Dissertation
zur Erlangung des Doktorgrades der Naturwissenschaften
an der Fakultät für Biologie
der Ludwig-Maximilians-Universität München

Diversity, Morphology, and Taxonomy of Selected Dinophytes

Juliane Kretschmann

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Juliane Kretschmann

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Erster Gutachter: Prof. Dr. Marc Gottschling
Zweiter Gutachter: Prof. Dr. Herwig Stibor

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Declaration

Erklärung

Ich erkläre hiermit, dass die Dissertation nicht ganz oder in wesentlichen Teilen einer anderen Prüfungskommission vorgelegt worden ist, und dass ich mich nicht anderweitig einer Doktorprüfung ohne Erfolg unterzogen habe.

Eidesstattliche Erklärung

Ich versichere hiermit an Eides statt, dass die vorgelegte Dissertation von mir selbstständig und ohne unerlaubte Hilfe angefertigt wurde.

Ohlstadt, den 08.06.2020 Juliane Kretschmann

Ort, Datum, Unterschrift

"Look at life all around; everything is growing,
everything is moving forward.
Therefore I recommend keeping in touch
with life and with art."

– Agrippina Vaganova

Summary

Dinophytes are unicellular eukaryotic algae and occur in nearly all marine and freshwater habitats worldwide. The biodiversity assessment of dinophytes started in the late 18th and early 19th century with light microscopy. For such historical descriptions, drawings have been mostly designated as types, because no original physical material is preserved. Based on these drawings many taxa cannot be unambiguously determined leading to an inconsistently use of names and a considerable taxonomic confusion. The International Code of Nomenclature for algae, fungi, and plants (ICN) provides the tool for designating an epitype for the clarification of such ambiguous historical names. An epitype is an interpretive, clarifying type ensuring a strong link between the species, its scientific name, its protologue and morphology as well as the genetic characterisation. Hence, epitypification has a great potential for a clarified taxonomy in various unicellular organismal groups.

The essential part of my project was the establishment of living dinophyte strains and ensuring constant access to fresh material, which allows detailed morphological investigations using light and scanning electron microscopy as well as molecular analyses. The established strains provided information for the specification of phylogenetic positions and resulted in the description of a new family, two new genera and the two new species, *Parvodinium marciniakii* and *P. trawinskii*. Furthermore, the established strains were used for the investigations on morphological and molecular intraspecific variability and for reliable inferences on the biogeography of dinophytes. Strains, established from samples collected at the type locality, that were morphologically consistent with corresponding protologues, were used for the taxonomic clarification of eight scientific names and the designation of interpretative epitypes. Taxonomic activity is usually the result of laborious work, which is associated with the gain of new morphological and molecular data.

Zusammenfassung

Dinophyten sind einzellige eukaryotische Algen, die in limnischen und marinen Habitaten weltweit zu finden sind. Die Erfassung ihrer Diversität begann bereits im späten 18. und frühen 19. Jahrhundert mittels Lichtmikroskopie. Für die meisten historischen Beschreibungen ist allerdings kein physisches Originalmaterial mehr erhalten und das Typusmaterial besteht aus Zeichnungen. Basierend auf diesen Zeichnungen können jedoch viele Taxa nicht zweifelsfrei bestimmt werden, was zu einer uneinheitlichen Verwendung der Namen und folglich zu einer erheblichen taxonomischen Verwirrung führt. Das grundlegende Werkzeug zur taxonomischen Klärung derartiger mehrdeutiger Namen gibt der International Code of Nomenclature for algae, fungi, and plants (ICN) mit der Möglichkeit einer Epitypisierung zur Hand. Ein Epityp ist interpretatives, klärendes Typusmaterial, das eine Verknüpfung zwischen der Art, ihrem wissenschaftlichen Namen, ihrem Protolog, der Morphologie sowie ihrer genetischen Information herstellt. Auf dem Weg zu einer zweifelsfreien Taxonomie innerhalb der Dinophyten, sowie zahlreicher weiterer einzelliger Organismengruppen, hat die Epitypisierung daher großes Potenzial.

Wesentlicher Teil meines Projektes war die Etablierung von lebenden Dinophytenstämmen, die die Grundlage für detaillierte morphologische Untersuchungen mittels Licht- und Rasterelektronenmikroskopie sowie für molekulare Analysen bildeten. Die Stämme lieferten Informationen über phylogenetische Positionen und führten zu den Beschreibungen einer neuen Familie, zweier neuer Gattungen sowie den neuen Arten *Parvodinium marciniakii* und *P. trawinskii.* Des Weiteren dienten die Stämme den Untersuchungen zur morphologischen und molekularen intraspezifischen Variabilität und ermöglichten zuverlässige Rückschlüsse über die Biogeographie. Stämme, die aus Proben etabliert werden konnten, die an Typuslokalitäten gesammelt wurden und morphologisch mit den entsprechenden Protologen übereinstimmten, dienten der Klärung von acht wissenschaftlichen Namen und der Benennung von interpretativen Epitypen. Taxonomische Aktivität ist gewöhnlich das Ergebnis mühevoller Arbeit, die mit dem Zugewinn neuer morphologischer und molekularer Daten einhergeht.

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List of publications

Publications included in this dissertation

GOTTSCHLING, M., **KRETSCHMANN, J.** & ŽERDONER ČALASAN, A. (2017) Description of Peridiniopsidaceae, fam. nov. (Peridiniales, Dinophyceae). *Phytotaxa* **299:** 293–296.
https://doi.org/10.11646/phytotaxa.299.2.16

GOTTSCHLING, M., ŽERDONER ČALASAN, A., **KRETSCHMANN, J.** & GU, H. (2017) Two new generic names for dinophytes harbouring a diatom as an endosymbiont, *Blixaea* and *Unruhdinium* (Kryptoperidiniaceae, Peridiniales). *Phytotaxa* **306:** 296–300.
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IZQUIERDO LÓPEZ, A., **KRETSCHMANN, J.**, ŽERDONER ČALASAN, A. & GOTTSCHLING, M. (2017) The many faces of *Peridinium cinctum* (Peridiniaceae, Peridiniales): Morphological and molecular variability in a common dinophyte. *European Journal of Phycology* **53:** 156–165.
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KRETSCHMANN, J., OWSIANNY, P.M., ŽERDONER ČALASAN, A. & GOTTSCHLING, M. (2018) The hot spot in a cold environment: Puzzling *Parvodinium* (Peridiniopsidaceae, Peridiniales) from the Polish Tatra Mountains. *Protist* **169:** 206–230.
https://doi.org/10.1016/j.protis.2018.02.004

KRETSCHMANN, J., ŽERDONER ČALASAN, A. & GOTTSCHLING, M. (2018) Molecular phylogenetics of dinophytes harboring diatoms as endosymbionts (Kryptoperidiniaceae, Peridiniales), with evolutionary interpretations and a focus on the identity of *Durinskia oculata* from Prague. *Molecular Phylogenetics and Evolution* **118:** 392–402.
https://doi.org/10.1016/j.ympev.2017.10.011

KRETSCHMANN, J., ŽERDONER ČALASAN, A., KUSBER, W.-H. & GOTTSCHLING, M. (2018) Still curling after all these years: *Glenodinium apiculatum* Ehrenb. (Peridiniales, Dinophyceae) repeatedly found at its type locality in Berlin (Germany). *Systematics and Biodiversity* **16:** 200–209. https://doi.org/10.1080/14772000.2017.1375045

KRETSCHMANN, J., ŽERDONER ČALASAN, A., MEYER, B. & GOTTSCHLING, M. (2020) Zero intercalary plates in *Parvodinium* (Peridiniopsidaceae, Peridiniales) and phylogenetics of *P. elpatiewskyi*, comb. nov. *Protist* **171** (in press). https://doi.org/10.1016/j.protis.2019.125700

ROMEIKAT, C., IZQUIERDO LÓPEZ, A., TIETZE, C., **KRETSCHMANN, J.** & GOTTSCHLING, M. (2019) Typification for reliable application of subspecific names within *Peridinium cinctum* (Peridiniales, Dinophyceae). *Phytotaxa* **424:** 147–157. https://doi.org/10.11646/phytotaxa.424.3.2

ŽERDONER ČALASAN, A., **KRETSCHMANN, J.**, FILIPOWICZ, N.H., IRIMIA, R.-E., KIRSCH, M. & GOTTSCHLING, M. (2019) Towards global distribution maps of unicellular organisms such as calcareous dinophytes based on DNA sequence information. *Marine Biodiversity* **49:** 749–758. https://doi.org/10.1007/s12526-018-0848-y

Additional publications

FELDBERG, K., VÁŇA, J., KRUSCHE, J., KRETSCHMANN, J., PATZAK, S.D.F., PÉREZ-ESCOBAR, O.A., RUDOLF, N.R., SEEFELDER, N., SCHÄFER-VERWIMP, A., LONG, D.G., SCHNEIDER, H. & HEINRICHS, J. (2016) A phylogeny of Cephaloziaceae (Jungermanniopsida) based on nuclear and chloroplast DNA markers. *Organisms Diversity & Evolution* **16:** 727–742.

GOTTSCHLING, M., CHACÓN, J., ŽERDONER ČALASAN, A., NEUHAUS, S., **KRETSCHMANN, J.**, STIBOR, H. & JOHN, U. (2020) Phylogenetic placement of environmental sequences using taxonomically reliable databases helps to rigorously assess dinophyte biodiversity in Bavarian lakes (Germany). *Freshwater Biology* **65:** 193–208.

HEIGL, H.M.L., **KRETSCHMANN, J.**, HILGER, H.H. & GOTTSCHLING, M. Flower and fruit anatomy of *Cordia nodosa* Lam. and *Varronia bonplandii* Desv. (Cordiaceae, Boraginales) with phylogenetic implication. *Organisms Diversity & Evolution* (in press).

ŽERDONER ČALASAN, A., **KRETSCHMANN, J.** & GOTTSCHLING, M. (2018) Absence of co-phylogeny indicates repeated diatom capture in dinophytes hosting a tertiary endosymbiont. *Organisms Diversity & Evolution* **18:** 29–38.

ŽERDONER ČALASAN, A., **KRETSCHMANN, J.** & GOTTSCHLING, M. Integrative taxonomy for sexually-deprived protists in the 21st century: A case study of *Trachelomonas* Ehrenb. (Euglenaceae) from Western Ukraine. *Taxon* (in press).

ŽERDONER ČALASAN, A., **KRETSCHMANN, J.** & GOTTSCHLING, M. (2019) They are young, and they are many: Dating freshwater lineages in unicellular dinophytes. *Environmental Microbiology* **21**: 4125–4135.

Declaration of contribution as a co-author

In this cumulative thesis, I present the results of my doctoral research, which was conducted under the supervision of Prof. Dr. Marc Gottschling at the Ludwig-Maximilians-University of Munich. The results of my research have been published in international peer-reviewed journals and are presented in the appendix of the thesis. All of them have resulted from collaborations with other scientists, and my contributions to each of them are as follows:

Publication 1

KRETSCHMANN, J., OWSIANNY, P.M., ŽERDONER ČALASAN, A. & GOTTSCHLING, M. (2018) The hot spot in a cold environment: Puzzling *Parvodinium* (Peridiniopsidaceae, Peridiniales) from the Polish Tatra Mountains. *Protist* **169:** 206–230. https://doi.org/10.1016/j.protis.2018.02.004

Own contribution: Strain isolation and cultivation (95%); morphological analysis (incl. images: 90%); type and epitype preparation (100%); manuscript preparation (35%).

Publication 2

KRETSCHMANN, J., ŽERDONER ČALASAN, A. & GOTTSCHLING, M. (2018) Molecular phylogenetics of dinophytes harboring diatoms as endosymbionts (Kryptoperidiniaceae, Peridiniales), with evolutionary interpretations and a focus on the identity of *Durinskia oculata* from Prague. *Molecular Phylogenetics and Evolution* **118:** 392–402. https://doi.org/10.1016/j.ympev.2017.10.011

Own contribution: Field work (50%); strain isolation and cultivation (95%); morphological analysis (incl. images: 90%); epitype preparation (100%); manuscript preparation (30%).

Publication 3

KRETSCHMANN, J., ŽERDONER ČALASAN, A., KUSBER, W.-H. & GOTTSCHLING, M. (2018) Still curling after all these years: *Glenodinium apiculatum* Ehrenb. (Peridiniales, Dinophyceae) repeatedly found at its type locality in Berlin (Germany). *Systematics and Biodiversity* **16:** 200–209. https://doi.org/10.1080/14772000.2017.1375045

Own contribution: Field work (40%); strain isolation and cultivation (95%); morphological analysis (incl. images: 90%); epitype preparation (100%); manuscript preparation (30%).

Publication 4

KRETSCHMANN, J., ŽERDONER ČALASAN, A., MEYER, B. & GOTTSCHLING, M. (2020) Zero intercalary plates in *Parvodinium* (Peridiniopsidaceae, Peridiniales) and phylogenetics of *P. elpatiewskyi*, comb. nov. *Protist* **171** (in press). https://doi.org/10.1016/j.protis.2019.125700

Own contribution: Field work (50%); strain isolation and cultivation (95%); morphological analysis (incl. images: 40%); manuscript preparation (20%).

Publication 5

GOTTSCHLING, M., **KRETSCHMANN, J.** & ŽERDONER ČALASAN, A. (2017) Description of Peridiniopsidaceae, fam. nov. (Peridiniales, Dinophyceae). *Phytotaxa* **299:** 293–296. https://doi.org/10.11646/phytotaxa.299.2.16

Own contribution: Study concept (30%); manuscript preparation (15%).

Publication 6

GOTTSCHLING, M., ŽERDONER ČALASAN, A., **KRETSCHMANN, J.** & GU, H. (2017) Two new generic names for dinophytes harbouring a diatom as an endosymbiont, *Blixaea* and *Unruhdinium* (Kryptoperidiniaceae, Peridiniales). *Phytotaxa* **306:** 296–300.

https://doi.org/10.11646/phytotaxa.306.4.6

Own contribution: Study concept (30%); manuscript preparation (15%).

Publication 7

IZQUIERDO LÓPEZ, A., **KRETSCHMANN, J.**, ŽERDONER ČALASAN, A. & GOTTSCHLING, M. (2017) The many faces of *Peridinium cinctum* (Peridiniaceae, Peridiniales): Morphological and molecular variability in a common dinophyte. *European Journal of Phycology* **53:** 156–165. https://doi.org/10.1080/09670262.2017.1397198

Own contribution: Field work (50%); strain isolation and cultivation (95%); morphological analysis (incl. images: 40%); manuscript preparation (20%).

Publication 8

ROMEIKAT, C., IZQUIERDO LÓPEZ, A., TIETZE, C., **KRETSCHMANN, J.** & GOTTSCHLING, M. (2019) Typification for reliable application of subspecific names within *Peridinium cinctum* (Peridiniales, Dinophyceae). *Phytotaxa* **424:** 147–157. https://doi.org/10.11646/phytotaxa.424.3.2

Own contribution: Field work (50%); strain isolation and cultivation (95%); morphological analysis (incl. images: 15%); manuscript preparation (10%).

Publication 9

ŽERDONER ČALASAN, A., **KRETSCHMANN, J.**, FILIPOWICZ, N.H., IRIMIA, R.-E., KIRSCH, M. & GOTTSCHLING, M. (2019) Towards global distribution maps of unicellular organisms such as calcareous dinophytes based on DNA sequence information. *Marine Biodiversity* **49:** 749–758. https://doi.org/10.1007/s12526-018-0848-y

Own contribution: Strain isolation and cultivation (30%); morphological analysis (incl. images: 90%); manuscript preparation (20%).

General introduction

Biodiversity and ecology

Dinophytes are a highly diverse group of unicellular eukaryotic algae (Fig. 1) that constitute a major component of the marine and freshwater phytoplankton and play as primary producers an important role in the global aquatic ecosystems (Fensome *et al.* 1993; Taylor *et al.* 2008). The word 'dino-' is derived from the ancient Greek '*δῖνος*' meaning 'whirling' and refers to the characteristic swimming behaviour of the motile cells (Bütschli 1885). Dinophytes are distributed worldwide and occur in nearly all marine and freshwater habitats from the polar regions to the tropics. However, the species richness in marine environments, including estuaries and brackish coastal waters, is remarkably higher than in freshwater environments.

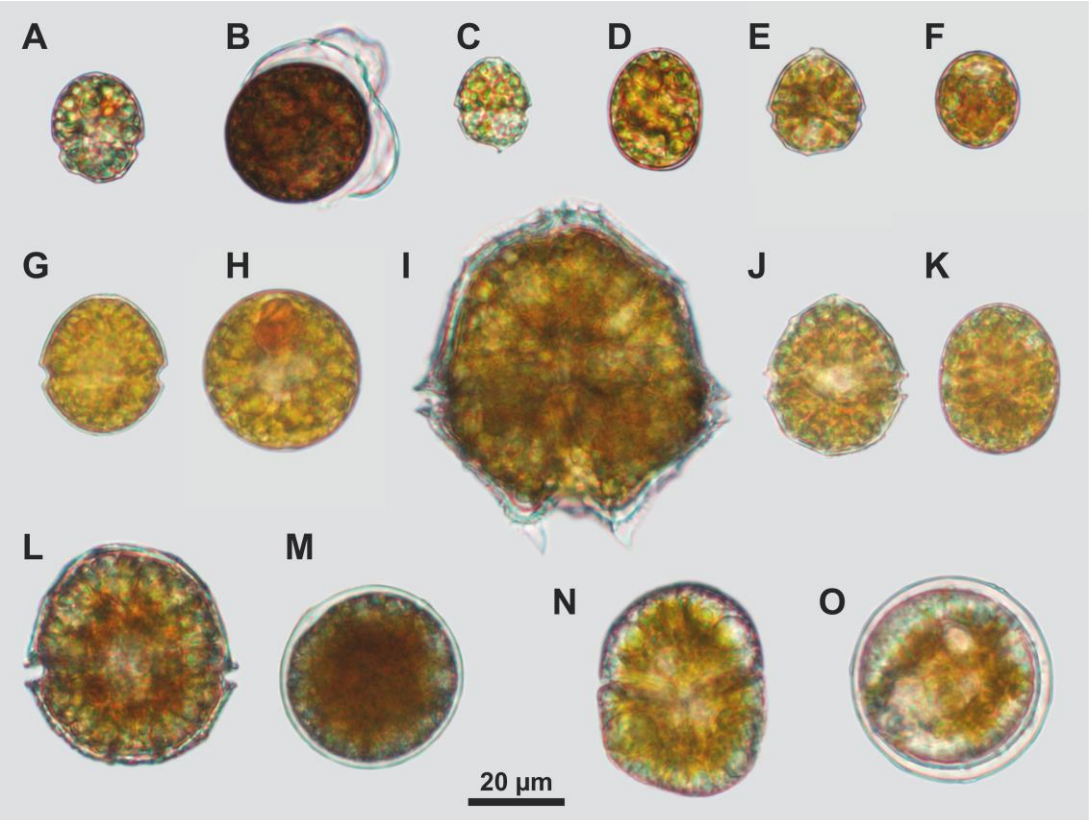

Figure 1: Morphological diversity of motile and immotile stages of selected freshwater dinophytes (all cells on the same scale). A–B *Parvodinium* cf. *umbonatum* (Peridiniopsidaceae). C–D *Parvodinium trawinskii* (Peridiniopsidaceae). E–F *Parvodinium mixtum* (Peridiniopsidaceae). G–H *Chimonodinium lomnickii* var. *wierzejskii* (Thoracosphaeraceae). I *Peridinium bipes* (Peridiniaceae). J–K *Palatinus apiculatus* (Peridiniopsidaceae). L–M *Peridinium cinctum* (Peridiniaceae). N–O *Spiniferodinium limneticum* (Gymnodiniaceae).

From more than 2000 extant species, approximately 350 species are described from freshwater habitats (Fensome *et al.* 1993; Taylor *et al.* 2008; Gómez 2012a; Mertens *et al.* 2012; Moestrup & Calado 2018).

Dinophytes exhibit an enormous diversity in lifestyle types and nutrition modes (Gómez 2012b). Roughly half of the known dinophyte species are phototrophic, possessing chloroplasts of multiple origins derived from red or green algae, cryptomonades, haptophytes, or diatoms that have been acquired through several endosymbiotic events (Keeling 2004). The other half lack chloroplasts and are heterotrophic predators on bacteria, phytoplankton (including other dinophytes), heterotrophic protists, metazoans, copepod eggs, and naupliar stages (Jacobson & Anderson 1986; Jeong 1999; Anderson & Menden-Deuer 2017). Additionally, some dinophytes are capable to combine photosynthesis with heterotrophy, termed mixotrophy (Stoecker 1999; Jeong *et al.* 2004; Fawcett & Parrow 2014).

The majority of dinophytes are free-living, but some species are ecto- or endoparasites with a wide host range including ciliates, other free-living dinophytes, invertebrates, and vertebrates (Chatton 1920; Coats 1999; Levy *et al.* 2007; Skovgaard *et al.* 2012; Jung *et al.* 2016). In some cases, they are capable to form mutualistic symbioses with various groups of protists and invertebrates such as cnidarians, sponges, and molluscs (Trench 1993; Stat *et al.* 2008; Annenkova *et al.* 2011; Hehenberger *et al.* 2016).

A few dinophytes are known to accumulate in masses causing algae blooms, discolouring the coastal water (Fraga *et al.* 1995; Pienaar *et al.* 2007; Sampedro *et al.* 2011; Tillmann *et al.* 2017) or lakes brownish or reddish (Horne *et al.* 1971; Moestrup *et al.* 2006; Takano *et al.* 2008; Zhang *et al.* 2016). Mass accumulation of toxin-producing dinophytes are of particular threat, because the toxins may accumulate in the food web and are responsible for poisoning symptoms of fish and shellfish as well as seabirds, marine mammals, and consequently humans (Anderson 1995; Van Dolah 2000; Tillmann *et al.* 2009). In the last few decades, mass accumulations of dinophytes appear to increase worldwide in frequency, intensity, and geographic distribution, largely explained as a result of anthropogenic eutrophication, introduction of invasive species as well as global

climate changes (Fraga & Bakun 1993; Hallegraeff 1993; Anderson 1995; Sellner *et al.* 2003).

Taxonomic unambiguity of scientific names is a necessary prerequisite for the fundamental understanding of biodiversity and the communication about organisms (Morrison *et al.* 2009; Steinicke 2014; Wilson 2017). Only reliable species determinations make it possible, for example, to distinguish between toxic and non-toxic species in order to avoid poisoning of humans and animals as well as to detect invasive or bloom-forming species in a timely manner. In addition, unambiguous names are necessary to make reliable inferences on species distribution, as biogeography has not been well understood so far due to misidentifications and unclear naming.

General morphology

The life history of dinophytes is complex and consists of various stages, including motile and immotile cells (Pfiester & Anderson 1987; Fensome *et al.* 1993; Rengefors & Kremp 2018), both showing a great morphological diversity (Fig. 1). The general morphology of motile dinophyte cells is summarised in Figure 2. A very detailed compilation of the morphological traits of dinophyte cells is given in Moestrup & Calado (2018), therefore only a brief overview is provided in this section.

