Epizoičke dijatomeje roda Poulinea na glavatim želvama iz Jadranskog mora

Kanjer, Lucija

University of Zagreb
Faculty of Science
Department of Biology

Lucija Kanjer

Epizoic diatom genus *Poulinea* from loggerhead sea turtles in the Adriatic Sea

Graduation Thesis

Zagreb, 2020.

This graduation thesis was made at the Laboratory of Algology, Division of Botany, Department of Biology at the Faculty of Science of the University of Zagreb under the guidance of Asst. Prof. Sunčica Bosak, Ph.D. The thesis is submitted to the Department of Biology at the Faculty of Science of the University of Zagreb for the purpose of acquiring a title/degree of Master of Science in Ecology and Nature Preservation.

ACKNOWLEDGMENTS

I am very thankful to my mentor Sunčica Bosak for being the best mentor not only for this thesis but all the way from the second year of my undergraduate study. Thank you for all the numerous great advice, inexhaustible patience, for always believing in me and challenging me to do better. I really appreciate all the opportunities I was given working alongside you all these years.

Special thanks to Klara Filek for her help in dealing with cultures and molecular techniques. I learned a lot from working with you. I really appreciate all your help during the beginning of your Ph.D. study because I know it was not always easy. Also, thanks to all other members of Laboratory of Algology at Division of Biology (University of Zagreb) for help in the laboratory and creating a warm and welcoming working environment.

Many thanks to Adriana Trotta and Professor Antonio Di Bello and all the staff of the "Sea Turtles Clinics" of the DVM at University of Bari "Aldo Moro" (Italy), for the loggerhead skin and carapace scrapings used for isolation of live cells and morphological analyses.

Big thanks to Karin Gobić Medica, Milena Mičić and other members of the staff from Marine Turtle Rescue Center in Aquarium Pula (Croatia) for the loggerhead skin and carapace scrapings used for morphological analyses.

Thanks to Bojan Lazar from Croatian Natural History Museum in Zagreb (Croatia) for skin scrapings from frozen loggerhead carcasses for diatom community and morphology analyses.

Thanks to prof. Bart Van de Vijver for providing SEM images and a great thanks to Matt Ashworth from University of Texas at Austin (USA) for generous help and conducting bioinformatical analyses and calculating molecular phylogenetic tree. Thanks to all members of TurtleBIOME project whom I did not mention by name and whose help is also of big value for this thesis.

This study was partially supported by Croatian Science Fund (HRZZ) Installation research Grant UIP-05-2017-5635 "Loggerhead sea turtle (*Caretta caretta*) microbiome: insight into endozoic and epizoic communities" - TurtleBIOME project.

Lastly, thanks to my "Flash" friends for all the fun times and for being my second family in Zagreb during the studies. Chris, thank you for believing in me and being there for me when I needed it. And the biggest thanks to my family for their unconditional love and support throughout all the years of my studies and without whom I would not be where I am today.

BASIC DOCUMENTATION CARD

University of Zagreb
Faculty of Science
Division of Biology

Graduation Thesis

EPIZOIC DIATOM GENUS *POULINEA* FROM LOGGERHEAD SEA TURTLES IN THE ADRIATIC SEA

Lucija Kanjer
Rooseveltov trg 6, 10000 Zagreb, Croatia

Genus *Poulinea* is a monospecific epizoic genus of marine diatoms found on sea turtles. It is a part of polyphyletic group of marine gomphonemoids, whose phylogenetical relationships are poorly understood. The aim of this study was to use a combined morphological and molecular approach to investigate *Poulinea* and related genera on loggerhead sea turtles from the Adriatic Sea. Firstly, the contribution of *Poulinea* cells in diatom epizoic communities was assessed using light microscope from seven loggerhead samples obtained in the period 1995-2004. Further on, morphology and morphometrics of *Poulinea*-like cells was assessed on scanning electron microscopy images taken from the first sample set with the addition of 31 loggerhead samples obtained from 2016 to 2019. Lastly, three strains of *Poulinea* were brought into laboratory cultures and information on their morphology and *rbcL*, *psbC* and SSU genes were obtained. *Poulinea* was recorded as a dominant taxon in the epizoic flora on the Adriatic loggerheads. However, four morphological groups of cells could be distinguished in the population of the *Poulinea*-like diatoms with only a single morphotype corresponding to the type species *Poulinea lepidochelicola*, while other groups showed higher variability and were generally more similar to *Chelonicola* spp. The phylogenetic analyses place all three cultured strains closely to other *Poulinea lepidochelicola* strains. This research offers new insights into the morphology and phylogeny of *Poulinea* and related taxa and sheds more light on the ecology of these small and poorly known epibionts.

(60 pages, 29 figures, 13 tables, 106 references, original in English)

Thesis deposited in the Central Biological Library.

Key words: epibiont, *Bacillariophyta*, *Caretta caretta*, polyphasic approach, morphology, phylogeny, culturing

Supervisor: Dr. Sunčica Bosak, Asst. Prof.

Reviewers: 1. Dr. Sunčica Bosak, Asst. Prof

2. Dr. Romana Gračan, Asst. Prof

3. Dr. Tomislav Ivanković, Asst. Prof

Thesis accepted: 06.02.2020.

TEMELJNA DOKUMENTACIJSKA KARTICA

Sveučilište u Zagrebu
Prirodoslovno matematički fakultet
Biološki odsjek

Diplomski rad

EPIZOIČKE DIJATOMEJE RODA *POULINEA* NA GLAVATIM ŽELVAMA IZ JADRANSKOG MORA

Lucija Kanjer
Rooseveltov trg 6, 10000 Zagreb, Hrvatska

Rod *Poulinea* je monospecifični rod dijatomeja koje žive na morskim kornjačama. Rod *Poulinea* spada u polifiletsku skupinu morskih epizoičkih gomfonemoidnih dijatomeja o kojoj nedostaju podaci o filogenetskim odnosima. Cilj ovog rada je kombiniranjem morfološkog i molekularnog pristupa istražiti rod *Poulinea* i srodne svojte na glavatim želvama iz Jadranskog mora. Relativna brojnost roda *Poulinea* u zajednici dijatomeja je istražena iz sedam uzoraka kože želvi prikupljenih u periodu 1995.-2004. koristeći svjetlosni mikroskop. Morfološke analize su napravljene na slikama skenirajućeg elektronskog mikroskopa koje su dobivene iz prvog seta uzoraka uz dodatak 31 uzorka sakupljenog u periodu 2016.-2019. Uzgojene su tri laboratorijske kulture vrste *Poulinea lepidochelicola* čija je morfologija analizirana te sekvencirani *rbcL*, *psbC* i SSU geni. Rezultati su pokazali da su dijatomeje roda *Poulinea* dominantne u epizoičkoj flori na jadranskim glavatim želvama. Međutim, morfološke analize su odvojile četiri različite grupe stanica, samo jedna morfološki odgovara tipskoj vrsti *Poulinea lepidochelicola*, dok ostale grupe pokazuju veću morfološku varijabilnost i sličnije su vrstama roda *Chelonicola*. Tri izolirane kulture prema morfologiji i filogeniji jasno se grupiraju s ostalim pripadnicima vrste *P. lepidochelicola* iz drugih krajeva svijeta. Ovo istraživanje nudi nove uvide u morfologiju i filogeniju roda *Poulinea* i poznavanje ekologije ovih malih i slabo poznatih epibionata.

(60 stranica, 29 slika, 13 tablica, 106 literaturna navoda, jezik izvornika: engleski)

Rad je pohranjen u Središnjoj biološkoj knjižnici.

Ključne riječi: epibiont, *Bacillariophyta*, *Caretta caretta*, polifazni pristup, morfologija, filogenija, kulture

Voditelj: Dr. sc. Sunčica Bosak, doc.

Ocjenitelji: 1. Dr. sc. Sunčica Bosak, doc.

2. Dr. sc. Romana Gračan, doc.

3. Dr. sc. Tomislav Ivanković, doc.

Rad prihvaćen: 06.20.2020.

TABLE OF CONTENTS

1. INTRODUCTION

1.1. Diatom biology

Diatoms are eukaryotic, unicellular, photoautotrophic microorganisms (Round, et al., 1990). They belong to the class *Diatomeae* Durtmortier 1821 (= *Bacillariophyta* Haeckel 1878) within the larger group Stramenopiles that forms cluster SAR together with superclasses Alveolata and Rhizaria (Adl, et al., 2019). Their plastids are of complex evolutional origin, gained via secondary endosymbiosis of green and red algal ancestor (**Figure 1**). Diatom assemblages can be recognized because of the specific brown color that is derived from pigments chlorophyll c_1 and c_3, diatoxantin (unique for diatoms), diadinoxantin, β-carotene and chlorophyll *a* (Benoiston, et al., 2017).

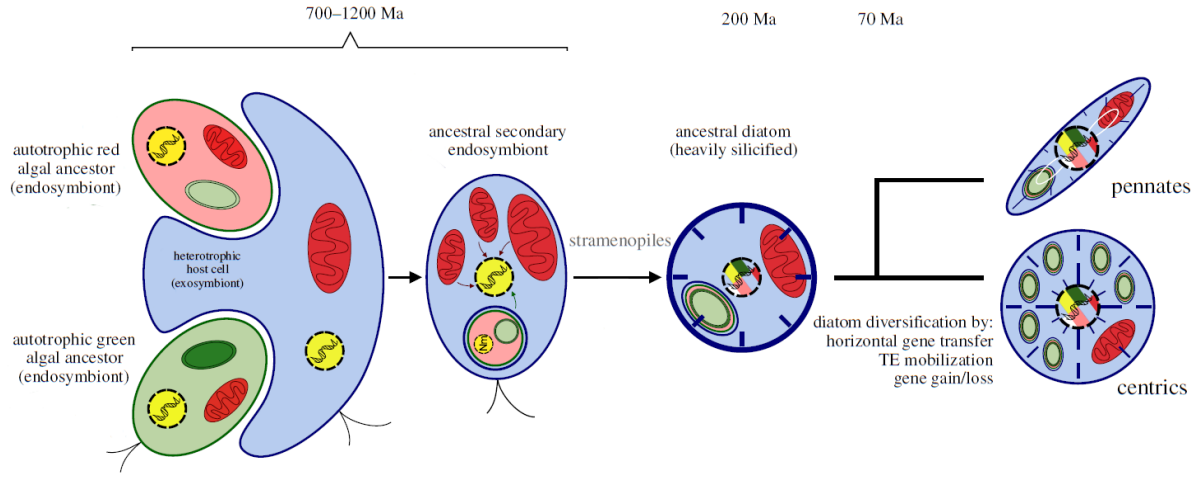

Figure 1. Origin of diatoms through secondary endosymbiosis (Benoiston, et al., 2017).

Hallmark of diatom cell is their uniquely structured cell wall made from organic matrix and silica, which is called frustule (Cox, 2014). Morphology of a frustule is used in traditional diatom taxonomy and systematics. Possession of silica frustule is one of the key reasons why diatoms are successful and dominant over other microalgae in their habitats. Silica frustule requires less energy to synthesize in comparison to the organic cell wall (Round, et al., 1990).

Diatoms are globally widespread in a variety of habitats. As primary producents they are the foundation of food webs in water ecosystems. They are responsible for 20% of the

oxygen produced on our planet (Mann, 1999). There are many ways in which diatoms can contribute to science and industry. In paleontology, diatoms are used for examining past marine/freshwater environments because their frustules remain in sediment and can indicate past environmental factors. It is believed that 90% of diatoms in marine environment are dissolved before burial (Round, et al., 1990). Nevertheless, marine diatom sedimentary record is still invaluable. Diatom sediments are especially well preserved and thus incredibly important for studying the history of freshwater lakes in temperate latitudes. There, diatoms can indicate changes in alkalinity and acidity. Studies of diatoms in recent sediments can be a great help in dating anthropogenic eutrophication, pollution or acidification of lake systems (Round, et al., 1990). In forensics diatoms are used to prove or exclude drowning as a cause of death, moreover diatom species composition in body can even identify in which water mass the body is drowned (Auer, 1991). Diatoms are also interesting in nanotechnology because they make their frustules using nano-scale structures (Bradbury, 2004; Mishra, et al., 2017). In industry diatomaceous earth is used for fine polishing, liquid filtration, food/drink processing, paint, cosmetics, insulation, toothpaste, etc. (Round, et al., 1990; Mishra, et al., 2017).

1.1.1. Frustule morphology

Siliceous part ($SiO_2 \times n\ H_2O$) of the diatom cell wall is called a frustule and its morphology is shown in **Figure 2**. It is composed of two thecae, epitheca which is slightly larger than the other, hypotheca. Each theca consists of one valve and many bands called copulae. The first copula adjacent to the valve is called valvocopula. All copulae together form mantle (lat. mantelum). A valve is made of the central smooth part (annulus) and pores or areolae (sing. areola). Through areolae, the cytoplasm can communicate with the environment. Areolae are organized in rows called striae (sing. stria) and between two striae is siliceous thickening called virga or costa (plur. virgae, costae). The raphe is a longitudinal gap through which cell excretes mucilage for moving on a substrate that only raphid pennate diatoms possess. Fascia is area of thickened silica in the center of a pennate diatom valve, it looks like large virga, area without areolae. Pore field is area with dense pores on one pole of the cell, its function is to produce mucilage or stalks for attaching to substrate. Septum is a sheet of silica extending from the inner wall of the copula, occluding only a part of its length. In contrast, pseudoseptum is the same structure but part of a valve, not copula. Terminal

silica flap is a pocket-like structure that hides distal raphe ends (Cox, 2014; Round, et al., 1990).

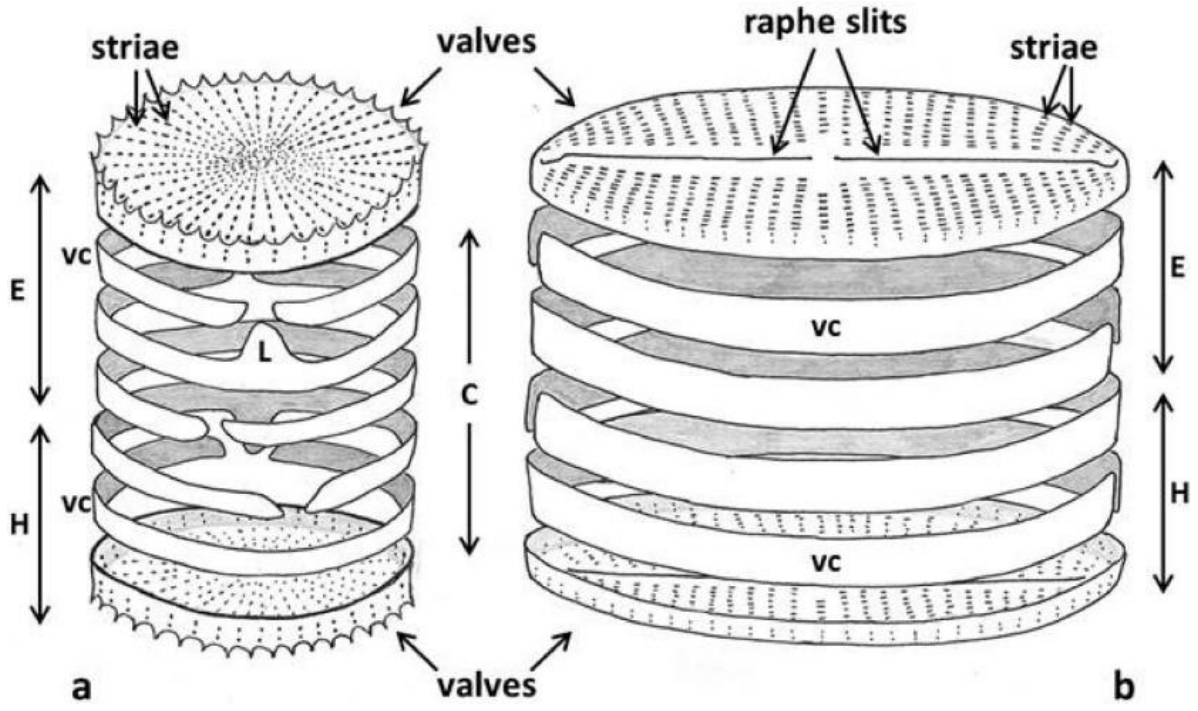

Figure 2. The basic morphology of a diatom cell; a – centric diatom scheme; b – pennate diatom scheme. E – epitheca, H – hypotheca, VC – valvocopula, C – copulae, L – ligula (Cox, 2014).

1.1.2. Evolution and phylogeny

With approx. 10 000 species and many more cryptic taxa (Mann, 1999; Mann & Vanormelingen, 2013) diatoms are part of heterokont algae group (Bhattacharya, et al., 1992; Leipe, et al., 1994; Medlin, et al., 1997) and have Bolidophyceae as their sister group (Guillou, et al., 1999) which can be seen on **Figure 3**. Studies that used molecular clock based on four genes (Kooistra & Medlin, 1996; Medlin, et al., 1997) show that earliest emergence of diatoms on the Earth was 240 Ma (average 165 Ma) and first fossil record of diatoms is dated to 180 Ma (Rothpletz, 1896).

Traditional division of diatoms in two groups, centric diatoms and pennate diatoms, was based on their symmetry, plastid shape and mode of sexual reproduction (Simonsen, 1979; Round, et al., 1990). Following that, centrics are oogamous, radially symmetric and have discoid plastids. In contrast, pennates are isogamous, bilaterally symmetric and have

less plate-like plastids. Moreover, Round et al. (1990) proposed the division of diatoms into three classes: Coscinodiscophyceae (centric diatoms), Fragilariophyceae (araphid pennate diatoms), and Bacillariophyceae (raphid pennate diatoms). Later was shown that both centrics and araphid pennates are paraphyletic groups (Medlin, et al., 1993; Sörhannus, et al., 1995) based on rRNA sequence comparison. In 2004, Medlin & Kaczmarska did a phylogenetic reconstruction of diatoms based on small subunit (SSU) of rRNA genes (18S and 16S). They proposed a new division of diatoms into three classes: Coscinodiscophyceae (radial centric diatoms), Bacillariophyceae (pennate diatoms) and formed new class Mediophyceae (bi- or multi- polar centric and some radial centric diatoms). The newest findings, based on concatenating SSU gene and six chloroplast genes, propose structural graduation theory: from radial centrics arose polar centrics, from polar centrics became araphid pennates and from them arose raphid pennates as youngest and only monophyletic group. A division of diatoms into 9 clades is proposed: three clades belonging to radial centric diatoms, three clades of polar centric diatoms, two clades of araphid pennates and single clade of raphid pennate diatoms as shown in **Figure 4** (Theriot, et al., 2015).

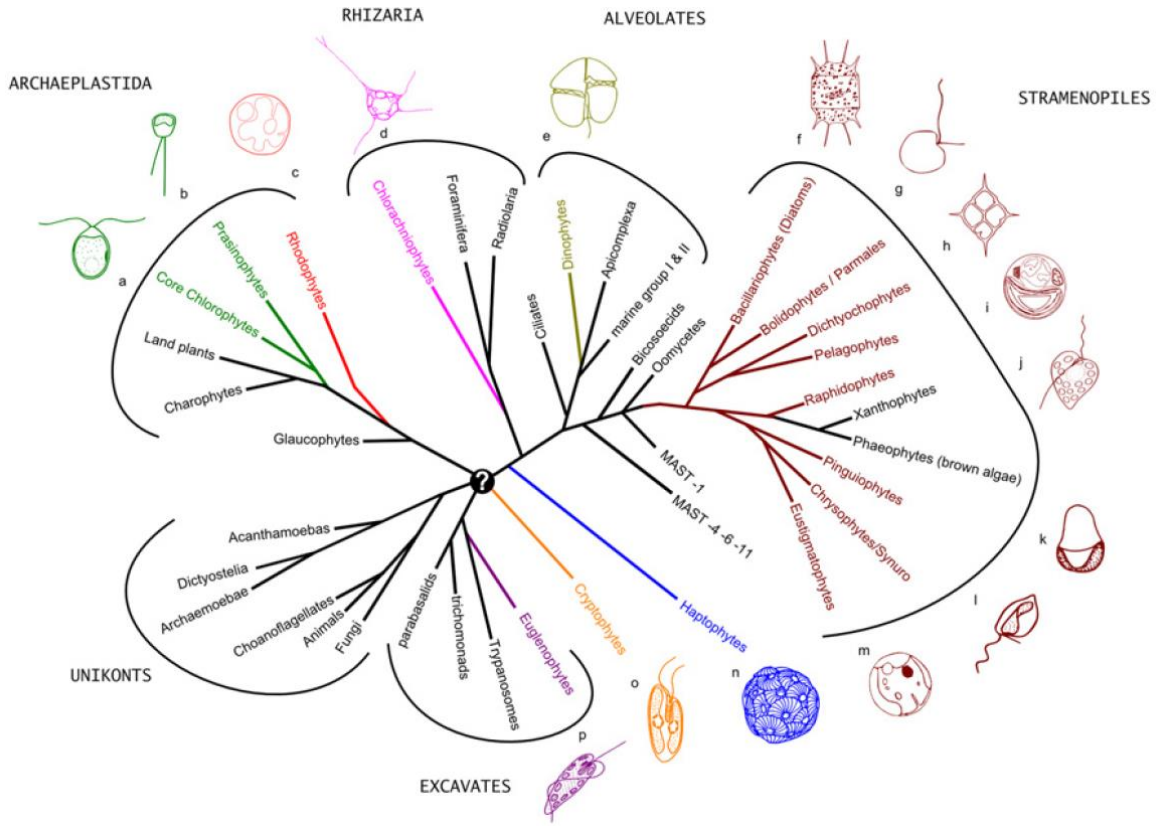

Figure 3. Schematic phylogenetic tree representing position of diatoms (f) among other major algal groups (Not, et al., 2012).

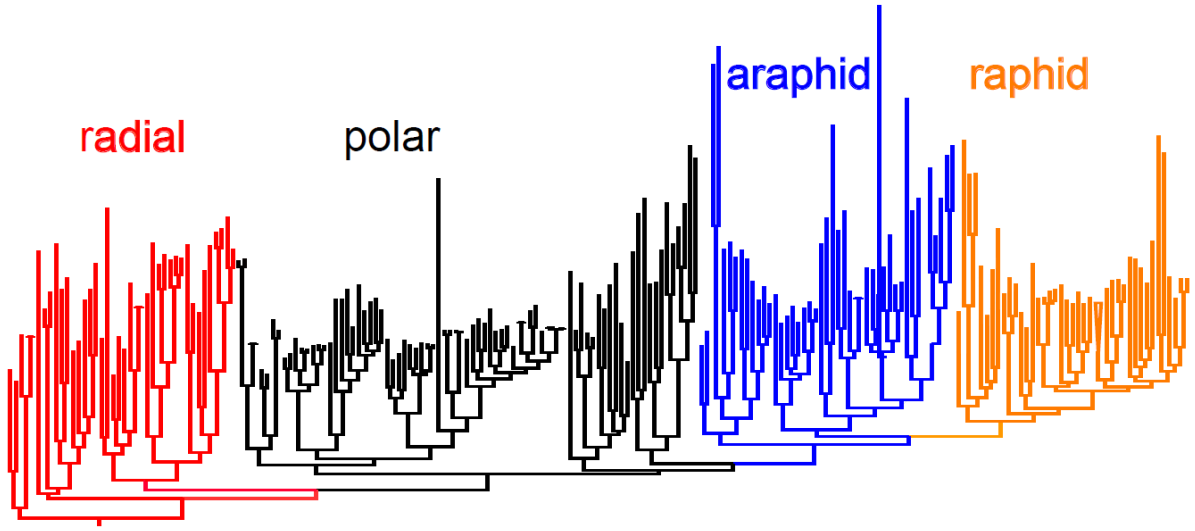

Figure 4. Molecular phylogenetic tree of diatoms showing 9 major clades belonging to radial centric diatoms (colored red), polar centric diatoms (black), araphid pennate diatoms (blue) and raphid pennate diatoms (orange) (Theriot, et al., 2015).

1.1.3. Life cycle

Diatoms are diploid organisms that have mainly asexual multiplication (mitosis). Their life cycle is composed from a long vegetative phase, that can last a few years and short phase of sexual reproduction in which they make gametes through meiosis, which lasts only a few days. During each multiplication, two new thecae must be made inside of the mother cell. New thecae are smaller than older thecae and thus with each new replication daughter cells are getting smaller until they get too small to function properly. The decrease in cell size is signal for producing gametes and beginning of sexual reproduction (Round, et al., 1990). There are three variations of diatom reproduction considering their gametes: isogamic (male and female gamete cannot be distinguished), anisogamic (motile male gametes, non-motile female gametes) and oogamic (small and motile male gamete and large non-motile female gamete). Radial centric diatoms have oogamic reproduction, polar centric diatoms have anisogamic reproduction and pennate diatoms have isogamic reproduction (**Figure 5**). Zygote is produced when two gametes are joined, and in diatoms, zygote is called auxospore. New, large initial (postsexual) cell with a frustule is being made inside auxospore. Asexual enlargement of a cell can also occur but is not that common and the cell does not undergo auxosporulation event. Diatoms can also form resting stages in unfavorable environmental conditions, but these stages are not called auxospores and no cell size restoration occurs (Kaczmarska, et al., 2013).

