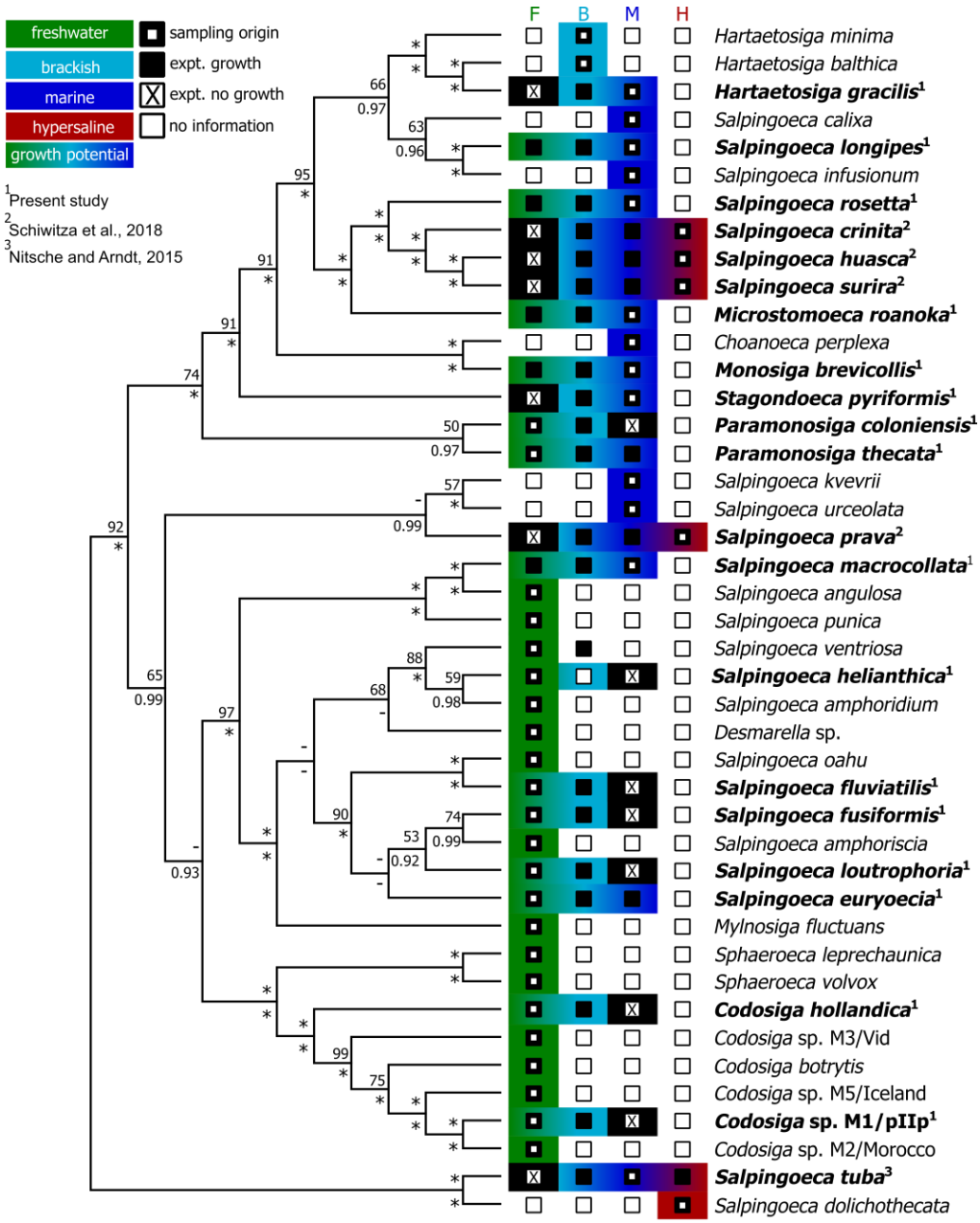

Figure 1. Phylogenetic cladogram of craspedid choanoflagellates with ecological traits regarding the environmental salinity range and origin. Empirical tested species given in bold. Legend of heat map in the upper left. Support values for ML/BI given at each branch. 100 % ML bootstrap percentage support (mlBP) and 1.00 BI posterior probabilities (biPP) are denoted by a *. Otherwise, mlBP and biPP values are given at each branch respectively. Support values below 50 % mlBP and 0.7 biPP are indicated by a -. Abbreviations and color code: F-freshwater (green), B-brackish water (turquois), M-marine water (blue), H-hypersaline water (red). The survival range of each species is color-coded. The salinity of the original sampling location is marked with a white dot.