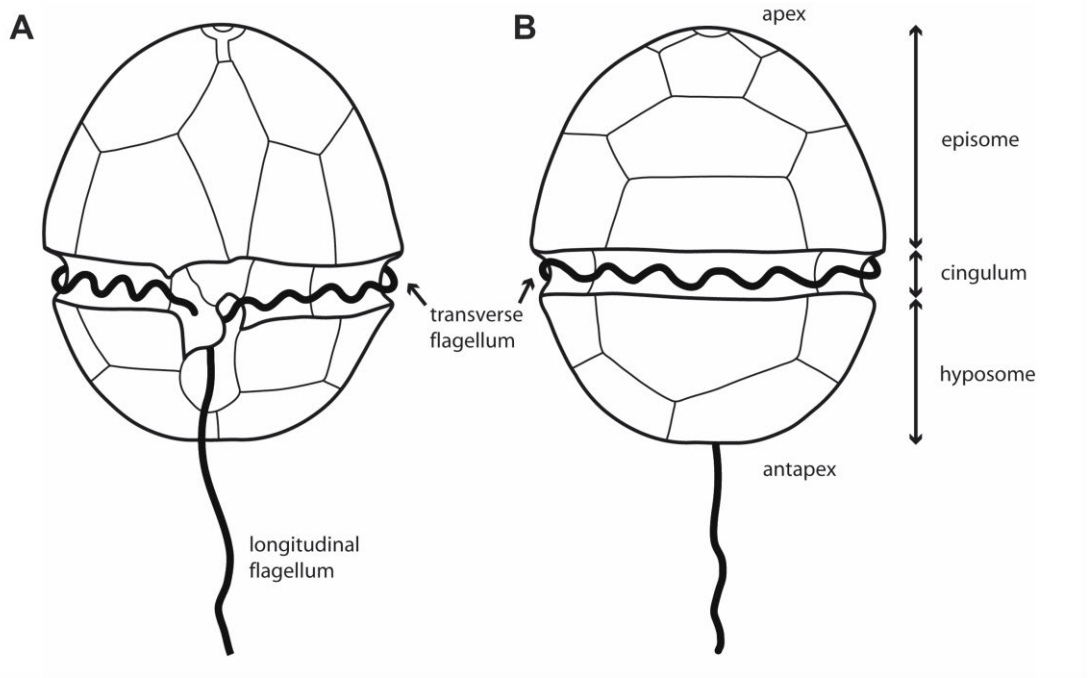

Figure 2: General morphology of a motile thecate cell. A ventral view. B dorsal view.

The size of motile cells ranges from a few µm to 100 µm in length, although a few species (e.g. *Noctiluca scintillans* Macartney Kof. & Swezy) can reach sizes up to 2 mm (Taylor 1980). Typically, motile dinophyte cells are surrounded by a transversal groove, termed cingulum, dividing the cell body into an epi- and hyposome (Fig. 2). A longitudinal groove on the hyposome, so-called sulcus, defines the ventral side of the cell. Motile cells have two morphologically differentiated flagella and comprise a specialised layer of amphiesmal vesicles directly beneath the plasma membrane, termed alveoli. The cellular alveoli sometimes contain cellulosic plates, that cover the whole cell surface, building some sort of an 'armour' termed theca. The

arrangement, number, and shape of such thecal plates serve as important diagnostic characters for species determination and taxonomy (e.g. Taylor 1980; Dodge 1985; Fensome *et al.* 1993). Dinophytes lacking such cellulosic plates are termed athecate, naked or unarmoured.

The immotile stages of dinophytes (Fig. 1) have been extensively studied for marine dinophyte species, whereas the immotile stages of freshwater species have received less attention (Mertens *et al.* 2012). Of the approximately 350 species of freshwater dinophytes, immotile cells have been described for only a quarter (Mertens *et al.* 2012). The immotile stages can be distinguished based on either ecological or morphological features (Fensome *et al.* 1993). The ecological and widespread term 'cysts' is generally adopted for cells lacking flagella and thus the ability of movement (Stosch 1973; Pfiester & Anderson 1987; Matsuoka & Fukuyo 2000). The distinction of cyst types is mainly based on their ecological functions. For example, resting cysts are defined as resting zygotes formed by fusion of gametes in the process of sexual reproduction (Matsuoka & Fukuyo 2000). However 'resting' as well as 'sexuality' are both ecological functions, also occuring independent of each other. Therefore, the mutual condition of functions is problematic, because the life histories are rarely investigated and thus the ecological functions of such cells as well as their ploidy level are largely unknown. In such cases, a morphological rather than ecological distinction can be used. Important morphological traits of immotile stages are colour, shape, surface ornamentation as well as shape and position of the archeopyle, the opening through which a motile cell or several of them germinate.

Phylogenetic systematics and classification

Together with their closest relatives, namely apicomplexans and ciliates, dinophytes belong to the alveolates, which are characterised by the presence of alveoli directly beneath the plasma membrane (Escalante & Ayala 1995; Harper *et al.* 2005; Adl *et al.* 2019). The 'core' dinophytes form a well-supported monophyletic group (Fensome *et al.* 1999; Leander & Keeling 2004; Costas & Goyanes 2005; Okamoto *et al.* 2012; Gu *et al.* 2013; Gottschling *et al.* 2020) based on both molecular and morphological apomorphies such as two morphologically differentiated flagella and the dinokaryotic nucleus with permanently condensed liquid crystalline chromosomes that lack the typical eukaryotic nucleosomes (Lin 2011; Wisecaver & Hackett 2011; Gornik *et al.* 2019).

Traditionally, dinophytes were classified based on morphological characters detectable by light microscopy. Traits such as cell size and shape, presence or absence of an apical pore and an eyespot, the size ratio of epi- to hyposome, girdle displacement, and position of the nucleus were considered as having diagnostic potential. Additionally, the arrangement of the amphiesmal vesicles (filled or not with cellulose thecal plates) as well as their number and shape were used as important diagnostic characters for species determination. Based on these traits extant dinophytes segregate into the following, morphologically well recognisable groups: dinophysoid, gonyaulacoid, gymnodinioid, peridinioid, prorocentroid, and suessioid (Taylor 1980; Fensome *et al.* 1993; Taylor 2004).

During the past three decades and in parallel to morphological investigations, molecular phylogenetic studies have greatly contributed to the knowledge about the relationships in dinophytes. Molecular phylogenetic trees early confirmed the monophyly of the Dinophysales, Gonyaulacales, and Suessiales (Saldarriaga *et al.* 2004). However, molecular trees obtained from a single locus are generally poorly resolved (Saldarriaga *et al.* 2004; Taylor 2004), and the morphologically well circumscribed Peridiniales and Prorocentrales are only monophyletic using concatenated sequences from nuclear (SSU, ITS, LSU, *hsp*90), mitochondrial (MT-CYB, MT-CO1), and/or plastid (*psb*A, *psb*C) loci (Zhang *et al.* 2007; Murray *et al.* 2009; Orr *et al.* 2012; Tillmann *et al.* 2012; Gottschling & McLean 2013; Gu *et al.*

2013). The athecate Gymnodiniales presents a monophyletic group once particular taxa such as Brachidiniaceae and Tovelliaceae are excluded (Kremp *et al.* 2005; Hansen & Daugbjerg 2011; Gottschling *et al.* 2012; Gu *et al.* 2013; Gottschling *et al.* 2020).

The further step towards a better understanding of dinophyte evolution marks the use of next-generation sequence data (NGS; Janouškovec *et al.* 2017; Price & Bhattacharya 2017). However, the phylogenetic relationship of the currently known dinophyte diversity is still not sufficiently clarified at present, due to the limited taxon sample (compared to the total diversity), insufficient sequence data (frequent single loci only), and a strong rate heterogeneity (Gottschling *et al.* 2012; Gu *et al.* 2013; Žerdoner Čalasan *et al.* 2019).

Current taxonomic state

Taxonomy is the science of identifying, describing, and classifying taxa. Each taxon is given a Latinised name according to a formal system of naming, termed nomenclature. Such work is essential for the assessment of biodiversity as well as the common understanding of organisms (Morrison *et al.* 2009; Steinicke 2014; Wilson 2017). In the fields of botany, mycology, and phycology, the formal scientific naming and publishing of taxa is governed by a set of rules and recommendations, namely the International Code of Nomenclature for algae, fungi, and plants (ICN, formerly ICBN; Turland *et al.* 2018). According to the ICN, each scientific species' name should be formally linked to a physical specimen, termed type, providing an objective and permanent link between a taxon and its name. The designated type material is essential to the application of names and ensures its uniqueness.

The biodiversity assessment of dinophytes started in the late 18th and early 19th century. Early species descriptions from, for example, Ch.G. Ehrenberg (1795–1876), F. von Stein (1818–1885), and E. Lindemann (1888–1945) are based on precise observations gained by light microscopy. However, such historical species' descriptions may cause difficulties for several reasons:

(1) Only in rare cases, physical original material is preserved. A taxonomically important example is the Ehrenberg Collection, containing several thousand microscopic preparations and drawings, deposited at the Museum für Naturkunde in Berlin (Fig. 3). However, the more common case is that no physical material is preserved, or the material is lost or destroyed such as in the case of J. Wołoszyńska (1882–1951), whose original material was destroyed during the Second World War (pers. comm. Prof. Dr. Konrad Wołowski in June 2016). For that reason, drawings have frequently served as types. However, such a drawing is always an interpretation of the species morphology by the drawer and is usually simplified (Gómez 2007).

(2) From a contemporary point of view, some historical descriptions lack information for a reliable species determination about crucial characters such as sizes of the cells (e.g. in Stein 1883) or thecal tabulation pattern, leading to

different understandings and misinterpretations of species by further authors (Gómez 2007; **publication 2**).

(3) Historical descriptions are based mostly on one or very few specimens found in freshly collected or fixed field samples. However, observations on single cells imply no evaluation of natural variability of morphological characters necessary to delimit taxa (Hoppenrath *et al.* 2013; Leliaert *et al.* 2014). First cultivation experiments were done by J. Wołoszyńska and E. Lindemann in the 1920's (Wołoszyńska 1925; Lindemann 1929).

(4) The life history of many dinophytes includes morphologically different stages. In some instances, these stages have been described as separate species, before their relationship was elucidated in the cultivation experiments (Wall & Dale 1967, 1968; Matsuoka 1988; Elbrächter *et al.* 2008). Different names for a single species are considered as heterotypic synonyms. According to the priority principles in the ICN, older names have priority over younger names (Turland *et al.* 2018).

Hence, in most cases the historical descriptions do not allow a reliable species identification, and the taxonomic and nomenclatural situation is unclear. The inconsistent use of names makes meaningful and taxonomically indisputable conclusions about biodiversity, ecology, and distribution impossible. Thus, the precise taxonomic identity of the majority of the dinophyte species remains to be clarified until now.

During the last decades, molecular approaches have gained importance for taxonomy. The use of a short standardised DNA sequence for species identification is called DNA barcoding (Thomas 2009; Vernooy *et al.* 2010). The basis for this approach is a large public DNA barcode reference library containing reliable DNA barcodes—preferable from type specimens. Unidentified specimens could be determined by finding the closest matching reference barcode in the library. For dinophytes, the ribosomal internal transcribed spacer (ITS) region has been proposed to serve as a species-specific DNA barcode (LaJeunesse 2001; Gottschling *et al.* 2005; Litaker *et al.* 2007; Stern *et al.* 2012).

Molecular sequence data have shown the existence of a large sequence diversity of molecular ribotypes in morphologically indistinguishable taxa. The research on so-called 'cryptic species' has increased in the past decades caused by an increasing

availability on DNA sequence data (Bickford *et al.* 2007). Cryptic speciation has also been reported for dinophytes (Montresor *et al.* 2003; Gottschling *et al.* 2005; Lilly *et al.* 2007; Genovesi *et al.* 2010; Gómez *et al.* 2011; Murray *et al.* 2012; Söhner *et al.* 2012; Anglès *et al.* 2017; Daugbjerg *et al.* 2019; **publication 7–9**). The discovery of such species complexes indicates that morphology-based species determinations should be handled with care and underlines the great importance to link a type specimen to DNA sequence data since species cannot be distinguished based on their morphology alone.

Epitypification as a taxonomic tool

For character-poor, unicellular organisms, such as dinophytes, a reliable link between the scientific species name and the genetic characterisation (i.e. DNA barcoding) is of particular importance and crucial for a robust taxonomy. However, the majority of dinophyte names are ambiguous, mostly because the types are not linked to DNA. There is an ongoing debate how to proceed with unreliable historical names and in hopes to reach a more stable taxonomy (Smith *et al.* 2016).

For the taxonomic clarification of ambiguous scientific names, the International Code of Nomenclature for algae, fungi and plants (ICN) provides the tool to designate an epitype (Greuter *et al.* 1994), described in Article 9.9 (Shenzhen Code): 'An epitype is a specimen or illustration selected to serve as an interpretative type when the holotype, lectotype, or previously designated neotype, or all original material associated with a validly published name, is demonstrably ambiguous and cannot be critically identified for purposes of the precise application of the name to a taxon.' (Turland *et al.* 2018). The designation of epitypes enables to achieve a status of a clear and stable taxonomy especially in unicellular organismal groups and strive towards keeping historic scientific names. However, this approach has not been applied for dinophytes frequently before the work presented here (Litaker *et al.* 2009; Zinßmeister *et al.* 2011; Nézan *et al.* 2012; Saburova *et al.* 2012).

The basic prerequisite to apply the epitypification approach is to collect living material at the type locality (or a locality that is as close as possible) and preferable in the same season (Hyde & Zhang 2008; Kretschmann *et al.* 2014, 2015a, 2015b; Gottschling *et al.* 2018, 2019; **publication 1–3, 8**). Established strains provide material for morphological re-investigations for contemporary light and scanning electron microscopy. Strains, which do not contradict the protologue (and preferably agree with previous interpretations) are used for the designation of interpretative epitypes in form of, for example, slides for light microscopy or preparations for scanning electron microscopy and are deposited in natural history collections. DNA barcoding of the epitypified strains is a key tool for reliable species determination and a valuable contribution for a clear taxonomy.

Aims of the thesis

1) Field work, strain isolation and cultivation

A fundamental step for my research is the extensive collection of freshwater tow and sediment samples. The established, preferably monoclonal dinophyte strains provide the material for comparative morphology and molecular investigations (**publication 1–9**). Moreover, maintenance of strains is of key importance for conservation, therefore corresponding strains are to be deposited in public culture collections, such as the Central Collection of Algal Cultures (CCAC), Culture Collection of Baltic Algae (CCBA), and Canadian Center for the Culture of Microorganisms (CCCM; **publication 1–3, 7–8**).

2) Detailed morphological investigation of cultivated material

The established strains are the basis for detailed morphological studies using light (incl. fluorescence) and scanning electron microscopy (**publication 1–4, 7–9**). The focus of my thesis is the comprehensive morphological investigation of strains to record different life history stages as well as intraspecific and intrastrain variability (**publication 1–4, 7–9**).

3) Taxonomic work

Taxonomic unambiguity of scientific names is a necessary prerequisite for the understanding of biodiversity and the communication about organisms. The investigated strains provide the basis for resolving the taxonomy of the species involved.

3.1 clarification of unreliable species names including the designation of epitypes (**publication 1–3, 8**)

3.2 description of new taxa and names (**publication 1, 5–6**)

3.3 clarification of phylogenetic positions (**publication 1, 4**)

4) Applied studies based on clarified taxonomy

Reliable species names and a clear taxonomy provides the basis for a wide range of other scientific studies such as:

4.1 inferences on biogeography (**publication 9**)

4.2 investigation of evolutionary ecology (**publication 2**)

4.3 inferences of the chloroplast origin in dinophytes harbouring diatoms

General discussion

Importance of (living) collections

Natural history collections (e.g. herbaria) document biodiversity over time and space and are as such irreplaceable and of inestimable value for the humankind (Michener 1970; Lane 1996; Powers *et al.* 2014). Each preserved specimen provides information as inherent part of itself, for example, information about anatomy, morphology, and genetics. Moreover, attached labels should provide additional information, for example, about collector(s), collecting date and locality, ecology, and/or biogeography (Lane 1996; Chavan & Krishnan 2003). Historically, an important role of collections was to store specimens to ensure the unambiguity of scientific names. Later, natural history collections have become an essential source for a wide range of scientific studies, such as spatial and temporal distribution of species (Lane 1996; James *et al.* 2018), phenological reconstructions (Borchert 1996; Lavoie & Lachance 2006; Zohner & Renner 2014) or environmental effects of climate change (Suarez & Tsutsui 2004; Lang *et al.* 2019). Furthermore, the stored specimens, especially of types, are used to gain sequence information for DNA barcoding (Pawlowski *et al.* 2012; international barcode of life project: https://ibol.org/).

For unicellular organisms such as dinophytes, historical material is preserved in very rare cases only. A taxonomically important example is the Ehrenberg Collection incorporated into the Museum für Naturkunde in Berlin (Fig. 3). Ehrenberg was one of the most important early scientists working on the taxonomy of microscopic organisms. The collection consists of several thousand raw samples,

Figure 3: Photographs of the Ehrenberg Collection during a trip to Berlin in June 2016. A shelve containing folders with stripes of Ehrenberg's mica preparations. B close up of a folder with mica preparations. C close up view of a strip and the labels with the taxon names.

approximately 40.000 of microscopic preparations (Fig. 3C) as well as several thousand drawings (Lazarus 1998; Lazarus & Jahn 1998). A recent example for the great value of the Ehrenberg collection is the taxonomic clarification of *Glenodinium apiculatum* Ehrenb. (**publication 3**). The species was described by Ehrenberg more than 180 years ago collected near Berlin. The published figures as well as original physical material mounted on a mica embedded in Canada Balsam (Fig. 3C) show dinophyte cells with distinctive multiple minute spines at the antapex. A newly established strain from the type locality was to a great extent consistent with the original material provided by Ehrenberg, thus the strain was used for further taxonomic purposes. Other examples for the taxonomic clarification of Ehrenberg's taxa are *Cryptomonas lima* Ehrenb. (McLachlan *et al.* 1997), *Peridinium acuminatum* Ehrenb. (Kretschmann *et al.* 2015a) as well as *Prorocentrum micans* Ehrenb. (Tillmann *et al.* 2019).

Another significant biological resource for scientific research are culture collections of living algae. The cultivation of dinophytes provides a consistent access to living material, which is necessary for morphological and molecular investigation using contemporary techniques. Therefore, a fundamental step during my project was the laborious collecting of field samples to obtain material for (preferably monoclonal) strain establishment. More than 240 sediment and/or water tow samples have been collected at 180 localities (110 localities were visited by me) mainly in Germany and Poland, but also in Austria, Czech Republic, Denmark, Ireland, Italy, Japan, and Romania. The isolation of single cells using microcapillary pipettes is challenging and time-consuming. The establishment of well growing monoclonal dinophyte strains is highly species dependent, however, on average successfully for around 5 to 15% only (personal experience). During the course of my study, I was successful in isolation and establishment of over 370 dinophyte strains from environmental samples under semi-sterile conditions. The strains were used for the description of new species (**publication 1**), the clarification of taxonomic identities (**publication 1–3, 8**) and specification of phylogenetic positions (**publication 1–2, 4**) as well as for investigations of morphological and molecular intraspecific variability (**publication 7**) and for inferences on the biogeography of dinophytes (**publication 9**).

According to the ICN, the type material of a taxon may not be a living organism or strain (Article 8.4: Shenzhen Code; Turland *et al.* 2018). Therefore, preparations of permanent slides for light microscopy were prepared and are currently deposited in the Centre of Excellence for Dinophyte Taxonomy (CEDiT; **publication 1–3**). Duplicates of these permanent slides are held in Berlin and Munich. Preparations for scanning electron microscopy (i.e. SEM stubs) are unpractical in terms of transport and storage, because the stubs must not tilt and must be protected against dust, water as well as general contact in any form. The significant difference of the epitypes to the historical types is now that the epitypes are linked to living material and thus enabling the generation of DNA sequences—the key tool for reliable species determination. In addition, the corresponding strains were transferred to public culture collections, such as the Central Collection of Algal Cultures (CCAC), Culture Collection of Baltic Algae (CCBA), and Canadian Center for the Culture of Microorganisms (CCCM) and are available for further investigations using methodologies such as transmission electron microscopy (TEM) and next-generation sequencing (NGS) approaches upon request.

Morphological and molecular intraspecific variability

Traditionally, dinophytes are classified based on morphological traits detectable by light microscopy. For thecate dinophytes, the plate pattern, in terms of the number, the arrangement as well as the shape of plates, plays an important role to delimitate taxa (e.g. Wołoszyńska 1916; Lindemann 1919, 1920; Balech 1980; Abé 1981; Hoppenrath 2017). A century ago, E. Lindemann was the first to assess morphological variability of the thecal plate pattern in *Peridinium cinctum* (O.F.Müller) Ehrenb. He used morphological traits of motile cells mainly the epithecal plate pattern as well as the general morphology to distinguish several forms and varieties, but he was also aware of the difficulties in distinguishing between new species, forms or varieties and individuals with an abnormal plate pattern (Lindemann 1917, 1920). However, since then, Lindemann's work has fallen into oblivion, but with the application of DNA sequencing, the combination of morphology and molecular characters enables new insights into intraspecific taxonomic delimitations.

For the investigation of morphological and molecular intraspecific variability, approximately 70 monoclonal strains of *P. cinctum* were used, which originated from samples collected across different freshwater sites in Central Europe (**publication 7**). Based on the molecular analysis, the existence of a large sequence diversity within the ITS region could be documented. Detailed morphological investigations of the plate pattern of *P. cinctum* showed a notable variability, not only between different strains, but also within monoclonal strains. Within the strains, distinct morphotypes could be identified using the epithecal plate pattern. However, the different ribotypes showed no clear correlation to the defined morphotypes (and vice versa) and/or geographic occurrences. Moreover, it remains unclear at present whether all the distinct ribotypes correspond to a single species *P. cinctum* or support the existence of cryptic species.

New taxa and names

Description of two new species

Freshwater dinophytes have been notoriously understudied in the past (Mertens *et al.* 2012; Thessen *et al.* 2012; Gómez 2014). Approximately 350 species are described from freshwater habitats (Taylor *et al.* 2008; Gómez 2012a; Mertens *et al.* 2012; Moestrup & Calado 2018), but numerous new freshwater species descriptions in recent years (e.g. Craveiro *et al.* 2013; Daugbjerg *et al.* 2014; Zhang *et al.* 2014; Li *et al.* 2015; Zhang *et al.* 2016; Takahashi *et al.* 2017; Pandeirada *et al.* 2019) indicate that the diversity of freshwater dinophytes has been underestimated. In addition, two species from freshwater environment have been described new to science during my project (**publication 1**). Both species, namely *Parvodinium marciniakii* Kretschmann, Owsianny, Zerdoner & Gottschling and *P. trawinskii* Kretschmann, Owsianny, Zerdoner & Gottschling, have been found in several lakes in the Polish Tatra Mountains. From this area, numerous dinophyte species have been described mostly dating back to the first half of the 20th century. However, the taxonomic identity of most of them remains unclear.

P. *marciniakii* and *P*. *trawinskii* are closely related, and their general morphology of the motile cells as well as molecular phylogenetics assigned the two new species to Peridiniopsidaceae. A reason, why these species have not been recognised before, is certainly its remote type locality, but may be also that both species are morphologically similar to a number of already known species of *Parvodinium* Carty and are difficult to distinguish by using light microscopy only. Furthermore, many species of *Parvodinium* are characterised by a unique and distinctive combination of traits rather than by a single autapomorphy. The description of two new species is another indication that the biodiversity assessment of dinophytes is likely not yet complete especially in unexplored remote areas such as water bodies at higher altitudes.

Phylogenetic position of Parvodinium elpatiewskyi

Historically, the classification of dinophytes was based entirely on morphological traits detectable by light microscopy. For thecate dinophytes, the plate pattern has been considered as a useful trait to delimitate and classify taxa. During the last decades, the molecular approaches have gained importance in classification. Detailed re-evaluations of morphological traits, combined with molecular phylogenetic analysis, provide data for the specification of the position within phylogenetic trees and result in the description of new genera (e.g. *Karenia* Gert Hansen & Moestrup: Daugbjerg *et al.* 2000; *Barrufeta* N.Sampedro & S.Fraga: Sampedro *et al.* 2011; *Nusuttodinium* Takano & T.Horig.: Takano *et al.* 2014; *Unruhdinium* Gottschling, *Blixaea* Gottschling: **publication 6**) or even families (e.g. Tovelliaceae: Lindberg *et al.* 2005; Peridiniopsidaceae: **publication 5**) as well as new combinations of species names (Kremp *et al.* 2005; Hansen *et al.* 2007; Kretschmann *et al.* 2014; Kretschmann *et al.* 2015a; **publication 4**).