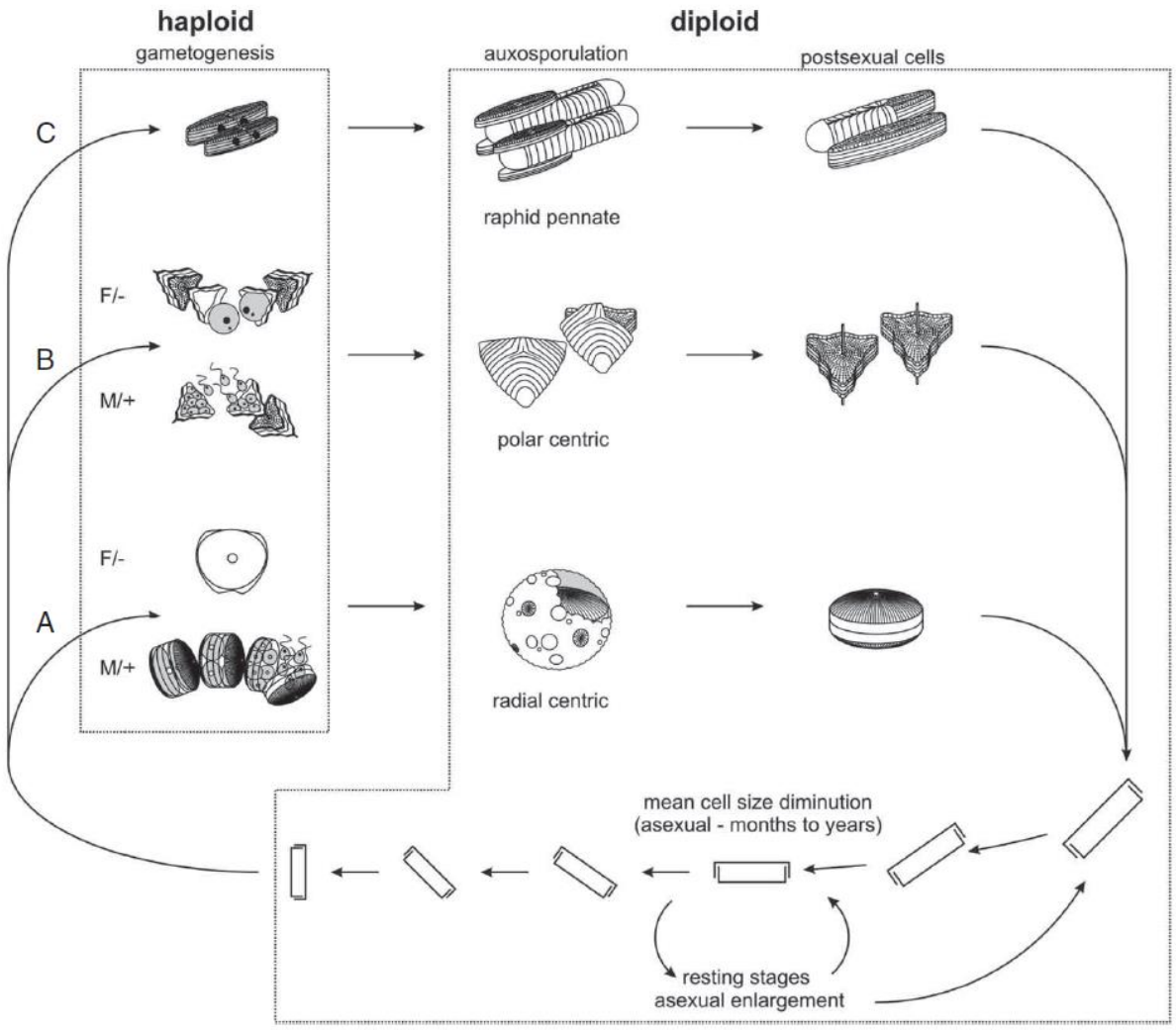

Figure 5. A summary of the principal features of the life cycle of diatoms in three main variants, namely oogamic (A), anisogamic (B) and isogamic (C). F/− & M/+ symbolize female/male or non-motile/motile gametes respectively (Kaczmarska, et al., 2013).

1.1.4. Ecology

Diatoms can be found in virtually all environments with water and light: oceans, freshwater, ice, and moist terrestrial environments. Considering their lifestyle, they can be either planktonic, living in open seas or benthic, living on various surfaces underwater. Furthermore, benthic diatom can be epipsammic (living in sand e.g. *Psammodiscus*), epilithic (on rocks), epiphytic (on algae or seagrass e.g. *Cocconeis*) and epizoic (on the surface of animals e.g *Epipelis*). Planktonic diatoms are far better studied but it is estimated that larger taxonomic diversity can be found in benthic diatoms (Round, et al., 1990).

Diatoms can also be a habitat for the growth of other, smaller algae, choanoflagellates or bacteria which find suitable habitat on diatom's exopolysaccharides (EPS). Some diatoms have cyanobacteria as endosymbionts for nitrogen fixation, and sometimes even diatoms can be endosymbionts in some Foraminifera and dinoflagellates (Round, et al., 1990). The especially interesting and novel field of research is diatom-bacteria interactions. It is believed that they coevolved to actively engage in complicated interactions that significantly modify each other's behavior and ultimately impact biogeochemical cycles. A relatively small set of bacterial taxa likely play a major/significant role in communicating with diatoms (*Alpha-*, *Beta-*, and *Gammaproteobacteria* and *Bacteroidetes* with representation by relatively few genera such as *Roseobacter*, *Sulfitobacter*, and *Flavobacterium*). Both diatoms and bacteria benefit from these interactions by better availability of vitamins, iron, and other trace elements, as well as dissolved carbon and nitrogen compounds (Amin, et al., 2012).

1.1.5. Diatom exopolysaccharides

Diatoms excrete exopolysaccharides (EPS) for growth on various surfaces, motility, and making of complex biofilms (Round, et al., 1990). EPS excreted by diatoms comes in various shapes and forms that range along a continuum from rigid stalks and pads, through mucilage in various stages of hydration (gels, slimes) to colloidal and dissolved carbohydrate components soluble in aqueous media. The state of the polymer is influenced by the chemical composition of the EPS as well as the interaction between molecules of EPS, the environment, and surrounding polymers (Underwood & Paterson, 2003).

Movement of diatoms is achieved by secreting EPS mucilage from raphe slit that hydrates, swells and adheres to the substratum. Then, the cell moves along the line of the raphe by intracellular microfilament bundles that connect the plasma membrane, mucilage and substratum together (Edgar & Pickett-Heaps, 1984). The physical structures produced by diatoms have been called stalks, pads and tubes, trails and extra-cellular matrix (Underwood & Paterson, 2003). The life form of a diatom highly depends on the physical structure of EPS that it produces. Diatoms can be solitary or colonial. Solitary diatoms can be free-living (mostly centric diatoms in plankton) or attached to a surface using different modes. They can be adnate which means that they are firmly attached by their valve face (e.g. *Cocconeis*) or by their girdle bands (e.g. *Amphora*). Second, they can be attached via mucilage pad on one pole and stick to the substrate (e.g., *Diatoma*, *Ulnaria*). The third mode of attachment is by

mucilage stalk excreted through specialized poroids located on apical pore fields. The stalk can be simple, that is, linked to one cell (e.g., *Achnanthidium*) or it can be linked to several cells (arbuscular colonies of *Gomphonema*). Some species of diatoms can link their cells in a colony by silica structures, mucilage or threads of polysaccharides. When a diatom forms a colony, it can be of various shapes, such as chain colony (e.g. *Thalassiosira*), ribbon colony (e.g. *Fragilariopsis*), zig-zag colony (e.g. *Diatoma*), rosette colony (e.g. *Ulnaria*), star colony (e.g. *Asterionella*), arbuscular colony (e.g. *Gomphonema*) and tube-dwelling colony (e.g. *Parlibellus*, *Berkeleya*). The latter ones are particularly interesting because those tubes can become so large that they can be seen macroscopically (Rimet & Bouchez, 2012; Round, et al., 1990).

1.2. Loggerhead sea turtle

There are currently seven species of sea turtles in the world's oceans: leatherback sea turtle (*Dermochelys coriacea*), green sea turtle (*Chelonia mydas*), loggerhead sea turtle (*Caretta caretta*), hawksbill sea turtle (*Eretmochelys imbricata*), olive ridley sea turtle (*Lepidochelys olivacea*), Kemp's ridley sea turtle (*Lepidochelys kempii*) and flatback sea turtle (*Natator depressus*). They are all reptiles belonging to the order Testudines, suborder Cryptodira. Sea turtles emerged on Earth in Late Jurassic, 150 million years ago (Wyneken, et al., 2013).

Loggerhead sea turtle (*Caretta caretta*) is the most frequent sea turtle in the Adriatic Sea (**Figure 6**) named after exceptionally large head with heavy strong jaws in comparison to their size. The carapace is bony, has no ridges and it is heart-shaped. Front flippers have two claws, while rear flippers can have two or three claws. Carapace color is red-brown while the body is yellow-brown. Carapace size of an adult loggerhead is between 80 and 100 cm long and they weight between 70 and 200 kg (Ernst & Lovich, 2009). These animals are carnivorous, and their diet mainly consists of shellfish, which they crush with strong jaws. A turtle's preferred habitats are coastal bays, estuaries and shallow waters along the continental shelf of all three oceans. They nest in an interval of 2 to 4 years, lay 3 to 6 nests per season, each nest containing between 100 and 126 eggs that incubate for 60 days. The estimated global population size is between 40 and 50 thousand nesting females (Wyneken, et al., 2013; Casale, et al., 2018).

Figure 6. Loggerhead sea turtle. Upper left image – adult loggerhead in Turtle Rescue Center in Aquarium Pula. Bottom left image – juvenile loggerhead. Right image – adult loggerhead with rich epizoic community composed of observable algae and barnacles on its carapace (photos: M. Babić).

1.2.1. Mediterranean loggerhead population

All loggerhead sea turtles found living in the Adriatic Sea are a part of a larger, Mediterranean population of loggerheads. In Mediterranean Sea, loggerheads are considered as a keystone species because they connect marine and land ecosystems (Casale, et al., 2018). This population includes numerous free-living healthy adults as well as injured or sick animals recovering in sea turtle clinics across the Mediterranean Sea. This provides great opportunities for studying loggerhead's biology (Trotta, 2020). There are 52 major nesting sites with 6571 average number of clutches each year. Of those nesting sites, 96% are in Greece, Turkey, Libya and Cyprus (**Figure 7**). No nesting activity has been observed for Algeria, Morocco, Monaco or the Eastern Adriatic (Albania, Bosnia and Herzegovina, Croatia, Montenegro, Slovenia) (Casale, et al., 2018). One of the distinctive features of Mediterranean loggerheads is that adult animals are on average smaller in size in comparison to adult animals in other populations. This phenomenon may be happening due to earlier sexual maturation and/or slower growth (Margaritoulis, et al., 2003; Casale, et al., 2011; Casale, et al., 2018). Reduction in size can also happen due to worsening of ecological factors (Casale, et al., 2011) and because of that sea turtles size reduction could act as bioindicator

for environmental changes such as marine pollution (Bjorndal, et al., 2017; Sydeman, et al., 2015) but there are no studies yet on this topic. Currently, positive trends in nest counts have been observed. This could implicate signs of population recovery and good work of protection measures over the last two-three decades since conservation activities started (Casale, et al., 2018).

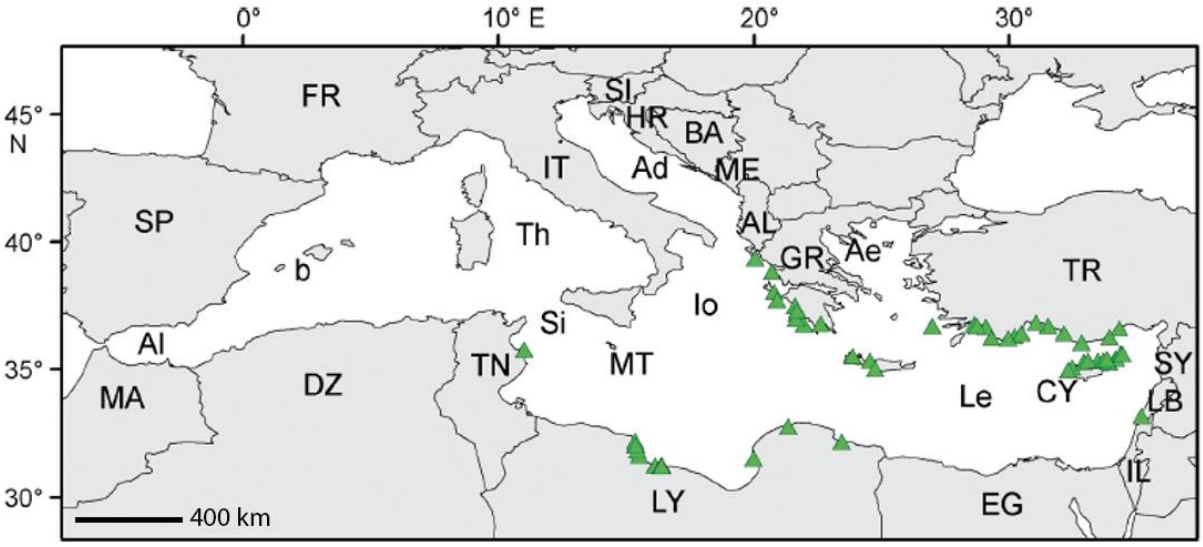

Figure 7. Major nesting sites of loggerhead sea turtles in the Mediterranean countries: AL:Albania; DZ:Algeria; BA:Bosnia and Herzegovina; HR:Croatia; CY:Cyprus; EG:Egypt; FR:France; GR:Greece; IL:Israel; IT:Italy; LB:Lebanon; LY:Lybia; MT:Malta; ME:Montenegro; MA:Morocco; SI:Slovenia; SP:Spain; SY:Syria; TN:Tunisia; TR:Turkey. Marine areas: Ad:Adriatic Sea; Ae:Aegean Sea; Al:Alboran Sea; Io:Ionian Sea; Le:Levantine Basin; Si:Sicilian Strait; Th:Tyrrhenian Sea; b:Balearic Islands (Spain). (Casale et al., 2018)

The Adriatic Sea is a suitable habitat for loggerheads in all stages of their life cycle. In deeper seas of the southern Adriatic young loggerheads are finding a suitable key development habitat in the first years of their lives (Casale, 2010). Juvenile loggerheads usually come to the Adriatic from the largest nesting site in the Mediterranean: Zakynthos, Greece. The north-central Adriatic Sea is the largest continental shelf in the Mediterranean which means that it is a good food source for both sub-adult and adult animals (Margaritoulis, et al., 2003; Lazar, et al., 2000). Adult animals visit the Adriatic mostly in late summer after nesting (Schofield, et al., 2013) or in winter season when they are then more exposed to the risk of bycatch with trawling (Schofield, et al., 2010; 2013; Casale, et al., 2012; 2018). Even though there are no loggerhead nesting sites in the Adriatic Sea, it is an important foraging and development habitat for loggerheads and a key part of their migratory route (Lazar, et al., 2004) as shown in **Figure 8**.

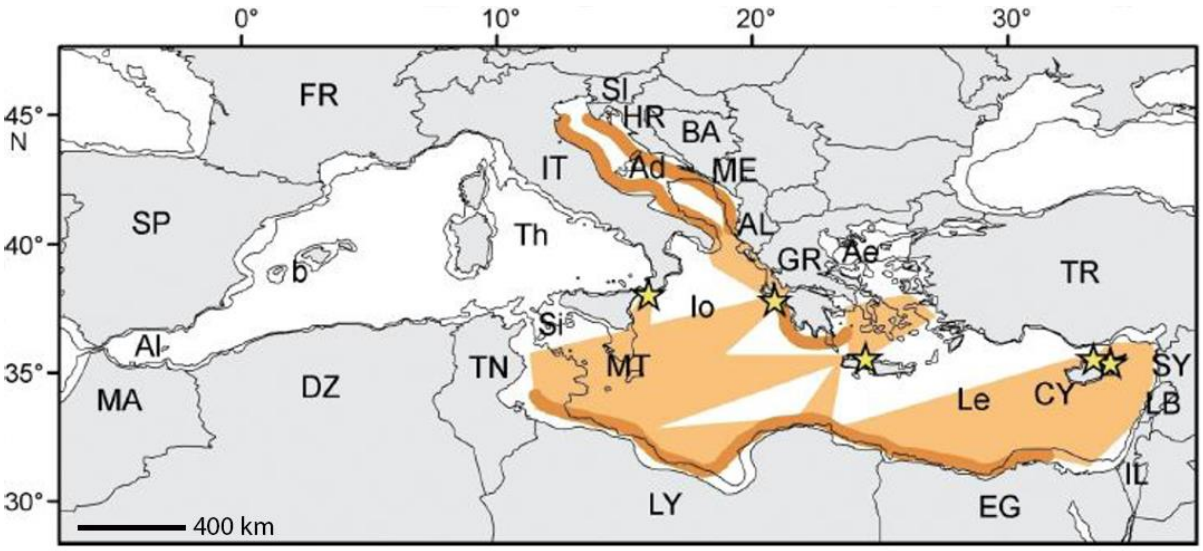

Figure 8. Main known migratory corridors for adult loggerheads (females and males) during reproductive migration from and to the breeding sites (yellow stars). Light brown areas represent migratory funnels in the open sea while darker strips represent paths along the coasts, typically in shallow waters. Country and Sea codes as in Fig. 7 (Casale et al., 2018).

1.2.2. Vulnerability and protection of loggerheads

Regarding non-anthropogenic factors, the greatest threat to loggerheads are predators who feed on their eggs and hatchlings. Those are animals such as red foxes, feral dogs, golden jackals, crabs, rats, and various birds. The rate of this kind of predation in unprotected nests ranges between 38% and 80% (Casale, et al., 2018; Witherington, et al., 2009). The major predators of juvenile loggerheads are animals such as great white sharks (*Carcharodon carcharias*) and Mediterranean monk seals (*Monachus monachus*). However, human's impact cannot be entirely excluded from this even though it is indirect. Cities and villages near loggerhead nests increase the number of animals that are possible land predators, like rats, etc. Moreover, female turtles could be pressured to choose beaches not suitable for nesting because of excessive presence of humans on the coastlines (Lutcavage, et al., 1997; Casale, et al., 2018).

There are various ways in which humans directly affect the loggerhead population's health and survival. The most severe threat is loggerheads being subject to fisheries bycatch which is believed to affect minimally 133 000 turtles in the Mediterranean each year (Cambiè, et al., 2010; Cambiè, et al., 2013; Wallace, et al., 2011; Casale, et al., 2007). These turtles often endure physical injury, drowning or decompression sickness which can cause gas embolism which can injure internal organs and cause death by vascular occlusion or

biochemical changes (Fahlman, et al., 2017). Loggerheads are also threatened by debris ingestion (Lazar & Gračan, 2011) and bioaccumulation and biomagnifying of organic and inorganic contaminants (Lutcavage, et al., 1997; Lazar, et al., 2011; Novillo, et al., 2017).

Because of the reasons mentioned above, loggerhead sea turtle as a species has been globally classified as "vulnerable" on the International Union for Conservation of Nature (IUCN) Red List of Threatened Species (Casale & Tucker, 2017). Moreover, loggerheads are also protected by the Endangered Species Act, Convention of International Trade in Endangered Species of Wild Fauna and Flora (CITES), Barcelona and Bern convention, as well as the European Habitat Directive (European Commision, 2014). In the Mediterranean Sea, the IUCN status of the subpopulation has been recently reclassified as "least concern" which is the result of various and extensive conservation activities. Those activities should not be reduced or abandoned now because Mediterranean loggerheads are considered to be "conservation dependent" and the good status of the population should not be considered as permanent (Casale, 2010; Casale, et al., 2014).

1.3. Sea turtle epibiosis

Any unprotected surface in marine habitat will eventually become home to various microorganisms, algae, and small invertebrates. This type of community on inanimate structures is called "fouling", and on living organisms, it is called "epibiosis", which is by definition an association between two or more living organisms. This relationship consists of host or "basibiont" who supports one or more colonizers called "epibionts". Epibiosis can be classified as mutualism, commensalism or parasitism depending on the ecological interactions between host and its epibionts (Wyneken, et al., 2013 and references therein).

Sea turtles can act as hosts for various epibiotic organisms. For example, over 200 taxa have been known to inhabit the surfaces of loggerhead sea turtle (Wyneken, et al., 2013). Most of them are unspecialized and can also be found on inanimate structures in the surrounding environment. This type of association is called "facultative commensalism" because host turtle receives little to no benefit and epibionts show no substrate specificity (Wahl & Mark, 1999). However, several epibionts are found to live almost exclusively on sea turtles. They are known as "obligate commensalists" because epibionts are dependent on the host for its survival, and the sea turtle is not affected with the presence of these epibionts

(Wyneken, et al., 2013). Coronuloid barnacle *Chelonobia tustudinaria* is an obligate epibiont predominantly occurring on sea turtles, but have also been found on crabs, sirenians, and crocodilians (Newman & Ross, 1976; Zardus & Hadfield, 2004; Wyneken, et al., 2013). Moreover, there are two species of obligately epibiotic crustaceans commonly found on sea turtles: ruby-eyed amphipod *Podocerus chelonophilus* and the robust tanaid *Hexapleomera robusta* (Moore, 1995; Wyneken, et al., 2013). Only one obligately epibiont macroalga, red alga *Polysiphonia carettia*, has been known to live only on sea turtles (Senties, et al., 1999). Parasitic epibionts have also been found on sea turtles. Although they are rare, these parasites cause serious health consequences for their hosts (Greenblatt, et al., 2004).

There are several reasons why epibionts would prefer to live on a turtle instead of on the inanimate surface. The major ones are reduced competition and predation which is highly emphasized in other marine benthic habitats (Wyneken, et al., 2013). Filter-feeding organisms can also benefit from the favorable feeding current on host turtles, while autotrophic epibionts such as algae can profit from increased light exposure (Shine, et al., 2010). Furthermore, by hitchhiking on migratory turtles, their epibionts can gain range expansion and increased genetic mixing. In this way, sea turtles can be considered as long-distance dispersal vectors for marine benthic organisms (Schärer & Epler, 2007; Harding, et al., 2011). However, sea turtle epibiosis can have a few drawbacks for epibionts. For example, a turtle can physically damage epibionts during mating or scraping carapace against a submerged structure. Some epibionts cannot handle desiccation or rapid changes in pressure, temperature, and salinity during turtle dives and migration. There is also a limited choice of potential mates and reduced food access. Those conditions can favor epibionts with asexual reproduction and dietary versatility (Wyneken, et al., 2013). Mostly, epibionts do not alter host turtle's life in a major positive or negative way. However, a turtle can experience some advantages of epibiosis through better camouflage which can be optical, chemical or electrical (Wyneken, et al., 2013). In extreme cases, epibionts can become costly for turtle host when they cause increased weight, drag and disruption of laminar flow over the carapace during swimming. This can cause exceptional energetic costs during long-distance migrations (Logan & Morreale, 1994). Sometimes, epizoic barnacles can become so deeply embedded in the host turtle's tissue that is creates wound on underlying bone. Moreover, some non-parasitic epibionts can act as vectors of pathogens (Greenblatt, et al., 2004; Wyneken, et al., 2013).

Although studying sea turtle epibiosis can never fully replace the use of tag-return data, satellite telemetry, stable-isotope analyses, or population genetics, it can provide a time- and cost-effective method to better understand geographic ranges, habitat preferences and migratory corridors of sea turtles. These data can be implemented in conservation measures of these vulnerable marine reptiles (Wyneken, et al., 2013).

1.3.1. Epizoic diatoms on sea turtles

Before the colonization of macroorganisms, all underwater surfaces undergo a similar sequence of events (Wahl, 1989). The first step is biochemical conditioning of substrate (absorbing dissolved macromolecules), then comes bacterial colonization and the last step is the attachment of unicellular eukaryotes such as diatoms, yeasts, and protozoa. These three steps in sea turtle epibiosis are far more unknown and unexplored in comparison to knowledge gained about macro-epibionts (Wyneken, et al., 2013). Recently there are more and more efforts to close the gap in our understanding of sea turtle micro-epibionts (Majewska, et al., 2015; Robinson, et al., 2016; Majewska, et al., 2017; Rivera, et al., 2018).