With our osmotolerance experiment (detailed in Figure 2) we could show that both, freshwater and marine species have the potential to cross the marine-freshwater boundary (marine species: *M. roanoka*, *M. brevicollis*, *S. longipes*, *S. macrocollata* and *S. rosetta*, freshwater species: *P. thecata*, *S. euryoecia*).

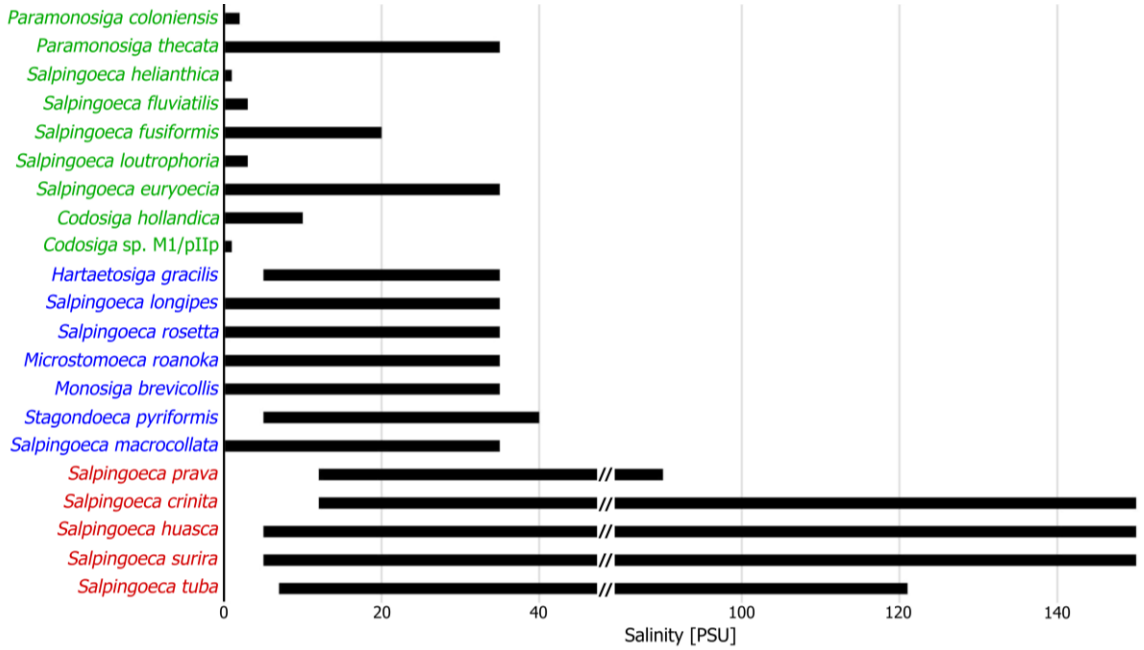

Figure 2. Salinity tolerance ranges of all tested choanoflagellate species. Color code of species origin: F-freshwater (green), M-marine water (blue), H-hypersaline water (red).

Discussion

Salinity was long thought to be the major abiotic factor limiting the dispersal of unicellular eukaryotes reflected by phylogenetic analyses based on housekeeping genes and distinct protist communities within marine or freshwater systems (Filker et al., 2017; Logares et al., 2007, 2009; Scheckenbach et al., 2006; von der Heyden et al., 2004). This perspective has been challenged as shown by freshwater alga which could experimentally be adapted to marine conditions (Lachapelle et al., 2015). By testing several freshwater and marine choanoflagellate species regarding their salinity tolerance, we could show that, also in the group of choanoflagellates, some species are euryoecious, able to survive both, freshwater and marine conditions. This new approach of verifying empirically the environmental distribution potential adds a new facet to our current understanding of protist dispersal.

So far, the assignment as a cosmopolitan or ubiquitous taxon mostly referred to the biogeographical distribution either within marine or freshwater habitats (Díez et al., 2001; Lefranc et al., 2005; Moon-van der Staay et al., 2001; Richards et al., 2005; Šlapeta et al., 2005), neglecting the occurrence of euryoecious species. A comparative study investigating protistan community structures from both aquatic