Until present, the widespread freshwater dinophyte *Parvodinium elpatiewskyi* (Ostenf.) Kretschmann, Zerdoner & Gottschling was placed in *Peridiniopsis* Lemmerm., based on the epithecal plate tabulation exhibiting no intercalary plates. However, their phylogenetic relationship has never been confirmed using molecular data. A molecular phylogenetic analysis with newly collected material showed that the species, having no intercalary plates on the epitheca, belongs to the Peridiniopsidaceae and is clearly assigned to *Parvodinium*. This taxon exhibits two intercalary plates and was as such (based solely on morphology) never considered to be a close relative of *Parvodinium elpatiewskyi*. Therefore, the combination of both, morphological and molecular data, is important to clarify unresolved phylogenetic positions.

Approaches to epitypification

Taxonomic work is essential for the fundamental understanding of biodiversity and the communication about organisms. However, there is an ongoing debate how to reach the objective of a clear and robust taxonomy and to avoid instability by the introduction of historic scientific names (Smith *et al.* 2016). Some researchers have advocated the view, that the potentially simplest and quickest solution is to eliminate historic species names from contemporary taxonomy and start accepting all the new names from a given date onwards (Smith *et al.* 2016). However, the principle of the taxonomic priority of older scientific names is deeply rooted in the ICN (Turland *et al.* 2018), therefore older names have priority over younger names, despite their current potentially wider usage. In addition, the ICN provides the key to achieve a status of a stable taxonomy, namely the designation of epitypes for unreliable species names (Article 9.9: Shenzhen Code; Turland *et al.* 2018). During the course of my project, I contributed to the taxonomic clarification of eight scientific names and the designation of interpretative epitypes (**publication 1–3, 8**). The most sensible approach for an adequate morphological and molecular re-investigation is to collect living material at the type locality or as close as possible and preferable at the same time of year (Hyde & Zhang 2008; **publication 1–3, 8**). Although the contemporary occurrence of dinophytes at their type localities have been rarely investigated, some species show a remarkably high site fidelity at localities, even if they were last collected and documented a century or more ago (Zinßmeister *et al.* 2011; Kretschmann *et al.* 2014, 2015a, 2015b). Moreover, during the course of my thesis, this approach has been successfully applied to *Glenodinium apiculatum*, *Glenodinium oculatum* F.Stein, three varieties of *P. cinctum* (*P. cinctum* var. *betacollineatum* Er.Lindem., *P. cinctum* var. *epsiloncollineatum* Er.Lindem., and *P. cinctum* var. *irregulatum* Er.Lindem.), *Peridinium eximium* Er.Lindem., and *Peridinium mixtum* Wołosz. ex Kretschmann, Owsianny, Zerdoner & Gottschling (and its two varieties *Peridinium mixtum* var. *remotum* Wołosz. ex Kretschmann, Owsianny, Zerdoner & Gottschling and *Peridinium mixtum* var. *conjunctum* Wołosz. ex Kretschmann, Owsianny, Zerdoner & Gottschling; **publication 1–3, 8**). Even though many localities around the world

have changed ecologically due to a anthropogenic impact, the approach of collecting at less modified localities (Litaker *et al.* 2009; Saburova *et al.* 2012; John *et al.* 2014), and with comparable ecological conditions of that time, does not appear as the most appropriate choice for the taxonomic clarification of unreliable species names. In most cases, the historical protologues do not provide ecological specifications for the type localities, therefore the exact ecological conditions are unknown and cannot be compared with that of more natural habitats of today. The ecological based approach is adequate for species descriptions with unknown, not precise, or destroyed type locality or after an exhaustive, but unsuccessful search of a species at its type locality.

Applied studies based on clarified taxonomy

Biogeography

The focus of my thesis has been on the morphology and taxonomy of freshwater dinophytes. Such taxonomic work is essential for the assessment of biodiversity as well as the common understanding of organisms. Moreover, a clear and robust taxonomy provides the basis for a wide range of other scientific applications such as the investigations of the biogeography of dinophytes, monitoring with contemporary techniques and reliable inferences of evolutionary relationships among marine and freshwater species.

In general, dinophytes are distributed worldwide, but the biogeography of unicellular organisms has been the subject of a recent debate. On the one hand, some researchers claim that all protists such as dinophytes are cosmopolitan organisms, and lack distinct distributions (Finlay 2002; Fenchel & Finlay 2004; Read *et al.* 2013), implying 'everything is everywhere' while on the other hand, researchers consider that protists actually consist of both wide-spread and endemic species (Coleman 2001; Foissner 2006; Bass *et al.* 2007; Bates *et al.* 2013; Kretschmann *et al.* 2015b). However, due to problems in reliable species identification and naming of dinophytes particularly in case of historical species descriptions, reliable conclusions about the biogeography are mostly impossible. Additionally, some dinophyte lineages underwent cryptic speciation resulting in morphologically indistinguishable species complexes (Montresor *et al.* 2003; John *et al.* 2014; LaJeunesse *et al.* 2018; Daugbjerg *et al.* 2019). Therefore, it is necessary to include genetic information for species identification as well as to use DNA-based records when assessing distribution (**publication 9**).

In the last decade, the knowledge on distribution of microorganisms has grown due to the development of new techniques such as high-throughput sequencing of environmental samples. Environmental sequencing is a cost-effective method to provide information about the molecular diversity of a given group and to draw conclusions about their distribution. However, reliable reference databases are required to link the molecular data to species names (Gottschling *et al.* 2020; **publication 1–3, 8**).

Evolutionary ecology

Evolutionary ecology links the fields of both ecology and evolutionary biology examining the evolutionary history of species. The quantification and dating of transitions from marine into freshwater environment are the basis for the understanding of the evolutionary processes leading to the diversification in dinophytes. The different physical and chemical properties of marine and freshwater habitats may act as a barrier limiting the frequency of transitions as well as the followed diversification of dinophytes (Logares *et al.* 2007). Based on molecular phylogenies, few monophyletic freshwater lineages that are distantly related to marine species have been considered as indication for rare crossings of the marine-freshwater boundary that has happened a long time ago (Logares *et al.* 2007). Moreover, fossils of putative freshwater dinophytes extends back until the Mesozoic (Gray & Taylor 1988; Batten 1989), but the precise systematic affiliation of such fossils has not yet been determined.

Molecular dated phylogenies help to shed light on such unresolved questions related to evolutionary events. However, such studies are still rare for dinophytes until now, because of restriction such as limited taxon sample, insufficient sequence data, and high heterogeneity in substitution rates (Saldarriaga *et al.* 2004; Murray *et al.* 2005; Gottschling *et al.* 2012; Gu *et al.* 2013; Žerdoner Čalasan *et al.* 2019). In phylogenetic trees comprising a broader taxon sample, marine to freshwater transitions are more frequent as previously assumed. The freshwater lineages consist mostly of small polyphyletic and only distantly related species groups, which implies independent, repeated colonisation events from the marine into the freshwater environment (Kretschmann *et al.* 2015b; Žerdoner Čalasan *et al.* 2019; **publication 2**). Molecular clock analysis dated such transitions within Gymnodiniacae and Peridiniales to 40 MYA. Since the Cretaceous, the marine to freshwater transitions have been independently taking place at different times and the followed diversification appears more gradual without noticeable major environmental impacts (Žerdoner Čalasan *et al.* 2019).

Chloroplast origin in dinophytes harbouring diatoms

Dinophytes are known to possess chloroplasts of multiple origins that have been acquired through several endosymbiotic events. The most common and widespread chloroplast type is derived from a red alga, through secondary endosymbiosis. Tertiary endosymbiosis is uniquely known from a few dinophytes and describes an engulfment of a secondary plastid-containing endosymbiont such as diatom algae in the Kryptoperidiniaceae. Chloroplasts are usually inherited from the mother cell to each daughter cell during cell division leading to congruent phylogenetic relationships between nuclear and chloroplast DNA sequences, termed co-phylogeny. Within the Kryptoperidiniaceae, a concordance between the phylogenies of the endosymbiotic diatoms and their hosting dinophytes would indicate a shared evolutionary history of the dinophytes and its harbouring diatoms. Molecular phylogenetic trees confirm the monophyly of all Kryptoperidiniacae as part of the Peridiniales (Pienaar *et al.* 2007; Takano *et al.* 2008; Saburova *et al.* 2012; Janouškovec *et al.* 2017; Price & Bhattacharya 2017; Yamada *et al.* 2017; Žerdoner Čalasan *et al.* 2018; **publication 2)**. However, molecular phylogenetic analysis of diatoms including all sequences derived from the endosymbionts of Kryptoperidiniaceae showed that almost all endosymbionts found their closest relatives in free-living diatoms and not in other harboured algae (Žerdoner Čalasan *et al.* 2018). This observation indicates multiple independent acquisition of endosymbiotic diatoms by hosting dinophytes through their evolutionary history and rejects the indication of co-phylogeny as main mechanism.

Conclusion and outlook

Reliable species identification and clarified taxonomy is essential for reliable and comparative studies of dinophyte species, because reliable species names form the basis for all further investigations or studies. To ensure reliable species identification and the consistent use of names, the ICN specifies that the application of scientific names is determined by nomenclatural types and each name must be correctly typified. However, the concept has limitations for unicellular organisms, especially for historic descriptions as the original material consists mostly of drawings. In many cases, historic type material is ambiguous because it does not provide sufficient information for unambiguous species determination especially in terms of morphological differentiation within cryptic species complexes. However, it is precisely these properties that make dinophytes a suitable group for epitypifying. Thus, epitypification is a key tool for reliable species determination ensuring an unambiguous links between the species, its scientific name, its protologue, morphology, and genetic characterisation. However, despite its great potential to clarify taxonomic confusions, in forms of synonyms and wrongly applied names, relatively few of such studies have used this approach in the past. In the course of my work I have succeeded in clarifying the identity of eight scientific names by means of epitypifying, but the taxonomic identity of the majority of the dinophyte species remains to be clarified until now.

References

ABÉ, T.H. (1981) *Studies on the family Peridinidae an unfinished monograph on the armoured dinoflagellata.* Abe Tōru Ikō Shuppankai, Tokyo.

ADL, S.M., BASS, D., LANE, C.E., LUKES, J., SCHOCH, C.L., SMIRNOV, A., AGATHA, S., BERNEY, C., BROWN, M.W., BURKI, F. *et al.* (2019) Revisions to the classification, nomenclature, and diversity of eukaryotes. *Journal of Eukaryotic Microbiology* **66:** 4–119.

ANDERSON, D.M. (1995) Toxic red tides and harmful algal blooms: A practical challenge in coastal oceanography. *Reviews of Geophysics* **33:** 1189–1200.

ANDERSON, S.R. & MENDEN-DEUER, S. (2017) Growth, grazing, and starvation survival in three heterotrophic dinoflagellate species. *The Journal of Eukaryotic Microbiology* **64:** 213–225.

ANGLÈS, S., REÑÉ, A., GARCÉS, E., LUGLIÈ, A., SECHI, N., CAMP, J. & SATTA, C.T. (2017) Morphological and molecular characterization of *Bysmatrum subsalsum* (Dinophyceae) from the western Mediterranean Sea reveals the existence of cryptic species. *Journal of Phycology* **53:** 833–847.

ANNENKOVA, N.V., LAVROV, D.V. & BELIKOV, S.I. (2011) Dinoflagellates associated with freshwater sponges from the ancient Lake Baikal. *Protist* **162:** 222–236.

BALECH, E. (1980) On thecal morphology of dinoflagellates with special emphasis on circular and sulcal plates. *Anales del Centro de Ciencas del Mar y Limnología, Universidad Nacional Autónoma de México* **7:** 57–67.

BASS, D., RICHARDS, T.A., MATTHAI, L., MARSH, V. & CAVALIER-SMITH, T. (2007) DNA evidence for global dispersal and probable endemicity of protozoa. *BMC Evolutionary Biology* **7:** 162.

BATES, S.T., CLEMENTE, J.C., FLORES, G.E., WALTERS, W.A., PARFREY, L.W., KNIGHT, R. & FIERER, N. (2013) Global biogeography of highly diverse protistan communities in soil. *The ISME Journal* **7:** 652–659.

BATTEN, D.J. (1989) Cretaceous freshwater dinoflagellates. *Cretaceous Research* **10:** 271–273.

BICKFORD, D., LOHMAN, D.J., SODHI, N.S., NG, P.K.L., MEIER, R., WINKER, K., INGRAM, K.K. & DAS, I. (2007) Cryptic species as a window on diversity and conservation. *TRENDS in Ecology and Evolution* **22:** 148–155.

BORCHERT, R. (1996) Phenology and flowering periodicity of Neotropical dry forest species: Evidence from herbarium collections. *Journal of Tropical Ecology* **12:** 65–80.

BÜTSCHLI, O. (1885) *Erster Band. Protozoa.* Klassen und Ordnungen des Thier-Reichs, Winter'sche Verlagshandlung, Leipzig.

CHATTON, É. (1920) *Les péridiniens parasites; morphologie, reproduction, éthologie.* Archives de zoologie expérimentale et générale, Librairie H. Soudier, Paris.

CHAVAN, V. & KRISHNAN, S. (2003) Natural history collections: A call for national information infrastructure. *Current Science* **84:** 34–42.

COATS, D.W. (1999) Parasitic life styles of marine dinoflagellates. *The Journal of Eukaryotic Microbiology* **46:** 402–409.

COLEMAN, A.W. (2001) Biogeography and speciation in the *Pandorina*/*Volvulina* (Chlorophyta) superclade. *Journal of Phycology* **37:** 836–851.

COSTAS, E. & GOYANES, V. (2005) Architecture and evolution of dinoflagellate chromosomes: An enigmatic origin. *Cytogenetic and Genome Research* **109:** 268–275.

CRAVEIRO, S.C., PANDEIRADA, M.S., DAUGBJERG, N., MOESTRUP, Ø. & CALADO, A.J. (2013) Ultrastructure and phylogeny of *Theleodinium calcisporum* gen. et sp. nov. a freshwater dinoflagellate that produces calcareous cysts. *Phycologia* **52:** 488–507.

DAUGBJERG, N., HANSEN, G., LARSEN, J. & MOESTRUP, Ø. (2000) Phylogeny of some of the major genera of dinoflagellates based on ultrastructure and partial LSU rDNA sequence data, including the erection of three new genera of unarmoured dinoflagellates. *Phycologia* **39:** 302–317.

DAUGBJERG, N., ANDREASEN, T., HAPPEL, E., PANDEIRADA, M.S., HANSEN, G., CRAVEIRO, S.C., CALADO, A.J. & MOESTRUP, Ø. (2014) Studies on woloszynskioid dinoflagellates VII. Description of *Borghiella andersenii* sp. nov.: Light and electron microscopy and phylogeny based on LSU rDNA. *European Journal of Phycology* **49:** 436–449.

DAUGBJERG, N., HANSEN, S.A. & RICHARDSON, K. (2019) Cryptic diversity of small-sized species of *Phalacroma* (Dinophysales, Dinophyceae) from Denmark Strait (Eastern Arctic Greenland). *Phycological Research* **67:** 244–249.

DODGE, J.D. (1985) The chromosomes of dinoflagellates. *International Review of Cytology* **94:** 5–19.

ELBRÄCHTER, M., GOTTSCHLING, M., HILDEBRAND-HABEL, T., KEUPP, H., KOHRING, R., LEWIS, J., MEIER, K.J.S., MONTRESOR, M., STRENG, M., VERSTEEGH, G.J.M. *et al.* (2008) Establishing an Agenda for Calcareous Dinoflagellate Research (Thoracosphaeraceae, Dinophyceae) including a nomenclatural synopsis of generic names. *Taxon* **57:** 1289–1303.

ESCALANTE, A.A. & AYALA, F.J. (1995) Evolutionary origin of *Plasmodium* and other Apicomplexa based on rRNA genes. *Proceedings of the National Academy of Sciences of the United States of America* **92:** 5793–5797.

FAWCETT, R.C. & PARROW, M.W. (2014) Mixotrophy and loss of phototrophy among geographic isolates of freshwater *Esoptrodinium/Bernardinium* sp. (Dinophyceae). *Journal of Phycology* **50:** 55–70.

FENCHEL, T. & FINLAY, B.J. (2004) The ubiquity of small species: Patterns of local and global diversity. *BioScience* **54:** 777–784.

FENSOME, R.A., TAYLOR, F.J.R., NORRIS, G., SARJEANT, W.A.S., WHARTON, D.I. & WILLIAMS, G.L. (1993) *A classification of living and fossil dinoflagellates.* Micropaleontology Special Publication no. 7, American Museum of Natural History, New York.

FENSOME, R.A., SALDARRIAGA, J.F. & TAYLOR, F.J.R. (1999) Dinoflagellate phylogeny revisited: Reconciling morphological and molecular based phylogeny. *Grana* **38:** 66–80.

FINLAY, B.J. (2002) Global dispersal of free-living microbial eukaryote species. *Science* **296:** 1061–1063.

FOISSNER, W. (2006) Biogeography and dispersal of micro-organisms: A review emphasizing protists. *Acta Protozoologica* **45:** 111–136.

FRAGA, S. & BAKUN, A. (1993) *Global climate change and harmful alagal blooms: The example of Gymnodinium catenatum on the Galician coast.* Toxic phytoplankton blooms in the sea, Elsevier, New York.

FRAGA, S., BRAVO, I., DELGADO, M., FRANCO, J.M. & ZAPATA, M. (1995) *Gyrodinium impudicum* sp. nov. (Dinophyceae), a non toxic, chain-forming, red tide dinoflagellate. *Phycologia* **34:** 514–521.

GENOVESI, B., SHIN-GRZEBYK, M.S., GRZEBYK, D., LAABIR, M., GAGNAIRE, P.A., VAQUER, A., PASTOUREAUD, A., LASSERRE, B., COLLOS, Y., BERREBI, P. *et al.* (2010) Assessment of cryptic species diversity within blooms and cyst bank of the *Alexandrium tamarense* complex (Dinophyceae) in a Mediterranean lagoon facilitated by semi-multiplex PCR. *Journal of Plankton Research* **33:** 405–414.

GÓMEZ, F. (2007) Synonymy and biogeography of the dinoflagellate genus *Histioneis* (Dinophysiales: Dinophyceae). *Revista de Biología Tropical* **55:** 459–477.

GÓMEZ, F., LÓPEZ-GARCÍA, P. & MOREIRA, D. (2011) Molecular phylogeny of dinophysoid dinoflagellates: the systematic position of *Oxyphysis oxytoxoides* and the *Dinophysis hastata* group (Dinophysales, Dinophyceae). *Journal of Phycology* **47:** 393–406.

GÓMEZ, F. (2012a) A checklist and classification of living dinoflagellates (Dinoflagellata, Alveolata). *CICIMAR Oceánides* **27:** 65–140.

GÓMEZ, F. (2012b) A quantitative review of the lifestyle, habitat and trophic diversity of dinoflagellates (Dinoflagellata, Alveolata). *Systematics and Biodiversity* **10:** 267–275.

GÓMEZ, F. (2014) Problematic biases in the availability of molecular markers in protists: The example of the dinoflagellates. *Acta Protozoologica* **53:** 63–75.

GORNIK, S.G., HU, I., LASSADI, I. & WALLER, R.F. (2019) The biochemistry and evolution of the dinoflagellate nucleus. *Microorganisms* **7:** 245.

GOTTSCHLING, M., KNOP, R., PLÖTNER, J., KIRSCH, M., WILLEMS, H. & KEUPP, H. (2005) A molecular phylogeny of *Scrippsiella sensu lato* (Calciodinellaceae, Dinophyta) with interpretations on morphology and distribution. *European Journal of Phycology* **40:** 207–220.

GOTTSCHLING, M., SÖHNER, S., ZINSSMEISTER, C., JOHN, U., PLÖTNER, J., SCHWEIKERT, M., ALIGIZAKI, K. & ELBRÄCHTER, M. (2012) Delimitation of the Thoracosphaeraceae (Dinophyceae), including the calcareous dinoflagellates, based on large amounts of ribosomal RNA sequence data. *Protist* **163:** 15–24.

GOTTSCHLING, M. & MCLEAN, T.I. (2013) New home for tiny symbionts: Dinophytes determined as *Zooxanthella* are Peridiniales and distantly related to *Symbiodinium*. *Molecular Phylogenetics and Evolution* **67:** 217–222.

GOTTSCHLING, M., TILLMANN, U., KUSBER, W.-H., HOPPENRATH, M. & ELBRÄCHTER, M. (2018) A Gordian knot: Nomenclature and taxonomy of *Heterocapsa triquetra* (Peridiniales: Heterocapsaceae). *Taxon* **67:** 179–185.

GOTTSCHLING, M., TILLMANN, U., KUSBER, W.-H., ELBRÄCHTER, M. & HOPPENRATH, M. (2019) To be or not to be: On the usefulness of infraspecific names in *Heterocapsa steinii* (Heterocapsaceae, Peridiniales). *Phytotaxa* **395:** 134–136.

GOTTSCHLING, M., CHACÓN, J., ŽERDONER ČALASAN, A., NEUHAUS, S., KRETSCHMANN, J., STIBOR, H. & JOHN, U. (2020) Phylogenetic placement of environmental sequences using taxonomically reliable databases helps to rigorously assess dinophyte biodiversity in Bavarian lakes (Germany). *Freshwater Biology***: 65:** 193–208.

GRAY, J. & TAYLOR, D.W. (1988) *Evolution of the freshwater ecosystem: The fossil record.* Palaeogeography, Palaeoclimatology, Palaeoecology, Elsevier Science Publishers B.V., Amsterdam.

GREUTER, W., BARRIE, F.R., BURDET, H.M., CHALONER, W.G., DEMOULIN, V., HAWKSWORTH, D.L., JØRGENSEN, P.M., NICOLSON, D.H., SILVA, P.C., TREHANE, P. *et al.* (1994) *International Code of Botanical Nomenclature (Tokyo Code). Adopted by the Fifteenth International Botanical Congress, Yokohama, Japan, August-September 1993. Regnum Vegetabile 131.* Koeltz Scientific Books, Königstein.

GU, H., KIRSCH, M., ZINSSMEISTER, C., SÖHNER, S., MEIER, K.J.S., LIU, T. & GOTTSCHLING, M. (2013) Waking the dead: Morphological and molecular characterization of

extant †*Posoniella tricarinelloides* (Thoracosphaeraceae, Dinophyceae). *Protist* **164:** 583–597.

HALLEGRAEFF, G.M. (1993) A review of harmful algal blooms and their apparent global increase. *Phycologia* **32:** 79–99.

HANSEN, G., BOTES, L. & DE SALAS, M. (2007) Ultrastructure and large subunit rDNA sequences of *Lepidodinium viride* reveal a close relationship to *Lepidodinium chlorophorum* comb. nov. (= *Gymnodinium chlorophorum*). *Phycological Research* **55:** 25–41.

HANSEN, G. & DAUGBJERG, N. (2011) *Moestrupia oblonga* gen. & comb. nov. (syn.: *Gyrodinium oblongum*), a new marine dinoflagellate genus characterized by light and electron microscopy, photosynthetic pigments and LSU rDNA sequence. *Phycologia* **50:** 583–599.

HARPER, J.T., WAANDERS, E. & KEELING, P.J. (2005) On the monophyly of chromalveolates using a six-protein phylogeny of eukaryotes. *International Journal of Systematic and Evolutionary Microbiology* **55:** 487–496.

HEHENBERGER, E., BURKI, F., KOLISKO, M. & KEELING, P.J. (2016) Functional relationship between a dinoflagellate host and its diatom endosymbiont. *Molecular Biology and Evolution* **33:** 2376–2390.