Until recently, epizoic diatoms on turtles have been not investigated at all because it was believed that diatom communities on turtles would be the same as diatom communities in nearby benthic environments. However, intensive research started with Wetzel et al. (2010) who described a new freshwater diatom species (*Luticola deniseae* CE Wetzel, Van de Vijver & Ector) found on red-headed amazon river turtle *Podocnemis erythrocephala* (Wetzel, et al., 2010). More recently, attention has been given to diatoms residing in biofilms formed on the skins and carapaces of sea turtles. It resulted in the description of two new genera, *Chelonicola* and *Poulinea*, from olive ridley sea turtles in Costa Rica (Majewska, et al., 2015a). Shortly after that *Tursiocola denysii* (Frankovich, et al., 2015) was described from the neck skin of loggerhead sea turtles in Florida Bay. From then up to today each year would result in several newly described species. New epizoic genus was described from loggerheads in Florida Bay, *Medlinella amphoroidea* (Frankovich, et al., 2016). Two new *Achnanthes* species, *Achnanthes elongata*, and *Achnanthes squaliformis* were discovered from olive ridley sea turtles in Costa Rica (Majewska, et al., 2017c). Two more *Tursiocola* species, *Tursiocola yin–yangii* and *Tursiocola guyanensis* were described from green turtles (*Chelonia mydas*) in French Guiana and Eastern Caribbean (Riaux-Gobin, et al., 2017a). Another species described from green turtles in Costa Rica is *Labellicula lecohuiana*

(Majewska, et al., 2017a). Riaux-Gobin, et al. (2017) described *Tripterion societatis* and *Chelonicola caribeana* from green turtles in the Eastern Caribbean and South Pacific. In 2019, altogether eight new epizoic species on sea turtles over the world have been described. Especially interesting new diatoms from loggerheads in the Adriatic Sea are *Catenula exigua* (Robert, et al., 2019) and *Planothidium kaetherobertianum* (Van de Vijer & Bosak, 2019). *Lucanicum ashworthianum* is a new species from loggerheads in South Africa (Majewska, et al., 2019). Finally, Majewska, et al. (2019) described altogether six new species of *Proschkinia*: *P. browderiana*, *P. lacrimula*, *P. maluszekiana*, *P. sulcata*, *P. torquata* and *P. vergostriata* from various areas around the world.

Poulinea and *Chelonicola* (**Table 1**) with its type species belong to the polyphyletic group of marine gomphonemoid diatoms (**Figure 9**). This group got its name because of similarity to freshwater diatom genus *Gomphonema*, although they are not related. This group is characterized by specific morphologic features like heteropolar valves, wedge cell shape and attachment to the surface with a valve footpole. Together with *Chelonicola* and *Poulinea*, in this group also belong genera like *Tripterion*, *Medlinella*, *Cuneolus*, *Gomphonemopsis*, *Gomphoseptatum*, and *Pseudogomphonema*. Problem of this group is that phylogenetic relationships are not well understood, and sole morphology is often not sufficient enough for solving this problem (Majewska, et al., 2018).

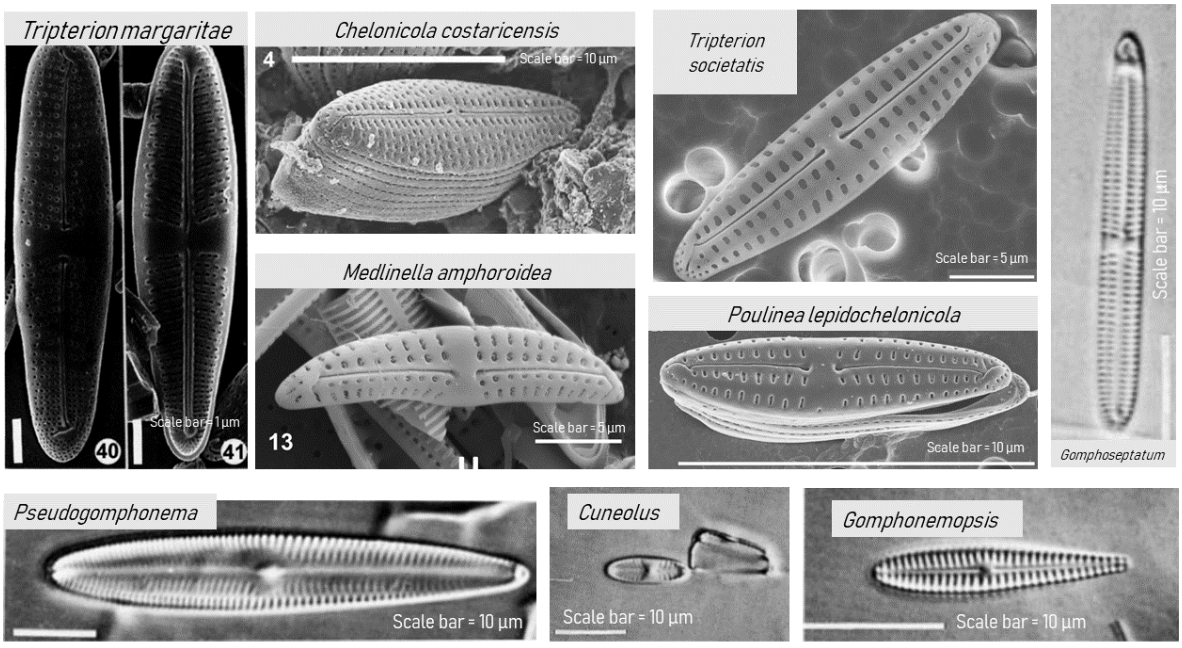

Figure 9. Genera and species of marine gomphonemoids group.

Table 1. Morphological differences between morphologically similar gomphonemoid genera *Poulinea* and *Chelonicola*.

Morphological feature	*Poulinea*	*Chelonicola*	Reference
length	5.2-10.0 µm	6.0–17.5 µm	*Majewska et al, 2015*
width	1.6–2.8 µm	1.7–3.1 µm	*Majewska et al, 2015*
areolae in 10 µm	25-36	36-47	*Majewska et al, 2015*
valve	at least one concave valve	not concave	*Majewska et al, 2015*
external areolae openings	elongate, parallel to stria direction	more or less circular	*Majewska et al, 2015*
girdle band poroids	similar to pores on the valve	different from valve pores	*Majewska et al, 2015*
internal raphe structure	with lateral fissure	with central fissure	*Majewska et al, 2015*
internal polar helictoglossae	Straight	twisted	*Majewska et al, 2015*
structure of a central area	Fascia	no fascia	*Majewska et al, 2015*
growth form	attached without a stalk	attached with a stalk	*Majewska et al, 2015*
number of areolae per stria	2 (3)	3 or more	*Frankovich et al, 2016*
girdle bands	>10, open, occasionally 2 irregular rows of poroids	open, up to 12, one row of poroids	*Riaux-Gobin, 2017*
structure of a central area	not specified	asymmetrically positioned	*Riaux-Gobin, 2017*
striae towards the foot pole	striae somewhat denser	not specified	*Riaux-Gobin, 2017*
terminal endings	covered by a large flap	no flap	*Riaux-Gobin, 2017*
apical pore field	present (but reduced)	no pore field	*Frankovich, (pers.comm.)*
septum	very shallow septa	deeper septa	*Frankovich, (pers. comm.)*

Genus *Poulinea* currently includes a single valid species, *Poulinea lepidochelicola* Majewska, De Stefano & Van de Vijver (Guiry & Guiry, 2020). Based on the original description, this species has cell length 5.2-10.0 μm, width 1.6–2.8 μm and 25–36 striae in 10 μm. It can be differentiated from other gomphonemoid diatoms by its unique set of morphological features. Cells have wedge-shaped to rectangular frustules in girdle view. Typically, one valve is concave while other is straight. Mantle is composed of ten or more open, perforated copulae of different width with occasionally two irregular rows of poroids. Septum is present on valvocopula at headpole and on the second copula at the footpole. Cells have heteropolar valves, headpole is broadly rounded while footpole is more acutely rounded. The raphe is straight or weakly curving, headpole raphe branch shorter than the footpole branch. Apical pore field is absent, but some closely space poroids near footpole are present. Wide fascia is present in the central area. External distal raphe ends are covered by large silica flaps. Striae are weakly radiate near the central area, composed of one row of two elongated areolae. Areolae occluded by hymens (Majewska, et al., 2015a).

After *Poulinea* description from olive ridley sea turtles, diatoms from this genus have been found on all seven sea turtle species across all of the world's oceans (Majewska, et al., 2018; Robinson, et al., 2016). Moreover, they have been observed in large abundances on loggerhead sea turtle, the most common turtle in the Adriatic Sea (Robert, 2019). However, apparently, there is great morphological variability between *Poulinea*-like diatoms that are found on sea turtles (Majewska, et al., 2018). As there are no published molecular sequences to the date, and we are lacking data to describe new species or to expand the description of the current *P. lepidochelicola* species, new combined morphological and molecular analyses are much needed.

1.4. The aim of the research

The main aim of this thesis is to investigate the diatoms belonging to the genus *Poulinea* and other marine epizoic gomphonemoids using a combined (polyphasic) approach that takes into account both their morphology and phylogenetic position. This is the first study that is focused on examination of the members of epizoic gomphonemoids from a variety of samples collected from loggerhead sea turtles residing in the Adriatic Sea. As the preliminary observations by Majewska, et al. (2018) showed a very large morphological variability between the specimens belonging to the geographically restricted diatom populations, there is a question if there is only one species with great morphological plasticity or if multiple species are present.

Specific aims of the thesis:

1. Assess the importance of genus *Poulinea* on loggerheads in the Adriatic Sea by examining their epibiotic diatom assemblages and the presence and role of *Poulinea* in those communities.
2. Contribute to the clarification of morphology confusion among marine epizoic diatoms by statistical analysis of their morphological features.
3. Obtain monocultures of *Poulinea* to visualize live cells, provide new DNA sequences and perceive the placement of *Poulinea* from Adriatic loggerheads on the molecular phylogenetic tree.

Three hypotheses can be put forward:

1. Genus *Poulinea* is a common member of diatom communities on loggerheads from the Adriatic Sea.
2. Two different species of the genus *Poulinea* are found on the carapaces and skins of loggerhead sea turtles in the Adriatic Sea based on morphology and morphometry.
3. Cultured *Poulinea* spp. strains belong to the species *Poulinea lepidochelicola* and will group with other *P. lepidochelicola* strains isolated from different seas on the molecular phylogenetic tree.

Moreover, the results of this research will contribute to the overall better knowledge of epizoic microbial communities associated with loggerhead sea turtles.

2. MATERIALS AND METHODS

2.1. Sampling

All samples have been collected from the loggerhead sea turtles found in the Adriatic Sea. In this thesis, I used three different sets of samples:

1) HPM (hrv. *HPM – Hrvatski prirodoslovni muzej*) set: skin scrapings collected from turtle heads stored in Croatian Natural History Museum (**Table S1**);
2) PTB (pre-TurtleBIOME) set: skin and carapace scrapings collected before 01/03/2018 from live loggerheads from Marine Turtle Rescue Center in Aquarium Pula (Croatia) (**Table S2**);
3) TB (TurtleBIOME) set: skin and carapace scrapings collected after 01/03/2018 (starting date of the project) from live loggerheads from Marine Turtle Rescue Center in Aquarium Pula, (Croatia) and Sea Turtle Clinic, Department of Veterinary Medicine, University of Bari "Aldo Moro" (Italy) (**Table S3**), these samples are collected under necessary permits as part of TurtleBIOME project – see acknowledgments.

HPM samples were collected from 14 beached turtles found across the eastern Adriatic coast in the period from 1995 to 2004. Each sample represents one turtle. After necessary analyses and autopsies, the loggerhead heads were preserved in the fridge at -20°C until epizoic sample collection. The sampling was done on 23/11/2016 using ethanol sterilized scissors, scalpels and tweezers. The surface skin from the neck and beak scrapings were collected from heads (**Figure 10B, C**) and stored in 250 mL plastic containers preserved with 4% formaldehyde at 4°C.

PTB set of samples consists of 7 samples. Each sample represents one turtle. Neck skin and carapace scrapings on a live animal were obtained with cotton swabs and clean toothbrushes, respectively. Samples were preserved in 4% formaldehyde and stored in 250 mL plastic containers at 4°C.

Samples from TB set include 25 samples that were sampled from 16 turtles. Here each sample represents either carapace (odd number of the sample) or skin scraping (even number of the sample). TB set was obtained from live turtles and was collected using the non-invasive method by brushing an area on the turtle neck/flipper skin and carapace with a single

use toothbrush (**Figure 10E, D**). For diatom morphology, samples were stored in 50 mL Falcon tube in 96% ethanol and put on ice (-20°C). For live-cell isolation and establishment of monocultures, samples were put in seawater and kept in a culture room (21°C on a 12:12 dark-light cycle).

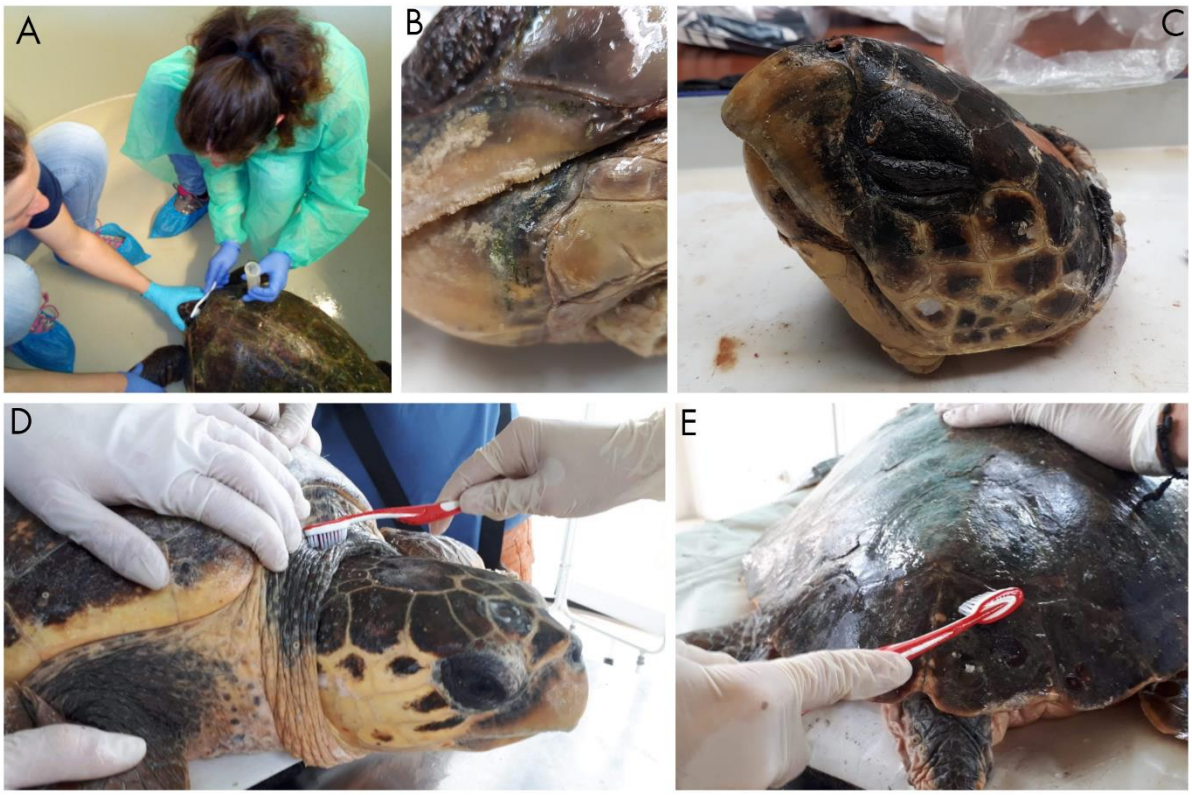

Figure 10. Sampling biofilm from loggerhead sea turtles. A - sampling in Aquarium Pula, Croatia (photo: R. Gračan). B – visible green biofilm on dead turtle's beak (photo: S. Bosak). C – picture of dead turtle head from Croatian Natural History Museum in Zagreb (photo: S. Bosak). D – scraping skin biofilm from the live turtle in Bari Sea Turtle Clinic, Italy (photo: A.Trotta). E – scraping carapace biofilm from live turtle in Bari turtle hospital, Italy (photo: A.Trotta).

2.2. Preparing samples for microscopic observation

In order to visualize details of a diatom cell, all organic matter must be removed by oxidation with only silica frustules remaining in the samples. Using Pasteur pipette, scissors and tweezers, I put part of the sample in 30% nitric acid (HNO_3) in 250 mL glasses. The sample to acid volume ratio was 1:5 to 1:10, depending on subjective assessment of organic matter in a sample (Graef, et al., 2013; Potapova, et al., 2014). Samples with acid were heated on a hot plate until all skin and carapace pieces were dissolved, approx. 1h (**Figure 11A**).

After cooling down, I added deionized water (dH_2O) to fill the glass and left it 48h in order to sediment diatom frustules. This method is used on epizoic environmental samples because it is harsh and cleans up organic matter really good. For monoculture samples I used another method with less harsh chemicals because the quantity of organic matter is lower in those samples. Monoculture samples were mixed with an oversaturated water solution of $KMnO_4$ in ratio 1:1 and left overnight (**Figure 11B**). Then I added the same volume of 35% hydrochloric acid (HCl). This was heated on ethanol burner until it changed color from brown to transparent (Simonsen, 1974; Hasle, 1978; Taylor, et al., 2007). After that, I removed supernatant using a vacuum water pump and left only 15 mL of cleaned diatom sample in acid. Samples were moved to 15 mL plastic Falcon epruvette and centrifuged for 15 min on 2500 rpm. After that, I removed supernatant and added up to 14 mL dH_2O. I repeated the sedimenting and washing process four more times or until pH value was close to 7, which I checked using indicator paper.

Figure 11. Light and electron microscopy and sample preparation. A – cleaning carapace sample from organic matter in HNO_3. B – cleaning culture samples in $KMnO_4$. C – preparing permanent microscopic slides for LM (photo: A. Žiroš). D – preparing samples on filters for SEM. E – observing samples on SEM (photo: S. Bosak).

For light microscopy I made permanent slides (**Figure 11C**). I put 2 mL dH_2O on cover glass (24×40 mm) and added 10-500 μL of cleaned sample, depending on the density of diatom in sample (white precipitate). I heated the cover glass on a hot plate until all water vaporized and left only diatom cells. I mounted those slides in Naphrax (Brunel Microscopes Ltd., UK) and again heated slides on the hotplate. After short cooling down, I put permanent slides in the slides box.

For scanning electron microscopy observations of the cultivated strains, I filtered 10-500 μL cleaned samples and 20 mL dH_2O through 3-μm Nucleopore (Nucleopore, Pleasanton, CA, USA) polycarbonate membrane filters (**Figure 11D**). Filters are stored in closed plastic Petri dishes. For SEM observation coating was done with palladium using a Precision Etching and Coating System, PECS II (Gatan Inc., CA, USA). Diatom specimens were analyzed with JEOL JSM-7800F scanning electron microscope in the Department of Physics, Centre for Micro and Nano Sciences and Technologies, University of Rijeka (**Figure 11E**). SEM images of HPM, PTB and TB set used for the morphometric and morphological analyses were obtained and shared by collaborators within TurtleBIOME project (see Acknowledgements).

2.3. Diatom community analysis

Diatom community was qualitatively and quantitatively analyzed using microscopic slides on light microscope Zeiss AxioVision A2 with 100× oil immersion and 40× objective and photographed using ZEN Imaging software 2.5 (Carl Zeiss Microscopy GmbH, Germany). Approx. 400 cells were counted and identified in a transect. Representation of a species in the diatom community is expressed as relative abundance. Relative abundance of a species is calculated as a species count divided by total cell count in a sample. Species were identified following Alvarez-Blanco & Blanco (2014), Witkowski, et al. (2000), and Al-Yamani & Saburova (2011). Only HPM samples were counted, the analyses of PTB and TB sample counts are out of the scope of this thesis.

2.4. *Poulinea* cell morphology analysis

Altogether 231 diatom cells from 34 turtle samples were analyzed for morphology (**Table 2**). I used SEM images because they show cells in much greater detail than LM images. Valve length, width, foot pole length, head pole length, number of copulae, number of areolae in stria, septum size and number of areolae in 10 μm were measured. I also recorded whether a cell has a pore field, at least one concave valve, areolae shape, shape of copula pores, presence of fascia and presence of terminal silica flap. Those characters were chosen because they are different between *Poulinea* and *Chelonicola* (**Table 1**), two similar genera. There are also some other characters that can distinguish between the two genera but due to my lack of experience I could not objectively notice the difference (for example internal raphe structure and internal polar helictoglossae) and it was not measured or recorded.

Table 2. Layout of the number of measured cells for morphological analyses from SEM images from different loggerhead samples. Total number of measured cells = 231; total number of samples = 34.

Sample	Number of cells measured	Sample	Number of cells measured
HPM9	39	TB13	8
HPM25	8	TB14	1
HPM69	17	TB19	2
PTB17	3	TB25	6
PTB24	44	TB31	1
PTB29	8	TB33	1
PTB31	5	TB49	5
PTB33	9	TB55	3
PTB42	7	TB56	1
TB3	11	TB73	1
TB4	1	TB74	7
TB5	2	TB89	5
TB7	1	TB90	14
TB8	4	TB115	3
TB9	7	TB116	1
TB11	2	TB117	1
TB12	2	TB118	1

After the measurements, I divided cells into four groups based on presence or absence of pore field and number of pores in a stria. I choose these characters because they are discrete variables, the most controversial in differentiating two genera and most easily spotted on all cells. I also recorded characters like length, width, footpole/headpole ratio, number of striae in 10 μm and they were used to calculate differences in-between the four groups. Characters like copula number, septum size, concave valve, presence of fascia and presence of terminal silica were not visible in the majority of SEM photograph hence are not good characters to base groups on them. Characters like areola shape, copula pores shape, presence of fascia and presence and terminal silica are not reliable as other characters because it is sometimes hard to decide which state of the character is present on each cell.

2.5. Establishing monocultures

For obtaining good monocultures I needed to isolate single diatom cell without any contaminates. I used a glass micropipette with a flexible latex tube attached to a mouthpiece (**Figure 12A**) under the inverted light microscope Olympus CKX41. Isolated diatom cells were transferred to f/2 + Si medium (Guillard, 1975; Guillard & Ryther, 1962) in separated sterile well plates or Petri dishes and in cell culture flasks (**Figure 12B**) (Andersen, 2005). Cultures were kept in the culture room (**Figure 12C**) at 21 °C on a 12:12 dark-light cycle at 15 μmol m^{-2} s^{-1}. Three strains (PMFTB0073, PMFTB0074, and PMFTB0077) were isolated from one skin sample of a loggerhead sea turtle named Iracus (sample TB90, **Table S3**). Strain PMFTB0073 and PMFTB0074 were isolated on 07/02/2019, and strain PMFTB0077 on 27/02/2019.

To obtain enough biomass (>100 mg) for DNA extraction cultures were grown in culture flasks and collected by scraping those flasks. Due to slow growth and tendency of benthic diatoms to attach to surfaces, cells were grown for several weeks and collected weekly. First, I inoculated the wanted culture into a cell culture flask. The culture was grown for 7-10 days before the cells adapt and become dense. I scraped dense cultures with >23 cm long plastic scrapers that are clean and sterile. I transferred all of the culture with media to sterile 50 mL Falcon tube. I added fresh media to the scraped flask, put back into the culture room and repeated procedure after 7 days. Scraped cells in Falcon tubes were left overnight in culture room to sediment. The next day, I carefully removed the supernatant by vacuum suction. I transferred sedimented cells to a sterile 15 mL Falcon tube and centrifuged at 3000

g for 10 min. After that, most of the supernatant was removed, approx. 100 µL was left to resuspend the sedimented cells. I transferred resuspended pellet to 2 mL Eppendorf tube and stored at -20°C. I repeated the collection of cells until >500 µL of pellet was collected.

Figure 12. Culturing diatoms. A – isolating single cells on inverted microscope using a micropipette and mouthpiece. B – growing diatom cultures in flasks for collecting biomass. C – culture room.

2.6. Molecular methods

DNA was extracted from the strains following the QIAGEN DNeasy Plant Mini Kit protocol with following modifications: in order to break the diatom cells 400 µL of AP1 buffer was added to the samples which were then vortexed horizontally for 10 minutes; after that, 4 µL of RNase A was added, the samples were vortexed briefly and incubated at 65 °C for 10 min. The rest of the DNA extractions steps were followed from the manufacturer's instructions. The extracted DNA was used directly in downstream analyses or stored at -20°C.