habitats could give broader insights into these adaptive mechanisms (in the context of salinity tolerant) (Simon et al., 2015) and reveal potential ubiquitous species which are able to cross the marine-freshwater boundary. Molecular analyses additionally comprising functional genes from osmotolerance pathways could contribute to a higher resolution regarding autecological traits, unveiling a potentially euryoecious characteristic within certain taxa. One major limitation in finding these ´trait-specific´ genes is the poor annotation for protist genomes in general. Comparative transcriptome analyses, displaying differential gene expression patterns in freshwater compared to marine treatments, might help to approach a suitable set of candidate genes for extended analyses. Possible candidate genes might be mitogen-activated protein kinases (MAPKs), which play a fundamental role in cellular stress responses, as described for *Saccharomyces cerevisiae* (Jansen et al., 2001; Maeda et al., 1995; Posas et al., 1996) and other fungi (Aggarwal and Mondal, 2009; Dragosits et al., 2010; Hernandez-Lopez et al., 2006). Although not functionally characterized, analyses of the ciliate *Tetrahymena thermophila* revealed the existence of several MAPKs and its expression, also under osmotic stress (Yıldız and Arslanyolu, 2014). Transcriptome investigations on halophilic protists discovered an increased expression of enzymes involved in synthesis and transport of organic osmolytes (Harding et al., 2016), which were further also detected by hydrogen-1 nuclear magnetic resonance spectroscopy (Weinisch et al., 2018).

This new perception has also severe implications for taxonomical studies, in particular for choanoflagellates. Historical species descriptions were based only on data of the original habitat and morphology (missing molecular information). According to the present policies of the ICZN (International Commission on Zoological Nomenclature), the sampling locality for a neotypification should be in close proximity to the original sampling site. Our present data show that euryoecious species, such as *S. euryoecia* (freshwater origin) and *S. macrocollata* (marine origin) indicate the need for an extension within the ICZN to allow for an adequat species description including species with a euryoecious character. In addition, adding autecological data of established species in culture will enhance phylogenetic analyses regarding ecological characteristics and our understanding of protist dispersal and hence the potential to cross the marine-freshwater boundary.

Author contributions

S.S., F.N. designed research, S.S. performed experiments and analyzed data. S.S wrote, F.N. improved the manuscript.

Acknowledgements

We thank Kathrin Sabrina Kiffer, Sarah Bank and Julia Engels who performed several autecological osmotolerance experiments whereby the idea to the manuscript rose. In addition, we thank Hartmut Arndt for proofreading. This work was supported by grants from the German Research Foundation to F.N. (NI1097/3-1) within the SPP 1991 "Taxon-omics".

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Part 2

Choanoflagellates from Extreme Hypersaline Environments of the Atacama Desert, Northern Chile

Chapter 4: Four New Choanoflagellate Species from Extreme Saline Environments: Indication for Isolation-Driven Speciation Exemplified by Highly Adapted Craspedida from Salt Flats in the Atacama Desert (Northern Chile)

Published publication

Schiwitza S., Arndt H., Nitsche F., **2018**. Four new choanoflagellate species from extreme saline environments: Indication for isolation-driven speciation exemplified by highly adapted Craspedida from salt flats in the Atacama Desert (Northern Chile). *European Journal of Protistology 66, 86-96. doi: 10.1016/j.ejop.2018.08.001*

Chapter 5: Mirroring the Effect of Geological Evolution: Protist Divergence in the Atacama Desert

Published publication

Arndt H., Ritter B., Rybarski A., **Schiwitza S.**, Dunai T., Nitsche F., **2020**. Mirroring the effect of geological evolution: Protist divergence in the Atacama Desert. *Global and Planetary Change 190, 103193. doi: 10.1016/j.gloplacha.2020.103193*

Chapter 6: Extended Divergence Estimates and Species Descriptions of New Craspedid Choanoflagellates from the Atacama Desert, Northern Chile

Published publication

Schiwitza S., Gutsche L., Freches E., Arndt H., Nitsche F., **2021**. Extended divergence estimates and species descriptions of new craspedid choanoflagellates from the Atacama Desert, Northern Chile. *European Journal of Protistology 79, 125798. doi: 10.1016/j.ejop.2021.125798*

Part 3

Nudiform Acanthoecids – an Unexpected Species Richness within Loricate Choanoflagellates

Chapter 7: First Description of an Euryoecious Acanthoecid Choanoflagellate Species, *Enibas tolerabilis* gen. et sp. nov. from a Salar in the Chilean Andes based on Morphological and Transcriptomic data

Published publication

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Chapter 8: A Needle in the Haystack – Mapping Sequences to Morphology Exemplified by the Loricate Choanoflagellate *Enibas thessalia* sp. nov. (Acanthoecida, Acanthoecidae)

Published publication

Schiwitza S., Nitsche F., **2021**. A needle in the haystack – Mapping sequences to morphology exemplified by the loricate choanoflagellate *Enibas thessalia* sp. nov. (Acanthoecida, Acanthoecidae). *Protist 172, 125782 doi: 10.1016/j.protis.2020.125782*