HOPPENRATH, M., CHOMÉRAT, N., HORIGUCHI, T., SCHWEIKERT, M., NAGAHAMA, Y. & MURRAY, S. (2013) Taxonomy and phylogeny of the benthic *Prorocentrum* species (Dinophyceae) — A proposal and review. *Harmful Algae* **27:** 1–28.

HOPPENRATH, M. (2017) Dinoflagellate taxonomy — a review and proposal of a revised classification. *Marine Biodiversity.* pp. 381–403. Springer-Verlag, Berlin Heidelberg.

HORNE, A.J., JAVORNICKY, P. & GOLDMAN, C.R. (1971) A freshwater 'red tide' on Clear Lake, California. *Limnology and Oceanography* **16:** 684–689.

HYDE, K.D. & ZHANG, Y. (2008) Epitypification: Should we epitypify? *Journal of Zhejiang University SCIENCE B* **9:** 842–846.

JACOBSON, D.M. & ANDERSON, D.M. (1986) Thecate heterotrophic dinoflagellates: Feeding behavior and mechanisms. *Journal of Phycology* **22:** 249–258.

JAMES, S.A., SOLTIS, P.S., BELBIN, L., CHAPMAN, A.D., NELSON, G., PAUL, D.L. & COLLINS, M. (2018) Herbarium data: Global biodiversity and societal botanical needs for novel research. *Applications in Plant Sciences* **6:** e1024.

JANOUŠKOVEC, J., GAVELIS, G.S., BURKI, F., DINH, D., BACHVAROFF, T.R., GORNIK, S.G., BRIGHT, K.J., IMANIAN, B., STROM, S.L., DELWICHE, C.F. *et al.* (2017) Major transitions in dinoflagellate evolution unveiled by phylotranscriptomics. *Proceedings of the National Academy of Sciences* **114:** E171–E180.

JEONG, H.J. (1999) The ecological roles of heterotrophic dinoflagellates in marine planktonic community. *The Journal of Eukaryotic Microbiology* **46:** 390–396.

JEONG, H.J., YOO, Y.D., KIM, J.S., KIM, T.H., KIM, J.H., KANG, N.S. & YIH, W. (2004) Mixotrophy in the phototrophic harmful alga *Cochlodinium polykrikoides* (Dinophycean): Prey species, the effects of prey concentration, and grazing impact. *The Journal of Eukaryotic Microbiology* **51:** 563–569.

JOHN, U., LITAKER, R.W., MONTRESOR, M., MURRAY, S., BROSNAHAN, M.L. & ANDERSON, D.M. (2014) Formal revision of the *Alexandrium tamarense* species complex (Dinophyceae) taxonomy: The introduction of five species with emphasis on molecular-based (rDNA) classification. *Protist* **165:** 779–804.

JUNG, J.H., CHOI, J.M., COATS, D.W. & KIM, Y.O. (2016) *Euduboscquella costata* n. sp. (Dinoflagellata, Syndinea), an intracellular parasite of the ciliate *Schmidingerella arcuata*: Morphology, molecular phylogeny, life cycle, prevalence, and infection intensity. *Journal of Eukaryotic Microbiology* **63:** 3–15.

KEELING, P.J. (2004) Diversity and evolutionary history of plastids and their hosts. *American Journal of Botany* **91:** 1481–1493.

KREMP, A., ELBRÄCHTER, M., SCHWEIKERT, M., WOLNY, J.L. & GOTTSCHLING, M. (2005) *Woloszynskia halophila* (Biecheler) comb. nov.: A bloom-forming cold-water dinoflagellate co-occurring with *Scrippsiella hangoei* (Dinophyceae) in the Baltic Sea. *Journal of Phycology* **41:** 629–642.

KRETSCHMANN, J., ZINSSMEISTER, C. & GOTTSCHLING, M. (2014) Taxonomic clarification of the dinophyte *Rhabdosphaera erinaceus* Kamptner, ≡ *Scrippsiella erinaceus* comb. nov. (Thoracosphaeraceae, Peridiniales). *Systematics and Biodiversity* **12:** 393–404.

KRETSCHMANN, J., ELBRÄCHTER, M., ZINSSMEISTER, C., SÖHNER, S., KIRSCH, M., KUSBER, W.-H. & GOTTSCHLING, M. (2015a) Taxonomic clarification of the dinophyte *Peridinium acuminatum* Ehrenb., ≡ *Scrippsiella acuminata* comb. nov. (Thoracosphaeraceae, Peridiniales). *Phytotaxa* **220:** 239–256.

KRETSCHMANN, J., FILIPOWICZ, N.H., OWSIANNY, P.M., ZINSSMEISTER, C. & GOTTSCHLING, M. (2015b) Taxonomic clarification of the unusual dinophyte *Gymnodinium limneticum* Wołosz. (Gymnodiniaceae) from the Tatra Mountains. *Protist* **166:** 621–637.

LAJEUNESSE, T.C. (2001) Investigating the biodiversity, ecology, and phylogeny of endosymbiotic dinoflagellates in the genus *Symbiodinium* using the its region: in search of a 'species' level marker. *Journal of Phycology* **38:** 866–880.

LAJEUNESSE, T.C., PARKINSON, J.E., GABRIELSON, P.W., JEONG, H.J., REIMER, J.D., VOOLSTRA, C.R. & SANTOS, S.R. (2018) Systematic revision of symbiodiniaceae highlights

the antiquity and diversity of coral endosymbionts. *Current Biology* **28:** 2570–2580.

LANE, M.A. (1996) Roles of natural history collections. *Annals of the Missouri Botanical Garden* **83:** 536–545.

LANG, P.L.M., WILLEMS, F.M., SCHEEPENS, J.F., BURBANO, H.A. & BOSSDORF, O. (2019) Using herbaria to study global environmental change. *New Phytologist* **221:** 110–122.

LAVOIE, C. & LACHANCE, D. (2006) A new herbarium-based method for reconstructing the phenology of plant species across large areas. *American Journal of Botany* **93:** 512–516.

LAZARUS, D. (1998) The Ehrenberg Collection and its curation. In: Williams, D.M. & Huxley, R. *Christian Gottfried Ehrenberg (1795–1876): The man and his legacy.* pp. 31–48. The Linnean Society, London.

LAZARUS, D. & JAHN, R. (1998) Using the Ehrenberg Collection. *Diatom Research* **13:** 273–291.

LEANDER, B.S. & KEELING, P.J. (2004) Early evolutionary history of dinoflagellates and apicomplexans (Alveolata) as inferred from Hsp90 and actin phylogenies. *Journal of Phycology* **40:** 341–350.

LELIAERT, F., VERBRUGGEN, H., VANORMELINGEN, P., STEEN, F., LÓPEZ-BAUTISTA, J.M., ZUCCARELLO, G.C. & DE CLERCK, O. (2014) DNA-based species delimitation in algae. *European Journal of Phycology* **49:** 179–196.

LEVY, M.G., LITAKER, R.W., GOLDSTEIN, R.J., DYKSTRA, M.J., VANDERSEA, M.W. & NOGA, E.J. (2007) *Piscinoodinium*, a fish-ectoparasitic dinoflagellate, is a member of the class Dinophyceae, subclass Gymnodiniphycidae: convergent evolution with *Amyloodinium*. *The Journal of Parasitology* **93:** 1006–1015.

LI, Z., SHIN, H.H. & HAN, M.-S. (2015) Morphology and phylogeny of a new woloszynskioid dinoflagellate *Tovellia paldangensis* sp. nov. (Dinophyceae). *Phycologia* **54:** 67–77.

LILLY, E.L., HALANYCH, K.M. & ANDERSON, D.M. (2007) Species boundaries and global biogeography of the *Alexandrium tamarense* complex (Dinophyceae). *Journal of Phycology* **43:** 1329–1338.

LIN, S. (2011) Genomic understanding of dinoflagellates. *Research in Microbiology* **162:** 551–569.

LINDBERG, K., MOESTRUP, Ø. & DAUGBJERG, N. (2005) Studies on woloszynskioid dinoflagellates I: *Woloszynskia coronata* re-examined using light and electron microscopy and partial LSU rDNA sequences, with description of *Tovellia* gen. nov. and *Jadwigia* gen. nov. (Tovelliaceae fam. nov.). *Phycologia* **44:** 416–440.

LINDEMANN, E. (1917) Beiträge zur Kenntnis des Seenplanktons der Provinz Posen. (Südwestposener Seengruppe) II. *Zeitschrift der Naturwissenschaftlichen Abteilung der Deutschen Gesellschaft für Kunst und Wissenschaft in Posen* **24:** 2–41.

LINDEMANN, E. (1919) Untersuchungen über Süßwasserperidineen und ihre Variationsformen. *Archiv für Protistenkunde* **39:** 209–262.

LINDEMANN, E. (1920) Untersuchungen über Süßwasserperidineen und ihre Variationsformen II. *Archiv für Naturgeschichte* **84:** 121–194.

LINDEMANN, E. (1929) Experimentielle Studien über die Fortpflanzungserscheinungen der Süßwasserperidineen auf Grund von Reinkulturen. *Archiv für Protistenkunde* **68:** 1–104.

LITAKER, R.W., VANDERSEA, M.W., KIBLER, S.R., REECE, K.S., STOKES, N.A., LUTZONI, F.M., YONISH, B.A., WEST, M.A., BLACK, M.N.D. & TESTER, P.A. (2007) Recognizing dinoflagellate species using ITS rDNA sequences. *Journal of Phycology* **43:** 344–355.

LITAKER, R.W., VANDERSEA, M.W., FAUST, M.A., KIBLER, S.R., CHINAIN, M., HOLMES, M.J., HOLLAND, W.C. & TESTER, P.A. (2009) Taxonomy of *Gambierdiscus* including four new species, *Gambierdiscus caribaeus*, *Gambierdiscus carolinianus*, *Gambierdiscus carpenteri* and *Gambierdiscus ruetzleri* (Gonyaulacales, Dinophyceae). *Phycologia* **48:** 344–390.

LOGARES, R., SHALCHIAN-TABRIZI, K., BOLTOVSKOY, A. & RENGEFORS, K. (2007) Extensive dinoflagellate phylogenies indicate infrequent marine-freshwater transitions. *Molecular Phylogenetics and Evolution* **45:** 887–903.

MATSUOKA, K. (1988) Cyst-theca relationships in the diplopsalid group (Peridiniales, Dinophyceae). *Review of Palaeobotany and Palynology* **56:** 95–122.

MATSUOKA, K. & FUKUYO, Y. (2000) *Technical guide for modern dinoflagellate cyst study.* WESTPAC-HAB/WESTPAC/IOC, Tokyo.

MCLACHLAN, J.L., BOALCH, G.T. & JAHN, R. (1997) Reinstatement of the genus *Exuviaella* (Dinophyceae, Prorocentrophycidae) and an assessment of *Prorocentrum lima*. *Phycologia* **36:** 38–46.

MERTENS, K.N., RENGEFORS, K., MOESTRUP, Ø. & ELLEGAARD, M. (2012) A review of recent freshwater dinoflagellate cysts: Taxonomy, phylogeny, ecology and palaeocology. *Phycologia* **51:** 612–619.

MICHENER, C.D. (1970) *Systematics in support of biological research.* Division of Biology and Agriculture, National Research Council, Washington, D. C.

MOESTRUP, Ø., HANSEN, G., DAUGBJERG, N., FLAIM, G. & D'ANDREA, M. (2006) Studies on woloszynskioid dinoflagellates II: On *Tovellia sanguinea* sp. nov., the

dinoflagellate responsible for the reddening of Lake Tovel, N. Italy. *European Journal of Phycology* **41:** 47–65.

MOESTRUP, Ø. & CALADO, A.J. (2018) *Dinophyceae.* Süßwasserflora von Mitteleuropa, Bd. 6 – Freshwater Flora of Central Europe, Vol. 6: Dinophyceae, Springer-Verlag, Berlin, Heidelberg.

MONTRESOR, M., SGROSSO, S., PROCACCINI, G. & KOOISTRA, W.H.C.F. (2003) Intraspecific diversity in *Scrippsiella trochoidea* (Dinopbyceae): Evidence for cryptic species. *Phycologia* **42:** 56–70.

MORRISON, W.R., LOHR, J.L., DUCHEN, P., WILCHES, R., TRUJILLO, D., MAIR, M. & RENNER, S.S. (2009) The impact of taxonomic change on conservation: Does it kill, can it save, or is it just irrelevant? *Biological Conservation* **142:** 3201–3206.

MURRAY, S., FLØ JØRGENSEN, M., HO, S.Y., PATTERSON, D.J. & JERMIIN, L.S. (2005) Improving the analysis of dinoflagellate phylogeny based on rDNA. *Protist* **156:** 269–286.

MURRAY, S., IP, C.L.-C., MOORE, R., NAGAHAMA, Y. & FUKUYO, Y. (2009) Are prorocentroid dinoflagellates monophyletic? A study of 25 species based on nuclear and mitochondrial genes. *Protist* **160:** 245–264.

MURRAY, S.A., GARBY, T., HOPPENRATH, M. & NEILAN, B.A. (2012) Genetic diversity, morphological uniformity and polyketide production in dinoflagellates (*Amphidinium*, Dinoflagellata). *PLoS One* **7:** e38253.

NÉZAN, E., TILLMANN, U., BILIEN, G., BOULBEN, S., CHÈZE, K., ZENTZ, F., SALAS, R. & CHOMÉRAT, N. (2012) Taxonomic revision of the dinoflagellate *Amphidoma caudata*: Transfer to the genus *Azadinium* (Dinophyceae) and proposal of two varieties, based on morphological and molecular phylogenetic analyses. *Journal of Phycology* **48:** 925–939.

OKAMOTO, N., HORAK, A. & KEELING, P.J. (2012) Description of two species of early branching dinoflagellates, *Psammosa pacifica* n. g., n. sp. and *P. atlantica* n. sp. *PLoS One* **7:** e34900.

ORR, R.J., MURRAY, S.A., STUKEN, A., RHODES, L. & JAKOBSEN, K.S. (2012) When naked became armored: An eight-gene phylogeny reveals monophyletic origin of theca in dinoflagellates. *PLoS One* **7:** e50004.

PANDEIRADA, M.S., CRAVEIRO, S.C., DAUGBJERG, N., MOESTRUP, O., DOMINGUES, P. & CALADO, A.J. (2019) Studies on woloszynskioid dinoflagellates X: Ultrastructure, phylogeny and colour variation in *Tovellia rubescens* n. sp. (Dinophyceae). *The Journal of Eukaryotic Microbiology* **66**: 937–953.

PAWLOWSKI, J., AUDIC, S., ADL, S., BASS, D., BELBAHRI, L., BERNEY, C., BOWSER, S.S., CEPICKA, I., DECELLE, J., DUNTHORN, M. *et al.* (2012) CBOL Protist Working Group: Barcoding eukaryotic richness beyond the animal, plant, and fungal kingdoms. *PLOS Biology* **10:** e1001419.

PFIESTER, L.A. & ANDERSON, D.M. (1987) Dinoflagellate reproduction. In: Taylor, F.J.R. *The biology of dinoflagellates. Botanical Monographs 21.* pp. 611–648. Blackwell Scientific Publications, London.

PIENAAR, R.N., SAKAI, H. & HORIGUCHI, T. (2007) Description of a new dinoflagellate with a diatom endosymbiont, *Durinskia capensis* sp. nov. (Peridiniales, Dinophyceae) from South Africa. *Journal of Plant Research* **120:** 247–258.

POWERS, K.E., PRATHER, L.A., COOK, J.A., WOOLLEY, J., BART JR., H.L., MONFILS, A.K. & SIERWALD, P. (2014) Revolutionizing the use of natural history collections in education. *Science Education Review* **13:** 24–33.

PRICE, D.C. & BHATTACHARYA, D. (2017) Robust Dinoflagellata phylogeny inferred from public transcriptome databases. *Journal of Phycology* **53:** 725–729.

READ, B.A., KEGEL, J., KLUTE, M.J., KUO, A., LEFEBVRE, S.C., MAUMUS, F., MAYER, C., MILLER, J., MONIER, A., SALAMOV, A. *et al.* (2013) Pan genome of the phytoplankton *Emiliania* underpins its global distribution. *Nature* **499:** 209–213.

RENGEFORS, K. & KREMP, A. (2018) Dinophyceae. In: Moestrup, Ø. & Calado, A.J. *Süßwasserflora von Mitteleuropa, Bd. 6 – Freshwater Flora of Central Europe.* pp. 27–36. Springer-Verlag, Berlin, Heidelberg.

SABUROVA, M., CHOMÉRAT, N. & HOPPENRATH, M. (2012) Morphology and SSU rDNA phylogeny of *Durinskia agilis* (Kofoid & Swezy) comb. nov. (Peridiniales, Dinophyceae), a thecate, marine, sand-dwelling dinoflagellate formerly classified within *Gymnodinium*. *Phycologia* **51:** 287–302.

SALDARRIAGA, J.F., TAYLOR, F.J.R., CAVALIER-SMITH, T., MENDEN-DEUER, S. & KEELING, P.J. (2004) Molecular data and the evolutionary history of dinoflagellates. *European Journal of Protistology* **40:** 85–111.

SAMPEDRO, N., FRAGA, S., PENNA, A., CASABIANCA, S., ZAPATA, M., GRÜNEWALD, C.F., RIOBÓ, P. & CAMP, J. (2011) *Barrufeta bravensis* gen. nov. sp. nov. (Dinophyceae): A new bloom-forming species from the northwest Mediterranean Sea. *Journal of Phycology* **47:** 375–392.

SELLNER, K.G., DOUCETTE, G.J. & KIRKPATRICK, G.J. (2003) Harmful algal blooms: Causes, impacts and detection. *Journal of Industrial Microbiology and Biotechnology* **30:** 383–406.

SKOVGAARD, A., KARPOV, S.A. & GUILLOU, L. (2012) The parasitic dinoflagellates *Blastodinium* spp. inhabiting the gut of marine, planktonic copepods: Morphology, ecology, and unrecognized species diversity. *Frontiers in Microbiology* **3:** 305.

SMITH, G.F., FIGUEIREDO, E. & MOORE, G. (2016) Increasing nomenclatural stability by preventing the introduction of long-forgotten names that will compete with ones in use: A solution must be found, and soon. *Taxon* **65:** 1385–1390.

SÖHNER, S., ZINSSMEISTER, C., KIRSCH, M. & GOTTSCHLING, M. (2012) Who am I — and if so, how many? Species diversity of calcareous dinophytes (Thoracosphaeraceae, Peridiniales) in the Mediterranean Sea. *Organisms Diversity & Evolution* **12:** 339–348.

STAT, M., MORRIS, E. & GATES, R.D. (2008) Functional diversity in coral-dinoflagellate symbiosis. *Proceedings of the National Academy of Sciences* **105:** 9256–9261.

STEIN, F.R.V. (1883) *Der Organismus der Infusionsthiere nach eigenen Forschungen in systematischer Reihenfolge bearbeitet 3.2.*, Engelmann, Leipzig.

STEINICKE, H. (2014) *Challenges and opportunities of integrative taxonomy for research and society. Taxonomic research in the era of OMICS technologies.*, Deutsche Akademie der Naturforscher Leopoldina, Halle/Saale.

STERN, R.F., ANDERSEN, R.A., JAMESON, I., KUPPER, F.C., COFFROTH, M.A., VAULOT, D., LE GALL, F., VERON, B., BRAND, J.J., SKELTON, H. *et al.* (2012) Evaluating the ribosomal internal transcribed spacer (ITS) as a candidate dinoflagellate barcode marker. *PLoS One* **7:** e42780.

STOECKER, D.K. (1999) Mixotrophy among dinoflagellates. *The Journal of Eukaryotic Microbiology* **46:** 397–401.

STOSCH, H.A.V. (1973) Observations on vegetative reproduction and sexual life cycles of two freshwater dinoflagellates, *Gymondinium pseudopalustre* Schiller and *Woloszynskia apiculata* sp. nov. *British Phycological Journal* **8:** 105–134.

SUAREZ, A.V. & TSUTSUI, N.D. (2004) The value of museum collections for research and society. *BioScience* **54:** 66–74.

TAKAHASHI, K., MOESTRUP, Ø., WADA, M., ISHIMATSU, A., NGUYEN, V.N., FUKUYO, Y. & IWATAKI, M. (2017) *Dactylodinium pterobelotum* gen. et sp. nov., a new marine woloszynskioid dinoflagellate positioned between the two families Borghiellaceae and Suessiaceae. *Journal of Phycology* **53:** 1223–1240.

TAKANO, Y., HANSEN, G., FUJITA, D. & HORIGUCHI, T. (2008) Serial replacement of diatom endosymbionts in two freshwater dinoflagellates, *Peridiniopsis* spp. (Peridiniales, Dinophyceae). *Phycologia* **47:** 41–53.

TAKANO, Y., YAMAGUCHI, H., INOUYE, I., MOESTRUP, Ø. & HORIGUCHI, T. (2014) Phylogeny of five species of *Nusuttodinium* gen. nov. (Dinophyceae), a genus of unarmoured kleptoplastidic dinoflagellates. *Protist* **165:** 759–778.

TAYLOR, F.J.R. (1980) On dinoflagellate evolution. *BioSystems* **13:** 65–108.

TAYLOR, F.J.R. (2004) Illumination or confusion? Dinoflagellate molecular phylogenetic data viewed from a primarily morphological standpoint. *Phycological Research* **52:** 308–324.

TAYLOR, F.J.R., HOPPENRATH, M. & SALDARRIAGA, J.F. (2008) Dinoflagellate diversity and distribution. *Biodiversity and Conservation* **17:** 407–418.

THESSEN, A.E., PATTERSON, D.J. & MURRAY, S.A. (2012) The taxonomic significance of species that have only been observed once: The genus *Gymnodinium* (Dinoflagellata) as an example. *PLoS One* **7:** e44015.

THOMAS, C. (2009) Plant bar code soon to become reality. *Science* **325:** 526.

TILLMANN, U., ELBRÄCHTER, M., KROCK, B., JOHN, U. & CEMBELLA, A. (2009) *Azadinium spinosum* gen. et sp. nov. (Dinophyceae) identified as a primary producer of azaspiracid toxins. *European Journal of Phycology* **44:** 63–79.

TILLMANN, U., SALAS, R., GOTTSCHLING, M., KROCK, B., O'DRISCOLL, D. & ELBRÄCHTER, M. (2012) *Amphidoma languida* sp. nov. (Dinophyceae) reveals a close relationship between *Amphidoma* and *Azadinium*. *Protist* **163:** 701–719.

TILLMANN, U., SÁNCHEZ-RAMÍREZ, S., KROCK, B. & BERNALES-JIMÉNEZ, A. (2017) A bloom of *Azadinium polongum* in coastal waters off Peru. *Revista de Biología Marina y Oceanografía* **52:** 591–610.

TILLMANN, U., HOPPENRATH, M. & GOTTSCHLING, M. (2019) Reliable determination of *Prorocentrum micans* Ehrenb. (Prorocentrales, Dinophyceae) based on newly collected material from the type locality. *European Journal of Phycology* **54:** 417–431.

TRENCH, R.K. (1993) Microalgal-invertebrate symbioses: A review. *Endocytobiosis and Cell Research* **9:** 135–175.

TURLAND, N.J., WIERSEMA, J.H., BARRIE, F.R., GREUTER, W., HAWKSWORTH, D.L., HERENDEEN, P.S., KNAPP, S., KUSBER, W.-H., LI, D.-Z., MARHOLD, K. *et al.* (2018) *International Code of Nomenclature for algae, fungi, and plants (Shenzhen Code). Adopted by the Nineteenth International Botanical Congress, Shenzhen, China, July 2017. Regnum Vegetabile 159.* Koeltz Botanical Books, Glashütten.