From the extracted DNA gene markers *rbcL*, *psbC* and SSU we amplified using polymerase chain reaction (PCR). For each 50 µL PCR reaction the following reagents were used: 25 µL of Takara Emeraldamp Master Mix 2x, 2 µL of DNA template, 1 µL of forward and 1 µL of reverse primers (0.2 µM final concentration) and 21 µL of sterile dH_2O. Primer

pairs used for initial PCR reactions for SSU were PT1/PT2, PT5/PT6 for *rbcL*, and PT8/PT9 for *psbC* gene. Subsequent nested PCR was performed for all the genes of interest with different primer pairs; PT3/PT4 for SSU, PT5/PT7 for *rbcL*, and PT10/PT11 for *psbC*. Initial PCR thermocycling conditions were set to 30 cycles: 10 sec at 98 °C for denaturation, 60 sec 60 °C for annealing, and elongation at 72 °C, 3 min for SSU and 2 min for *rbcL* and *psbC*. Nested PCR was prepared in the same way as initial PCR but with DNA template from the initial PCR reaction. Thermocycling conditions remained similar except for the annealing temperature which was changed at 65 °C for SSU, 52 °C for *rbcL* and 54 °C for *psbC*. PCR products were visualized on 1% agarose gel by electrophoresis. In order to prepare the agarose gel, 45 mL of TBE buffer was mixed with 0.48 g of agarose and heated in the microwave until agarose dissolved. After dissolving the agarose 3 μL of Midori green dye was added, the gel was poured in a gel tray with a gel comb and was used after it solidified. The samples and 200 bp DNA ladder (Takara) were loaded into the wells and ran for 20 min at 100 V. The DNA in the gel was visualized under UV light. Samples with successful amplification were then purified using Macherey-Nagel NucleoSpin Gel and PCR Clean-up kit and protocol. Purified SSU, *rbcL* and *psbC* DNA was then sent for Sanger sequencing (Macrogen) with the following primers: SSU PT3, PT4, PT16-24; *rbcL* PT5, PT7, PT12, PT14; *psbC* PT5, PT7, PT12, PT14 (see **Table S4**).

Following phylogenetic analyses were done by Matt Ashworth from the University of Texas in Austin (USA). Phylogenetic analysis of the DNA sequence data was conducted using a concatenated three-gene dataset: nuclear-encoded SSU rRNA, *rbcL*, and *psbC*. SSU sequences were aligned using the SSUalign program (Nawrocki, 2009), with the covariance model based on the ten diatoms included with the program download and twenty-three additional diatoms. Initially eight separate partitions were created for the data: SSU paired and unpaired sites and the first, second, and third codon positions for each of the protein-coding genes (*rbc*L and *psb*C). Partition Finder results (Lanfear, et al., 2014), using the AICc criterion, suggested not combining any of the partitions in the analysis. This dataset and partitioning scheme were analyzed using the GTR+G model under maximum likelihood using RAxML ver. 8.2.9 (Stamakis, 2014) compiled as the thread-AVX version on an Intel i7 based processor in Linux Mint 18. 24 replicates each were run with 500 rapid bootstrap (BS) replicates with maximum likelihood (ML) optimization. BS support was assessed using the BS replicates from the run, producing the optimal ML score.

2.7. Data analysis

I recorded *Poulinea* cell morphology measurements using the program MS Excel 2013. I have used programming language R and R Studio interface for calculating descriptive statistics and plotting the results. I used package ggplot2 for boxplots, dot-plots and bubble-plots. The Analysis of Variance (ANOVA) and Student's t-test were calculated in MS Excel 2013 to analyze and visualize resemblance between groups. Hierarchical cluster analysis (CLUSTER) was used in Primer-e Version 7 (Clarke & Gorley, 2015). I used only data for continuous variables (length - L, width – W, length/width ratio – LW, footpole/headpole ratio – FH, and striae density in 10 μm – S) without missing values. CLUSTER was calculated based on Euclidian distance on group average mode. Also, I used Principal Components Analysis (PCA) to visualize how the samples will group and to know which variables are responsible for the separation. Only continuous variables (L, W, LW, FH, and S) with no missing data were used. Prior to PCA, I transformed data using square root transformation. After the removal of missing values, 128 cells from 27 turtle samples (**Table 3**) were left and analyzed using PCA and CLUSTER analyses.

Table 3. Layout of the number of analyzed cells for PCA and CLUSTER analysis from different loggerhead samples. Total number of analyzed cells = 128; total number of samples = 28.

Sample	Number of analyzed cells	Sample	Number of analyzed cells
HPM9	19	TB9	6
HPM25	8	TB11	1
HPM69	6	TB12	1
PTB17	1	TB13	3
PTB24	21	TB19	1
PTB29	2	TB25	4
PTB31	5	TB33	1
PTB33	7	TB49	2
PTB42	5	TB55	3
TB3	4	TB74	4
TB4	1	TB89	5
TB5	1	TB90	11
TB7	1	TB115	3
TB8	1	TB116	1

Not all analyses could be performed on every set of samples. The detailed layout is shown in **Table 4**. Diatom community analysis was done only on samples from HPM set while analyzing communities of PTB and TB samples is out of the scope of this thesis and these results will be analysed in separate publications in the future. For morphological analyses I used SEM images from all three sets (HPM, PTB, and TB) and the exact number of measured cells from each sample is shown in **Table 2**. Measurements from those cells are used for descriptive statistics and plots. From those measurements, I removed any missing data for PCA and CLUSTER analyses to obtain reliable results from these analyses. Removal of missing data resulted in a reduced number of samples from which the data came from (**Table 3**). Lastly, the three isolated monocultures of interest were successfully obtained only from one sample from the TB set, and that is TB90.

Table 4. The layout of performed analyses and samples used for this study.

Analysis	Turtle sample set	Description
Diatom community species identification	HPM	14 turtle samples, 7 samples contained diatoms (HPM9, HPM25, HPM33, HPM48, HPM69, HPM70 and HPM71)
Morphology: descriptive statistics and plots	HPM, PTB, and TB	231 cells from 34 samples (see **Table 2**)
Morphology: PCA and CLUSTER	HPM, PTB, and TB	128 cells from 28 samples (see **Table 3**)
Culturing, DNA isolation and phylogeny	TB	3 cultured strains (PMFTB0073, PMFTB0074, and PMFTB0077) from the sample TB90

Additionally, it is important to note that sampling, photographing of the SEM images and constructing molecular phylogenetic tree were done by collaborators within TurtleBIOME project (see acknowledgments).

3. RESULTS

3.1. Diatom community analysis

Seven out of 14 museum samples contained diatoms. Genus *Poulinea* was dominant on 6 out of 7 samples but was present in all analyzed samples. The relative abundance of *Poulinea* cells and other genera is shown in **Figure 13**.

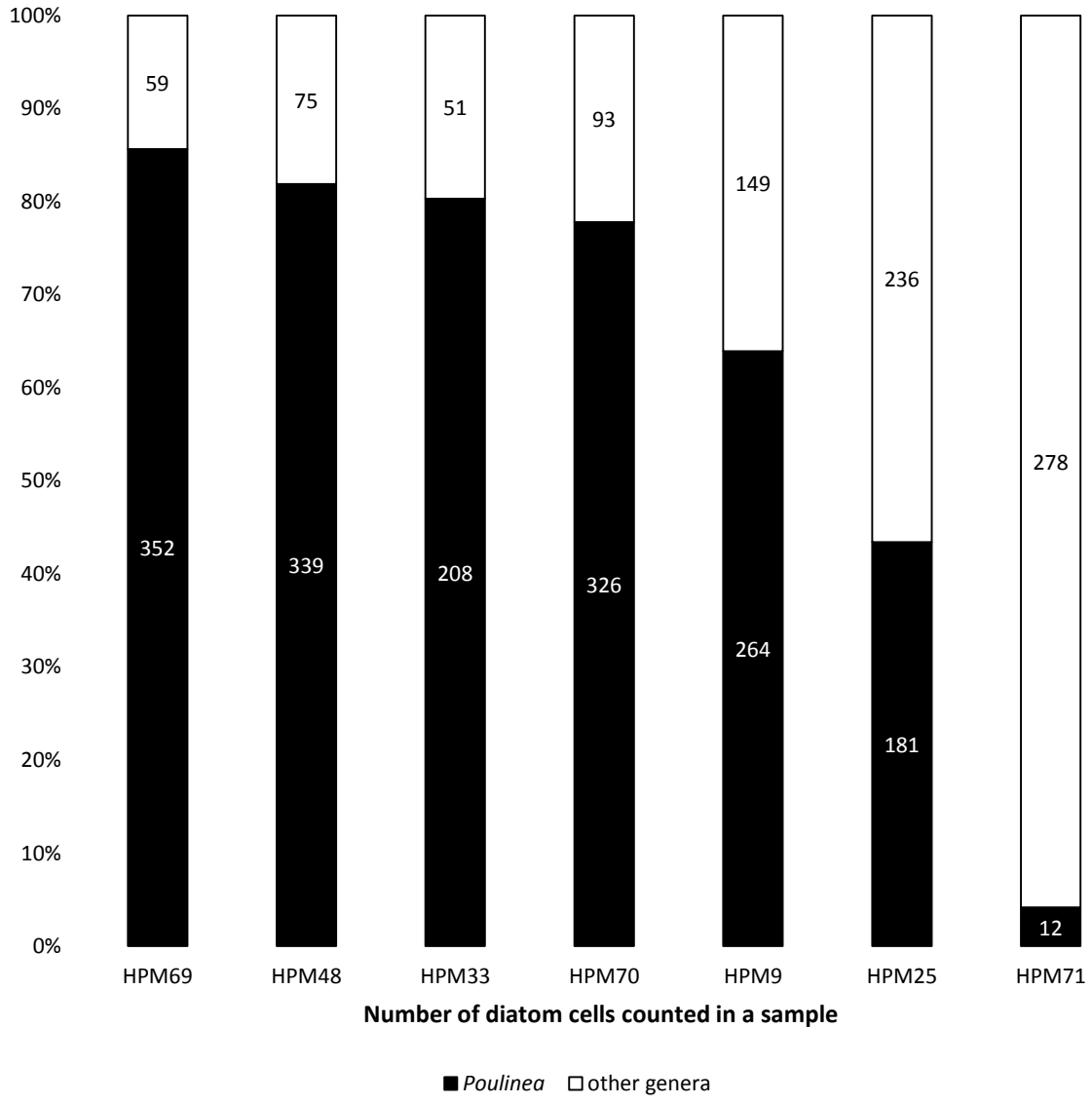

Figure 13. The relative abundance of *Poulinea* spp. and other genera on turtle head scrapings from HPM samples. Numbers on the bars represent the number of cells counted in each category on 400 cells in transect.

Altogether 35 genera were observed in HPM samples. The average number of genera per turtle is 12.4. Turtle HPM9 had the least genera, only six. The highest number of genera was observed in the sample HPM70, 17 genera. Together with *Poulinea,* only *Navicula* and *Amphora* occurred in all samples. Genera *Astartiella*, *Dimmeregrama,* and *Haslea* were found only on turtle HPM69. Genera *Bidulphia*, *Denticula,* and *Melosira* were found only on turtle HPM70. Genera *Cyclophora* and *Fragilariopsis* were found only on turtle HPM25. *Psammodyction* and *Stauroneis* were found only on turtle HPM48. *Bacillaria*, *Mastogloia* and *Tursiocola* were found only on turtle HPM33. Genus *Proschkinia* was found only on turtle HPM71.

Achnantes elongata, *Catenula* sp. and *Tursiocola* sp. are epizoic genera that are found on HPM samples. *A. elongata* was found on samples HPM9, HPM25, HPM48, HPM33 and HPM71. *Catenula* sp. was found on samples HPM70 and HPM33. *Tursiocola* sp. was found on sample HPM33. All other genera are typical benthic genera.

3.2. Morphological measurements

Four morphological diatom groups were established based on presence of apical pore field and number of areolae per stria. They are named G1, G2, G3 and G4 and description of their morphological features is shown in **Table 5**. SEM images of the most representative cells from each group are shown in **Figure 14** for G1, in **Figure 15** for G2, in **Figure 16** for G3, and in **Figure 17** for G4.

Table 5. Diatom groups and their characteristics.

group	Pore field	Number of areolae per stria
G1	No	2
G2	No	3 or more
G3	Yes	2
G4	Yes	3 or more

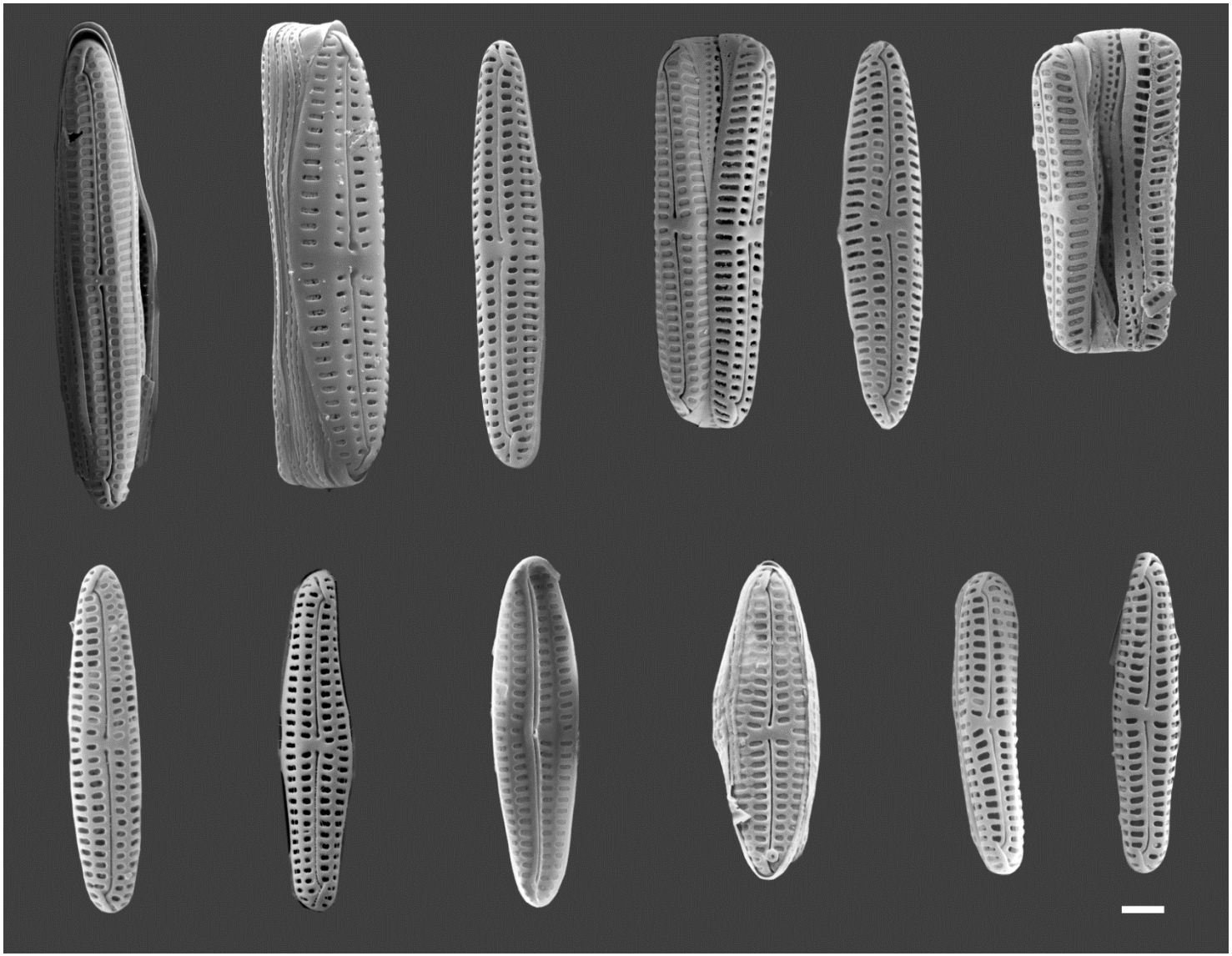

Figure 14. Scanning electron microscopy images of diatoms cells from group 1 (G1), scale bar = 1 μm.

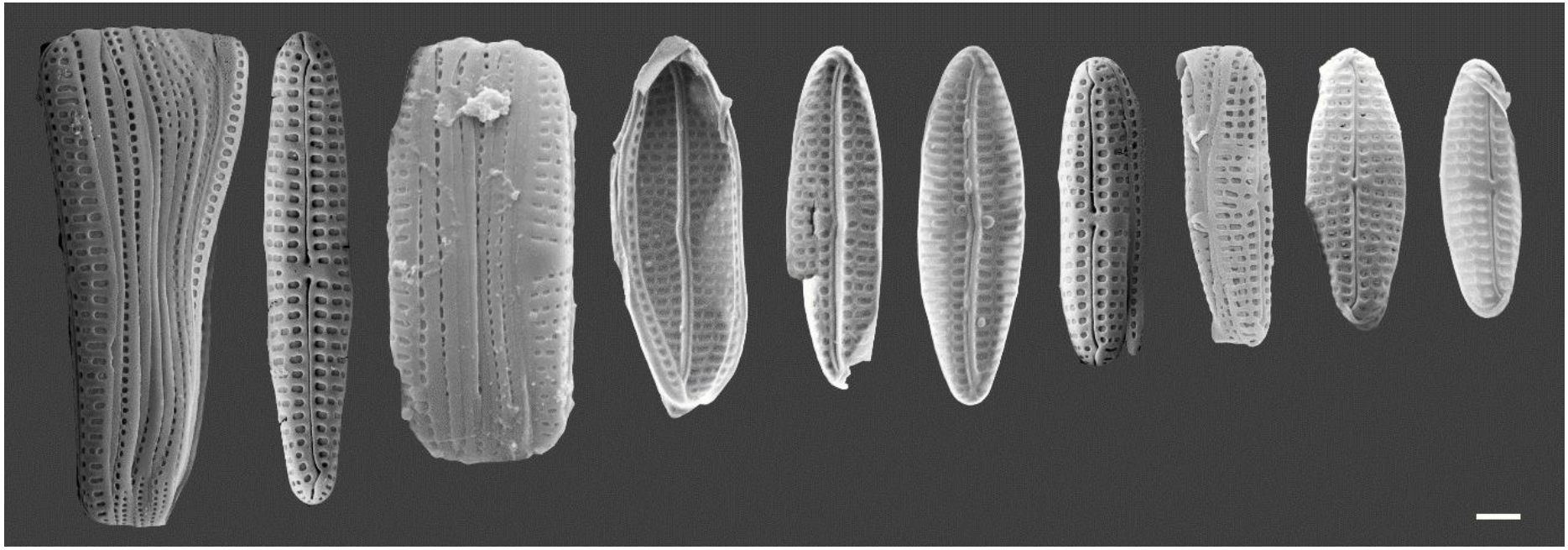

Figure 15. Scanning electron microscopy images of diatoms cells from group 2 (G2), scale bar = 1 μm.

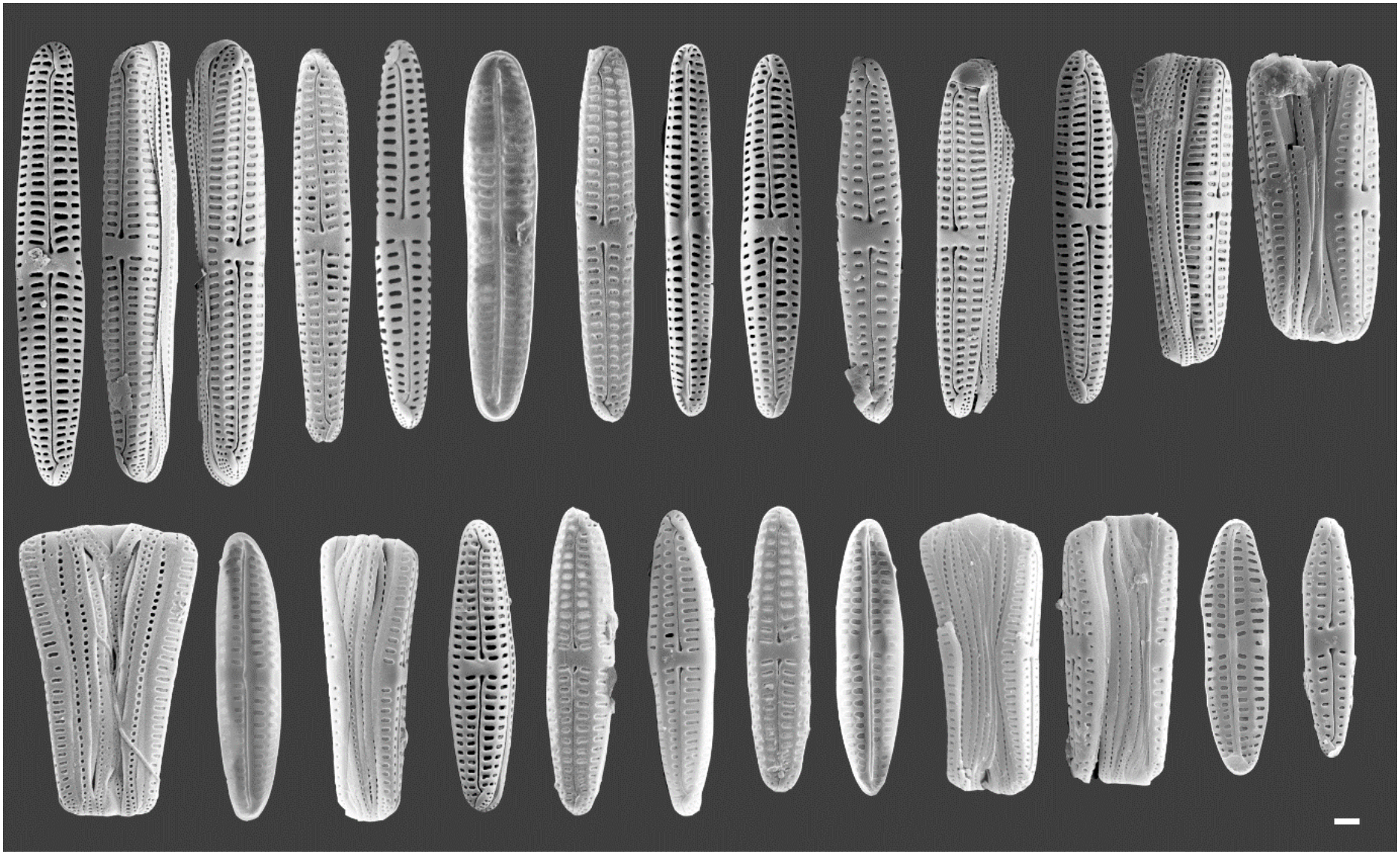

Figure 16. Scanning electron microscopy images of diatoms cells from group 3 (G3), scale bar = 1 µm.

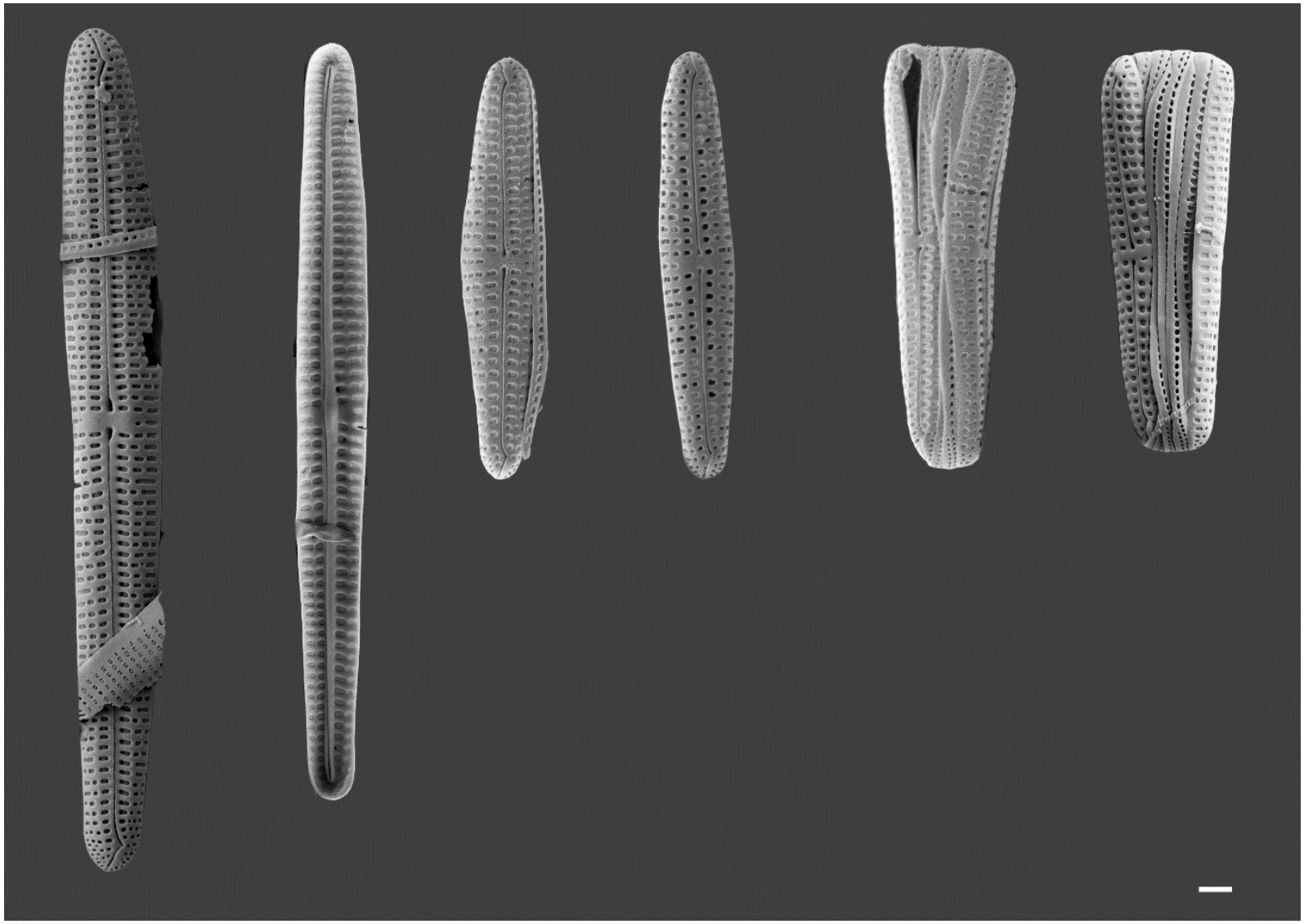

Figure 17. Scanning electron microscopy images of diatoms cells from group 4 (G4), scale bar = 1 μm.