Conclusive Summary and Perspectives

Traditional taxonomy, mainly based on morphological characteristics, pioneered already in the 19th century for the understanding of protist diversity and systematics. Only in the late 20th century, molecular based studies, derived from research on prokaryote diversity (Amann et al., 1995; Stackebrandt and Rainey, 1995), found their way into research on protists (Cavalier-Smith, 1993; Sogin et al., 1986; Woese et al., 1990). The amplification and analysis of the ribosomal DNA, a multicopy gene present in all eukaryotes, helped to refine phylogenetic relationships between protistan groups. Today, multigene analyses aim to resolve the question of the last common ancestor of eukaryotic life, but also the origin of multicellularity (Adl et al., 2012; Burki et al., 2020; Strassert et al., 2019). In particular, the evolution of multicellularity within the Opisthokonta, comprising metazoans, is a current focus of evolutionary biology (Maldonado, 2004; Medina et al., 2003; Richter and King, 2013). To understand the processes which led to multicellularity, the ecology of the closest related group to the metazoans, the Choanoflagellatea, is of particular interest. In this context, it is essential to study a broad variety of choanoflagellate species in culture in order to obtain not only morphological and molecular but also ecological data. This will substantially help to improve the understanding of diversification processes within choanoflagellates and environmental factors which have led to the evolution of multicellularity.

The present thesis extends our current knowledge on the diversity and ecology of choanoflagellates, by investigations of several diverse aquatic habitats using a combined approach of generating morphological, extended molecular and ecological data. This integrative taxonomy is of urgent need to get a comprehensive insight into choanoflagellate evolution and a revised, reliable systematic. Applying this integrative taxonomic approach allowed to extend the knowledge on protist diversity comprising adaptation to extreme habitats, drivers for diversification and insights into evolution of these closest related unicellular relatives to metazoans. These cultivation-based studies enabled the integration of historical species descriptions with matching molecular data for a revised systematics, including now also an ecological perspective.

Exemplified by long-term investigations at the River Rhine in Cologne, a well-studied aquatic habitat, the still underestimated role of choanoflagellate species within the microbial food web could be demonstrated in this thesis. Although protist diversity in the River Rhine was previously studied (Norf and Foissner, 2010; Scheckenbach et al., 2006; Stoupin et al., 2012), additional six

different craspedid choanoflagellates (*Paramonosiga coloniensis* sp. nov., *Salpingoeca amphoridium* James-Clark, 1867, *S. amphoriscia* sp. nov., *S. angulosa* de Saedeeler, 1927, *S. fluviatilis* sp. nov. and *S. loutrophoria* sp. nov.) were isolated and described. As four of the six described species were new to science, these results support the hypothesis that species richness within protists is broadly underestimated. By comprehension of previously described species, a salpingoecid population of *S. amphoridium* from the River Rhine and another salpingoecid species, *S. angulosa*, previously reported by de Saedeeler (1927) from Belgium, were redescribed and neotypified respectively, based on distinctive morphological traits (Chapter 1). This study depicts the importance to reinvestigate the morphologically described type species, such as *S. gracilis* James-Clark, 1867 by applying molecular methods to obtain a higher phylogenetic resolution. Only recently, the splitting of the *Codosiga* species complex into two monophyletic genera, *Codosiga* (freshwater) and *Hartaetosiga* (marine), based on molecular and ecological data, demonstrated the significance of molecular sequencing methods to distinguish morphotypes or cryptic species complexes (Carr et al., 2017). The newly established, strictly marine genus *Hartaetosiga* could be extended within our survey along a transect of the Atlantic Ocean. The finding and description of several strains of the new species, *H. australis* sp. nov., extends our knowledge on the monophyletic character of this genus. With our dataset of several strains and their spatial distribution in the Southern Hemisphere, further data on the biogeography of protists was added (de Vargas et al., 1999; Dolan, 2005; Foissner, 2008) (Chapter 2). As shown in this study, common phylogenetic analyses on other protistan taxa support these data of distinct freshwater and marine clades (Logares et al., 2007; Scheckenbach et al., 2006; von der Heyden et al., 2004). The empirical dataset, obtained by testing the salinity tolerances of several craspedid species, evenly represented from marine and freshwater, gave a new perception of the phyloecological clusters (Chapter 3). The survival of several cultured species within both salinity conditions will be the basis for further molecular studies using a comparative transcriptomic method to elucidate the molecular background and mechanisms of the potential to cross the marine-freshwater boundary. As salinity is not the only abiotic factor influencing the distribution and dispersal of protist species, the tool of comparative transcriptomics is a powerful method to further study other factors, e.g. temperature tolerances and food preferences, to shed light on the ecology and biogeography of protists, in particular choanoflagellate species.