WOŁOSZYŃSKA, J. (1925) Przyczynki do znajomości polskich brózdnic słodkowodnych. (Beiträge zur Kenntnis der Süsswasser-Dinoflagellaten Polens). *Acta Societatis Botanicorum Poloniae* **3:** 49–64.

VAN DOLAH, F.M. (2000) Marine algal toxins: Origins, health effects, and their increased occurrence. *Environmental Health Perspectives* **108:** 133–141.

VERNOOY, R., HARIBABU, E., MULLER, M.R., VOGEL, J.H., HEBERT, P.D., SCHINDEL, D.E., SHIMURA, J. & SINGER, G.A. (2010) Barcoding life to conserve biological diversity: Beyond the taxonomic imperative. *PLoS Biololgy* **8:** e1000417.

WALL, D. & DALE, B. (1967) The resting cysts of modern marine dinoflagellates and their palaeontological significance. *Review of Palaeobotany and Palynology* **2:** 349–354.

WALL, D. & DALE, B. (1968) Quaternary calcareous dinoflagellates (Calciodinellideae) and their natural affinities. *Journal of Paleontology* **42:** 1395–1408.

WILSON, E.O. (2017) Biodiversity research requires more boots on the ground. *Nature Ecology & Evolution* **1:** 1590–1591.

WISECAVER, J.H. & HACKETT, J.D. (2011) Dinoflagellate genome evolution. *Annual Review of Microbiology* **65:** 369–387.

WOŁOSZYŃSKA, J. (1916) Polskie Peridineae słodkodne. *Bulletin international de l'Académie des sciences de Cracovie* **Série B:** 259–285.

YAMADA, N., SYM, S.D. & HORIGUCHI, T. (2017) Identification of highly divergent diatom-derived chloroplasts in dinoflagellates, including a description of *Durinskia kwazulunatalensis* sp. nov. (Peridiniales, Dinophyceae). *Molecular Biology and Evolution* **34:** 1335–1351.

ŽERDONER ČALASAN, A., KRETSCHMANN, J. & GOTTSCHLING, M. (2018) Absence of co-phylogeny indicates repeated diatom capture in dinophytes hosting a tertiary endosymbiont. *Organisms Diversity & Evolution* **18:** 29–38.

ŽERDONER ČALASAN, A., KRETSCHMANN, J. & GOTTSCHLING, M. (2019) They are young, and they are many: Dating freshwater lineages in unicellular dinophytes. *Environmental Microbiology* **21:** 4125–4135.

ZHANG, H., BHATTACHARYA, D. & LIN, S. (2007) A three-gene dinoflagellate phylogeny suggests monophyly of Prorocentrales and a basal position for *Amphidinium* and *Heterocapsa*. *Journal of Molecular Evolution* **65:** 463–474.

ZHANG, Q., LIU, G. & HU, Z. (2014) Description of a new freshwater bloom-forming dinoflagellate with a diatom endosymbiont, *Peridiniopsis minima* sp. nov. (Peridiniales, Dinophyceae) from China. *Algological Studies* **145:** 119–133.

ZHANG, Q., ZHU, H., HU, Z. & LIU, G. (2016) Blooms of the woloszynskioid dinoflagellate *Tovellia diexiensis* sp. nov. (Dinophyceae) in Baishihai Lake at the eastern edge of Tibetan Plateau. *Algae* **31:** 205–217.

ZINSSMEISTER, C., SÖHNER, S., FACHER, E., KIRSCH, M., MEIER, K.J.S. & GOTTSCHLING, M. (2011) Catch me if you can: The taxonomic identity of *Scrippsiella trochoidea* (F.Stein) A.R.Loebl. (Thoracosphaeraceae, Dinophyceae). *Systematics and Biodiversity* **9:** 145–157.

ZOHNER, C.M. & RENNER, S.S. (2014) Common garden comparison of the leaf-out phenology of woody species from different native climates, combined with herbarium records, forecasts long-term change. *Ecology Letters* **17:** 1016–1025.

APPENDICES

Publication 1

The hot spot in a cold environment:
Puzzling *Parvodinium* (Peridiniopsidaceae, Peridiniales)
from the Polish Tatra Mountains

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ORIGINAL PAPER

The Hot Spot in a Cold Environment: Puzzling *Parvodinium* (Peridiniopsidaceae, Peridiniales) from the Polish Tatra Mountains

Juliane Kretschmann[a], Paweł M. Owsianny[b], Anže Žerdoner Čalasan[a,c], and Marc Gottschling[a,1]

[a]Department Biologie, Systematische Botanik und Mykologie, GeoBio-Center, Ludwig-Maximilians-Universität München, Menzinger Str. 67, D-80 638 München, Germany
[b]Nadnotecki Institute UAM in Piła, Adam Mickiewicz University, ul. Kołobrzeska 15, PL-64 920 Piła, Poland
[c]Department of Botany, School of Biology and Chemistry, University of Osnabrück, Barbarastr. 11, D-49 076 Osnabrück, Germany

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Because of a great variety of remote localities and cold habitats, the Tatra Mountains are home to many freshwater protist lineages. Dinophytes have been subjected to a number of studies from this area dating mostly to the first half of the 20th century, but their true diversity remains elusive until today. We collected water tow samples at five lakes in the Tatra Mountains in order to establish monoclonal strains. We found four lineages that were distinctive in terms of morphology and DNA sequence data and that could be assigned to peridinialean *Parvodinium*. These four species can be readily distinguished based on a general shape, size, thecal plate tabulation pattern and presence or absence of an antapical protuberance. The plate overlap pattern is considered conserved at higher taxonomic levels, and the divergent keystone Plate 3′ in *Parvodinium marciniakii*, sp. nov., thus appears as a striking diagnostic character. For taxonomic conclusion, we describe two species new to science and validate three old scientific names (i.e., one species and two varieties). Our study underlines that the biodiversity assessment, particularly of species adapted to cold environments, is anything but completed as shown from remote and unexplored European landscapes such as the Tatra Mountains.

Key words: Biodiversity; dinoflagellates; epitype; molecular phylogenetics; morphology; Poland.

Introduction

The North European Plain is characterised by the presence of extensive lake districts and ice age river valleys as parts of the Baltic Ridge (being a

[1]Corresponding author;
e-mail gottschling@bio.lmu.de (M. Gottschling).

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belt of terminal moraines). With 7,081 lakes covering an area of over 1 ha (Choiński 2006), Poland has a great number of isolated water bodies on a relatively small area. Its geological structure has been shaped primarily by the continental collision of Europe and Africa over the past 60 million years (Passendorfer in Mirek 1996). The Pleistocene glaciations of northern Europe (Lindner et al. 2004; Pelzer 1991) had later on a strong impact on community dynamics. This is true in particular for (unicellular) freshwater organisms, when they are subjected to enforced dispersal/migration and to speciation due to habitat fragmentation/isolation in a relatively short period of time. Before exploring any specific ecological or phylogenetic research question in this area, it is of fundamental necessity to revise and clarify the taxonomy of the organisms under investigation.

Unambiguous scientific names are prerequisite for meaningful and taxonomically indisputable conclusions about biology and potential conservation strategies of a certain taxon, but we lack basic data on reliable species numbers and lists of correct scientific names (i.e., a taxonomy adjusted to synonyms avoiding pseudospecies). Dinophytes are no exception. Not less than ten taxa at the species level (and some more below the species level) have been described from various lakes in the Tatra Mountains (Wołoszyńska 1916, 1919, 1936), but the taxonomic identity of the majority of them remains obscure. Most of them are considered endemic to the Tatra area (Kawecka in Mirek 1996) such as gymnodinialean *Spiniferodinium limneticum* (Wołosz.) Kretschmann & Gottschling, which is so far unknown from localities outside the Tatra Mountains (Kretschmann et al. 2015b).

Freshwater dinophytes have been notoriously understudied in the past (Gómez 2014; Mertens et al. 2012; Popovský and Pfiester 1990; Thessen et al. 2012). Many of the species exhibiting a theca composed of cellulosic plates are found in two distinct lineages of the Peridiniales, namely the Peridiniaceae and the Peridiniopsidaceae. The latter may appear morphologically heterogeneous at first sight, but their representatives exhibit consistently maximally two intercalary plates as well as six cingular plates (Bourrelly 1968; Carty 2008; Craveiro et al. 2009; Gottschling et al. 2017; Kretschmann et al. 2018; Popovský and Pfiester 1986) contrasting the three intercalary plates and five cingular plates in Peridiniaceae. As an integral element of the Peridiniopsidaceae, ten species of *Parvodinium* Carty are currently accepted (Carty 2008). However, α-taxonomy is challenging in *Parvodinium* (last but not least also because of its small cell size), and the exact number of species is thus unknown at present. *Parvodinium* includes some frequently encountered species such as *Parvodinium inconspicuum* (Lemmerm.) Carty and *Parvodinium umbonatum* (F.Stein) Carty, but a reasonable number of imperfectly known taxa such as "*Peridinium*" *minimum* A.J.Schill. and "*Peridinium*" *tatricum* Wołosz. could also belong to it (the latter two names are currently regarded as synonyms of *P. umbonatum*: Popovský & Pfiester, 1990, but their precise taxonomic status has not been worked out yet).

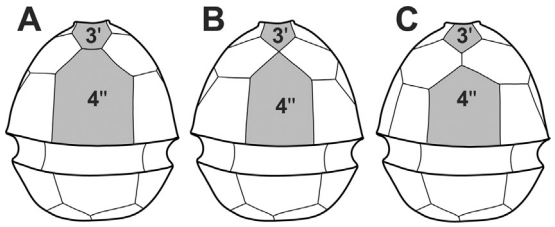

Figure 1. Schematic drawing of the three different dorsal epithecal conformations (after Carty 2008; Lefèvre 1932; Lindemann 1918a,b; Popovský 1968). **A**. <*conjunctum*> tabulation type. **B**. <*contactum*> tabulation type. **C**. <*remotum*> tabulation type.

Despite their small size, all species of *Parvodinium* are characterised by the presence of two anterior intercalary plates. Three distinct conformations of the dorsal epitheca have been known so far (Carty 2008; Lefèvre 1932; Lindemann 1918a,b; Popovský 1968; Fig. 1). In the <*conjunctum*> tabulation, the third apical plate shares one plate side with the fourth precingular plate in a way that both intercalary plates are separated and are regularly pentagonal in shape. Plates 3′ and 4″ are separated by the two anterior intercalary plates in the <*remotum*> tabulation, leading to shared plate sides and irregularly hexagonal shapes of the anterior intercalary plates. In the <*contactum*> tabulation, Plates 3′ and 4″ as well as both intercalary plates all meet at a certain point of the epitheca, so that the sutures between the plates form a cross on the dorsal cell side. All three different tabulation types may occur within a monoclonal strain (of, e.g., *P. umbonatum*: Elbrächter and Meyer, 2001), indicating that this trait varies to a certain extent and is not necessarily diagnostic to delimitate taxa of *Parvodinium*.

The plate overlap (or imbrication) pattern is a useful trait to determine plate homologies and to infer phylogenetic relationships of dinophytes (Below 1987; Netzel and Dürr 1984). For example, the fourth precingular plate is the keystone plate

(i.e., a plate overlapping all adjacent plates) in representatives of the Peridiniales with seven precingular plates [including *P. umbonatum*: Elbrächter and Meyer, 2001, and *Scrippsiella acuminata* (Ehrenb.) Kretschmann, Zinssmeister, S.Soehner, Elbr., Kusber & Gottschling: Kretschmann et al. 2015a], while those of Amphidomataceae and Gonyaulacales have the third precingular plate playing the role of the keystone plate (Dodge 1988; Fensome et al. 1993; Tillmann et al. 2012, 2014). Anyhow, the imbrication pattern is investigated for a handful of dinophyte species only, thus we are far away from any general assessment of its significance for taxonomy and phylogenetic relevance. In this study, we provide detailed descriptions of species assigned to *Parvodinium* that we collected at remote and unexplored localities in the Polish Tatra Mountains. We aim at a better knowledge of this ecologically important though imperfectly known group of freshwater dinophytes.

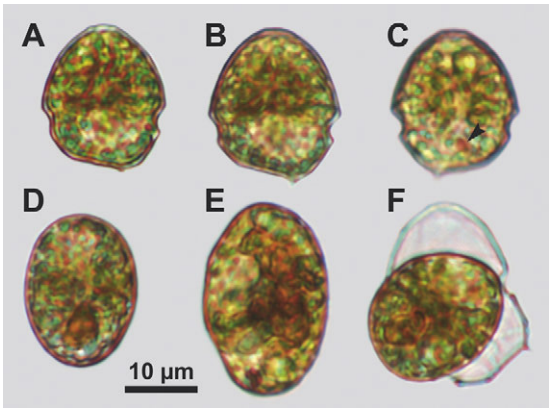

Figure 2. Motile thecate and immotile cells of *Parvodinium marciniakii*, sp. nov. (GeoM*709; LM; all at the same scale). **A–B**. motile thecate cells in ventral view. **C**. motile thecate cell in dorsal view. Black arrowhead indicates the red eyespot. **D–E**. immotile coccoid cells. **F**. immotile coccoid cell with theca remnant.

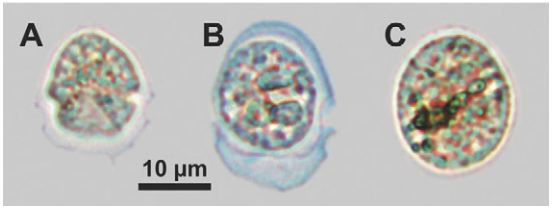

Figure 3. Motile and immotile stages (stained with astra blue and eosin) prepared as a holotype of *Parvodinium marciniakii*, sp. nov. (GeoM*709; LM; all at the same scale). **A**. motile thecate cell. **B**. immotile coccoid cell with theca remnant. **C**. immotile coccoid cell.

Results

Morphology of *Parvodinium marciniakii,* sp. nov.

The strains GeoM*701 and GeoM*750 (collected at Długi Staw Gąsienicowy) as well as GeoM*708 and GeoM*709 (collected at Zielony Staw Gąsienicowy) grew especially well in WC medium at 12 °C and were morphologically indistinguishable. They exhibited both motile thecate cells (Figs 2A–C, 3A, 4A–C, G) and immotile coccoid cells (Figs 2D–F, 3B–C, 4F), but the motile cells were predominant. The coloration of the motile cells was golden-brown in the upper part of the theca whereas in the lower part of the cell, content was brown-hyaline and showed a small, red area (interpreted as eyespot) in the sulcal region (Fig. 2A–C). The dinokaryon with distinctly condensed chromosomes was located mostly in the hypotheca.

Thecate cells were widely through very widely ovoid, and the epitheca was larger than the hypotheca (Figs 2A–C, 3A, 4A–C). The shape of the epitheca was semi-elliptical in outline with an obtuse apex. The hypotheca was semi-circular through trapezoidal in outline and showed a single, antapical spine. The size of the motile cells ranged from 18–24 µm (GeoM*709; mean: 22 µm; median: 22 µm; sd: 1 µm; n = 50) in length and from 15–21 µm (GeoM*709; mean: 18 µm; median: 17 µm; sd: 1 µm; n = 50) in width. The cingulum was excavated, and it surrounded the motile cell with a descendent displacement of approximately one half of its own width (Fig. 4A). The sulcus was likewise excavated, extending slightly into the epitheca. It widened towards the posterior end of the cell and reached down nearly to the antapex (Fig. 4A).

The motile cells were covered by a theca (astra blue staining indicated their cellulosic composition). The cell surface of the thecal plates was smooth and scattered randomly with small, circular pores (probably openings of trichocysts). The thecate plate formula was pp, cp, x, 4′, 2a, 7″, 6c, 5s, 5‴, 2⁗ (Figs 4A–E, G–I, 14A–D). The arrangement of the epithecal plates was symmetric and showed three different tabulation types, with the <*conjunctum*> tabulation being predominant. The quantification of the tabulation types is given in Table 2.

The apical pore complex consisted of a nearly circular apical pore plate, a cover plate and a canal (or x or preapical) plate (Fig. 4H). The cingulum was composed of six plates of different size. The

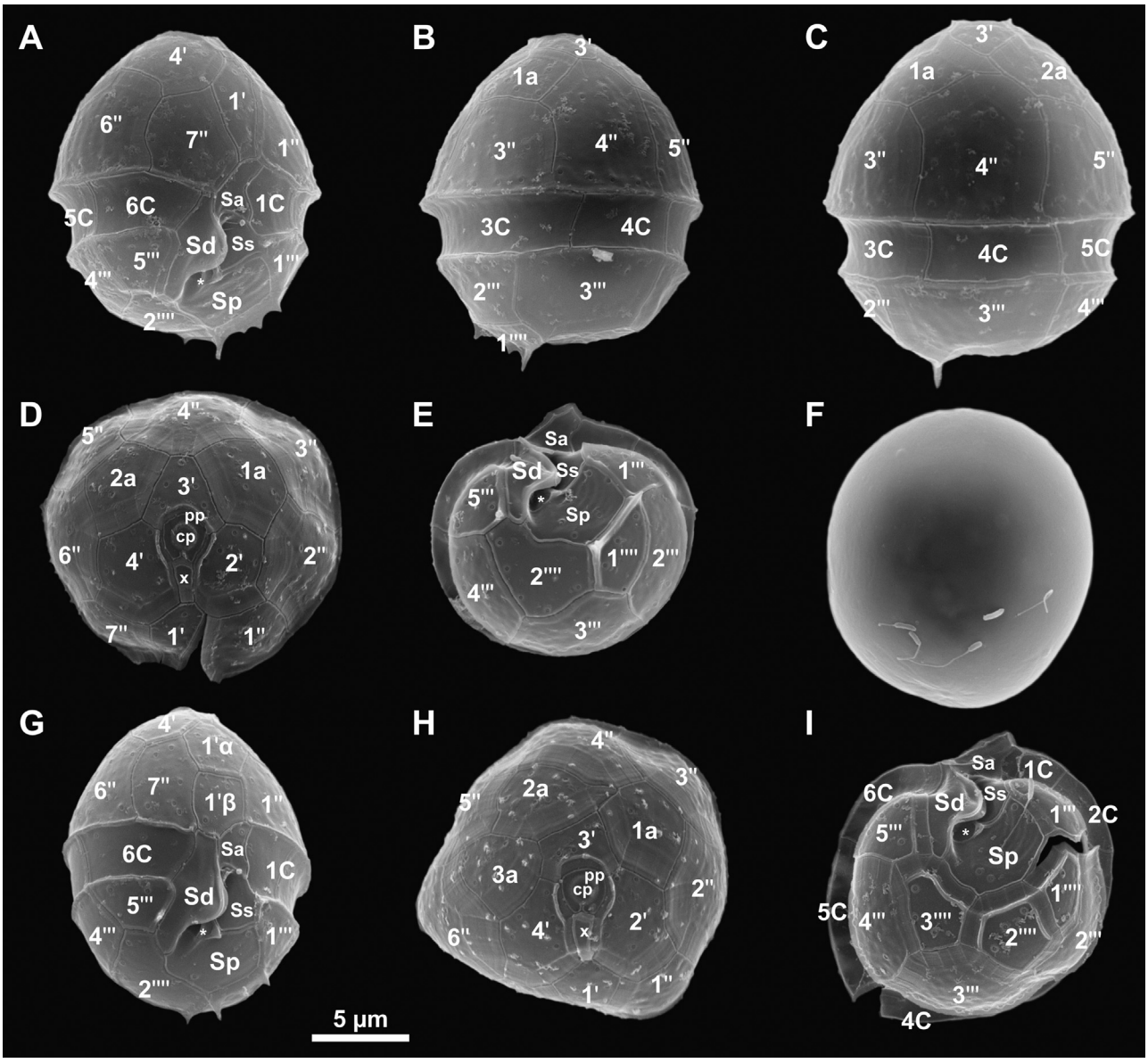

Figure 4. Motile thecate and immotile cells of *Parvodinium marciniakii*, sp. nov. (**A–E**, **G–H**. GeoM*709; **F**, **I**. GeoM*708; SEM; all at the same scale). **A–E**. thecae showing the tabulation pattern (asterisks indicate the sulcal plate Sm). **A**. ventral view. **B–C**. dorsal view showing the <*conjunctum*> tabulation type. **D**. apical view showing the <*conjunctum*> tabulation type. **E**. antapical view. **F**. immotile coccoid cell showing a smooth surface. **G–J**. examples of variations in thecal plate pattern (asterisks indicate the sulcal plate Sm). **G**. subdivision of the apical Plate 1′. **H**. additional anterior intercalary plate. **I**. additional antapical plate. Abbreviations: cp: closing plate. n′: apical plate. n″: precingular plate. n‴: postcingular plate. n⁗: antapical plate. na: anterior intercalary plate. nC: cingular plate. pp: pore plate. Sa: anterior sulcal plate. Sd: right sulcal plate. Sm: median sulcal plate. Sp: posterior sulcal plate. Ss: left sulcal plate. x: canal (preapical) plate.

sulcus consisted of five plates, where the plates Sm and Ss were small and partially covered by the large plate Sd. The thickened left edge of the Sd plate extended towards the middle of the sulcus and covered the flagellar pores. The Sp plate was relatively large and reached down to the antapex. The arrangement of the hypothecal plates was nearly symmetrical and composed of five postcingular plates varying slightly in size and two antapical plates, where the antapical Plate 2⁗ was slightly larger than the Plate 1⁗. The posterior spine of various shapes and lengths was an extension of the

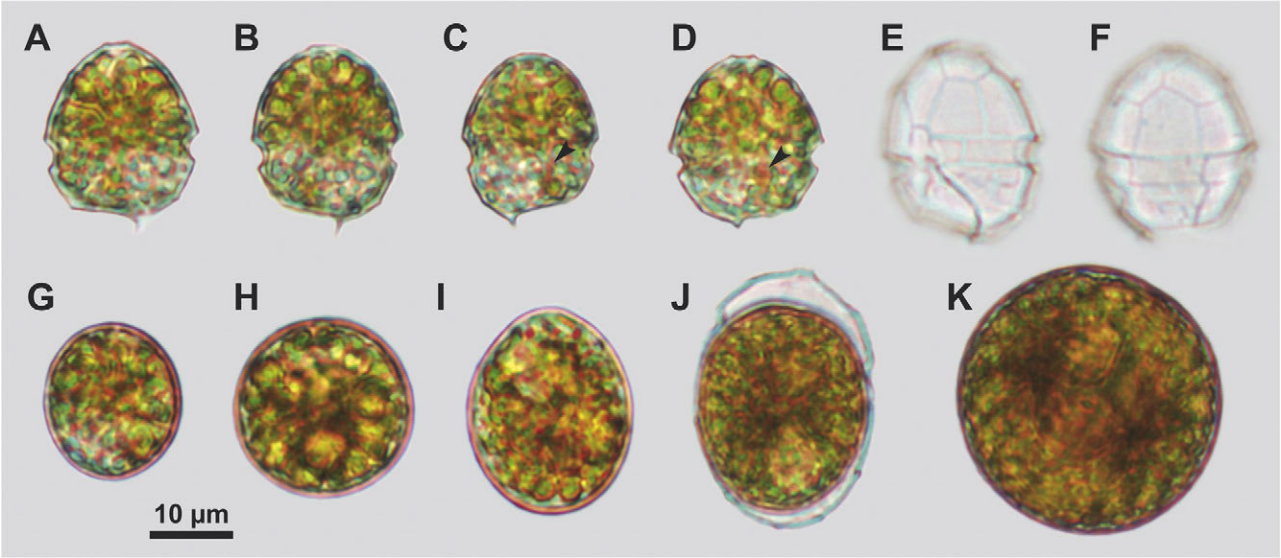

Figure 5. Motile thecate and immotile cells of *Parvodinium trawinskii*, sp. nov. (GeoM*753; LM; all at the same scale). **A–D**. motile thecate cells. **A–B**. ventral view. **C–D**. dorsal view (black arrowheads indicate the eyespot). **E–F**. empty thecae. **E**. ventral-lateral view. **F**. dorsal view showing the <*remotum*> tabulation type. **G–K**. immotile coccoid cells showing variation in size and shape. **J**. coccoid cell with theca remnant.

antapical Plate 1′′′′ and emerged from its margin at the contact site with the sulcal plate Sp and the antapical Plate 1′′′′ (Fig. 4A–C, E). In the cultivated strains, only a few deviations from the typical plate pattern were observed regarding epi- or hypothecal plates (Fig. 4G–I).