Cell length values are higher in groups G3 and G4 than G1 and G2. Same pattern can be observed for cell width and length/width values. In contrast number of striae in 10 μm is higher in G1 and G2 than in G3 and G4. Average value for footpole/headpole ratio is the highest in G4 and lowest in G2, but there is little difference for this value between groups. There were too little data for G2 and G4 (only for one cell each group), but G1 and G3 have similar number of copulae although there were also very little data for those groups, too. Exact values are shown on boxplots in **Figure 18** and written in **Table 6** and **Table 7**.

Table 6. Descriptive statistics (maximum, minimum, average and standard deviation) for cell length (L), cell width (W) and length to width ratio for four diatom groups (G1, G2, G3, and G4).

	Cell length (L)				**Cell width (W)**				**Length/width ratio (LW)**			
group	**G1**	**G2**	**G3**	**G4**	**G1**	**G2**	**G3**	**G4**	**G1**	**G2**	**G3**	**G4**
max	14.34	11.28	25.14	25.83	3.78	2.87	6.21	3.08	7.55	5.60	10.14	10.61
min	5.21	4.12	7.56	9.33	1.29	1.57	1.86	1.32	2.08	2.18	1.90	3.53
mean	8.47	7.43	13.08	15.28	1.92	2.09	2.62	2.35	4.69	3.63	5.17	7.66
SD	1.83	1.81	2.66	6.50	0.42	0.35	0.57	0.57	1.06	0.89	1.39	2.82
N	86	26	99	10	52	17	56	7	52	17	54	7

Table 7. Descriptive statistics (maximum, minimum, average and standard deviation) for footpole to headpole ratio (FH), number of copulae (C) and number of striae in 10 μm for four diatom groups (G1, G2, G3 and G4).

	Footpole/headpole ratio (FH)				**Number of copulae (C)**				**Number of striae in 10 μm (S)**			
group	**G1**	**G2**	**G3**	**G4**	**G1**	**G2**	**G3**	**G4**	**G1**	**G2**	**G3**	**G4**
max	1.28	1.09	1.25	1.15	12	9	12	7	60	44	31	32
min	0.81	0.80	0.70	0.98	4	9	5	7	16	20	18	18
mean	1.01	0.99	1.05	1.07	6.94	9	7.67	7	35.87	35.46	24.41	27.90
SD	0.08	0.07	0.08	0.05	2.13		1.41		6.55	5.84	2.63	4.09
N	86	26	97	10	18	1	36	1	86	26	101	10

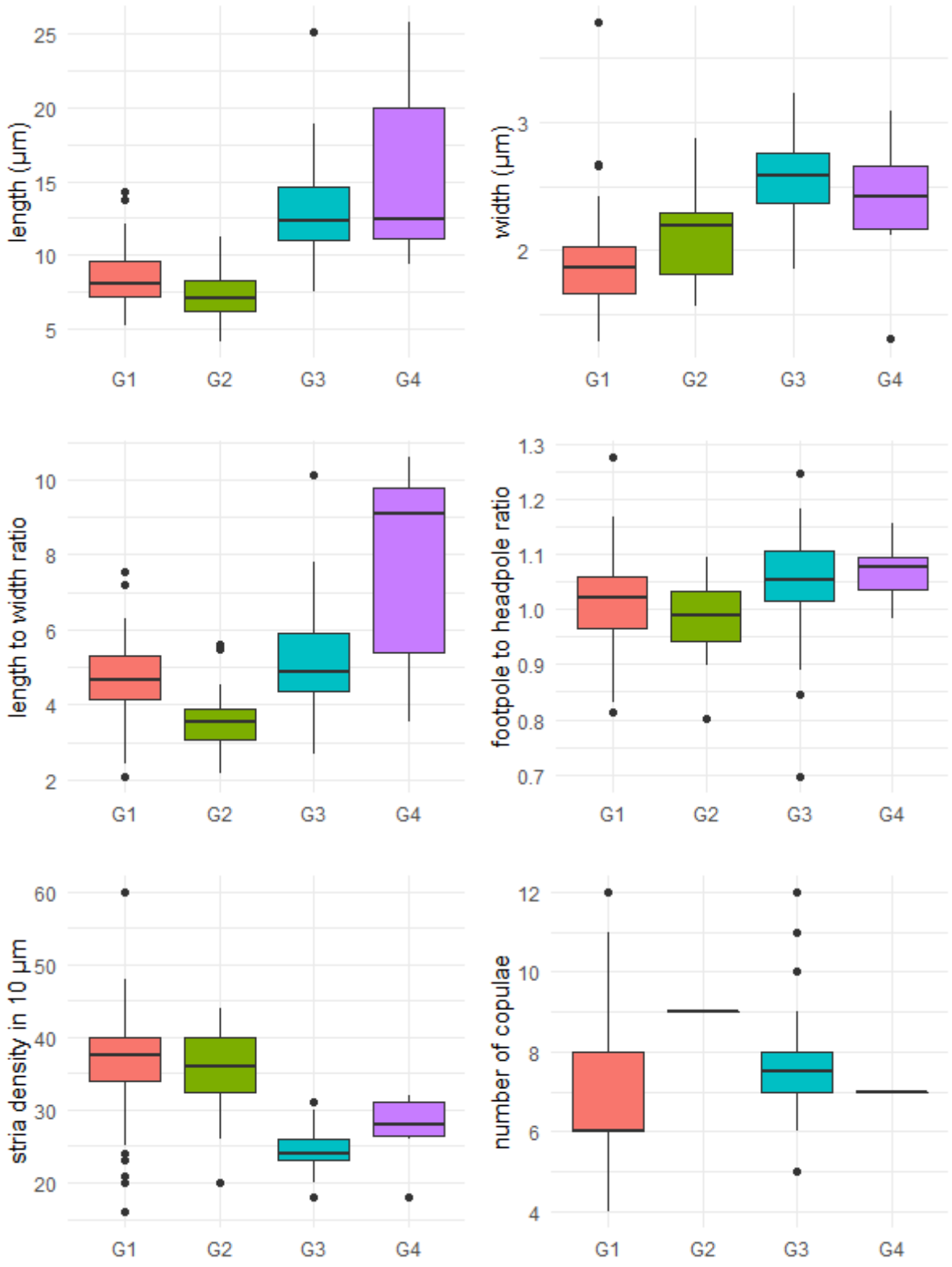

Figure 18. Box and whiskers graphical representation of morphological characteristics of four different diatom groups (G1, G2, G3, and G4). Upper left: cell length in µm. Upper middle: cell width in µm. Upper right: cell length to width ratio. Bottom left: striae density in 10 µm. Bottom middle: footpole to headpole length ratio. Bottom right: number of copulae.

After performing the ANOVA test for L, LW, FH and S variables I found out that there is a significant difference between groups considering every variable that I tested. We reject null hypothesis that there is no difference in cell length between groups with p-value 5.07×10^{-33}, for cell width 2.27×10^{-10}, length to width ratio 4.8×10^{-9}, footpole to headpole ratio 2.82×10^{-5} and striae density 2.62×10^{-39}. To find out exactly between which groups there are differences I used Student's t-test. For this test null hypothesis is that there are no differences between groups. To rule out the null hypothesis absolute value of absolute t-statistic must be lower than the value of t critical two-tail value, as well as p-value must be lower than alpha (0.05). For cell length, there are differences between every group except between G3 and G4 (**Table 8**). Differences in cell width were confirmed by the t-test only between G1 and G3, and G2 and G3 (**Table 9**). Differences in L/W ratio are confirmed for groups G1 and G2, G1 and G4, G2 and G3, and G2 and G4 (**Table 10**). For the F/H ratio significant differences exist for G1 and G3, G1 and G4, G2 and G3 and G2 and G4 (**Table 11**). Significant differences between groups based on striae density exist for every pair of groups except for G1 and G2 (**Table 12**).

Table 8. Matrix with Student's t-test results (t-stat) for cell length (L) values between groups G1, G2, G3 and G4. Red-colored numbers indicate values of t-statistics where the null hypothesis is rejected, meaning there are differences between two groups based on t critical two-tail value, p-value and alpha value = 0.05.

	G1	G2	G3	G4
G1				
G2	2.545			
G3	-13.863	-12.680		
G4	-3.304	-3.767	-1.066	

Table 9. Matrix with Student's t-test results (t-stat) for cell width (W) values between groups G1, G2, G3 and G4. Red-colored numbers indicate values of t-statistics where the null hypothesis is rejected, meaning there are differences between two groups based on t critical two-tail value, p-value and alpha value = 0.05.

	G1	G2	G3	G4
G1				
G2	-1.700			
G3	-7.317	-4.660		
G4	-1.960	-1.136	1.181	

Table 10. Matrix with Student's t-test results (t-stat) for length to width ratio (LW) values between groups G1, G2, G3, and G4. Red-colored numbers indicate values of t-statistics where the null hypothesis is rejected, meaning there are differences between two groups based on t critical two-tail value, p-value and alpha value = 0.05.

	G1	G2	G3	G4
G1				
G2	4.110			
G3	-1.906	-5.371		
G4	-2.742	-3.707	-2.301	

Table 11. Matrix with Student's t-test results (t-stat) for footpole to headpole ratio (FH) values between groups G1, G2, G3, and G4. Red-colored numbers indicate values of t-statistics where the null hypothesis is rejected, meaning there are differences between two groups based on t critical two-tail value, p-value and alpha value = 0.05.

	G1	G2	G3	G4
G1				
G2	1.718			
G3	-3.517	-4.513		
G4	-2.987	-3.958	-0.754	

Table 12. Matrix with Student's t-test results (t-stat) for striae density in 10 µm (S) values between groups G1, G2, G3 and G. Red colored numbers indicate values of t-statistics where null hypothesis is rejected, meaning there are differences between two groups based on t critical two tail value, p-value and alpha value = 0.05.

	G1	G2	G3	G4
G1				
G2	0.305			
G3	15.217	9.411		
G4	5.404	4.374	-2.645	

After removal of missing data, 128 measurement data was added to PCA and CLUSTER analysis. Out of those data, 52 were from G1, 17 from G2, 52 from G3 and 7 from G4. Figure **19** shows chart on principal components PC1 on x-axis and PC2 on y-axis. Cumulative percentage of variation explained by PC1 and PC2 is 95.4%. PC1 explains 74.4% variability, while PC2 explains 21.1% of variability. Eigenvectors on PC1 with absolute value >0.5 are S (-0.758) and L (0.599) while PC2's eigenvectors with absolute value >0.5 are S (-0.603), L (-0.570) and LW (-0,545).

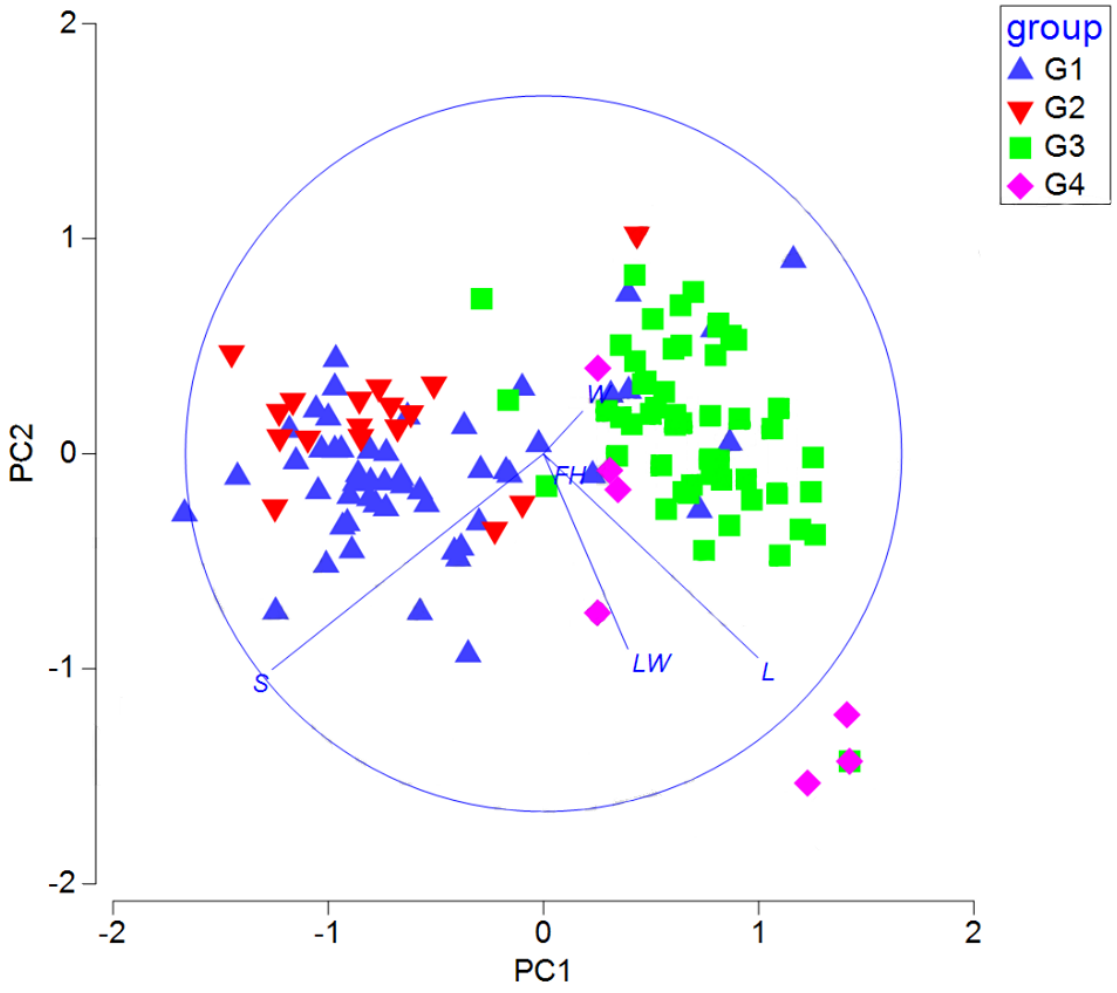

Figure 19. Principal Component Analysis of diatom cell measurements. PC1 74.4% variation, PC2 21.1% variation, cumulative variation of PC1 and PC2 is 95.4%. Each mark represents one diatom cell. Variables: L – cell length (µm), W – cell width (µm), LW – cell length to width ratio, FH – cell footpole to headpole length ratio, S – number of striae in 10 µm.

CLUSTER dendrogram shows separation of two main branches. First one (left on **Figure 20**) is mix of all four groups, but mostly G1 and G2. Second branch (right on **Figure 20**) contains mostly diatom cells from G3. G4 data are scattered on dendrogram without a regular distrubution.

After running PCA and CLUSTER analysis I found out that variables L and S are the ones that explain the separation of diatom groups into G1, G2, G3, and G4. Distribution of groups among samples is shown in **Figure 21**. The distribution of these variables can be shown on plots in **Figure 22** and **Figure 23**.

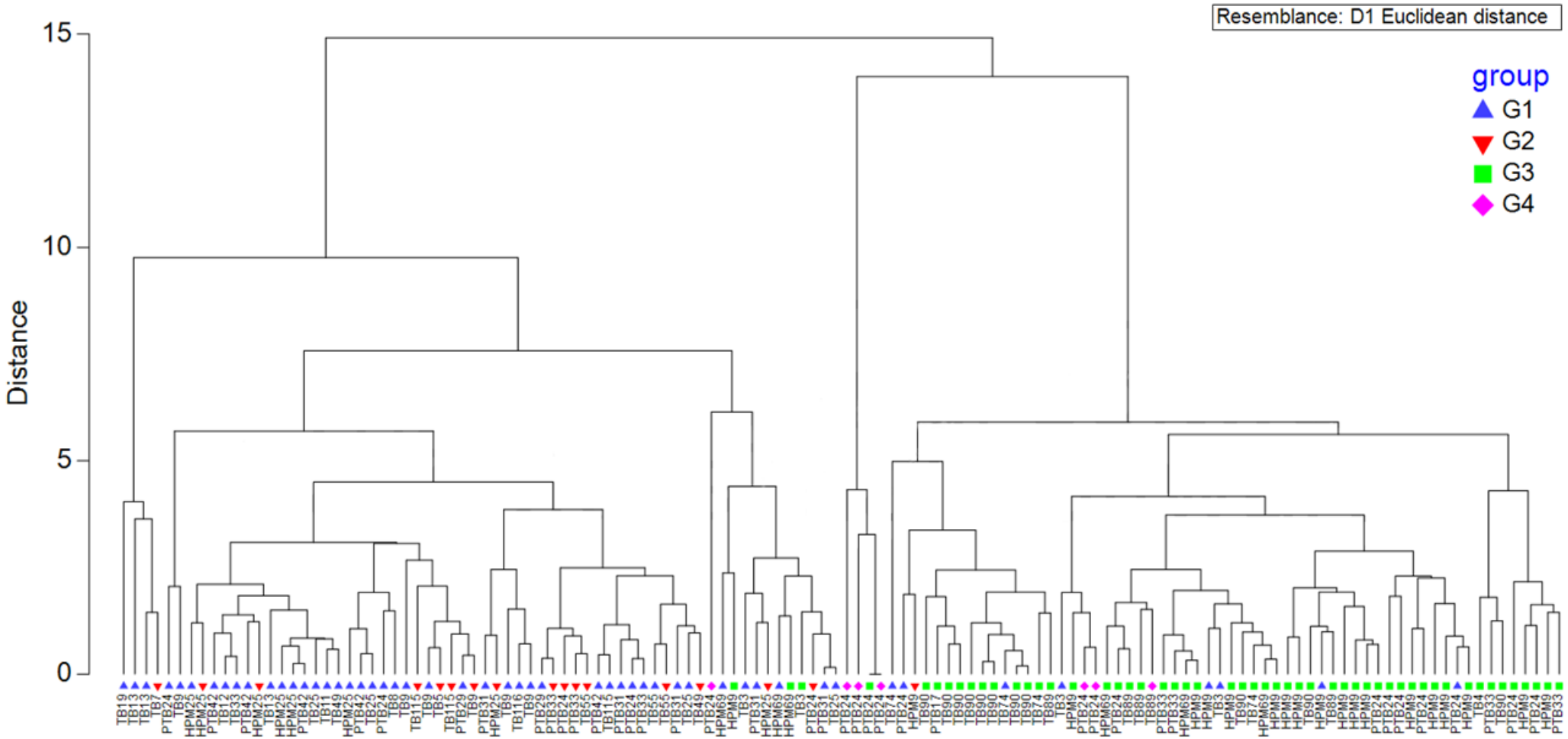

Figure 20. Hierarchical cluster analysis (CLUSTER) dendrogram. Each mark represents one diatom cell. X-axis show to which sample cell belongs to, shape of the mark represents to which group it belongs, and on y-axis value of Euclidean distance is shown.

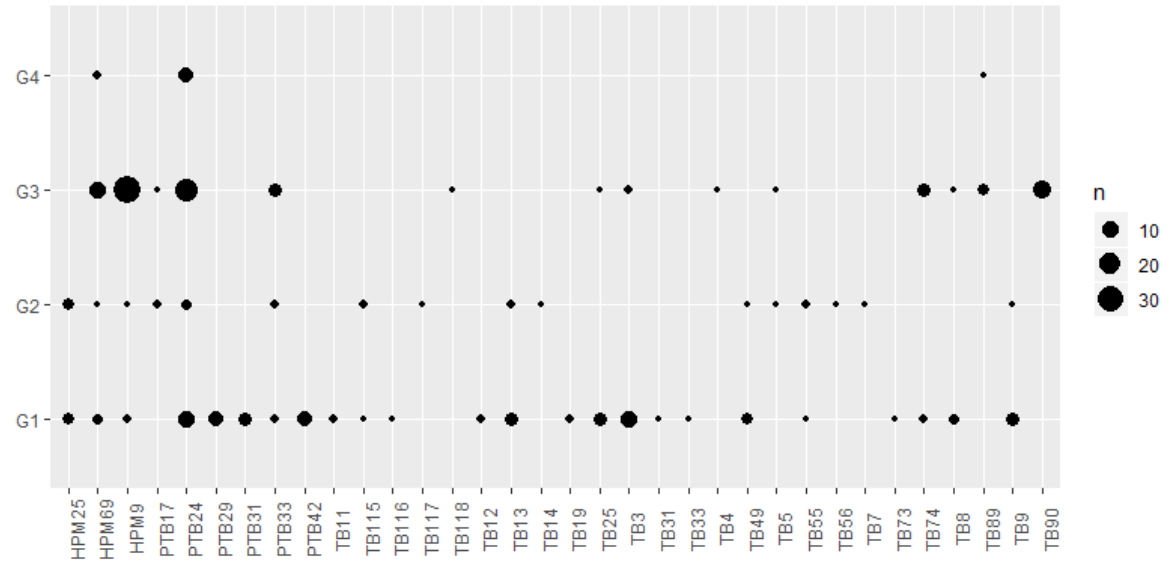

Figure 21. Dot plot showing the distribution of groups G1, G2, G3, and G4 in each sample. The size of each dot corresponds to the number of cells.

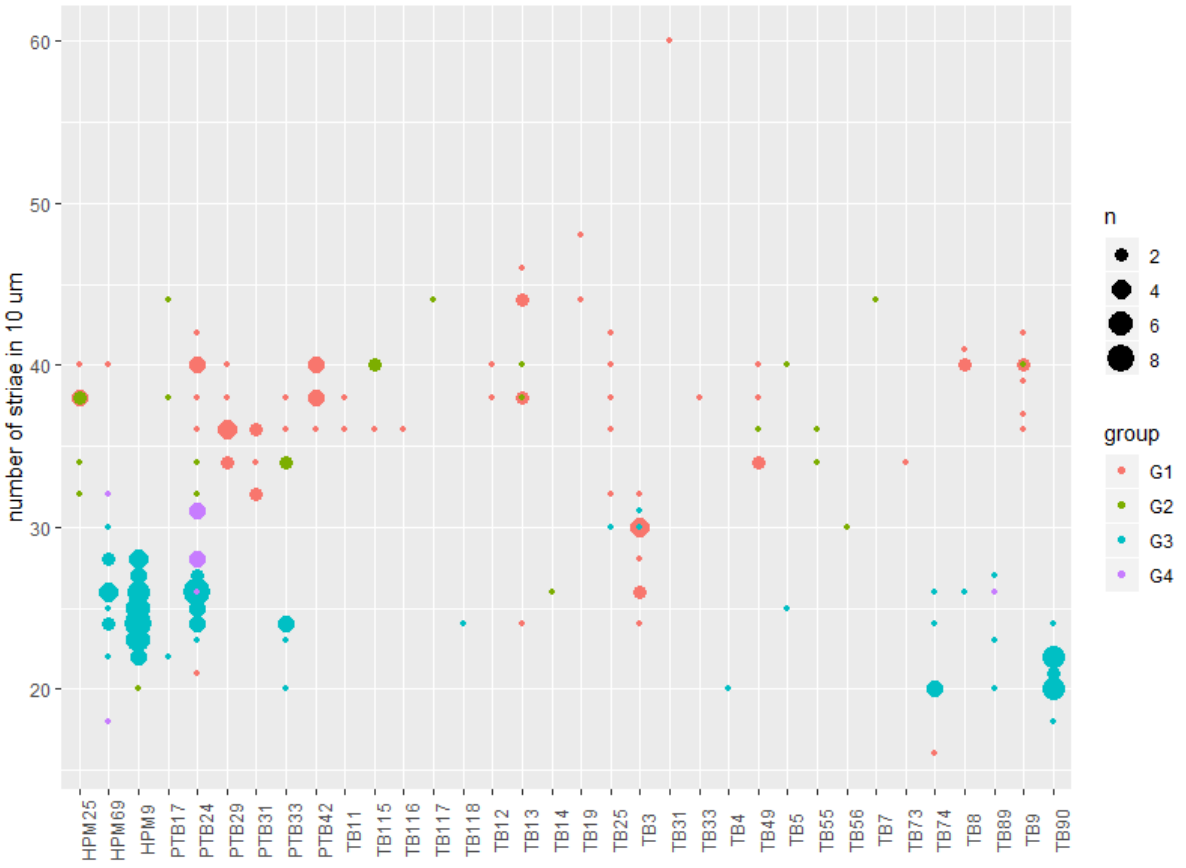

Figure 22. Bubble plot showing the number of cells with the corresponding number of striae in 10 µm in each sample. The size of each dot corresponds to the number of cells. The color of each dot corresponds to the group which each cell belongs to.