The selecting force of environments was shown to be especially expressed under extreme environmental conditions, as several studies indicate a unique protist community in these habitats (Alexander et al., 2009; Park et al., 2006; Park

and Simpson, 2015; Schoenle et al., 2021). Hypersaline environments were shown to harbour a high degree of molecular novelty, also for choanoflagellates (Heidelberg et al., 2013; Paul Antony et al., 2013; Triadó-Margarit and Casamayor, 2013). Within this present study, seven new craspedids (*S. aroma* sp. nov., *S. crinita* sp. nov., *S. guzmanae* sp. nov., *S. huasca* sp. nov., *S. llamariensis* sp. nov., *S. prava* sp. nov., *S. surira* sp. nov.) were isolated and described from aquifers and hypersaline water bodies of the Atacama Desert in Northern Chile (Chapter 4, 6). These first records of protists from salt flats from this extreme environment already got attention in studies on the microbial community composition at Salar de Huasco, where until now only the prokaryotic diversity was investigated (Dorador et al., 2020). A comparison of the V9 dataset of the global survey of Tara Oceans (de Vargas et al., 2015) with our molecular data revealed that none of these organisms was previously recorded from marine habitats, indicating their geographical restriction. The athalassic salt flats within the Atacama Desert differ regarding their chemical composition of salt structuring components and the deposition of toxic elements, such as arsenic (Demergasso et al., 2007; Risacher et al., 1999; Tapia, 2018), among each other and most important, to marine conditions. High UV radiation likely led to high mutation rates which favoured a strong speciation process, selected by varying salinities. This hypothesis was supported by divergence time estimates in which the craspedid strains of the Atacama Desert were relatively young compared to their closest marine relatives (Chapter 5, 6). In addition, the molecular clock data indicate a strong correlation of biological colonisation and diversification processes to geological event, i.e. the formation of the salt flats (Ritter et al., 2018; Sáez et al., 1999). Adaptation mechanisms to such extreme conditions, like hypersalinity and/or the presence of arsenic are already well-studied for prokaryotes on a genetic level (Dorador et al., 2008; Kartal et al., 2006; Lara et al., 2012; Padan et al., 1989; Steil et al., 2003). Within the present studies, first ecological experiments to test for the survival range were performed by exposing species to varying salinities. In addition, first transcriptomic data were established as a reference for most species isolated from the Atacama Desert. These data will be the basis for further comparative analyses to unravel the eukaryotic adaptation to these challenging conditions. As horizontal gen transfer was already discussed for choanoflagellates (Yue et al., 2013), analyses of the bacterial community might be a key factor to decipher the underlying mechanisms. The prokaryotic influence on choanoflagellates was previously shown to be an important driver for their ecology and evolution (Alegado et al., 2012; Rossiter and Wuest, 2017; Woznica et al., 2017).

The high molecular novelty of choanoflagellates in the Atacama Desert was also of note for the family of nudiform reproducing choanoflagellates, the

Acanthoecidae. This novelty resulted in the isolation and description of one new nudiform genus comprising the species *Enibas tolerabilis* gen. et sp. nov., characterised by a distinct morphology. Phylogenetic analyses clearly allowed the erection of a new genus (Chapter 7). The following study on the genus *Enibas* strongly indicated, that the species richness of nudiform reproducing species was highly underestimated (Chapter 8). Within these phylogenetic analyses, a high number of environmental eukaryotic sequences clustered within the clade of *Enibas* (Behnke et al., 2006, 2010; Oikonomou et al., 2012), extending the group by a multiple. The targeted resampling approach of one of these sequences at Lake Karla in Greece (Oikonomou et al., 2012) successfully matched morphological data to the corresponding sequence resulting in the description of *E. thessalia* sp. nov., the second morphologically described species of this new genus. This approach demonstrated the importance to reinvestigate habitats with phylogenetically interesting sequence data to verify the phylogenetic relationships on a taxonomical basis. The use of a single cell isolation approach has a great applicability and acceptance also for other protistan taxa. As described species and related environmental sequences from the genus *Enibas* mainly originate from extreme habitats, it might be hypothesized that members of the genus pose exceptional and unusual requirements regarding the habitat. Both described species were isolated in water bodies with low salinity and loads of heavy metal (Hermosilla et al., 2019; Skordas et al., 2015). Salinity tolerance experiments on the type species revealed a euryoecious character, able to survive from freshwater up to hypersaline conditions. Until now, loricates were mainly recorded from brackish to marine habitats with only two freshwater exceptions (Nitsche, 2014; Paul, 2012), making this new genus of great interest for further ecological studies.

The striking morphological resemblance of the newly erected genus *Enibas* to the tectiform genus *Stephanoeca* supported the paraphyletic character of this genus. Only recently, with morphological species descriptions on further *Stephanoeca* species, Thomsen and Østergaard (2019) supported the hypothesis about the possibility of an ancestral type of lorica as it is now present in both families. Within the study mentioned above, the verification of a tectiform division in species of *Stephanoeca* is discussed critically. The list of data regarding morphology and molecular biology confirming the tectiform character of all assigned species is very limited, indicating the need for a revision. For a conclusive revision of this genus, a molecular characterisation of the type species, *S. ampulla* Ellis, 1929 would be required to verify its phylogenetic position.