Along the thecal plate boundaries, an overlap pattern of adjacent plates could be inferred (Figs 4A–E, G–I, 14A–D). Generally, it followed an imbricate pattern from dorsal towards ventral direction. In the epithecal plate series, the dorsal-lateral precingular Plate 3′′ was the keystone plate whereas in the cingulum, it was the Plate 4C. The keystone plate of the hypotheca was postcingular Plate 3′′′. In the cultivation plates, numerous empty thecae were observed indicating cell division by eleutheroschisis. Thecate cells opened (mostly) along the upper ridge of the cingulum (i.e., the cingulum was attached to the hypotheca) to release dividing or ecdysing cells (Figs 2F, 3B, 4D–E, H–I). Single coccoid cells developed intrathecately and were released after shedding of the theca (Figs 2F, 3B). Coccoid cells were golden-brown in colour and mostly widely ovoid through elongated in shape (Fig. 2D–F). The size was variable and ranged from 18–32 µm in length (GeoM*709; mean: 22 µm; median: 21 µm; SD: 4 µm; n = 50), 14–20 µm in width (GeoM*709; mean: 17 µm; median: 17 µm; SD: 1 µm; n = 50) and had a smooth surface (Fig. 4F). The cytoplasm of the coccoid cells was filled with numerous brown granules and contained frequently a large, red accumulation body (Fig. 2D).

Morphology of *Parvodinium trawinskii,* sp. nov.

The strains GeoM*702, GeoM*703, GeoM*704, GeoM*749 and GeoM*753 (all collected at Długi Staw Gąsienicowy) grew especially well in WC medium at 12 °C and were morphologically indistinguishable. They exhibited motile thecate cells (Figs 5A–D, 7A–D, G, I) as well as immotile coccoid cells (Figs 5G–K, 6C-D, 7H), but the motile cells were predominant. In the epitheca of the motile cells, the cell content was yellow through golden-brown in colour and densely filled with numerous granules. The dinokaryon with distinctly condensed chromosomes was located in the hypotheca, which led to a hyaline appearance of the lower cell part (Fig. 5A–D). In the sulcal region, a red area (interpreted as eyespot) was observed (Fig. 5C–D).

Thecate cells were ovoid and the epitheca was larger than the hypotheca (Figs 5A–F, 6A–B, 7A, D). The shape of the epitheca was semi-elliptical in outline with a flattened tip. The hypotheca was semi-circular through trapezoidal in outline and showed a single, antapical spine (Figs 5A–D, 7A, C–D, F). The size of the motile cells ranged from 21–26 µm (GeoM*753; mean: 24 µm; median: 24 µm; sd: 1 µm; n = 50) in length and from 18–23 µm (GeoM*753; mean: 20 µm; median:

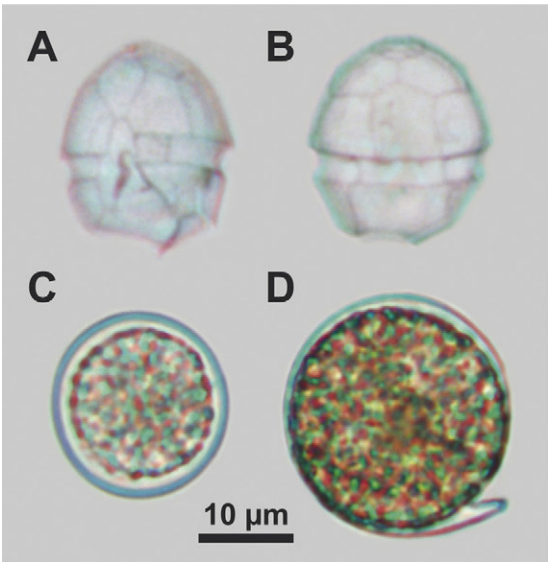

Figure 6. Motile and immotile stages (stained with astra blue and eosin) prepared as a holotype of *Parvodinium trawinskii*, sp. nov. (GeoM*753; LM; all at the same scale). **A**. empty theca in ventral view. **B**. empty theca in dorsal view showing the <*remotum*> tabulation type. **C–D**. immotile coccoid cells.

20 µm; sd: 1 µm; n = 50) in width. The cingulum was excavated and surrounded the motile cell with a descendent displacement of approximately one half of its own width (Figs 5E–F, 6A, 7A). The sulcus was likewise excavated, extended into the epitheca, widened towards the posterior end of the cell and reached down to the antapex.

The motile cells were covered by a theca, which possessed numerous, small pits on the cell surface organised in vertical rows (Fig. 7A–G, I). Additionally, few, small circular pores (probably openings of trichocysts) were irregularly scattered over the thecal plates. The thecate plate formula was pp, cp, x, 4′, 2a, 7″, 6c, 5s, 5‴, 2⁗ (Figs 5E–F, 6A–B, 7A–G, I, 14E–H). The arrangement of the epithecal plates was symmetric and showed mostly the <*remotum*> tabulation type (Figs 5F, 6B, 7B–E, G, Table 2). Both anterior intercalary plates were hexagonal in shape and the length of the common suture varied barely. The cingulum was composed of six plates of similar size except for Plate 1C, which was smaller. The sulcus consisted of five plates, where the plates Sm and Ss were small and partially covered by the large Sd plate. The left edge of the Sd plate, and the posterior end of the Sa plate, was covered by flagellar pores. The Sp plate was relatively large and reaching all the way up to the antapex. The arrangement of the hypothecal plates was nearly symmetrical. The hypotheca was composed of five postcingular and two antapical plates of almost the same size. The posterior spine of various shapes and lengths emerged from the thecal plate margin between the sulcal plate Sp and antapical Plate 1⁗ as well as from the margin between the two antapical plates. In the cultivated strains, only a few deviations from the typical epithecal plate pattern (such as fusion of plates: Fig. 7I) were observed.

The plate overlap pattern in epithecal, cingular and hypothecal plate series followed the general gradient from dorsal towards ventral site (Figs 7A–G, I, 14E–H). In the epitheca, the dorsal precingular Plate 4″ was the keystone plate, whereas the keystone plate function in the cingular series belonged to the dorsal Plate 4C. The keystone plate of the hypotheca was postcingular Plate 3‴. Numerous empty thecae on the bottom of the cultivation plates indicated a cell division by eleutheroschisis. Dividing or ecdysing cells exited thecate cells through an opening on the hypotheca. Single coccoid cells developed intrathecately and were released after shedding of the theca (Fig. 5J). Coccoid cells were ovoid through spherical in shape (Figs 5G–K, 6C–D, 7H) and variable in size ranging from 21–34 µm in length (GeoM*753; mean: 26 µm; median: 24 µm; SD: 3 µm; n = 50), 17–33 µm in width (GeoM*753; mean: 23 µm; median: 22 µm; SD: 3 µm; n = 50) and had a smooth surface (Fig. 7H). The cytoplasm of the coccoid cells was filled with numerous golden-brown through brown granules of varying size and usually contained a large, red accumulation body (Fig. 5G–K).

Morphology of *Parvodinium mixtum,* sp. nov., and its varieties *P. mixtum* var. *remotum,* var. nov., and *P. mixtum* var. *conjunctum,* var. nov.

The single cells of the strains GeoM*695, GeoM*706, Geo*707, GeoM*751 and GeoM*752 (collected at Zielony Staw Gąsienicowy), GeoM*716, GeoM*717, GeoM*720 and GeoM*746 (collected at Litworowy Staw) as well as GeoM*710, GeoM*711, GeoM*754 and GeoM*755 (collected at Morskie Oko) grew especially well in WC medium at 12 °C (GeoM*695 and GeoM*720 also at 18 °C) and were indistinguishable in their gross morphology. However, all (notably monoclonal) strains showed variability regarding different tabulation types. The strains exhibited different frequencies of such tabulation types (Table 2), hence the strains were determined as *P. mixtum*, sp. nov., var. *mixtum* (GeoM*706 and GeoM*720), *P. mixtum* var. *conjunctum*, var. nov. (GeoM*695; GeoM*711), and *P. mixtum* var. *remotum*, var.

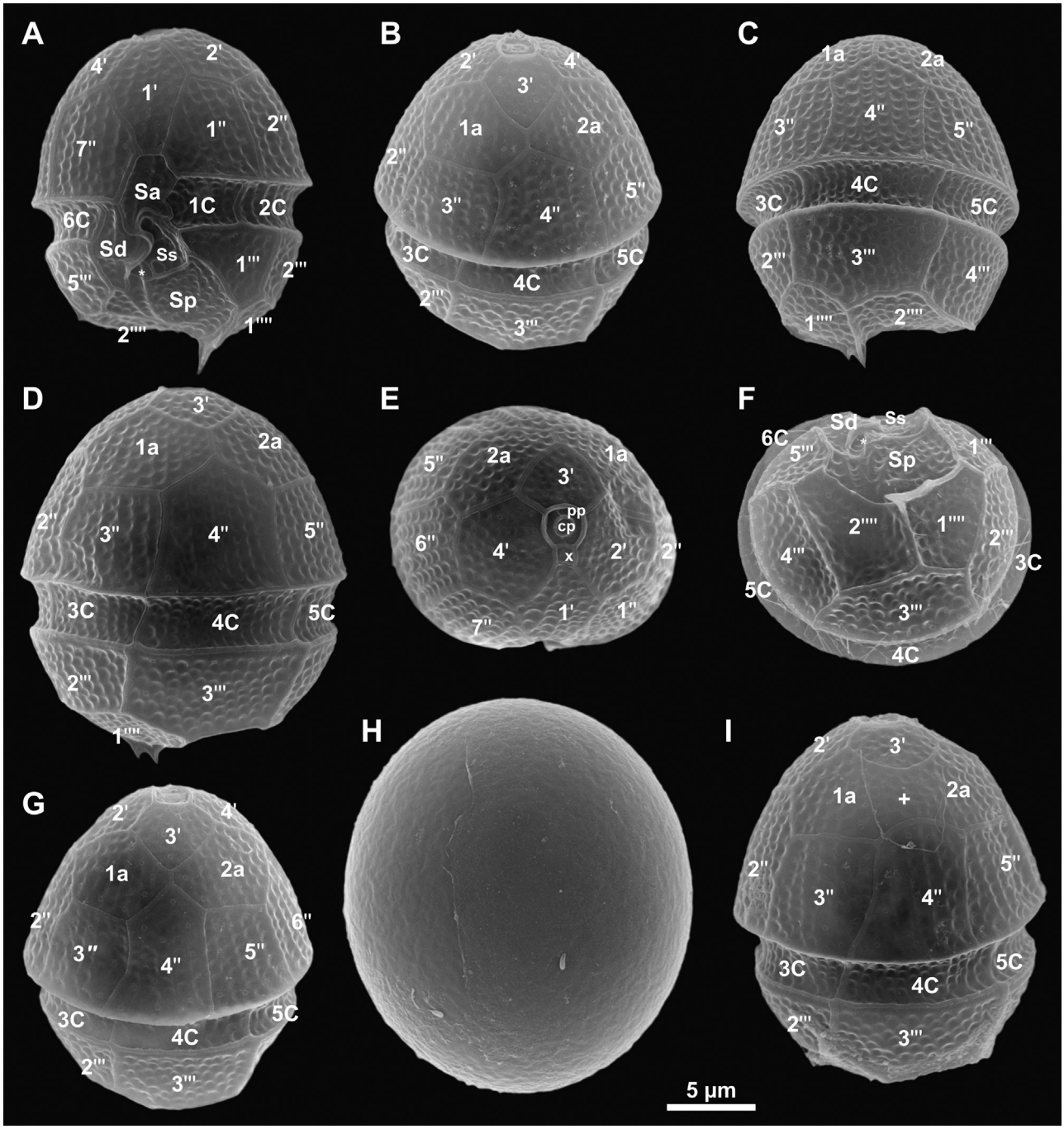

Figure 7. Motile thecate and immotile cells of *Parvodinium trawinskii*, sp. nov. (**A–F**; GeoM*753; **G–I**. GeoM*749; SEM; all at the same scale). **A–G**. motile thecate cells showing the tabulation pattern (asterisks indicate the sulcal plate Sm). **A**. ventral view. **B–D**. dorsal view showing the <*remotum*> tabulation type. **E**. apical view showing the <*remotum*> tabulation type. **F**. antapical view. **G**. dorsal view showing the <*remotum*> tabulation type. **H**. immotile coccoid cell showing a smooth surface. **I**. unusual plate pattern showing the presence of only one anterior intercalary plate (assumed to be the result of the fusion of both intercalary plates: the missing suture is indicated by '+'). Abbreviations: cp: closing plate. n′: apical plate. n″: precingular plate. n‴: postcingular plate. n⁗: antapical plate. na: anterior intercalary plate. nC: cingular plate. pp: pore plate. Sa: anterior sulcal plate. Sd: right sulcal plate. Sm: median sulcal plate. Sp: posterior sulcal plate. Ss: left sulcal plate. x: canal (preapical) plate.

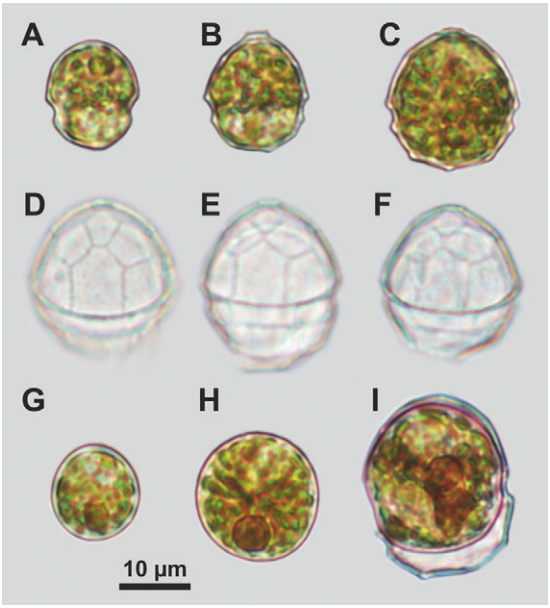

Figure 8. Motile thecate and immotile cells of *Parvodinium mixtum*, sp. nov., var. mixtum (GeoM*720; LM; all at the same scale). **A–C**. motile thecate cells showing variation in size and shape. **D–F**. empty thecae in dorsal view showing different tabulation types. **D**. <*conjunctum*> tabulation type. **E–F**. <*remotum*> tabulation type. **G–I**. immotile coccoid cells showing variation in size. **I**. coccoid cell with theca remnant.

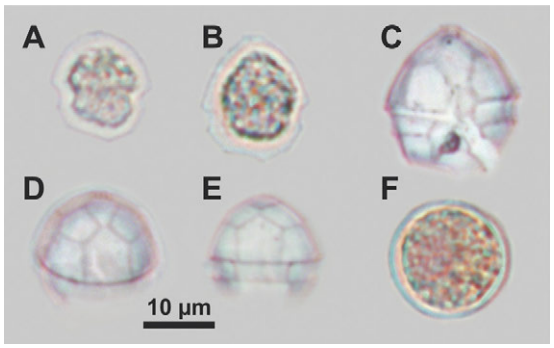

Figure 9. Motile and immotile stages (stained with astra blue and eosin) prepared as a holotype of *Parvodinium mixtum*, sp. nov., var. mixtum (GeoM*720; LM; all at the same scale). **A–B**. motile theca in ventral view. **C–E**. empty thecae. **C**. ventral view. **D**. dorsal view showing the <*conjunctum*> tabulation type. **E**. dorsal view showing the <*remotum*> tabulation type. **F**. immotile coccoid cell.

nov. (GeoM*717). Because of the variability within monoclonal strains, they were all considered to represent a single species, namely *P. mixtum*, sp. nov., which is characterised further below.

All strains exhibited both motile thecate cells (Figs 8A–C, 9A–B, 10, 11A–C, H–I, 12A, G, 13A–H) and immotile coccoid cells (Figs 8G–I, 9F, 11E–F, L–M, 12F, L), but the motile cells were predominant. These were golden-brown in colour and densely filled with numerous granules. The dinokaryon with distinctly condensed chromosomes was located mostly in the hypotheca (Fig. 11A–B) and only occasionally positioned in the epitheca. In the sulcal region, a red area (interpreted as eyespot) was observed (Fig. 11C).

Thecate cells were ovate in outline and had a slightly larger epitheca. The shape of the epitheca was semi-elliptical in outline with a slightly acuminate apex. The hypotheca was hemispherical without postcingular or antapical protuberances (Figs 8A–C, 9A–B, 10A–B, 11A–C, H–I, 12A, G, 13A, E). In strain GeoM*720, the size of the motile cells ranged from 14–23 µm (mean: 19 µm; median: 19 µm; sd: 2 µm; n = 50) in length and from 12–21 µm (mean: 17 µm; median: 17 µm; sd: 2 µm; n = 50) in width, and the other strains showed comparable measures (documented, but not explicitly reported here). The cingulum was excavated, and it surrounded the motile cell with a descendent displacement of approximately one third of its own width (Figs 9C, 10A–B, 12B, H, 13A, E). The epi- and hypothecal cingular edges had short and slightly jagged lists (Fig. 10A–B, D–I). The sulcus was likewise excavated and extended into the epitheca. It widened towards the posterior end of the cell and reached down to the antapex (Figs 9C, 10A–B, 12B, H, 13A, E).

The motile cells were covered by a theca built of thick, cellulosic plates (Figs 8D–F, 9C–E, 10, 11D, J–K, 12B–E, H–K, 13A–H) that were particularly more pronounced than in *P. marciniakii*, sp. nov. The cell surface of the thecal plates showed a light reticulate ornamentation and was irregularly scattered with small circular pores (probably openings of trichocysts) on the thecal plates. The sutures between the plates varied in their thickness and were cross-striated (Figs 10, 13A–H).

The thecate plate formula was pp, cp, x, 4′, 2a, 7″, 6c, 5s, 5‴, 2⁗ (Figs 8D–F, 9C–E, 10, 11D, J–K, 12B–E, H–K, 13A–H, 14I–L). The arrangement of the epithecal plates was symmetric and showed all three different tabulation types (<*conjunctum*>: Figs 8D, 9D, 10G–I, 11J, 12I–J, 13F–G, <*contactum*>: Fig. 10F; <*remotum*>: Figs 8E–F, 9E, 10D–E, 11D, 12C–D, 13B–C). The quantification of the tabulation types for several strains is given in Table 2. The apical pore complex consisted of a nearly cir-

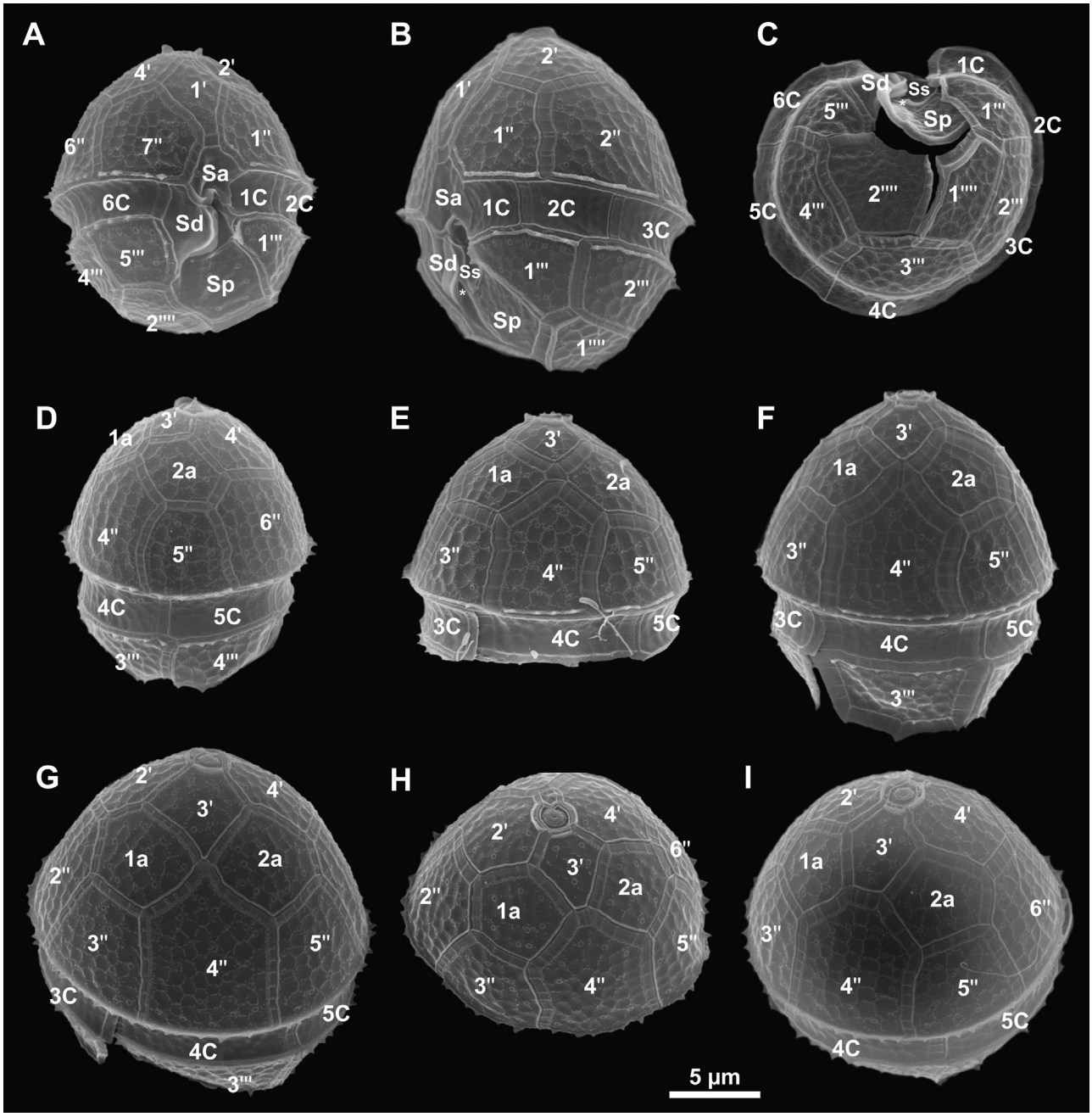

Figure 10. Thecae of *Parvodinium mixtum* var. *mixtum*, var. nov., showing the plate tabulation pattern (GeoM*720; SEM; all at the same scale). **A**. ventral view. **B**. lateral view (asterisk indicates the sulcal plate Sm). **C**. antapical view (asterisk indicates the sulcal plate Sm). **D–I**. dorsal view on epitheca showing the presence of all three tabulation types. **D–E**. <*remotum*> tabulation type. **F**. <*contactum*> tabulation type. **G–I**. <*conjunctum*> tabulation type. Abbreviations: n′: apical plate. n″: precingular plate. n‴: postcingular plate. n⁗: antapical plate. na: anterior intercalary plate. nC: cingular plate. Sa: anterior sulcal plate. Sd: right sulcal plate. Sm: median sulcal plate. Sp: posterior sulcal plate. Ss: left sulcal plate.