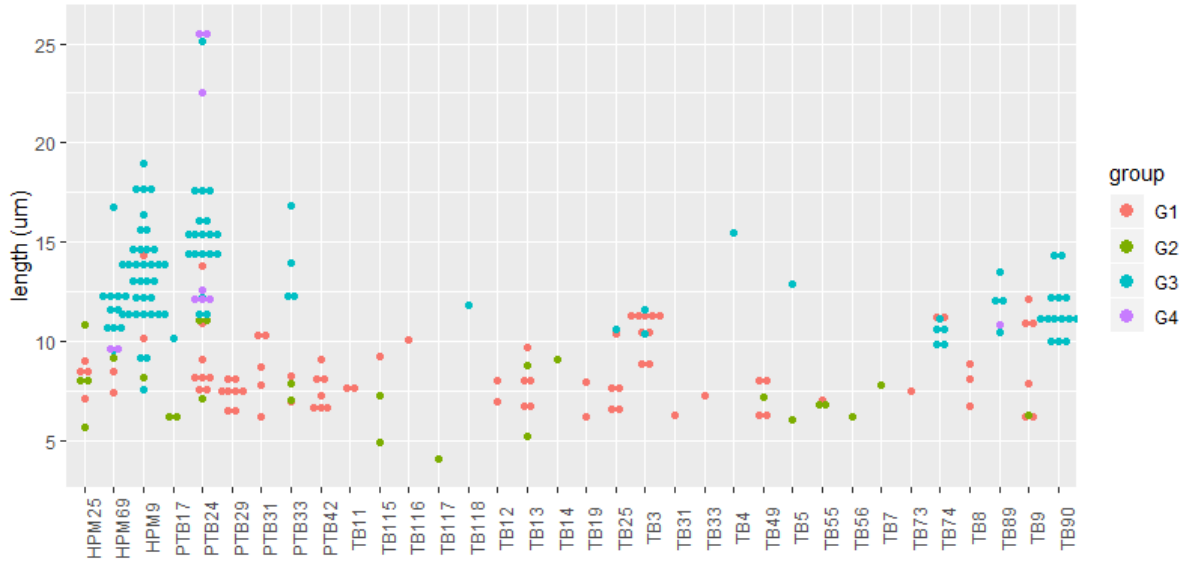

Figure 23. Dot plot showing the number of cells corresponding cell length in µm in each sample. The size of each dot corresponds to the number of cells. The color of each dot corresponds to the group which each cell belongs to.

Discrete variables (Fa, TSF, AS and CP) were not included in PCA or CLUSTER analysis, but the distribution among groups is shown in **Figure 24**. In G1 78% out of 82 cells had fascia (F), in G2 52% out of 25 cells, G3 100% out of 104 cells and in G4 90% out of 10 cells poses a fascia. Regarding terminal silica flap (TSF), I recorded that 91% out of 74 cells in G1 poses it, in G2 80% out of 15, 100% out of 65 cells in G3 have it and 75% out of 4 cells in G4. In G1 87% out of 89 cells had elongated rather than round areolae, in G2 only 15% out of 26 cells had elongate areolae, 95% out of 106 cells in G3 had elongate areolae and in G4 only 20% out of 10 cells had elongate areolae. Considering their size, shape and density 54% out of 52 cells in G1 had different copula pores than valve pores (areolae), in G2 29% out of 14 cells different CP, 76% out of 55% cells had different CP, and lastly, in G4 0% out of 10 cells had different CP from areolae.

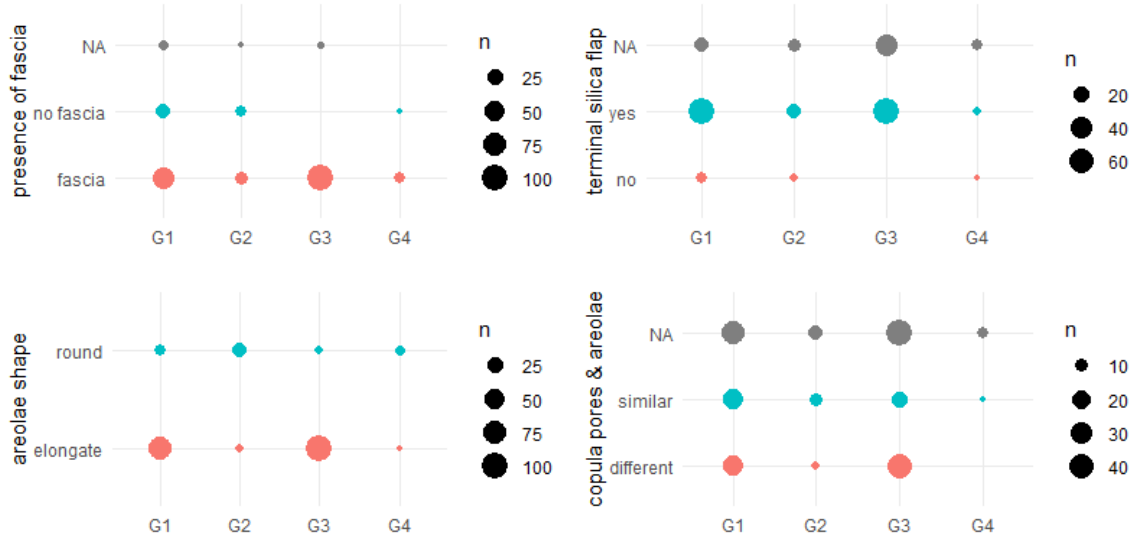

Figure 24. Discrete variables distribution among groups.

3.3. Monoculture morphology and phylogeny

After extensive effort, three strains of *Poulinea lepidochelicola* (PMFTB0073, PMFTB0074 and PMFTB0077) were obtained from skin sample TB90. Culturing *Poulinea* cells enabled me to observe their live behavior and see their chloroplasts (**Figure 25**). Cells are both moving and attached on a long mucilage stalk. They are moving shortly after inoculation and start to form stalks when culture density is high.

Considering their morphology, they belong to group G3 because they have an apical pore field and 2 areolae per stria. They have pronounced fascia and terminal silica flap, their areolae are elongated and differ from copula pores (**Figure 26**, **Figure 27** and **Figure 28**). Descriptive statistics of measurements of length, with, length to width ratio, footpole to headpole ratio, number of copulae, number of striae in 10 µm and septum size are shown in **Table 13**.

Table 13. Descriptive statistics (maximum, minimum, average and standard deviation) for cell length (L), cell width (W), length to width ratio (LW), footpole to headpole ratio (FH), number of copulae (C), number of striae in 10 µm (S) and septum size (SS) for *Poulinea lepidochelicola* cultured strains PMFTB0073, PMFTB0074, and PMFTB0077.

	L (µm)	W (µm)	LW	FH	C	S	SS (µm)
max	15.03	6.17	6.85	1.29	7	26	1.42
min	10.72	1.68	1.91	0.56	6	20	0.69
average	12.36	2.39	5.52	1.10	6.67	22.44	1.00
SD	1.01	0.86	1.01	0.12	0.58	1.32	0.21
N	36	23	23	36	3	36	9

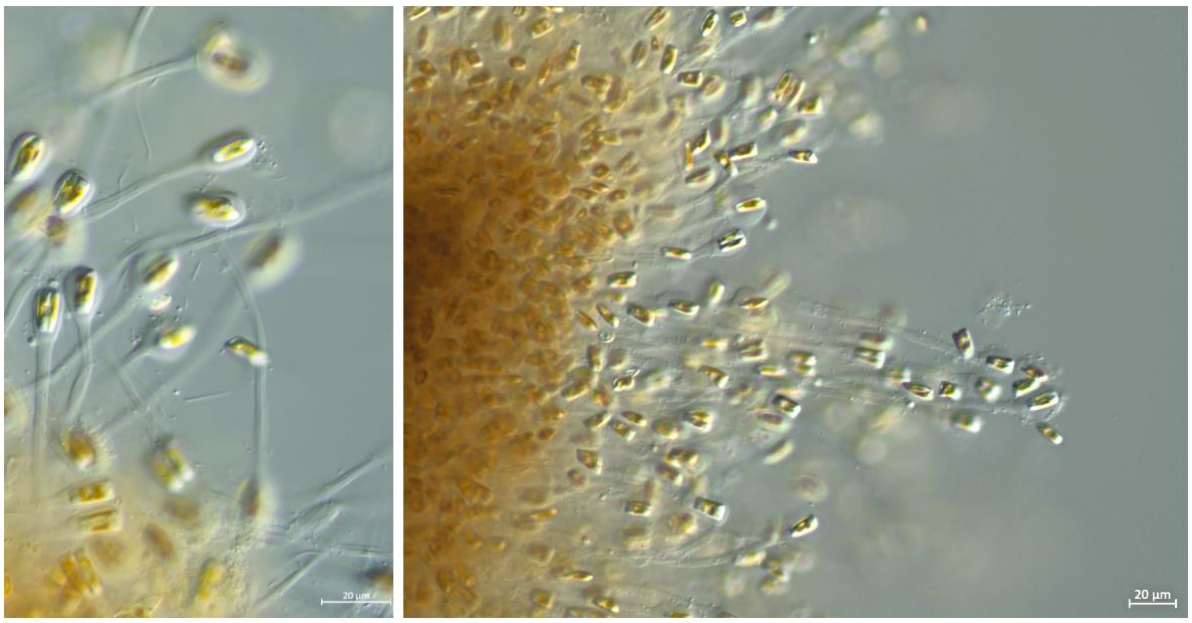

Figure 25. Light microscopy images of *Poulinea lepidochelicola* cultured strains. Left image – strain PMFTB0074 showing single cells on long stalks. Right image – strain PMFTB0073 large colony of cells with stalks.

After the construction of molecular phylogenetic tree (**Figure 29**), the three strains grouped with other *P. lepidochelicola* strains and it is well supported with a bootstrap value of 100%. Its sister clade is a clade that has *Medlinella amphoroidea* and *Chelonicola caribeana*, which are also marine epizoic gomphonemoid diatoms isolated from sea turtles. This grouping is also well supported with a bootstrap value of >95%.

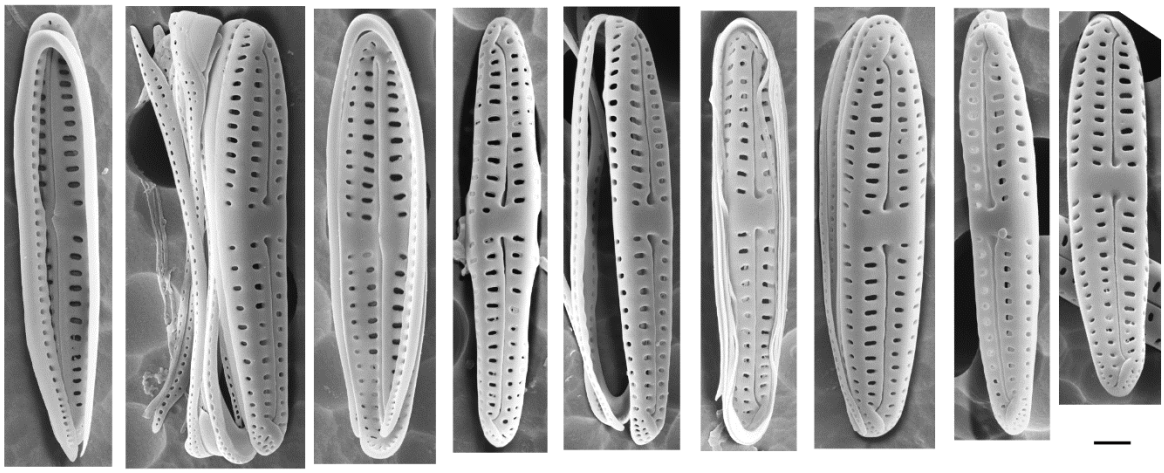

Figure 26. Scanning electron microscopy images of *Poulinea lepidochelicola* strain PMFTB0073. Black line in the bottom right corner represents 1 μm.

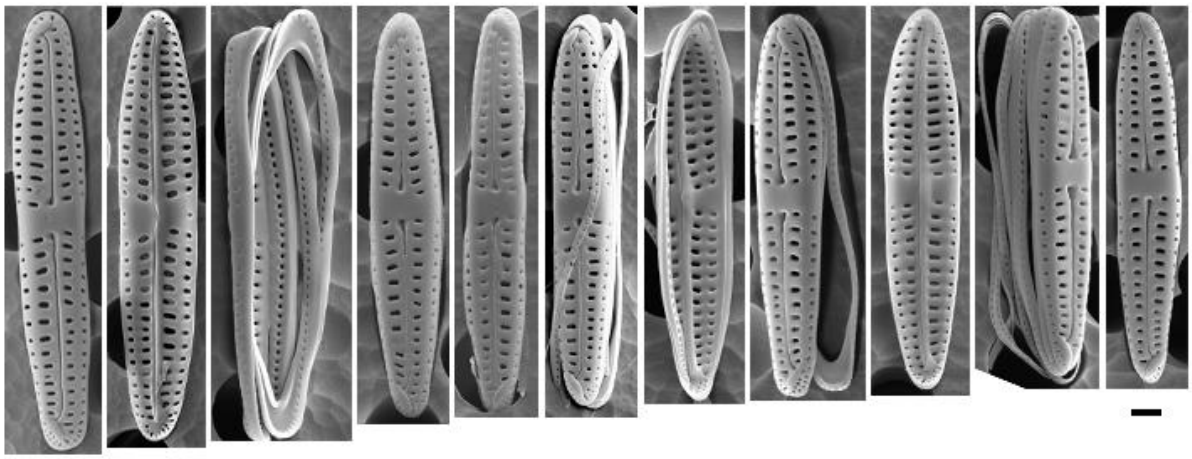

Figure 27. Scanning electron microscopy images of Poulinea lepidochelicola strain PMFTB0074. Black line in the bottom right corner represents 1 μm.

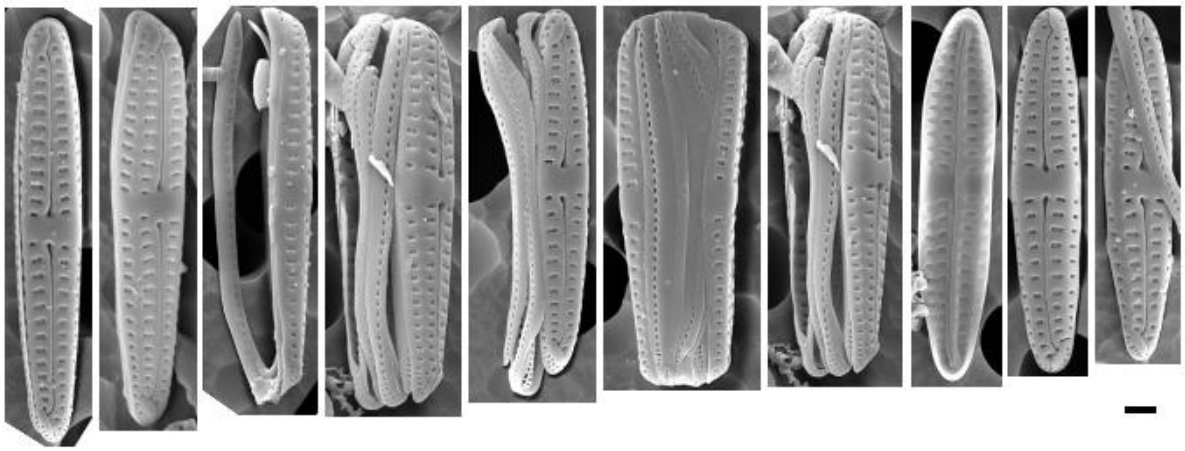

Figure 28. Scanning electron microscopy images of Poulinea lepidochelicola strain PMFTB0077. Black line in the bottom right corner represents 1 μm.

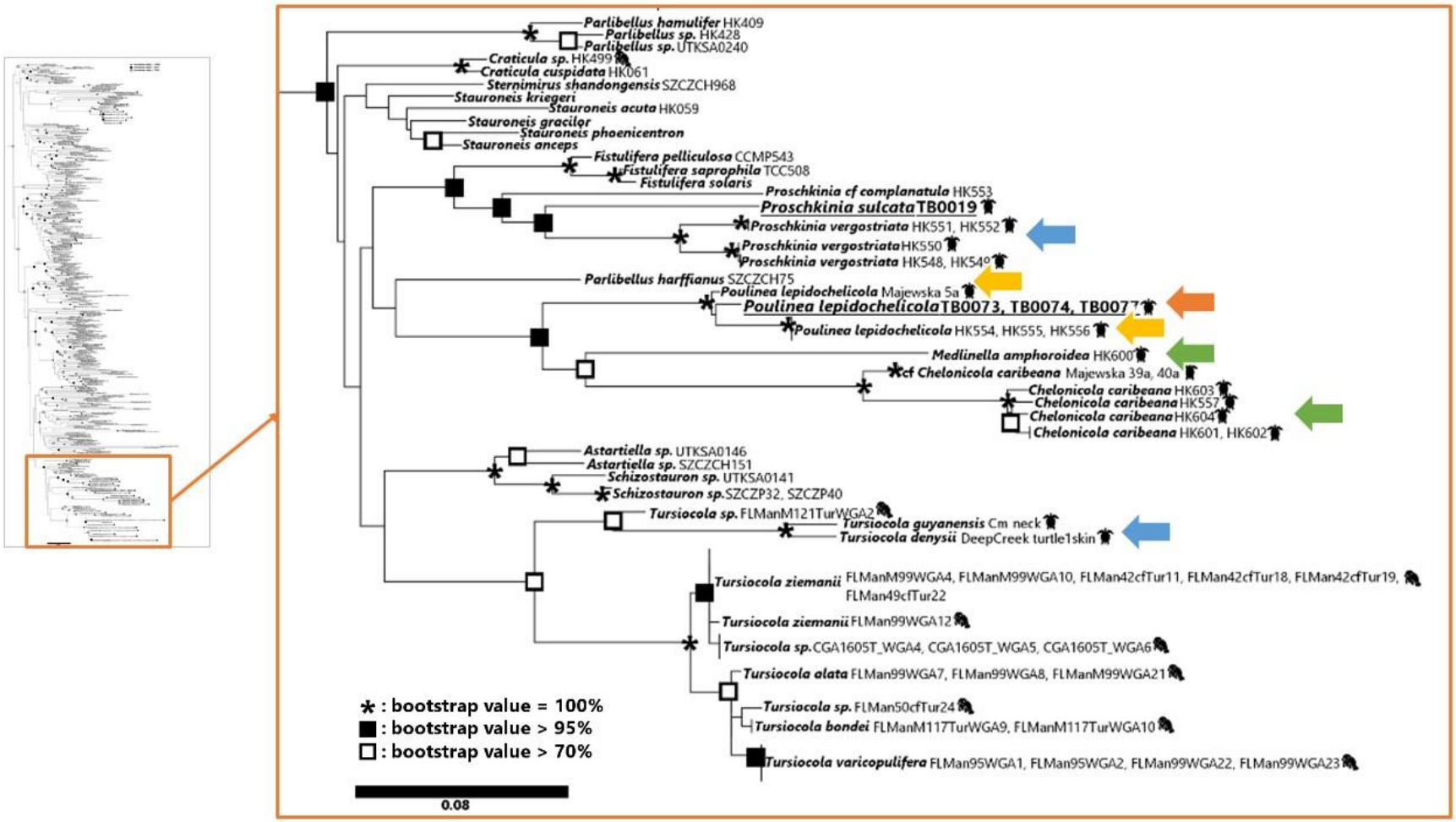

Figure 29. Phylogenetic tree diagram based on Maximum Likelihood analysis of concatenated nuclear-encoded ribosomal SSU and chloroplast-encoded *rbcL* and *psbC* markers. Strain identifier follows taxon name when known. Bootstrap support at nodes have been simplified into four categories: the three icons shown in the legend for 100%, >95%, >70%, and then unmarked for any node with <70% support. Strains isolated from epizoic material are shaded and coded with an icon specific to their host taxon (manatee or sea turtle). Orange arrow represents mine *P. lepidochelicola* strains; yellow arrows represent other *P. lepidochelicola* strains; green arrows represent other marine epizoic gomphonemoid clades; blue arrows represent other clades isolated from sea turtles.

4. DISCUSSION

4.1. Role of genus *Poulinea* in diatom communities on sea turtles

The main goal of the diatom community analysis of HPM samples was to assess the importance of genus *Poulinea* on turtles in the Adriatic Sea. Although the sample size was small (14 turtles), collected turtles date back to almost 25 years in the past and come from different locations, providing a great temporal and spatial scale of the study. The fact that no diatom was found in 50% of the samples can be explained by a number of reasons. The most probable one is that the samples were old and not primarily intended for collecting and studying biofilm and diatoms, therefore while handling them it is possible that their biofilm was unintentionally scraped or washed off. Other reasons for absence of diatoms can come from the scarcity of sample material and the probability that diatoms have never inhabited that area of the turtle in the first place. Those kinds of bare areas without epibionts in-between areas with high densities of diatoms are spotted before by Majewska, et al. (2017b). Nevertheless, in the remaining seven samples *Poulinea* was observed in every one of them with relative abundance ranging from 5% to 80% to in a sample. This is congruent with previous findings in the study on green turtles from Iran and Costa Rica where *Poulinea* was also observed in every sample with relative abundance 25.3-70.8% and 9.1-34%, respectively (Majewska, et al., 2017b). Study of diatoms from olive ridley sea turtles reports 100% prevalence of "*Tripterion* sp.2" amongst samples with 12-32.3% relative abundance in a sample (Majewska, et al., 2015b). "*Tripterion* sp.2" has later been described as *Poulinea lepidochelicola* (Majewska, et al., 2015a). Moreover, Majewska, et al. (2018) collected samples from all seven sea turtle species from all three oceans and found *Poulinea* on every species from every ocean. Only one published study dealt with the community structure of loggerheads from the Adriatic Sea and it reports 5-10% relative abundance of *Poulinea* (Robert, 2019). Results from this thesis and from other studies show that *Poulinea* is universally present on sea turtles in the world, as well as the Adriatic Sea. Moreover, *Poulinea* often shows dominance in diatom communities on sea turtles, it seems to be important part of sea turtle epizoic communities and should not be overlooked.

It is interesting that relative abundances of *Poulinea* show such large variations among turtles. According to Robinson, et al. (2016), foraging habitats of an individual turtle are related to the diversity of macroepibionts of several sea turtle species. Thus, turtles with a lower diversity of foraging habitats might be expected to have a lower diversity of epibionts.

Majewska et al. (2017b) hypothesized that the same mechanisms play a key role in the relative abundance of *Poulinea* on a turtle. In that study, Iranian turtles showed species-poor diatom communities, little to no bacterial biofilm observed and a higher relative abundance of *Poulinea*. In contrast, in the same study, turtles from Costa Rica showed higher diatom species diversity, rich bacterial biofilms and lower relative abundance of *Poulinea*. Bearing that in mind, results from this thesis could indicate that turtles from HPM set exhibited different foraging behavior, but no definite conclusion can be made about a turtle's habitat with studying only one turtle from one location. Since Adriatic loggerhaeds are dominantly foraging on shallow, easy available, rich, and diverse benthic communities in the north-central Adriatic, the varation in abundance of an epibiont species, and consequently *Poulinea,* are not a surprising result (Gračan, pers. comm.). In before mentioned study of Majewska et al. (2017b), the autors also noticed negative correlation between abundance of *Poulinea* and amount of bacterial biofilm suggesting that *Poulinea* spp. prefer to attach direcly onto a turtle host, indicating the truly epizoic behaviour of genus *Poulinea*.