Only recently, data on protist diversity exponentially increased by the use of modern molecular techniques, in particular high-throughput sequencing (HTS). These methods rely on the amplification of specific marker regions of the SSU rDNA, i.e. the hypervariable region V4 or V9 to delineate differences within protistan taxa (Dunthorn et al., 2012; Hu et al., 2015; Stoeck et al., 2010). This approach offered the opportunity for diversity estimates of the protist community from aquatic and terrestrial environments worldwide, including extreme habitats (Bates et al., 2013; de Vargas et al., 2015; Hohlfeld et al., 2021; Mahé et al., 2017; Schoenle et al., 2021). One major limitation of this method is related to the development of universal eukaryotic primers. Currently applied primers are selective and do not amplify several protistan taxa, distorting the perspective on diversity patterns in certain habitats. The analyses of metabarcoding data also strongly depends on the quality and reliability of reference databases. For this purpose, the initiation of projects which focus on the curation of databases based on nucleotides or protein-coding genes was fundamental to increase the power of HTS-based studies (del Campo et al., 2018; Guillou et al., 2012; Richter et al., 2020). With special emphasis on choanoflagellates, the evaluation of these metabarcoding data is still challenged, in particular diversity studies which are based on the V9 region of the SSU rDNA. As early molecular studies resulted only in short amplicons missing the V9 region (Nitsche, 2014; Nitsche et al., 2007; Nitsche and Arndt, 2008), only few choanoflagellates are represented in reference databases, heavily biasing the interpretation of metabarcoding data. In addition, the extended dataset on choanoflagellates, e.g. the commonly distributed marine genus *Hartaetosiga* (see Chapter 2), revealed the limited applicability of the universal primer set for the V9 region, the most popular amplicon for environmental studies.

Facing these challenges, the present thesis focused on the establishment of reliable reference data for choanoflagellate taxa based on detailed morphological, molecular and also ecological data to build a solid and reliable basis for HTS-analyses. By expanding the molecular dataset of choanoflagellates, in combination with sound morphological and ecological data, it will be possible to generate reference alignments for e.g. phylogenetic placement analyses of HTS-data (Dunthorn et al., 2014). In particular, the use of the V4 region is a promising approach to gain further insights regarding molecular phylogenetic relationships (Choi and Park, 2020). Additionally, the development of group specific primer sets (Blandenier et al., 2017; Fiore-Donno et al., 2018) will refine metabarcoding approaches and eliminate the bias caused by universal eukaryotic primers.

As choanoflagellates phylogenetically form the sister clade to the Metazoa, molecular investigations on the diversity of choanoflagellates are essential to

understand their relationship and also the evolution of multicellularity (Carr et al., 2008; Medina et al., 2003; Richter and King, 2013). Genomic and transcriptomic data already revealed an exclusively shared gene pool of choanoflagellates and Metazoa, e.g. genes for cell adhesion and signalling processes (Fairclough et al., 2013; King et al., 2008; Richter and King, 2013). With our study, the number of high-quality transcriptomes was doubled by adding 17 choanoflagellate strains. Extending molecular information based on genomic and transcriptomic data will help to understand the diversification of protistan lineages. In addition, this dataset is useful to infer divergence time estimates by applying molecular clock analyses, to date the origin of eukaryotes and in particular multicellularity (Parfrey et al., 2011).

The present thesis underlines the significance of integrative taxonomy by generating reliable species descriptions based on morphological, molecular and ecological data. This approach is more important than ever as massive amounts of sequences are generated by HTS-methods. These data rely on a verified reference database for comprehensive analyses. Still, cultivation-based approaches are very selective as only a small fraction of the protistan community can be cultivated (Jeuck et al., 2017). With regards to choanoflagellates, especially new methods have to be established to increase the number of loricate cultures, which seem to be very fragile and susceptible when cultivated by dilution series or micromanipulation (Nitsche, 2014). The establishment of clonal cultures offers the opportunity to get deeper understandings of molecular adaptation mechanisms based on transcriptome analyses. Only a combination of traditional and modern methods will elucidate the diversity of the eukaryotic microbial biosphere.

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Subpublications and Records of Achievement

Part 1

Chapter 1: **Schiwitza S.**, Lisson H., Arndt H., Nitsche F., **2020**. Morphological and molecular investigation on freshwater choanoflagellates (Craspedida, Salpingoecidae) from the River Rhine at Cologne (Germany). *European Journal of Protistology 73, 125687. doi: 10.1016/j.ejop.2020.125687*

The study was designed and written by the author of the present thesis under guidance of Prof. Dr. Hartmut Arndt and Dr. Frank Nitsche. Results were achieved by the author and coauthor Helene Lisson.