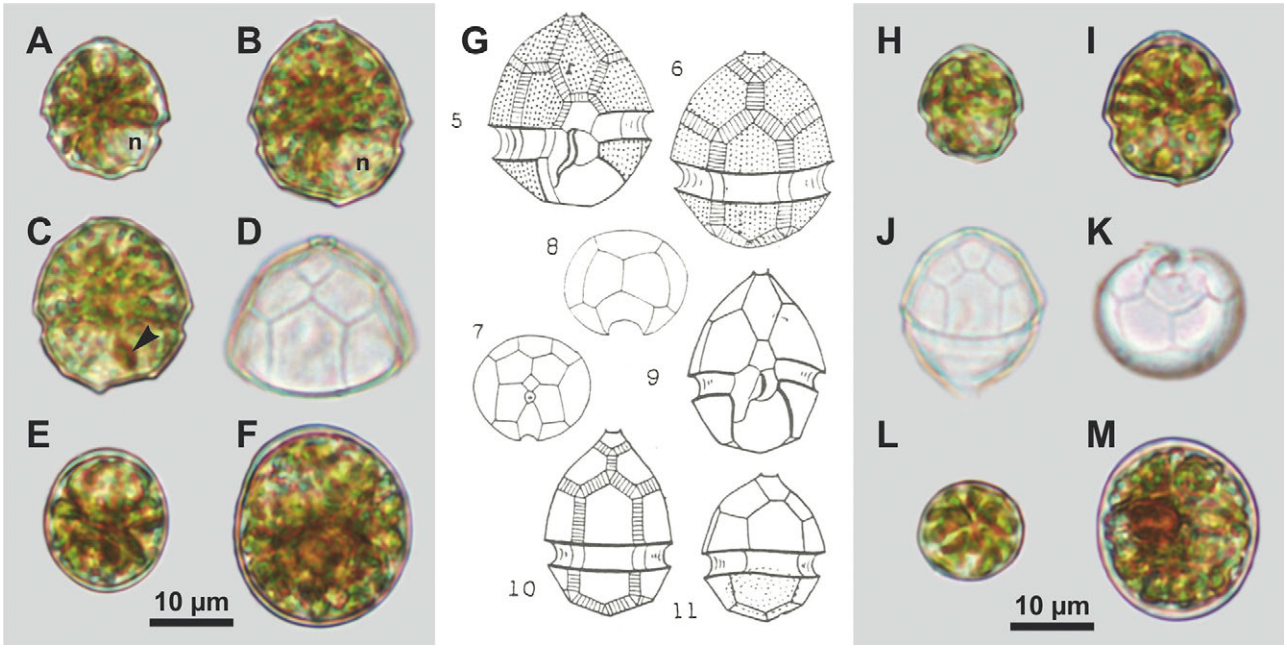

Figure 11. Varieties of *Peridinium mixtum*, sp. nov. (**A**–**F**, **H**–**M**; LM; all at the same scale). **A–F**. *Parvodinium mixtum* var. *remotum*, var. nov. (GeoM*717). **A–C**. motile thecate cells. **A–B**. ventral view. **C**. dorsal view (black arrowhead indicates the red eyespot). **D**. empty epitheca showing the <*remotum*> tabulation type. **E–F**. immotile coccoid cells. **G**. original drawings reproduced from Wołoszyńska (1952: pl. XII) showing *Peridinium mixtum* tab. *remotum* (pl. XII 5–10) and *Peridinium mixtum* tab. *conjunctum* (pl. XII 11). Figures 6 and 11 correspond to the holotypes of *Peridinium mixtum* var. *remotum*, var. nov., and *Peridinium mixtum* var. *conjunctum*, var. nov., respectively (accessed from https://pbsociety.org.pl/journals/index.php/asbp/article/view/asbp.1952.020/6455). **H–M**. *Parvodinium mixtum* var. *conjunctum*, var. nov. (GeoM*711). **H–I**. motile thecate cells in ventral view. **J–K**. empty thecae. **J**. dorsal view showing the <*conjunctum*> tabulation type. **K**. antapical view. **L–M**. immotile coccoid cells showing variation in size and shape. Abbreviation: n: nucleus.

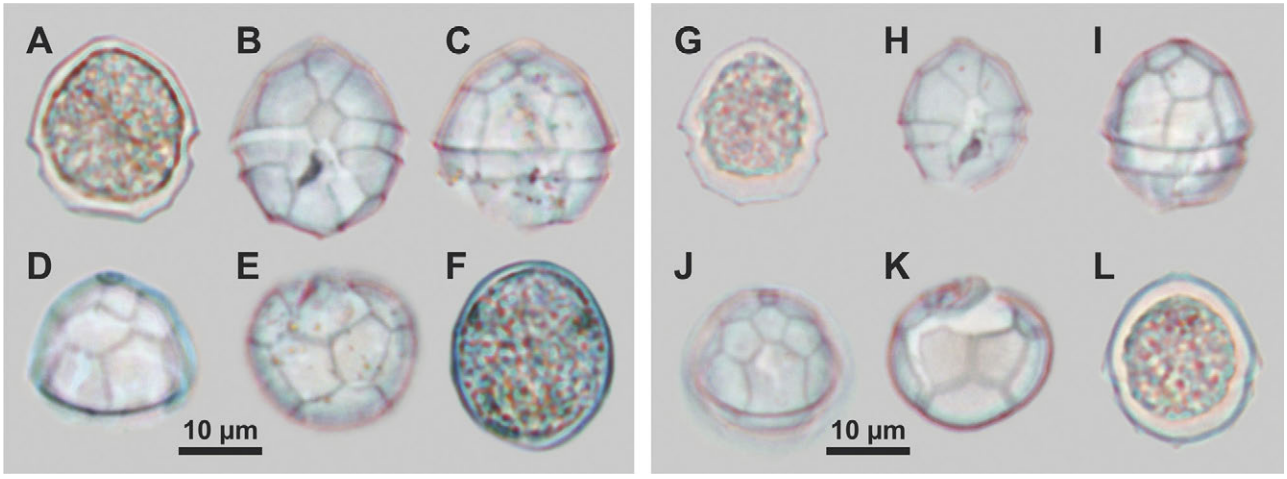

Figure 12. Motile and immotile stages (stained with astra blue and eosin) prepared as the epitype of *Peridinium mixtum* var. *remotum*, var. nov. (**A–F**; GeoM*717; LM) and *Peridinium mixtum* var. *conjunctum*, var. nov. (**G–L**; GeoM*711; LM; all at the same scale). **A**. motile thecate cell. **B**–**E**. empty thecae. **B**. ventral view. **C–D**. dorsal view showing the <*remotum*> tabulation type. **E**. antapical view. **F**. immotile coccoid cell. **G**. motile thecate cell. H–K. empty thecae. **H**. ventral view. **I**. dorsal-lateral view showing the <*conjunctum*> tabulation type. **J**. dorsal view showing the <*conjunctum*> tabulation type. **K**. antapical view. **L**. immotile coccoid cell with theca remnant.

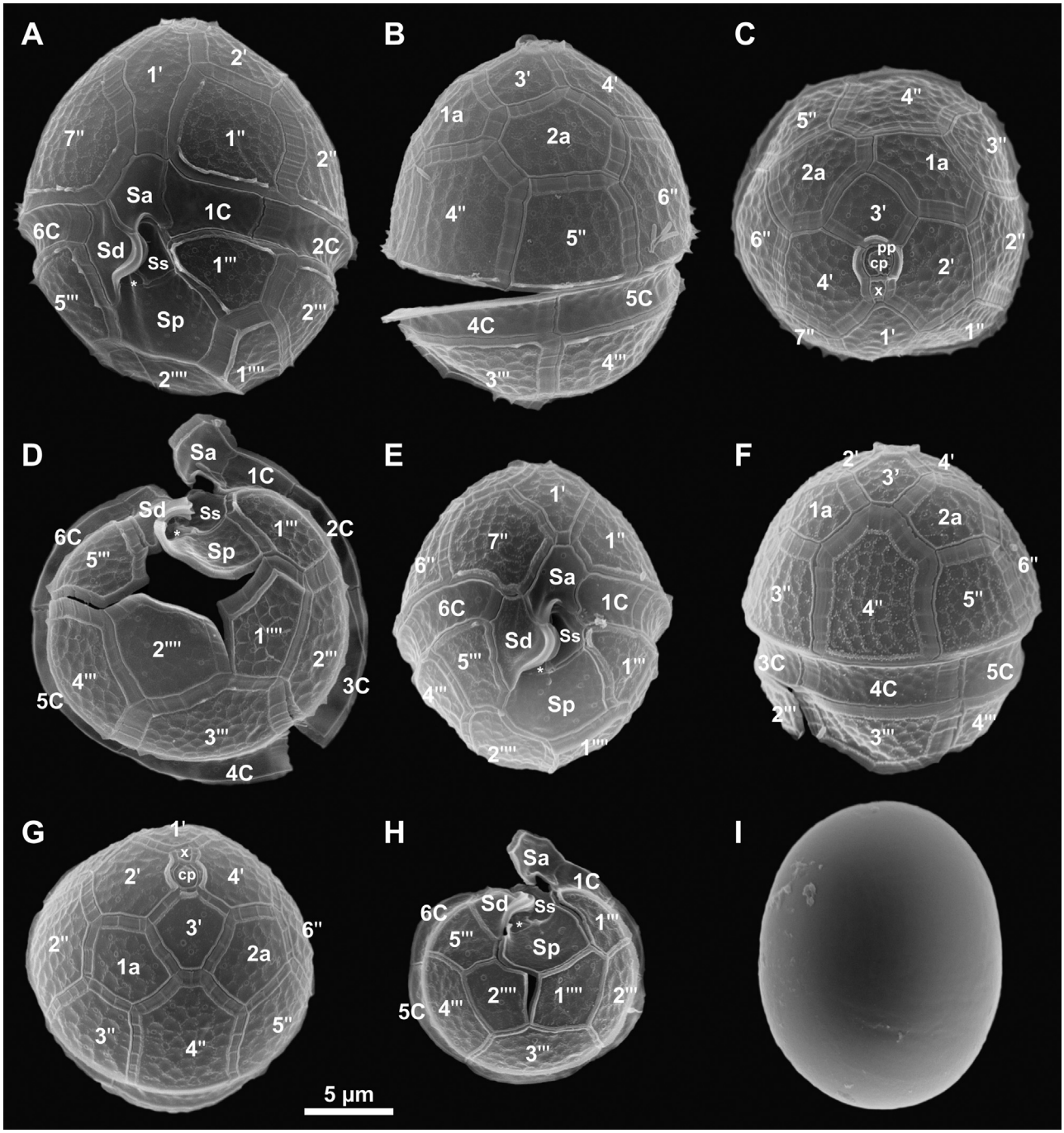

Figure 13. Motile and immotile stages of *Parvodinium mixtum* var. *remotum*, var. nov. (**A–D**; GeoM*717; SEM) and *Parvodinium mixtum* var. *conjunctum*, var. nov. (**E–I**; GeoM*711; SEM; all at the same scale). **A–H**. thecae (asterisks indicate the sulcal plate Sm). **A**. ventral view. **B**. dorsal-lateral view showing the <*remotum*> tabulation type. **C**. apical view showing the <*remotum*> tabulation type with an unusual plate overlapping of the two anterior intercalary plates. **D**. antapical view. **E**. ventral view. **F–G**. dorsal view showing the <*conjunctum*> tabulation type. **H**. antanpical view. **I**. immotile coccoid cell showing a smooth surface. Abbreviations: cp: closing plate. n′: apical plate. n″: precingular plate. n‴: postcingular plate. n⁗: antapical plate. na: anterior intercalary plate. nC: cingular plate. pp: pore plate. Sa: anterior sulcal plate. Sd: right sulcal plate. Sm: median sulcal plate. Sp: posterior sulcal plate. Ss: left sulcal plate. x: canal (preapical) plate.

cular apical pore plate, a cover plate and a canal (or X or preapical) plate (Fig. 13C). The cingulum was composed of six plates of different sizes. The sulcus consisted of five plates, where the plates Sm and Ss were small and partially covered by the large plate Sd. The thickened, left edge of the Sd plate, and the posterior end of the Sa plate, extended towards the middle of the sulcus and covered the flagellar pores. The Sp plate was relatively large and reached the antapex. The arrangement of the hypothecal plates was nearly symmetrical and composed of five postcingular plates of similar size and two antapical plates, where the antapical Plate 2′′′′ was slightly larger than the Plate 1′′′′.

Along the boundaries of the thecal plates, an overlap of adjacent plates was visible (Figs 10, 13A–H, 14I–L). Generally, it followed an imbricate pattern from dorsal towards ventral site: In the epitheca, the dorsal precingular Plate 4′′ was the keystone plate, as it was Plate 4C in the cingulum plate series. The keystone plate of the hypotheca was postcingular Plate 3′′′. Motile cells having a <*remotum*> tabulation type mostly showed an overlap of the intercalary Plate 1a over Plate 2a (Fig. 13B) but occasionally, Plate 1a was overlapped by Plate 2a (Fig. 13C).

Numerous empty thecae were observed either at the bottom of the cultivation plates or floating in the medium indicating cell division by eleutheroschisis. Dividing or ecdysing cells exited thecate cells through an opening on the hypotheca (Figs 9C, 12C). Single coccoid cells developed intrathecately and were released after shedding of the theca (Figs 8I, 12L). Coccoid cells were coloured golden-brown through yellow-hyaline and were mostly widely ovoid in shape (Figs 8G–I, 11E–F, L–M, 12F, L, 13I). The size was variable and ranged from 13–22 μm in length (GeoM*720; mean: 18 μm; median: 18 μm; SD: 2 μm; n = 50), 12–21 μm in width (GeoM*720; mean: 16 μm; median: 16 μm; SD: 2 μm; n = 50) and had a smooth surface (Fig. 13I). The cytoplasm of the coccoid cells was filled with numerous, brown granules and frequently contained a large, red accumulation body (Figs 8G–I, 11E–F, L–M).

Morphology of *Parvodinium* cf. *umbonatum*

The strains GeoM*791, GeoM*792, GeoM*795, GeoM*796 and GeoM*797 (all collected at Toporowy Staw Niżni), grew especially well in WC medium at 18 °C and were morphologically indistinguishable. They exhibited motile thecate cells (Fig. 15A–B, D–E) as well as immotile coccoid cells (Fig. 15C), but the motile cells were predominant. The thecate cells were yellow through golden-brown in colour and frequently contained a large, red ccumulation body in the epitheca. The overall shape was ovoid and the epitheca was larger than the hypotheca (Fig. 15A–B). The epitheca was broadly rounded in outline with a slightly obtuse apex at the position of the apical pore, which was slightly displaced to the left lateral cell side. The hypotheca was semi-circular through trapezoidal in outline and was more narrow than the epitheca. The size of the motile cells ranged from 21–30 μm (GeoM*795; mean: 26 μm; median: 26 μm; sd: 2 μm; n = 50) in length and from 17–25 μm (GeoM*795; mean: 21 μm; median: 21 μm; sd: 2 μm; n = 50) in width. The cingulum was excavated, and surrounded the cell with a descendent displacement comprising of approximately one third of its own width (Fig. 15D).

The motile cells were covered by a theca, with irregularly scattered small circular pores (probably openings of trichocysts) on the plate surface. The thecate plate formula was pp, cp, x, 4′, 2a, 7′′, 6c, 5s, 5′′′, 2′′′′ (Fig. 15D–E). The arrangement of the epithecal plates was more or less symmetric and within cultivated strains, all three different tabulation types were found. Numerous empty thecae indicated a cell division by eleutheroschisis. Coccoid cells were reddish-brown through dark brown in colour, ovoid through spherical in shape (Fig. 15C) and variable in size ranging from 21–38 μm in length (GeoM*795; mean: 29 μm; median: 29 μm; SD: 3 μm; n = 50), 20–35 μm in width (GeoM*795; mean: 27 μm; median: 26 μm; SD: 4 μm; n = 50) and had a smooth surface.

Molecular diversity within *Parvodinium*

In total, sequences were generated and deposited as 37 new GenBank entries in the course of the study (Supplementary Material Table S1). The SSU + ITS + LSU alignment was 1,796 + 696 + 2,460 bp long and comprised 97 + 346 + 274 parsimony informative sites (14%, mean of 10.10 per terminal taxon) as well as 1,521 distinct alignment patterns. Figure 16

shows the best-scoring Maximum Likelihood (ML) tree (−ln = 19,175.85) recovering the Peridiniopsidaceae as monophyletic (100LBS, 1.00BPP), including *Palatinus* (100LBS, 1.00BPP), *Parvo-*

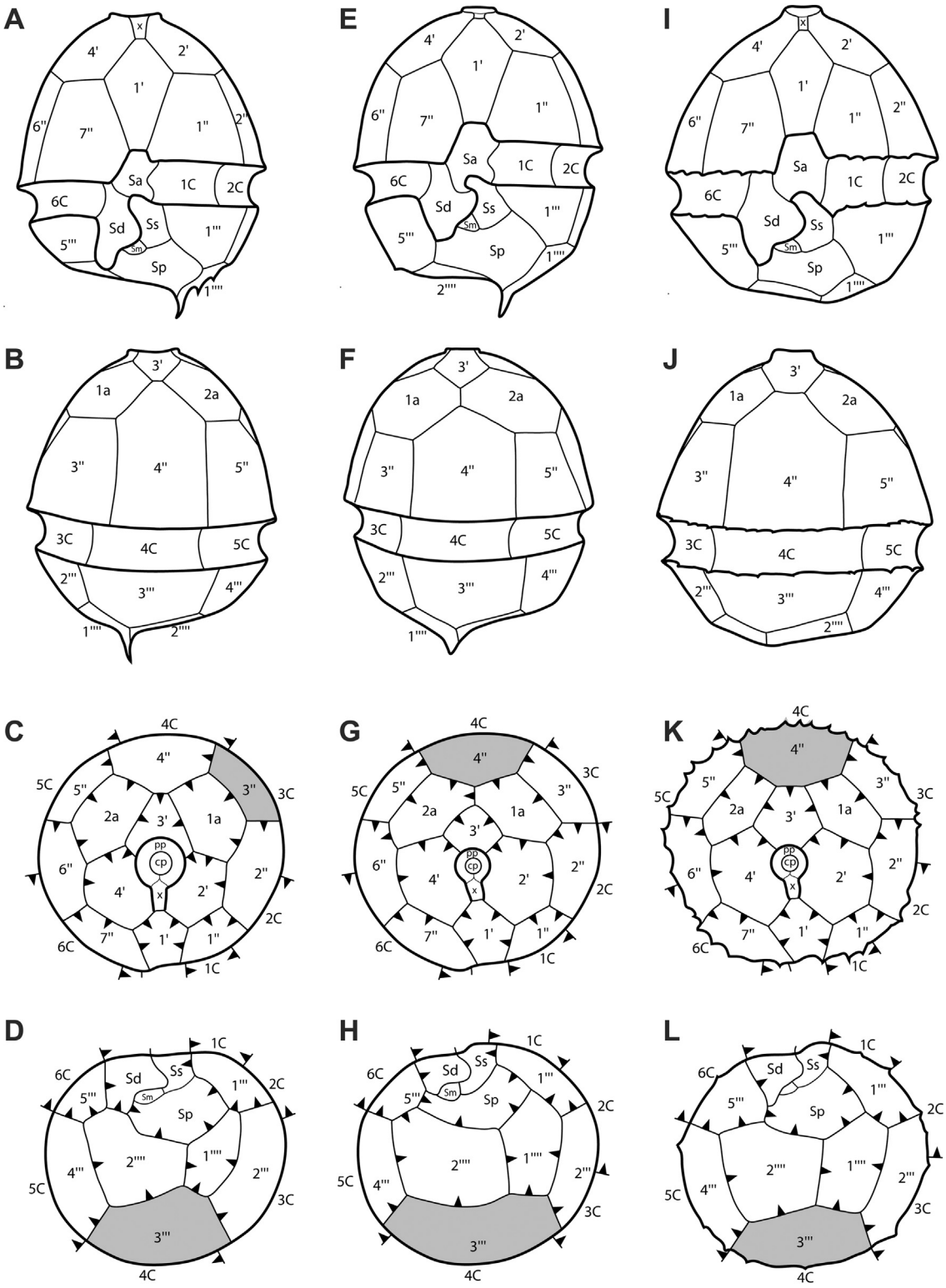

Figure 14. Schematic drawings of the thecal plate pattern. **A–D**. *Parvodinium marciniakii*, sp. nov. **A**. ventral view. **B**. dorsal view. **C**. apical view. **D**. antapical view. **E–H**. *Parvodinium trawinskii*, sp. nov. **E**. ventral view. **F**. dorsal view. **G**. apical view. **H**. antapical view. **I–L**. *Parvodinium mixtum*, sp. nov. **I**. ventral view. **J**. dorsal view. **K**. apical view (note that the <*conjunctum*> tabulation type is depicted, but the <*contactum*> and the <*remotum*>

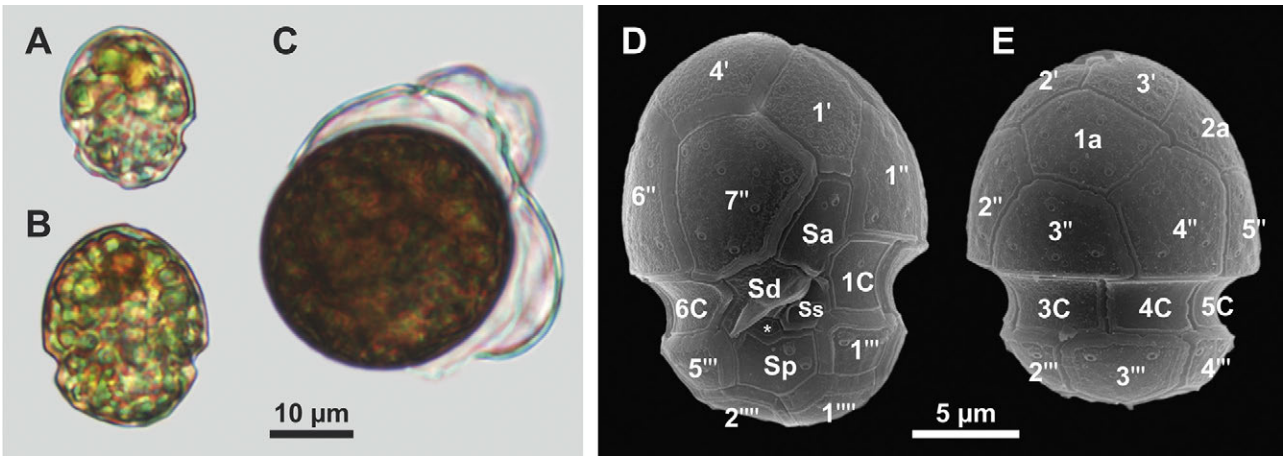

Figure 15. Motile thecate and immotile cells of *Parvodinium* cf. *umbonatum* (GeoM*795; **A–C**: LM; all at the same scale; **D–E**: SEM; all at the same scale). **A–B**. motile thecate cells. **C**. immotile coccoid cell. **D**. motile thecate cell in ventral view. **E**. motile thecate cell in dorsal view.

dinium (100LBS, .91BPP) and *Peridiniopsis* (single accession). Furthermore, *Palatinus* and *Parvodinium* also appeared to be closely related (92LBS, .92BPP).

Strains established in this study from five different lakes in the Polish Tatra Mountains were placed in four distinct lineages of *Parvodinium*, each of them showing low genetic variability (without any correlation to predominant tabulation types). Three of such lineages, namely *P. marciniakii*, sp. nov. (100LBS, 1.00BPP), *P. trawinskii*, sp. nov. (100LBS, 1.00BPP) and *P. mixtum*, sp. nov. (97LBS, 1.00BPP), were inferred from sequences unknown to science until the present study, while the strains collected at Toporowy Staw Niżni constituted a clade with accessions assigned to, and with the morphology of, *P. umbonatum* (100LBS, 1.00BPP). In the molecular phylogeny, *P. marciniakii*, sp. nov., and *P. trawinskii*, sp. nov., were the only lineages of *Parvodinium* exhibiting distinct antapical spines, but were only distantly related in the molecular tree. There was some size overlap between species of *Parvodinium*, but cells from *Parvodinium* excluding *P. centenniale* and *P. umbonatum* were significantly smaller than those of the other Peridiniopsidaceae ($p \leq 0.001$; $n_1 = 300$, $n_2 = 50$).