4.2. Comparison of morphological groups

After the description of *Poulinea* and *Chelonicola,* there have been many findings of *Poulinea* diatoms who do not quite match the original description (Majewska, et al., 2018). For example, many of them had an apical pore field even though the original description says *Poulinea* does not have it. There has also been finding of *Poulinea*-like cells with three instead of two areolae per striae (Ashworth, pers. comm.). Same confusion with morphology is present in this research's dataset. That is why the aim of morphology analysis in this thesis is to provide new data and possibly some clarification of this issue.

In the dataset used for morphology measurements, group G3 resembles the most original *P. lepidochelicola* description. It consists of cells that have an apical pore field and two rows of areolae per stria. Additionally, cultured strains that all have the same morphology as G3, and molecular evidence confirm that it is *P. lepidochelicola*. Nevertheless, cells of G3 are larger in length (7.56-25.14 μm) and width (1.86-6.21 μm) than originally described *P. lepidochelicola* cells (L: 5.2–10.0 μm; W: 1.6–2.8 μm). Footpole to headpole ratio (FH) is recorded as a measure of valve heteropolarity because the original description of *P. lepidochelicola* says that valves are heteropolar. Cells of G3 show a slight degree of valve heteropolarity with mean FH value of 1.05, which is congruent with *P. lepidochelicola*.

Regarding the number of copulae, G3 has from 5 to 12 (7.67 on average) even though the original population of *P. lepidochelicola* has more than ten. The number of striae in 10 μm in G3 are 18-31, slightly different than *P. lepidochelicola* (25-26). Pronounced fascia had all of the cells in G3, which is also a characteristic of *P. lepidochelicola*. As well as that, majority of cells (91%) have terminal silica flap and elongate pores (95%) like *P. lepidochelicola*. Altogether, only for this group can be said that belongs to the species *P. lepidochelicola* because of the molecular evidence. In spite of that, not all morphological measurements match the originally described population of *P. lepidochelicola*, especially presence of the apical pore field which is not present in the originally described population of *P. lepidochelicola*. Hence, these results broaden the understanding of the morphological features of *P. lepidochelicola*.

Group G2 is characterized by no pore field and three areolae per stria, this matches the original description of *Chelonicola costaricensis* (Majewska, et al., 2015a), but there are also some *Poulinea*-like cells in the group that have pronounced fascia and other features that are not characteristic for *Chelonicola*. Cell length of G2 (4.12-11.28 μm) is similar to *P. lepidochelicola* (5.2–10.0 μm) and *C. costaricensis* (6.0-17.5 μm) as well as cell width: G2 (1.57-2.87 μm), *P. lepidochelicola* (1.6–2.8 μm), *C. costaricensis* (1.7–3.1 μm). The mean FH value (0.99) for G2 does not indicate pronounced valve heteropolarity. Only one measurement was made for the number of copulae (9), and both *P. lepidochelicola* and *C. costaricensis* are described to have 10 or more. Regarding the number of striae, the mean value of G2 (35.46) and range (20-44) are in the middle of the range of *C. costaricensis* (36-47) and to *P. lepidochelicola* (25-36). Pronounced fascia has 52% of the group, which is also non-informative for the identification of this group. Terminal silica flap possess 80% of the cells in this group, which a characteristic of *P. lepidochelicola*. The majority (85%) of cells in G2 have round areolae like *C. costaricensis* rather than elongate like *P. lepidochelicola*. All in all, the majority of measured cells in this group can be designated as *C. costaricensis*, mainly because of the absence of apical pore field, three rows of areolae per striae and number of striae in 10 μm. Since this species is only recently described it is not unusual to find cells that do not quite match the original description of a species. That is why these results will help to give a better understanding of the species morphology.

Group G1 is classified as cells which have no pore field and two areolae per stria. Considering the fact that it groups together with *C. costaricensis* in PCA and CLUSTER analysis, I suggest that it belongs to the genus *Chelonicola*, not *Poulinea*. Besides *C.*

costaricensis, there is currently only one more species from this genus, *C. caribeana*. Cell length (5.21-14.34 μm) is more or less congruent with cell length of *C. caribeana* (4.5-13.1 μm). Cell width (1.29-3.78 μm) is also similar to *C. caribeana* (0.7-1.8 μm). The average value of FH for G1 is 1.01, while for C. caribeana is 1.02. The number of copulae (4-12, mean 6.94) is similar to that of *C. caribeana* (<8). G1 has a wide range of striae density (16-60, 35.87 on average) than while *C. caribeana* (38-55). This character is important because it is consistent within the group and is one of the characters most that PCA analysis is based on. The majority of cells in G1 have developed fascia (78%), terminal silica flap (91%), elongate pores (87%) which is congruent to *C. caribeana* description. Considering all measured morphological features, I suggest that the majority of cells in this group belong to genus *Chelonicola*, species *C. caribeana*. Even though not all morphological features match the originally described *C. caribeana* population, the overall morphology of the group matches the above-mentioned species. Moreover, the morphology of the G1 is congruent with other *C. caribeana* strains on the molecular phylogenetic tree (**Figure 29**) (Ashworth, et al., 2019a).

Group G4 is consists of cells that have apical pore field and three rows of areolae per stria. This combination of features is known for neither *Poulinea* nor *Chelonicola*. Even so, diatoms with this kind of morphology appeared in the dataset. The results show that this group has overall longer cells (9.33-25.83 μm) than originally described population of *P. lepidochelicola* or *C. costaricensis*, but similar to G3. G4 have narrower cells that other groups (see LW values in **Table 6**). Those cells also show some degree of heteropolarity (average 1.07) and number of striae in 10 μm (18-32) are more consistent with that of the originally described population of *P. lepidochelicola* (25-36) and cells from G3 (18-31). The majority of cells have fascia (90%), terminal silica flap (75%) like *P. lepidochelicola* and small, round areolae (80%) like *C. costaricensis*. Keeping everything in mind, G4 is probably a mix of different taxa of marine gomphonemoids, not only *Poulinea* or *Chelonicola*. Along with that, it had too few cells (10) to clearly state which genera would prevail in the group. Cells from G4 do not group together in PCA or CLUSTER analysis. Therefore, each cell should be identified separately, which would be out of the scope of this thesis.

With the start of this thesis, based on preliminary research, it was believed that all of the cells in this dataset belong to the genus *Poulinea*. However, with more and more data I came to the conclusion that there are in fact one species of *Poulinea*, *P. lepidochelicola* (G3) and two species of *Chelonicola*: *C. costaricensis* (G2) and *C. caribeana* (G1). The results of the statistical analysis of this thesis indicate that the key features in separating these two genera

are not number of rows of areolae in a stria (as previously believed) but rather the presence of apical pore field, striae density and cell length. According to that, cells from genus *Poulinea* have apical pore filed, lower striae density and are overall larger, while cells from genus *Chelonicola* do not possess apical pore field, have denser striae and are overall smaller in length.

However, the possibility of morphological variation due to phenotypic plasticity of these *Poulinea*-like and *Chelonicola*-like cells should not be excluded. Kociolek & Stoermer (2010) analyzed and reviewed the three main reasons for morphological variability in diatoms: development, genetics and environment. Furthermore, Riaux-Gobin, et al. (2019) reported and analyzed the phenotypic variability of epizoic diatom *Olifantella gorandiana* from sea turtles. They discussed the possibility of physiological or environmental factors as reasons for the species plasticity in these epizoic habitats. A similar case could be occurring with genera *Poulinea* and *Chelonicola*, but to prove that, more research in this area is needed, especially in molecular genetics and phylogeny of these epizoic taxa.

4.3. Significance of cultured *Poulinea lepidochelicola* strains

Regarding live-cultured strains of *P. lepidochelicola*, I observed interesting behavior of the strains during isolation, culturing and re-inoculations. They were actively moving when cell density in a flask was low. As the number of cells grows cells were predominantly found stationary on a stalk. It has been previously observed that the movement of usually stalk-producing diatom species is behavior they only express in cultures in laboratory conditions (Majewska & Ashworth, pers. comm.). However, it is possible that *P. lepidochelicola* cells are moving when they aim to colonize new surfaces, but on turtles are mainly observed in stalk-forming mode because of the already well-established colonies. Regarding stalks, they require a high amount of produced EPS. It has been observed that specific interactions between bacteria and diatoms that produce high amounts of EPS exist. Since *Poulinea* is only recently discovered and described, there is no data on possible effect diatoms have on bacteria and *vice versa*. However, in the study of Majewska, et al. (2017b) authors observed and addressed the possibility that high amounts of bacterial biofouling could prevent *Poulinea* cells from attachment on a turtle skin or carapace. This may suggest that *Poulinea* cells prefer direct attachment to a turtle, proposing the truly epizoic nature of genus *Poulinea*. However, the ability to grow and form stalks in Petri dishes and culture flasks and not only

on a turtle's skin or carapace suggests that they do not need an animal surface to survive. This fact brings up the possibility of specific interactions of *Poulinea* and bacteria that are co-cultured with it that may enable their survival in laboratory conditions (Ashworth, 2019b). To test this hypothesis effort should be made to grow *Poulinea* in axenic cultures and compare them with cultures which grown with bacterial "contamination".

Apart from microscopy, in recent years different approach in identifying diatom species in environmental samples based on DNA has been developed and adopted (Taberlet, et al., 2012). Metabarcoding is based on combining DNA barcoding and high throughput sequencing. Currently, only one study published results of metabarcoding for epizoic diatoms on sea turtles (Rivera, et al., 2018). They got different results from diatom community analysis by microscopy versus metabarcoding. Only 29% of OTUs could be identified at the species or genus level using metabarcoding while using microscopy 93% of taxa are identified at the species or genus level. Lack of DNA reference sequences in the diatom barcoding library is causing this incongruity of the two methods. Epizoic diatoms are especially poorly represented in those barcoding reference libraries because some of them have only recently been described and some are still undescribed (Ashworth, 2019b). One of the accomplishments of this thesis are also *P. lepidochelicola* DNA sequences which can be incorporated in those sequence libraries and increase the percentage of identified species when analyzing communities by metabarcoding. Metabarcoding of epizoic samples on sea turtles is only in the beginning stages, but it is promising powerful method due to analysis of many samples at low cost, sample treatments (e.g., extraction, PCR, sequencing, and bioinformatics) do not require rare experts in diatom taxonomy and it also allows cryptic diatom diversity to be revealed, a difficult undertaking using microscopy (Rivera, et al., 2018). However, some aspects of microscopy in diatom community research cannot be replaced by metabarcoding. That is why combined microscopy and metabarcoding studies that result in photo-vouchered sequence data and reference databases for metabarcoding are important and should be the base of diatoms community analysis in the future (Ashworth, 2019b).

5. CONCLUSION

The main accomplishments of this study are statistical analyses of morphological features of genera *Poulinea* and *Chelonicola* which are done for the first time for these genera and second, cultivating three strains of *Poulinea lepidochelicola* and isolating their molecular markers which will be included in diatom DNA barcoding libraries.

The specific conclusions connected with the hypotheses of this thesis are:

1. Genus *Poulinea* is commonly present on loggerheads from the Adriatic Sea. The genus is found in high abundance and often dominant in those epizoic diatom communities.
2. Three statistically different groups of *Poulinea*-like cells are found living on loggerheads from the Adriatic Sea. One group is assorted in genus *Poulinea* as species *Poulinea lepidochelicola* (group G3) and two groups are assorted in genus *Chelonicola* as species *Chelonicola costaricensis* (group G1) and *Chelonicola caribeana* (group G2). There is also fourth, small group of *Poulinea*-like cells (group G4) but they do not belong to either *Poulinea* or *Chelonicola*.
3. Three isolated *P. lepidochelicola* strains are morphologically and genetically congruent with species *P. lepidochelicola* from other seas and other sea turtle species.

Based on the results and conclusions from this study, there is a lack of knowledge about morphology and even more so about the genetics of these epizoic taxa. Therefore, more studies with the combined, polyphasic approach are needed to understand complex relationships between the marine epizoic gomphonemoid diatoms on sea turtles. To accomplish that, more effort should be put into isolating taxa like *Poulinea*, *Chelonicola*, *Medlinella*, and *Tripterion* in monocultures and obtaining their molecular markers. This will enable to construct larger and more reliable molecular phylogenetic trees. Additionally, the result of these studies should be photo-vouchered sequence data and reference databases which can be used in metabarcoding these epizoic habitats. Furthermore, monocultures of these diatoms can be used in investigating their physiology and ecology which could shed more light onto understanding epizoic diatom relationship with its turtle host. As a consequence, these epizoic diatoms could tell us more about the behavior of sea turtles and help to protect them better in their natural habitat like the Adriatic Sea.

6. LITERATURE

Adl, S. M. et al., 2019. Revisions to the Classification, Nomenclature, and Diversity of Eukaryotes. *Journal of Eukaryotic Microbiology 66*, pp. 4-119.

Alvarez-Blanco, I. & Blanco, S., 2014. *Benthic diatoms from Mediterranean coasts.* Stuttgart: Bibliotheca Diatomologica 60. Gebr. Borntraeger Verlagsbuchhandlung.

Al-Yamani, F. Y. & Saburova, M. A., 2011. *Illustrated guide on the benthic diatoms of Kuwait's marine environment.* Safat: Kuwait Institute for Scientific Research.

Amin, S. A., Parker, M. S. & Armbrust, E. V., 2012. Interactions between Diatoms and Bacteria. *Microbiology and Molecular Biology Reviews 76*, pp. 667-684.

Andersen, R. A., 2005. *Algal culturing techniques.* Oxford: Elsevier Academic Press.

Ashworth, M. et al., 2019a. *Progress in characterizing diatom assemblages on aquatic vertebrates: lessons and applications.* Unpublished poster, Southeastern Phycological Colloquium, USA.

Ashworth, M. P., 2019b. On the shoulders of giants: what epizoic diatoms are teaching us about diatom evolution. In: S. Bosak & Z. Ljubešić, eds. *Seventh European Phycological Congress Programme and Book of Abstracts.* Oral presentation: European Journal of Phycology, Taylor & Francis.

Auer, A., 1991. Qualitative Diatom Analysis as a Tool to Diagnose Drowning. *The American Journal of Forensic Medicine and Pathology 12*, p. 213–218.

Benoiston, A.-S.et al., 2017. The evolution of diatoms and their biogeochemical functions. *Philosophical Transactions of the Royal Society*.

Bhattacharya, D. et al., 1992. Algae containing chlorophylls a + c are paraphyletic: molecular evolutionary analysis of the Chromophyta. *Evolution 47*, pp. 1808-1817.

Bjorndal, K. A. et al., 2017. Ecological regime shift drives declining growth rates of sea turtles throughout the West Atlantic. *Global Change Biology 23*, pp. 4556-4568.

Bradbury, J., 2004. Nature's Nanotechnologists: Unveiling the Secrets of Diatoms. *PLoS Biology 2*, p. 1512–1515.

Cambiè, G., Caminas, J. A., Franquesa, R. & Mingozzi, T., 2010. Fishing activity and impacts along the main nesting area of loggerhead sea turtle Caretta caretta in Italy: overwhelming discrepancy with the official data. *Scientia Marina 74*, pp. 275-285.

Cambiè, G. et al., 2013. Identifying and mapping local bycatch hotspots of loggerhead sea turtles using a GIS-based method: Implications for conservation. *Marine Biology 160*, pp. 653-665.

Casale, P., 2010. Sea turtle by-catch in the Mediterranean. *Fish and Fisheries 12*, p. 299–316..

Casale, P., Aprea, A., Deflorio, M. & De Metrio, G., 2012. Increased by-catch rates in the Gulf of Taranto, Italy, in 20 years: a clue about sea turtle population trend?. *Chelonian Conservation Biology 11*, pp. 239-243.

Casale, P. et al., 2018. Mediterrananean sea turtles: current knowledge and priorities for conservation and research. *Endangered Species Reseach 36*, pp. 229-267.

Casale, P. et al., 2007. Incidental catch of marine turtles by Italian trawlers and longlines in the central Mediterranean. *Aquatic Conservation: Marine and Freshwater Ecosystem 17*, pp. 686-701.

Casale, P. et al., 2014. Annual survival probabilities of juvenile loggerhead sea turtles indicate high anthropogenic impact on Mediterranean population. *Aquatic Conservation: Marine and Freshwater Ecosystem 25*, pp. 690-700.

Casale, P., Mazaris, A. & Freggi, D., 2011. Estimation of age at maturity of loggerhead sea turtles Caretta caretta in the Mediterranean using length-frequency data. *Endangered Species Research 13*, pp. 123-129.

Casale, P. & Tucker, A. D., 2017. *Caretta caretta (amended version of 2015 assessment). The IUCN Red List of Threatened Species 2017: e.T3897A119333622.*. [Online] Available at: http://dx.doi.org/10.2305/IUCN.UK.2017-2.RLTS.T3897A119333622.en. [Accessed 29. 12. 2019.].

Clarke, K. R. & Gorley, R. N., 2015. *PRIMER v7: User Manual/Tutorial.* Devon: PRIMER-E Ltd.

Cox, E. J., 2014. Diatom identification in the face of changing species concepts and evidence of phenotypic plasticity. *Journal of Micropalaeontology 33*, pp. 111-120.

Cupul-Magaña, F. A. R.-D. A. E.-G. a. C. R.-N., 2011. First report of marine barnacles Lepas anatifera and Chelonibia testudinaria as epibionts on American crocodile (Crocodylus acutus). *Herpetology Notes 4*, pp. 213-214.

Edgar, L. A. & Pickett-Heaps, J. D., 1984. Diatom locomotion. *Phycology Research 3*, pp. 47-88.

Ernst, C. H. & Lovich, J., 2009. *Turtles of the United States and Canada.* 2nd edition ed. s.l.:JHU Press.

European Commision, 2014. *Council Directive 92/43/EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora,* Brussels: Official Journal of the European Communities.

Fahlman, A. et al., 2017. Defining risk variables causing gas embolism in loggerhead sea turtles Caretta caretta caught in trawls and gillnets. *Scientific Reports 7*.

Frankovich, T. A. et al., 2016. Medlinella amphoroidea gen. et sp. nov. (Bacillariophyta) from the neck skin of Loggerhead sea turtles (Caretta caretta). *Phytotaxa 272*, pp. 101-114.

Frankovich, T. A., Sullivan, M. J. & Stacy, N., 2015. Tursiocola denysii sp. nov. (Bacillariophyta) from the neck skin of Loggerhead sea turtles (Caretta caretta). *Phytotaxa 234*, pp. 227-236.

Graef, C. L., Kociolek, J. P. & Rushfort, S. R., 2013. New and interesting diatoms (Bacillariophyta) from Blue Lake Warm Springs, Tooele Vounty, Utah. *Phytotaxa 153*, pp. 1-38.

Greenblatt, R. J. et al., 2004. The Ozobranchus leech is a candidate mechanical vector for the fibropapilloma-associated turtle herpesvirus found latently infecting skin tumors on Hawaiian green turtles (Chelonia mydas). *Virology 1*, pp. 101-110.

Guillard, R. R. L., 1975. Culture of phytoplankton for feeding marine invertebrates. In: W. L. Smith & M. H. Chanley, eds. *Culture of Marine Invertebrate Animals.* New York, USA: Plenum Press, pp. 26-60.

Guillard, R. R. L. & Ryther, J. H., 1962. Studies of marine planktonic diatoms. I. Cyclotella nana Hustedt and Detonula confervacea Cleve. *Canadian Journal of Microbiology*, pp. 229-239.

Guillou, L. et al., 1999. Bolidomonas, a new genus with two species belonging to a new algal class: the Bolidophyceae. *Journal of Phycology 35*, pp. 368-381.

Guiry, M. D. & Guiry, G. M., 2020. *AlgaeBase. World-wide electronic publication, National University of Ireland, Galway.* [Online] Available at: https://www.algaebase.org [Accessed 16 January 2020].

Harding, J. M. et al., 2011. Sea turtles as potential dispersal vectors for non-indigenous species: The veined rapa whelk as an epibiont of loggerhead sea turtles. *Southeastern Naturalist 10*, pp. 233-244.

Hasle, G. R., 1978. Diatoms. In: A. Sourina, ed. *Phytoplankton Manual*. Paris: UNESCO, pp. 136-142.

Kaczmarska, I. et al., 2013. Proposals for a terminology for diatom sexual reproduction, auxospores and resting stages. *Diatom Research*.

Kociolek, J. P. & Stoermer, E. F., 2010. Variation and polymorphism in diatoms: the triple helix of development, genetics and environment. A review of the literature. *Vie et Milieu - Life and Environment 60*, pp. 75-87.

Kooistra, W. H. C. F. & Medlin, L. K., 1996. Evolution of the diatoms (Bacillariophyta). IV. A reconstruction of their age from small subunit rRNA coding regions and the fossil record. *Molecular Phylogenetics and Evolution 6*, pp. 391-407.

Lanfear, R. et al., 2014. Selecting optimal partitioning schemes for phylogenomic datasets. *BMC Evolutionary Biology 14*, pp. 1-82.

Lazar, B. & Gračan, R., 2011. Ingestion of marine debris by loggerhead sea turtles, Caretta caretta, in the Adriatic Sea. *Marine Pollution Bulletin 62*, pp. 43-47.

Lazar, B., Margaritoulis, D. & Tvrtković, N., 2000. Migration of loggerhead sea turtles (Caretta caretta) into the Adriatic sea. In: F. A. Abreu-Grobois, R. Briseno-Dueras, R. Marquez & L. Sarti, eds. *Proceedings of the 18th Annual Symposium on Sea Turtle Biology and Conservation.* Miami: NOAA Technical Memorandum NMFS-SEFSC-346, p. 101.

Lazar, B., Margaritoulis, D. & Tvrtković, N., 2004. Tag recoveries of the loggerhead sea turtle Caretta caretta in the eastern Adriatic Sea: implications for conservation. *Journal of the Marine Biological Association of the United Kingdom 84*, pp. 475-480.

Lazar, B. et al., 2011. Accumulation of organochlorine contaminants in loggerhead sea turtles, Caretta caretta, from the eastern Adriatic Sea. *Chemosphere 82*, p. 121–129.

Leipe, D. D. et al., 1994. The stramenopiles from a molecular perspecti ve: 16S-like rRNA sequences from Labyrinthuloides minuta and Cafeteria roenbergensis. *Phycologia 33*, pp. 369-377.

Logan, P. & Morreale, S. J., 1994. Hydrodynamic drag characteristics of juvenile L. kempi, C. mydas, and C. caretta. In: B. A. Schroeder & B. E. Witherington, eds. *Proceedings of the 13th Annual Symposium on Sea Turtle Biology and Conservation.* Miami, FL: NOAA-Technical Memorandum NMFS-SEFSC-341, pp. 248-252.

Lutcavage, M., Plotikin, P., Witherington, B. & Lutz, P. L., 1997. Human Impact on Sea Turtle Survival. In: L. P. a. M. J.A., ed. *The Biology of Sea Turtles*. Boca Raton, FL: CRC Press, pp. 387-410.

Majewska, R. et al., 2019. Six new epibiotic Proschkinia (Bacillariophyta) species and new insights into the genus phylogeny. *European Journal of Phycology* , pp. 1-23.

Majewska, R. et al., 2017c. Two new epizoic Achnanthes species (Bacillariophyta) living on marine turtles from Costa Rica. *Botanica Marina DOI 10.1515/bot-2016-0114*, pp. 1-16.

Majewska, R., De Stefano, M. & Van de Vijer, B., 2017a. Labellicula lecohuiana, a new epizoic diatom species living on green turtles in Costa Rica. *CNova Hedwigia, Beiheft 146*, pp. 23-31.

Majewska, R. et al., 2015a. Chelonicola and Poulinea, two new gomphonemoid diatom genera (Bacillariophyta) living on marine turtles from Costa Rica. *Phytotaxa 233*, pp. 236-250.

Majewska, R., Robert, K., Van de Vijer, B. & Nel, R., 2019. A new species of Lucanicum (Cyclophorales, Bacillariophyta) associated with loggerhead sea turtles from South Africa. *Botany Letters DOI: 10.1080/23818107.2019.1691648*, pp. 1-8.

Majewska, R., Santoro, M., Bolaños, F. & Chaves, G., 2015b. Diatoms and other epibionts associated with Olive Ridley (Lepidochelys olivacea) sea turtles from the Pacific coast of Costa Rica. *PLoS ONE 10(6): e0130351*.

Majewska, R. et al., 2018. *Morphological variations in sea turtle-associated "gomphonemoid" diatoms.* Berlin, 25th International Diatom Symposium.

Majewska, R. et al., 2017b. Shared epizoic taxa and differences in diatom community structure between green turtles (Chelonia mydas) from distant habitats. *Microbial Ecology 47*, p. 69–978.