Chapter 2: **Schiwitza S.**, Spruck C., Nitsche F., **manuscript**. Extending the genus *Hartaetosiga* (Choanoflagellatea, Craspedida, Salpingoecidae) by species from a transect across the Atlantic Ocean.

The study was planned by the author of the present thesis together with Dr. Frank Nitsche. The author performed transcriptome analyses and wrote the manuscript. Cultures and morphological data were also obtained by the coauthor Christiane Spruck.

Chapter 3: **Schiwitza S.**, Nitsche F., **manuscript**. The potential to cross the marine-freshwater boundary – a case study on craspedid choanoflagellates.

The study was planned by the author of the present thesis together with Dr. Frank Nitsche. The author performed all experiments and wrote the manuscript.

Part 2

Chapter 4: **Schiwitza S.**, Arndt H., Nitsche F., **2018**. Four new choanoflagellate species from extreme saline environments: Indication for isolation-driven speciation exemplified by highly adapted Craspedida from salt flats in the Atacama Desert (Northern Chile). *European Journal of Protistology 66, 86-96. doi: 10.1016/j.ejop.2018.08.001*

The study was designed and written by the author of the present thesis under guidance of Prof. Dr. Hartmut Arndt and Dr. Frank Nitsche. Cultures, morphological and ecological data were gained during author's master thesis.

Further molecular and phylogenetic analyses were performed during the PhD thesis.

Chapter 5: Arndt H., Ritter B., Rybarski A., **Schiwitza S.**, Dunai T., Nitsche F., **2020**. Mirroring the effect of geological evolution: Protist divergence in the Atacama Desert. *Global and Planetary Change 190, 103193. doi: 10.1016/j.gloplacha.2020.103193*

The study was designed by Prof. Dr. Hartmut Arndt and Dr. Frank Nitsche. The author provided molecular transcriptomes for analyses and helped with the data collection and proof reading of the manuscript.

Chapter 6: **Schiwitza S.**, Gutsche L., Freches E., Arndt H., Nitsche F., **2021**. Extended divergence estimates and species descriptions of new craspedid choanoflagellates from the Atacama Desert, Northern Chile. *European Journal of Protistology 79, 125798. doi: 10.1016/j.ejop.2021.125798*

The study was planned by the author of the present thesis together with Prof. Dr. Hartmut Arndt and Dr. Frank Nitsche. The author performed molecular analyses and wrote the manuscript. Morphological and ecological data were also obtained by the coauthors Lennart Gutsche and Eric Freches.

Part 3

Chapter 7: **Schiwitza S.**, Arndt H., Nitsche F., **2019**. First description of an euryoecious acanthoecid choanoflagellate species, *Enibas tolerabilis* gen. et sp. nov. from a salar in the Chilean Andes based on morphological and transcriptomic data. *European Journal of Protistology 67, 106-113. doi: 10.1016/j.ejop.2018.11.004*

The study was designed and written by the author of the present thesis under guidance of Prof. Dr. Hartmut Arndt and Dr. Frank Nitsche. Cultures and morphological data were gained during author's master thesis. Molecular transcriptome and phylogenetic analyses were performed during the PhD thesis.

Chapter 8: **Schiwitza S.**, Nitsche F., **2021**. A needle in the haystack – Mapping sequences to morphology exemplified by the loricate choanoflagellate *Enibas thessalia* sp. nov. (Acanthoecida, Acanthoecidae). *Protist 172, 125782 doi: 10.1016/j.protis.2020.125782*

The conception and writing was conducted by the author of the present thesis under guidance of Dr. Frank Nitsche. All results were gained by the author.

Erklärung gemäß § 7 Absatz 8 der Promotionsordnung

„Hiermit versichere ich an Eides statt, dass ich die vorliegende Dissertation selbstständig und ohne die Benutzung anderer als der angegebenen Hilfsmittel und Literatur angefertigt habe. Alle Stellen, die wörtlich oder sinngemäß aus veröffentlichten und nicht veröffentlichten Werken dem Wortlaut oder dem Sinn nach entnommen wurden, sind als solche kenntlich gemacht. Ich versichere an Eides statt, dass diese Dissertation noch keiner anderen Fakultät oder Universität zur Prüfung vorgelegen hat; dass sie - abgesehen von unten angegebenen Teilpublikationen und eingebundenen Artikeln und Manuskripten - noch nicht veröffentlicht worden ist sowie, dass ich eine Veröffentlichung der Dissertation vor Abschluss der Promotion nicht ohne Genehmigung des Promotionsausschusses vornehmen werde. Die Bestimmungen dieser Ordnung sind mir bekannt. Darüber hinaus erkläre ich hiermit, dass ich die Ordnung zur Sicherung guter wissenschaftlicher Praxis und zum Umgang mit wissenschaftlichem Fehlverhalten der Universität zu Köln gelesen und sie bei der Durchführung der Dissertation zugrundeliegenden Arbeiten und der schriftlich verfassten Dissertation beachtet habe und verpflichte mich hiermit, die dort genannten Vorgaben bei allen wissenschaftlichen Tätigkeiten zu beachten und umzusetzen. Ich versichere, dass die eingereichte elektronische Fassung der eingereichten Druckfassung vollständig entspricht."