Mann, D. G., 1999. The species concept in diatoms. *Phycologia 38*, pp. 437-495.

Mann, D. G. & Vanormelingen, P., 2013. An Inordinate Fondness? The number, distrubutions and origins of diatom species. *Journal of Eukyryotic Microbiology 60*, pp. 414-420.

Margaritoulis, D. et al., 2003. Loggerhead turtles in the Mediterranean Sea: present knowledge and conservation perspectives. In: B. A. a. W. B., ed. *Biology and conservation of loggerhead sea turtles.* Washington: Smithsonian Institution Press, pp. 175-198.

Medlin, L. K. & Kaczmarska, I., 2004. Evolution of the diatoms: V. Morphological and cytological support for the. *Phycologia 43*, pp. 245-270.

Medlin, L. K. et al., 1997. Phylogenetic relationships of the 'golden algae' (haptophytes, heterokonts, chrysophytes) and their plastids. *Plant Systematics and Evolution 11*, pp. 187-210.

Medlin, L. K., Williams, D. M. & Sims, P. A., 1993. The evolution of the diatoms (Bacillariophyta). I. Origin of the group and assessment ofthe monophyly of its major divisions. *European Journal of Phycology 28*, pp. 261-275.

Mishra, M. et al., 2017. All New Faces of Diatoms: Potential Source of Nanomaterials and Beyond. *Frontiers in Microbiology 8*, p. 1239.

Moore, P. G., 1995. Podocerus chelonophilus (Amphipoda: Podoceridae) associated with epidermal lesions of the loggerhead turtle, Caretta caretta (Chelonia). *Journal of the Marine Biological Association of the United Kingdom 75*, pp. 253-255.

Nawrocki, E. P., 2009. *Structural RNA homology search and alignment using covariance models.* PhD thesis, Washington University in Saint Louis: School of Medicine.

Newman, W. A. & Ross, A., 1976. Revision of the balanomorph barnacles; including a catalog of the species.. *San Diego Society of Natural History 9*, pp. 1-108.

Not, F. et al., 2012. Diversity and ecology of eukaryotic marine phytoplankton. *Advances in Botanical Research 64*, pp. 1-64.

Novillo, O., Pertusa, J. F. & Tomás, J., 2017. Exploring the presence of pollutants of sea: Monitoring heavy metals in loggerhead turtles (Caretta caretta) from the Western Mediterranenan. *Science of the Total Environment 598*, pp. 1130-1139.

Potapova, M. G., Hamilton, P. B., I, K. L. & Sosina, N. K., 2014. New and rare diatom (Bacillariophyta) species from a mountain lake in Eastern Siberia. *Phytotaxa 156*, pp. 100-116.

Riaux-Gobin, C., Witkowski, A., Chevallier, D. & Daniszewska-Kowalczyk, G., 2017a. Two new Tursiocola species (Bacillariophyta) epizoic on green turtles (Chelonia mydas) in French Guiana and Eastern Caribbean. *Journal of the Czech Phycological Society 17*, pp. 150-163.

Riaux-Gobin, C. et al., 2019. Diatom phenotypic plasticity: Olifantiella gorandiana epizoic on ‘G5-Manahere’ (Society Archipelago, South Pacific), a case study. *Phytotaxa 415*, pp. 89-104.

Riaux-Gobin, C., Witkowski, A., Kociolek, P. J. & Ector, L., 2017b. New epizoic diatom (Bacillariophyta) species from sea turtles in the Eastern Caribbean and Sout Pacific. *Diatom Research 32*, pp. 109-125.

Rimet, F. & Bouchez, A., 2012. Life-forms, cell-sizes and ecological guilds of diatoms in European rivers. *Knowledge and Management of Aquatic Ecosystems 406*.

Rivera, S. F. et al., 2018. DNA metabarcoding and microscopic analyses of sea turtles biofilms: Complementary to understand turtle behavior. *PLoS ONE 13(4): e0195770*.

Robert, K., 2019. *Ecology and diversity of epizoic diatom communities on marine turtles.* University of Antwerp: Master Thesis.

Robert, K., Bosak, S. & Van de Vijer, B., 2019. Catenula exigua sp. nov., a new marine diatom (Bacillariophyta) species from the Adriatic Sea. *Phytotaxa 414*, pp. 113-118.

Robinson, N. J. et al., 2016. Assortative epibiosis of leatherback, olive ridley and green sea turtles in the Eastern Tropical Pacific. *Journal of Marine Biologogical Association of United Kingdom*, pp. 1-8.

Robinson, N. J. et al., 2016. Epibiotic diatoms are universally present on all sea turtle species. *PLoS ONE 11(6): e0157011*.

Rothpletz, A., 1896. Uber die Flysch-Fucoiden und einige andere fossile Algen, sowie tiber liasische, Diatomeen ftihrende Hornschwamme. *ZeitschriJt der Deutsche Geologische Gesellschaft 48*, pp. 910-914.

Round, F. E., Crawford, R. M. & Mann, D. G., 1990. *The Diatoms: Biology and morphology of the genera.* s.l.:Cambridge University Press.

Schärer, M. T. & Epler, J. H., 2007. Long-range dispersal possibilities via sea turtle—A case study for Clunio and Pontomyia (Diptera: Chironomidae) in Puerto Rico. *Entomological News 118*, p. 273–277.

Schofield, G., Dimadi, A., Fossettes, S. & Katsalidis, K. A., 2013. Satellite tracking large numbers of individuals to infer population level dispersal and core areas for the protection of the endangered species. *Diversity Distribution 19*, pp. 834-844.

Schofield, G. et al., 2010. Inter annual variability in the home range of breeding turtles: implications for current and future conservation management. *Biology Conservation 143*, pp. 722-730.

Schrichte, D., 2004. *Information About Sea Turtles: Loggerhead Sea Turtle. Sea Turtle Conservancy.* [Online]
Available at: https://conserveturtles.org/information-sea-turtles-loggerhead-sea-turtle/ [Accessed 28. 12. 2019.].

Senties, G. A., Espinoza-Avalos, J. & Zurita, J. C., 1999. Epizoic algae of nesting sea turtles Caretta caretta (L.) and Chelonia mydas (L.) from the Mexican Caribbean. *Bulletin of Marine Science 64*, pp. 185-188.

Shine, R., Brischoux, F. & Pile, A. J., 2010. A seasnake’s colour affects its susceptibility to algal fouling. *Proceedings of the Royal Society B 277*, p. 2459–2464.

Simonsen, R., 1974. *The Diatom Plankton of the Indian Ocean Expedition of RV "Meteor" 1964-1965.* s.l.:Gebrüder Borntraeger .

Simonsen, R., 1979. The diatom system: ideas on phylogeny. *Bacillaria 2*, pp. 9-71.

Sorhannus, U., Gasse, F., Perasso, R. & Baroin-Tourancheau, A., 1995. A preliminary phylogeny of diatoms based on 28S ribosomal RNA sequence data. *Phycologia 34*, pp. 65-73.

Stamakis, A., 2014. RAxML Version 8: A tool for Phylogenetic Analysis and Post-Analysis of Large Phylogenies. *Bioinformatics 30*, pp. 1312-1313.

Sydeman, W. J., Poloczanska, E., Reed, T. E. & Thompson, S. A., 2015. Climate change and marine vertebrates. *Science 350*, pp. 772-777.

Taberlet, P. et al., 2012. Towards next-generation biodiversity assessment using DNA metabarcoding. *Molecular Ecology 21*, pp. 2045-2050.

Taylor, J. C., Harding, W. R. & Archibald, C. G. M., 2007. A methods manual for the collection, preparation and analysis of diatoms samples Version 1.0. *Water Research Commission Report TT 281/07, Pretoria*.

Theriot, E. C. et al., 2015. Dissecting signal and noise in diatom chloroplast protein encoding genes with phylogenetic information profiling. *Molecular Phylogenetics and Evolution*.

Trotta, A., 2020. *Microbial communities and antimicrobial resistance: an insight on bacterial world of sea turtles (Caretta caretta) from the Mediterranean sea.* PhD thesis ed. University of Bari "Aldo Moro": Department of veterinary medicine (DVM).

Underwood, G. J. & Paterson, D. M., 2003. The importance of extracellular carbohydrate production by marine epipelic diatoms. *Advances in Botanical Research 40*, pp. 184-231.

Van de Vijer, B. & Bosak, S., 2019. Planothidium kaetherobertianum, a new marine diatom (Bacillariophyta) species from the Adriatic Sea. *Phytotaxa 425*, pp. 105-112.

Wahl, M., 1989. Marine epibiosis. I. Fouling and antifouling: Some basic aspects. *Marine Ecology Progress Series 58*, pp. 175-189.

Wahl, M. & Mark, O., 1999. The predominately facultative nature of epibiosis: Experimental and observational evidence. *Marine Ecology Progress Series 187*, p. 59–66.

Wallace, B. P. et al., 2011. Global conservation priorities for marine turtles. *PLoS One 6:e24510*.

Wetzel, C. E., Van de Vijer, B. & Ector, L., 2010. Luticola deniseae sp. nov. a nem epizoic diatom from the Rio Negro (Amazon hydrographical basin). *Vie et Milieu - Life and environment 60*, pp. 177-184.

Witherington, B. E., Kubilis, P., Brost, B. & Meylan, A., 2009. Decreasing annual nest counts in a globally important loggerhead sea turtle population. *Ecology Applications 19*, pp. 30-54.

Witkowski, A. H., Lange-Bertalot, H. & Metzeltin, D., 2000. *Diatom flora of marine coasts. Annotated Diatom Micrographs.*. 1 ed. Koenigstein: Koeltz Scientific Books.

Wyneken, J., Lohmann, K. J. & Musick, J. A., 2013. *The biology of Sea Turtles.* Volume 3 ed. New York: CRC Press, Taxlor & Francis group.

Zardus, J. D. & Hadfield, M. G., 2004. Larval development and complemental males in Chelonibia testuditestudinaria, a barnacle commensal with sea turtles. *Journal of Crustacean Biology 24*, pp. 409-421.

7. SUPPLEMENTS

List of supplements:

Table S1. Information about loggerheads from HPM (Hrvatski prirodoslovni muzej, engl. Croatian Natural History Museum) set of samples. SCCL = standard curved carapace length, CCW = curved carapace width.

Table S2. Information about loggerheads from PTB (pre-TurtleBIOME) set of samples. SCCL = standard curved carapace length, MCCL = minimal curved carapace length, CCW = curved carapace width.

Table S3. Information about loggerheads from TB (TurtleBIOME) set of samples. SCCL = standard curved carapace length, MCCL = minimal curved carapace length, CCW = curved carapace width.

Table S4. The list of primers used for initial PCR, nested PCR and sequencing of SSU, *rbcL* and *psbC* molecular markers of cultured diatom strains PMFTB0073, PMFTB0074 and PMFTB0077.

Table S1. Information about loggerheads from HPM (Hrvatski prirodoslovni muzej, engl. Croatian Natural History Museum) set of samples. SCCL = standard curved carapace length, CCW = curved carapace width.

Sample code	Locality of finding	Date of finding	Method	Body condition	SCCL (cm)	CCW (cm)	sex
HPM3	Mali Lošinj, Kvarner (Croatia)	10/05/2002	stranded	?	60.7	56.8	?
HPM9	Piran Bay (Slovenia)	1995	stationary net	?	26.6	25.0	male
HPM23	Neretva, Komin (BiH)	21/06/2001	stationary gill nets	?	57.5	53.0	female
HPM24	Dugi Rat, Dugi otok (Croatia)	04/02/2002	floating in the sea	moderately decomposed	58.6	51.5	female
HPM25	Palagruza (Croatia)	23/04/2002	floating in the sea	moderately decomposed	84.5	75.0	female
HPM31	Prevlaka, Konavle, (Croatia)	20/09/2002	longline	fresh dead	41.4	37.1	male
HPM33	Lokrum, Dubrovnik (Croatia)	15/08/2002	gill net	?	40.4	37.0	female
HPM44	Zabudarski, Lošinj (Croatia)	01/12/2003	stranded in the beach	Severely decomposed	63.0	58.8	female
HPM48	Poreč (Croatia)	19/10/2002	stranded	moderately decomposed	79.2	69.2	female
HPM67	Pula (Croatia)	01/06/2003	floating in the sea	fresh dead	58.2	53.2	male
HPM68	North Adriatic (Croatia)	?	?	moderately decomposed	47.7	41.8	male
HPM69	Medulin (Croatia)	21/05/2004	stranded	moderately decomposed	51.3	46.3	female
HPM70	Krk (Croatia)	02/06/2004	?	moderately decomposed	38.2	35.5	female
HPM71	Mali Lošinj, Kvarner (Croatia)	19/05/2004	?	moderately decomposed	32.7	28.8	male

Table S2. Information about loggerheads from PTB (pre-TurtleBIOME) set of samples. SCCL = standard curved carapace length, MCCL = minimal curved carapace length, CCW = curved carapace width.

Sample code	Type of sample	Sea turtle name	Duration of rehabilitation	After rehabilitation	Locality of finding	Method	SCCL (cm)	MCCL (cm)	CCW (cm)	Weigth (kg)	Body conditon
PTB17	carapace	Bolko	17/06/2016 - 14/10/2016	released into nature	Lošinj	Floating on sea surface	32.2	31.6	29.5	3.45	debilitated syndrome
PTB24	skin	Shigy Lola	2/12/2016 - 16/06/2017	released into nature	Lošinj	Floating on sea surface	24.1	24.5	23	0.9	debilitated syndrome
PTB29	carapace	Marko	16/01/2017 - 16/06/2017	released into nature	Zadar, Karin Sea	stranded on a beach	58	56.5	54	21.4	hypothermic
PTB31	carapace	Raslinka	30/01/2017 - 16/06/2017	released into nature	Šibenik, Prokljan	stranded on a beach	62.5	60.8	60	48.2	hypothermic
PTB33	carapace	Miro	19/01/2017 - 16/06/2017	released into nature	Zadar, Karin Sea	stranded on a beach	64	63	57	28	hypothermic
PTB42	skin	Tilago	24/02/2017 -?	remained in the rehabilitation centre	Kornati	Floating on sea surface	64	63	58	28.2	carapace injury

Table S3. Information about loggerheads from TB (TurtleBIOME) set of samples. SCCL = standard curved carapace length, MCCL = minimal curved carapace length, CCW = curved carapace width.

Sample code	Type of sample	Sampling date	Sea turtle name	Duration of rehabilitation	After rehabilitation	Locality of finding	Method	SCCL (cm)	MCCL (cm)	CCW (cm)	Weigth (kg)	Body conditon
TB3	carapace	28/12/2017	Stela	22/12/2017-08/06/2018	released into nature	Veli Lošinj, Croatia	floating on sea surface	68	?	65	42.1	hypothermic
TB4	skin	28/12/2017										
TB5	carapace	4/16/2018	Cuki	29/03/2018 - 08/06/2018	released into nature	20 miles SW from Pula, Croatia	gill net	64	62.3	57	33	hypothermic
TB7	carapace	4/16/2018	Rada	16/04/2018 - 08/06/2018	released into nature	Telašćica, Dugi otok, Croatia	floating on sea surface	69	67.8	68	42	?
TB8	skin	4/16/2018										
TB25	carapace	8/6/2018										
TB9	skin	16/4/2018	Neven	25/06/2018 - 08/06/2019	released into nature	Medulin Bay, Croatia	gill net	36.5	35.4	34.2	5	?
TB19	carapace	1/6/2018										
TB11	carapace	28/5/2018	Mimi	14/04/2018 - 08/06/2020	released into nature	20 miles SW from Pula, Croatia	trawl net	69.5	69	67	42	hypothermic
TB12	skin	28/5/2018										
TB13	carapace	1/6/2018										
TB14	skin	1.6.2018.										
TB31	carapace	11/12/2018	Merry Fisher	08/12/2018 - 4/11/2019	released into nature	Korčula, Croatia	floating on sea surface	70	69	63.5	40	head and carapace injury

Sample code	Type of sample	Sampling date	Sea turtle name	Duration of rehabilitation	After rehabilitation	Locality of finding	Method	SCCL (cm)	MCCL (cm)	CCW (cm)	Weigth (kg)	Body conditon
TB33	carapace	21/12/2018	Martina	21/12/2018	died	?	swallowed fishing hook	38	27	25.5	?	poor
TB49	carapace	9/1/2019	Tarcontes	09/01/2019 - 11/01/2019	released into nature	Barletta, Bari, Italy	trawl net	50.7	50	51	31	gas embolism
TB55	carapace	10/1/2019	Reti	10/1/2019	released into nature	Barletta-Trani, Bari, Italy	trawl net	72	66.5	65.5	42	good
TB56	skin	10/1/2019										
TB73	carapace	17/01/2019	Murrana	17/01/2019 - 21/01/2019	released into nature	Barletta, Bari, Italy	trawl net	63	58.1	58	29.5	gas embolism
TB74	skin	17/01/2019										
TB89	carapace	22/01/2019	Iracus	22/01/2019	released into nature	Bisceglie, Bari, Italy	trawl net	72	66.1	66.5	45.6	good
TB90	skin	22/01/2019										
TB115	carapace	8/5/2019	Žal	02/05/2019 - 14/06/2019	released into nature	Albanež, Kamenjak, Croatia	gill net	53.5	52.5	51	20	?
TB116	skin	8/5/2019										
TB117	carapace	9/6/2019	Samba	08/06/2019 - 21/06/2019	died	Ston, Croatia	floating on sea surface	74	71	70	43	head and carapace injury
TB118	skin	9/6/2019										

Table S4. The list of primers used for initial PCR, nested PCR and sequencing of SSU, *rbcL* and *psbC* molecular markers of cultured diatom strains PMFTB0073, PMFTB0074 and PMFTB0077.

Primer ID	Name	Sequence	Description	Source
PT1	SSU1+	AACCTGGTTGATCCTGCCAGT	SSU initial PCR forward primer	Theriot et al. (2015)
PT2	SSUB-	CCTTCTGCAGGTTCACCTAC	SSU initial PCR reverse primer	Theriot et al. (2015)
PT3	SSU11+	TGATCCTGCCAGTAGTCATACGCT	SSU nested PCR forward primer	Theriot et al. (2015)
PT4	SSU1672-	TAGGTGCGACGGGCGGTGT	SSU nested PCR reverse primer	Theriot et al. (2015)
PT5	rbcL40+	GGACTCGAATYAAAAGTGACCG	rbcL initial and nested forward primer	Theriot et al. (2015)
PT6	rbcl1444-	GCGAAATCAGCTGTATCTGTWG	rbcL initial PCR reverse primer	Theriot et al. (2015)
PT7	rbcL1255-	TTGGTGCATTTGACCACAGT	rbcL nested PCR reverse primer	Theriot et al. (2015)
PT8	psbC+	CACGACCWGAATGCCACCAAT	psbC initial PCR forward primer	Theriot et al. (2015)
PT9	psbC-	ACAGGMTTYGCTTGGTGGAGTGG	psbC initial PCR reverse primer	Theriot et al. (2015)
PT10	psbC22+	CGTGGTGATACATAGTTA	psbC nested PCR forward primer	Theriot et al. (2015)
PT11	psbC1154-	GCDCAYGCTGGYTTAATGG	psbC nested PCR reverse primer	Theriot et al. (2015)
PT12	rbcL404+	GCTTTACGTTTAGAAGATATG	rbcL sequencing forward	Theriot et al. (2015)
PT13	rbcL587-	GTCTAAACCACCTTTTAAMCCTTC	rbcL sequencing reverse	Theriot et al. (2015)
PT14	psbC221+	ACGCATTGTTTCACCACC	psbC sequencing forward	Theriot et al. (2015)
PT15	psbC857-	CTTTGGTTATGACTGGCGTG	psbC sequencing reverse	Theriot et al. (2015)
PT16	SSU850+	GGGACAGTTGGGGGTATTCGTA	SSU sequencing forward	Theriot et al. (2015)
PT17	SSU1004+	CGAAGATGATTAGATACCATCG	SSU sequencing forward	Theriot et al. (2015)
PT18	SSUE4+	CAGAGGTGAAATTCT	SSU sequencing forward	Theriot et al. (2015)
PT19	SSUE7-	TCAGGCTCCCTCTCCGG	SSU sequencing reverse	Theriot et al. (2015)
PT20	SSUE8-	ACCGCGGCKGCTGGC	SSU sequencing reverse	Theriot et al. (2015)
PT21	SSUE9-	AGAATTTCACCTCTG	SSU sequencing reverse	Theriot et al. (2015)
PT22	SSUE11-	CGGCCATGCACCACC	SSU sequencing reverse	Theriot et al. (2015)
PT23	SSU870-	TACGAATACCCCCAACTGTCCC	SSU sequencing reverse	Theriot et al. (2015)
PT24	SSU1147-	AGTTTCAGCCTTGCGACCATAC	SSU sequencing reverse	Theriot et al. (2015)

8. CURRICULUM VITAE

Lucija Kanjer

address: Braće Leonardelli 31, 52100 Pula
e-mail: lkanjer@stud.biol.pmf.hr

Education

date	September 2017 – February 2020
institution	University of Zagreb, Faculty of Science, Department of Biology
program	**Ecology and Nature Preservation, marine module** (graduate study)

date	September 2014 – September 2017
institution	University of Zagreb, Faculty of Science, Department of Biology
program	**Biology** (undergraduate study)

Experience

date	April 2019 – November 2019
institution	Botanical Garden at Faculty of Science, University of Zagreb
description	**Educator and tourist guide** (job)

date	August 2018 – September 2018
institution	Centre ALGATECH, Institute of Microbiology (Czech Republic)
description	**Methods in aquatic microbiology** (Erasmus+ internship)

date	March 2018 – June 2018
institution	Laboratory for aquaculture and pathology of aquatic organisms, Ruđer Bošković Institute
description	**General microbiology techniques** (internship)

date	March 2016 – June 2016
institution	Department of Biology, Faculty of Science, University of Zagreb
internship	**Plankton diatom and dinoflagellates species identification** (internship)

Activities and projects

2019	Member of technical staff at Seventh European Phycology Congress – EPC7 in Zagreb
2017-2019	Algology Group leader at Biology Students Association – BIUS
2018	Member of Steering Committee at Biology Students Association – BIUS
2016-2019	Participant in educational-research projects “Insula Auri 2019”, “Insula Tilagus 2017” and “Mura-Drava 2016” organized by Biology Students Association – BIUS

2014-2019 Volunteer at the yearly science-popularization event "Biology Night" at Division of Biology, Faculty of Science, University of Zagreb

Publications

Kanjer, Lucija; Filek, Klara; Matek, Antonija; Majewska, Roksana; Van de Vijver, Bart; Ashworth, Matt P.; Gračan, Romana; Lazar, Bojan; Bosak, Sunčica (2019). Diatom genus *Poulinea* as epibiont on Adriatic loggerhead sea turtles. Sixth Croatian Botanical Symposium Book of Abstracts, eds: Jasprica, Nenad; Car, Ana. Zagreb: Hrvatsko Botaničko Društvo, p. 17-17 (oral presentation).

Kanjer, Lucija; Mucko, Maja; Car, Ana; Bosak, Sunčica (2019). Epiphytic diatoms on *Posidonia oceanica* (L.) Delile leaves from eastern Adriatic Sea. Natura Croatica 28; p. 1-20 doi:10.20302/NC.2019.28.1 (scientific article).

Filek, Klara; **Kanjer, Lucija**; Matek, Antonija; Trotta, Adriana; Majewska, Roksana; Ashworth, Matt P.; Van de Vijver, Bart; Bosak, Sunčica (2019). A polyphasic approach for identification of epibiotic diatoms associated with loggerhead sea turtles in Adriatic Sea. The Molecular Life of Diatoms - Programme and Abstract Book, ed: Mock, Thomas. Norwich, UK: EMBO Press, p. 105-105 (poster).

Bosak, Sunčica; Lazar, Bojan; Gračan, Romana; **Kanjer, Lucija**; Van de Vijver, Bart; Majewska, Roksana (2018). Epizoic diatoms associated with the neck skin of adriatic loggerhead seaturtle. Book of Abstracts, 6th Mediterranean Conference on Marine Turtles, eds: Lazar, Bojan; Jančić, Matic. Zagreb: Croatian Natural History Museum, p. 76-76 (poster).

Skills

English	Understanding C1, speaking B2, writing C1
German	Understanding B1, speaking A2, writing A2
Digital skills	MS Office (Word, Excel, PowerPoint), WordPress, R Studio, Adobe Photoshop, CorelDRAW, Primer
Other	Driving license B category

Awards

County of Istria excellence stipend (2019)
City of Pula excellence stipend (2016, 2017 and 2018)

Memberships

Biology Students Association – BIUS (2015-2019)
Young European Biologist – YEB (2017-2018)