Unterschrift:

Datum: 01.06.2021, Köln

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Education

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10/2015-04/2018	**M.Sc. in Biological Sciences** at University of Cologne, Germany
10/2012-09/2015	**B.Sc. in Biology** at University of Cologne, Germany
08/2002-06/2011	**Abitur** at Alte Landesschule, Korbach, Germany

Employments

since 06/2018	**PhD student** in the group of Prof. Dr. Hartmut Arndt, University of Cologne, Institute of Zoology, General Ecology
01/2017-04/2018	**Research assistance** in the group of Prof. Dr. Hartmut Arndt, University of Cologne, Institute of Zoology, General Ecology
11/2015-07/2016	**Research assistance** in the group of Prof. Dr. Eric von Elert, University of Cologne, Institute of Zoology, Aquatic Chemical Ecology
03/2014-07/2014	**CEPLAS research fellowship** in the group of Dr. Tamara Gigolashvili, University of Cologne, Botanical Institute

External Internships and Expeditions

05/2019	**Atlantic expedition MSM82/2** on the RV Maria S. Merian "Mapping Sequences to Protist Morphospecies"
03/2018	Expedition to the **Atacama Desert**, Chile within the CRC 1211 "Earth – Evolution at the Dry Limit"
07/2017-08/2017	**Atlantic expedition M139** on the RV Meteor "Deep Microbes and Bright Flows"
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11/2016-01/2017	**Leibniz-Institute for Zoo and Wildlife Research**, Berlin, Germany "Investigations on the behavior of *Tachyglossus aculeatus lawesii*, a short-beaked echidna from New Guinea for reproduction success"
03/2015-04/2015	**Nationalpark Kellerwald-Edersee**, Bad Wildungen, Germany "Moss- and Lichenmonitoring"

Conferences and Workshops

23-25/02/2021	**40th Meeting of the German Society for Protozoology (DGP)**, Duisburg-Essen, Germany, online. Talk: "Underestimated species richness of nudiform choanoflagellates?"
22/02/2021	Workshop on digital microscopy, BIIGLE, online
05/12/2019	Training course on real-time PCR gene expression and microarray transcriptome analysis, Cologne, Germany
28-29/11/2019	Workshop on Applied Machine Learning in Taxonomy, Munich, Germany
28/07-02/08/2019	**VIII European Congress of Protistology -ISOP Joint Meeting**, Rome, Italy. Talk: "Spotlight on nudiform choanoflagellates – an evolutionary paradox"
28-29/06/2019	**Taxon-omics SPP 1991 Annual Meeting**, Munich, Germany. Talk: "Integrative taxonomy of protists exemplified by choanoflagellates"
24-27/05/2019	**7th Choanoflagellates & Friends Meeting**, Barcelona, Spain. Talk: "Spotlight on nudiform choanoflagellates – an evolutionary paradox"
20-22/02/2019	**38th Meeting of the German Society for Protozoology (DGP)**, Vienna, Austria. Talk: "Hidden diversity of choanoflagellates from extreme saline environments – Investigation of new species originating from the Atacama Desert"
19/02/2019	Workshop on parasite microscopy, Vienna, Austria
23-27/09/2018	**German Society for Limnology (DGL)**, Kamp-Lintfort, Germany. Poster presentation: "Morphology, systematics and autecology of choanoflagellates from the Atacama Desert"
19-22/08/2018	**II Joint Congress on Evolutionary Biology**, Montpellier, France. Poster presentation: "Morphology, systematics and autecology of choanoflagellates from the Atacama Desert"

Society Membership

German Society for Protozoology (DGP)
International Society of Protistologists (ISOP)

Awards

28/07-02/08/2019	**Holz-Conner Award** at the VIII European Congress of Protistology -ISOP Joint Meeting, Rome, Italy
20-22/02/2019	**Oral Presentation Award 1st Place** at the 38th Meeting of the German Society for Protozoology (DGP), Vienna, Austria