Discussion

Dinophyte Diversity in the Tatra Mountains

A hot spot is circumscribed as a biogeographic region with significant levels of biodiversity that is threatened with destruction (Mittermeier et al. 2005). The Tatra Mountains, with their numerous bodies of water exhibiting a great variety of ecological licences, may fulfil such criteria for cold-adapted freshwater organisms. A considerable number of dinophytes have been described from there, many of which are unknown from outside the mountain range (Kretschmann et al. 2015b; Wołoszyńska 1916, 1919, 1935, 1936). Larger parts of the mountains are under effective natural protection; however, severe threat is presented last but not least by the ongoing global warming (particularly if the organisms are adapted to cold water habitats). That this Central European ecosystem (at least in the microbiome sense) remains imperfectly known at present is also illustrated by our descriptions of not less than three dinophyte species new to science, collected only within two days in autumn of 2015.

Some of the new species are not documented from a single, but several lakes in the Tatra Moun-

types also occur). **L**. antapical view. Abbreviations: cp: closing plate. n′: apical plate. n″: precingular plate. n‴: postcingular plate. n⁗: antapical plate. na: anterior intercalary plate. nC: cingular plate. pp: pore plate. Sa: anterior sulcal plate. Sd: right sulcal plate. Sm: median sulcal plate. Sp: posterior sulcal plate. Ss: left sulcal plate. x: canal (preapical) plate. Arrowheads in **C–D**, **G–H** and **K–L** indicate plate overlap pattern. Keystone plates of the pre- and postcingular plate series are shaded in grey.

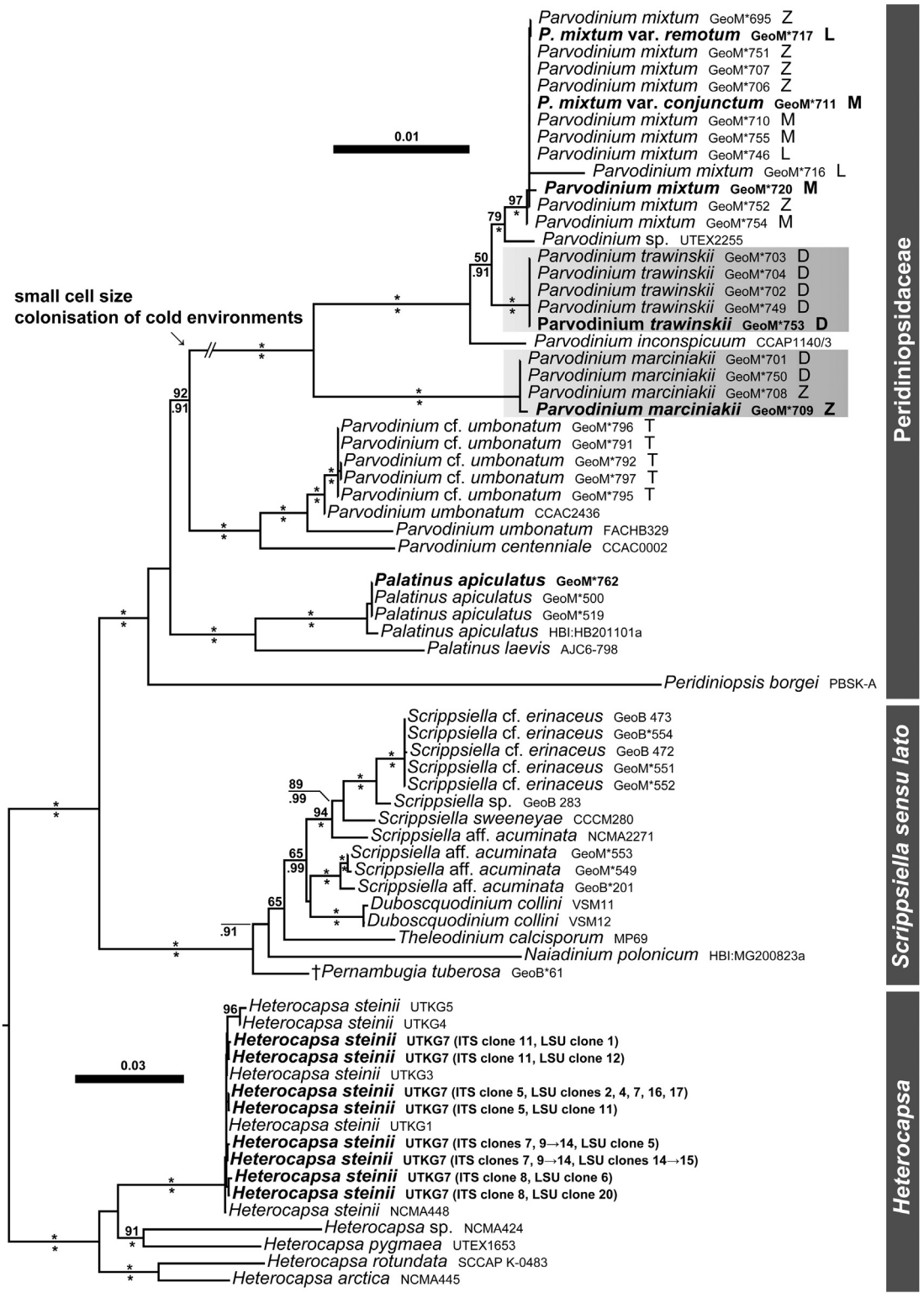

Figure 16. Maximum Likelihood (ML) tree (−ln = 19175.85) of 71 peridinialean operational taxonomic units (OTUs) under the GTR + Γ substitution model. Typified OTUs are highlighted in bold, and branch lengths are drawn to scale, with the scale bar indicating the number of nucleotide substitutions per site. The numbers on the branches are statistical support values (above: ML bootstrap values, values <50 are not shown; below:

tains (Supplementary Material Table S1). Thus, several similar species, presumably with similar ecological requirements, can be found at the same locality, which challenges explanations for their evolutionary origin (being supposedly not allopatric). As likely endemics, all the new taxa are hitherto exclusively known from lakes in the Tatra Mountains. Their geographical origin remains unknown as well, as most Tatra lakes are younger than the last glacial period. If the sampled lakes are all younger than 10,000 years (Klimaszewski in Mirek 1996; Łajczak in Mirek 1996), then the inhabiting species must have originated somewhere else. Therefore, the extant distribution must be a result of dispersal caused by, for example, water connections (Wit-Jóźwik 1974; Wit-Jóźwik and Ziemońska in Trafas 1985) or birds (Cichocki 2015), which can play the role of vectors (Padisák 2009) when flying from valley to valley (and concomitantly from lake to lake). The importance of Tatra lakes having karstic origin (Łajczak in Mirek 1996) and/or ice-free valleys (Marks 2004; Makos et al. 2013) as possible refugia for survival during glaciation remains to be worked out in future research.

Interpretations of the Molecular Tree

The general morphology of the motile cells (including the thecal plate pattern), as well as molecular phylogenetics of all Tatra strains under investigation, confirm their correct systematic placement to the Peridiniopsidaceae, and more specifically to *Parvodinium*. Two intercalary plates as well as six cingular plates are both found in all of our morphologically investigated strains. Within Peridiniopsidaceae, *Parvodinium* includes the smallest dinophytes known so far. However, cells of strains assigned to *P. centenniale* and *P. umbonatum* (i.e., ours as well as CCAC strains: Supplementary Material Tabel S1, *pers. comm.* B. Melkonian; FACHB238: Zhang et al. 2011) are significantly larger than those of our three new species and *P. inconspicuum* (Tardio et al. 2009). Size is not necessarily indicative for relationships in the dinophytes, but the small cell size in this latter subset of *Parvodinium* can be considered apomorphic. To the contrary, species of *Parvodinium* exhibiting antapical protuberance do not constitute a monophyletic group in the molecular tree. Presence or absence of such trait thus appears diagnostic to delimit species but is of limited value for phylogenetic inference, as it is also the case within, for example, the Amphidomataceae (Tillmann et al. 2014). The small cell size in the subset of *Parvodinium* may correlate with the ecological preference for cold habitats (Fig. 16). The precise collection circumstances of the strains UTEX2255 (locality unknown) and CCAP1140/3 (from Northern Germany; Supplementary Material Table S1) are unknown, but all the other representatives of the group have been collected from water, whose temperature does not exceed 10 °C, and our strains grow best at 12 °C.

Species delimitation in *Parvodinium* is challenging (Carty 2008; Popovský and Pfiester 1986). Anyhow, many taxa have already been described, frequently without clear documentation of diagnostic traits. Moreover, a considerable number of names are crosswise transferred between taxa, eventually pending on individual concepts and/or interpretations and occasionally violating information from the protologue. Overall, the species under investigation here are very similar, but *P. marciniakii*, sp. nov., and *P. trawinskii*, sp. nov., (both exhibiting an antapical spine) are clearly separated because of such a distinctive and persisting trait. Plate overlap patterns are considered conserved at higher taxonomic levels (Elbrächter and Meyer 2001; Netzel and Dürr 1984; Tillmann and Elbrächter 2010), and this is – to the best of our knowledge – the first time that two different keystone plates are diagnostic to distinguish taxa at the species level (Fig. 14). Before, an inverted plate ('flip-flop') overlap between the cingular plates C3 and C4, and thus a rare case of intra-specific variability in this respect, has been documented in *Heterocapsa steinii* Tillmann, Gottschling, Hoppenrath, Kusber & Elbr. only (Tillmann et al. 2017). Furthermore, *Parvodinium marciniakii*, sp. nov., is different in the separation of Plates 5‴ and Sp, which are adjacent in *P. mixtum*, sp. nov., and *P. trawinskii*, sp. nov. (Fig. 14).

Variability of the Epithecal Conformation

Regarding the conformation of the two epithecal Plates 3′ and 4″, previous authors have distinguished tabulation types such as <*conjunc-*

Bayesian posterior probabilities, values <.90 are not shown; asterisks indicate maximal support). Accessions exhibiting antapical protuberances are highlighted in grey boxes, and the node with the apomorphic reduction of cell size is also indicated. Abbreviations: D: Długi Staw Gąsienicowy. M: Morskie Oko. T: Toporowy Staw Niżni. L: Litworowy Staw Gąsienicowy. Z: Zielony Staw Gąsienicowy.

tum>, <*contactum*> and <*remotum*> (Lefèvre 1932; Lindemann 1918a,b; Fig. 1). It is worth mentioning that none of these nor any of the consecutive authors (Carty 2008; Elbrächter and Meyer 2001) have considered this trait significant in order to distinguish species. We confirm this appraisal by showing that all forms can be present in the same (monoclonal) strain (not only of *P. mixtum*, sp. nov., but also of *P.* cf. *umbonatum*). Such observations are indicative for an affiliation with the same isolated reproductive unit and thus the species status, respectively. It is tempting to speculate whether the three different phenotypes correspond with an expression of dominant/recessive inheritance with incomplete dominance (Mendel 1866). To the best of our knowledge, this would be the first mention of such a connection in the dinophytes.

Several taxa of *Parvodinium* including "*P.*" *tatricum* (Wołoszyńska 1916) are documented from the Polish Tatra Mountains. However, this species is significantly larger (i.e., longer than 35 μm) than any of the organisms investigated in the present study. After Jadwiga Wołoszyńska's death in 1951, a number of further *Parvodinium* taxa from the Tatra Mountains have been introduced (Wołoszyńska 1952), but they all lack a description and are therefore not validly published. However, their taxonomic concepts are clear from J. Wołoszyńska's excellent drawings exhibiting minute details (Fig. 11G), and several of our strains can be reliably assigned to the names, introduced in her publications. In *P. mixtum*, sp. nov., for example, she was aware of different conformations of the epitheca, which is expressed in the proposed epithets, <*conjunctum*> and <*remotum*>. In some of our strains, one of the two types is predominant (e.g., <*conjunctum*> in GeoM*711, <*remotum*> in GeoM*717) and today, we can use this distinction for the validation of the two varieties (in the sense of inventorying distinct phenotypes present in a population) based on J. Wołoszyńska's observation more than half a century ago (see Taxonomic activity). For *P. mixtum* itself, no illustration is available and for typification of the taxon, we used a strain exhibiting both types to an equal amount (see Taxonomic activity).

Delimitations of the New Species from the Historical Names (Table 3; Supplementary Material Figs S1–S2)

Many Peridiniopsidaceae show a variously spinulose hypotheca (Carty 2008; Craveiro et al. 2009; Kretschmann et al. 2018; Popovský and Pfiester 1986), and also *Parvodinium* includes representatives with one (or only occasionally with more) distinct, spine-like protuberance(s). *Parvodinium marciniakii*, sp. nov., and *P. trawinskii*, sp. nov., differ from other 'armed' *Parvodinium* taxa based on the smaller size [larger in *Parvodinium deflandrei* (M.Lefèvre) Carty and "*P.*" *tatricum*] and the wider (instead of the slender shape as in "*P.*" *tatricum*) and rounder shapes in outline [instead of the pentagonal shapes of *Parvodinium africanum* (Lemmerm.) Carty, *P. deflandrei* and "*Peridinium*" *marchicum* Lemmerm.]; these two new taxa further have a more regularly formed epitheca [not as conical as in *P. africanum*, *P. deflandrei, Parvodinium goslaviense* (Wołosz) Carty, "*P.*" *marchicum*, "*Peridinium*" *munusculum* Er. Lindem. and "*P.*" *tatricum*], possess antapical plates of similar size (and not unequal size as in *P. africanum* and "*P.*" *marchicum*), and the cingulum is in a sub-median position (and not median as in *P. africanum*, "*P.*" *marchicum*, "*P.*" *munusculum* and "*P.*" *tatricum*); *Parvodinium marciniakii*, sp. nov., and *P. trawinskii*, sp. nov., show only a single protuberance (instead of two or three spines as in *P. deflandrei*, *P. inconspicuum*, "*P.*" *marchicum*, "*P.*" *munusculum* and "*P.*" *tatricum*), and they perform photosynthesis [instead of being heterotrophic as *P. goslaviense*; Lefèvre 1927; Lemmermann 1899, 1910; Lemmermann in West 1907; Lindemann 1918a,b; Wołoszyńska 1916].

Parvodinium mixtum, sp. nov. (including the new/old varieties), does not have any antapical protuberance and differs from other such species of *Parvodinium* based on its smaller size [larger in *Parvodinium centenniale* (Playfair) Carty, "*Peridinium*" *dzieduszyckii* Wołosz., "*Peridinium*" *linzium* Er.Lindem., *Parvodinium lubieniense* (Wołosz.) Carty and *Parvodinium morzinense* (M.Lefèvre) Carty] and its ovate shape in outline (neither circular as in *P. centenniale* nor heptagonal as in "*Peridinium*" *minimum* A.J.Schill.); it is also wider (and not as slender as in *Parvodinium belizense* Carty and "*Peridinium*" *orrei* Huitf.-Kaas), has a more regularly shaped epitheca (neither as conical as in *P. belizense*, "*P.*" *dzieduszyckii*, *P. lubieniense,* "*P.*" *minimum* and "*P.*" *orrei* nor as dome-shaped as in *P. umbonatum*) and has a wider cingulum (narrower in *P. centenniale* and *P. umbonatum*) in a sub-median position (not in a median position as in "*P.*" *dzieduszyckii*, "*P.*" *linzium* and *P. lubieniense*); it possess antapical plates of similar size (and not unequal size as in "*P.*" *dzieduszyckii* and *P. belizense*) and has straight sutures (instead of the arcuate sutures in *P. morzinense*; Carty and Wujek 2003; Huitfeldt-Kaas 1906; Lefèvre 1925; Lemmermann 1910; Lindemann 1918b; Playfair 1920; Schilling 1891; Stein 1883; Wołoszyńska

1916). "*Glenodinium*" *pusillum* Penard is also considered to be a part of *Parvodinium* (otherwise having consistently two intercalary plates: Carty 2008; Gottschling et al. 2017), but there is no intercalary plate depicted in the figures provided in the protologue (Penard, 1891). This allows doubts upon the correct assignment of this species to *Parvodinium*, but it still separates "*G.*" *pusillum* from *P. mixtum*, sp. nov.

Conclusion

Correlation between morphology and molecular phylogenetics is present and allows for taxonomic delimitation. However, many species of *Parvodinium* are characterised by a distinctive and unique combination of traits rather than a single autapomorphy (Table 3). In terms of DNA sequence data, variability within species of *Parvodinium* appears rather low, contrasting the intraspecific divergence observed in *Peridinium cinctum* (O.F.Müll.) Ehrenb. (Izquierdo López et al. in press) or *Alexandrium ostenfeldii* (Paulsen) Balech & Tangen (Kremp et al. 2014). The unexpected diversity of *Parvodinium* found in remote places in the Tatra Mountains may result from recent radiations as it has been shown in *Apocalathium* Craveiro, Daugbjerg, Moestrup & Calado (Annenkova et al. 2015) or *Nusuttodinium* Y.Takano & T.Horig. (Onuma et al. 2015; Takano et al. 2014) from other parts of the world. In any case, the biodiversity assessment for organisms from the Tatra Mountains is not completed yet, and it is only a matter of time until many more species from this remote region are going to be discovered.

Taxonomic Activity

1. *Parvodinium marciniakii* Kretschmann, Owsianny, Zerdoner & Gottschling, sp. nov.—TYPE [slide with non-fossil specimens]: Poland. Lesser Poland, Tatra, Zielony Staw Gąsienicowy, 22 Sep 2015: P.M. Owsianny, G. Marciniak & K. Trawiński PL021 [J. Kretschmann GeoM*709] (**holotype, designated here**: CEDiT-2018H79!, **isotypes, designated here**: B 40 0042053! M-0289940!) [http://phycobank.org/100118].

Description: Dinophytes small, phototrophic, thecate, the thecal plate pattern obscure and only diffusely seen in light microscopy. Cells 18–24 μm long, 15–21 μm wide, widely through very widely ovoid, with a characteristic, short, spine-like protu-

Table 1. Major geographical, morphological and habitat characteristics of the investigated localities in the Tatra Mountains.

lake name	geographic coordinates[4]	altitude [m a.s.l.][1]	lake volume [10^3 m^3]	lake area [ha]	max depth [m]	temperature [°C][4]	pH[4]	conductivity [μS cm^{-1}][4]
Długi Staw Gąsienicowy	49°13′39.0′′N, 20°00′31.5′′E	1784	81[3]	1.59[1]	10.6[1]	6.75	6.35	18.8
Zielony Staw Gąsienicowy	49°13′44.3′′N, 20°00′1.1′′E	1672	261[3]	3.84[1]	15.1[1]	7.88	6.83	25.2
Litworowy Staw Gąsienicowy	49°14′0.5′′N, 19°59′47.7′′E	1618	2.72[3]	0.40[3]	1.1[3]	7.58	6.85	19.5
Morskie Oko	49°12′3.6′′N, 20°04′15.9′′E	1395	9904.3[2]	33.39[2]	51.8[2]	8.49	7.12	31.5
Toporowy Staw Niżni	49°16′59.5′′N, 20°01′51.0′′E	1089	11.7[3]	0.62[1]	5.7[1]	9.45	5.75	18.0

[1]Kopáček et al. (2006).
[2]Choiński and Strzelczak (2011).
[3]data from the Tatra National Park.
[4]field data.

Table 2. Phenotypic variability in the type material of Tatra *Parvodinium* species. The predominant phenotype is highlighted in bold.

	Strain No.	<*conjunctum*> tabulation	<*contactum*> tabulation	<*remotum*> tabulation	
Parvodinium marciniakii, sp. nov.	GeoM*709	**86.7%**	2.1%	11.2%	n = 196
Parvodinium trawinskii, sp. nov.	GeoM*749	**98.0**%	1.6%	0.4%	n = 245
	GeoM*753	**99.6**%	0.2%	0.2%	n = 524
Parvodinium mixtum, sp. nov.	GeoM*720	**47.2%**	6.4%	46.4%	n = 551
	GeoM*706	29.6%	5.3%	65.1%	n = 524
P. mixtum var. *remotum*, var. nov.	GeoM*717	**18.6%**	3.1%	**78.3%**	n = 603
P. mixtum var. *conjunctum*, var. nov.	GeoM*711	**85.4%**	3.0%	11.6%	n = 562
	GeoM*695	**88.8%**	3.7%	7.5%	n = 510

berance of Plate 1‴. Tabulation formula: APC (pp, cp, x), 4′, 2a, 7″, 6c, 5s, 5‴, 2⁗; the Plates 3′ and 4″ predominantly adjacent; the plates Sp and 5‴ not adjacent; the epithecal keystone plate: 3″.

Note: A detailed description for the strain, from which type material was prepared, is provided in the Results section and a diagnosis in the Discussion section.

Etymology: The epithet honours our friend and member of our research team, Grzegorz Marciniak, acknowledging his long-term and enthusiastic support of field work in the Tatra Mountains. Grzegorz Marciniak is likewise one of the collectors of the new species.

2. *Parvodinium trawinskii* Kretschmann, Owsianny, Zerdoner & Gottschling, sp. nov.—TYPE [slide with non-fossil specimens]: Poland. Lesser Poland, Tatra, Długi Staw Gąsienicowy, 22 Sep 2015: P.M. Owsianny, K. Trawiński & G. Marciniak PL019 [J. Kretschmann GeoM*753] (**holotype, designated here**: CEDiT-2018H80!, **isotypes, designated here**: B 40 0042054! M-0289941!) [http://phycobank.org/100119].

Description: Dinophytes small, phototrophic, thecate, the thecal plate pattern distinct. Cells 21–26 μm long, 18–23 μm wide, ellipsoid, with flattened hypotheca and a characteristic, short, spine-like protuberance of Plate 1‴. Tabulation formula: APC (pp, cp, x), 4′, 2a, 7″, 6c, 5s, 5‴, 2⁗; the Plates 3′ and 4″ predominantly separated; the plates Sp and 5‴ adjacent; the epithecal keystone plate: 4″.

Note: A detailed description for the strain, from which type material was prepared, is provided in the Results section and a diagnosis in the Discussion section.

Etymology: The epithet honours our friend and member of our research team, Krzysztof Trawiński, acknowledging his long-term and enthusiastic support of field work in the Tatra Mountains. Krzysztof Trawiński is likewise one of the collectors of the new species.

3. *Parvodinium mixtum* Wołosz. ex Kretschmann, Owsianny, Zerdoner & Gottschling, sp. nov. *Peridinium mixtum* Wołosz., not validly published (ICN Art. 38.1.), Acta Societatis Botanicorum Poloniae 21: 315. 1952.—TYPE [slide with non-fossil specimens]: Poland. Lesser Poland, Tatra, Litworowy Staw Gąsienicowy, 22 Sep 2015: P.M. Owsianny PL018 [J. Kretschmann GeoM*720] (**holotype, designated here**: CEDiT-2018H81!, **isotypes, designated here**: B 40 0042055! M-0289942!) [http://phycobank.org/100120]. Note that no original material (particularly no illustration) can be associated with this name.

Description: Dinophytes small, phototrophic, thecate, the thecal plate pattern distinct. Cells 14–23 μm long, 12–21 μm wide, very widely ovoid, the antapex without protuberance. Tabulation formula: APC (pp, cp, x), 4′, 2a, 7″, 6c, 5s, 5‴, 2⁗; the Plates 3′ and 4″ adjacent or separated to similar amounts; the plates Sp and 5‴ adjacent; the epithecal keystone plate: 4″.

Note: A detailed description for the strain, from which type material was prepared, is provided in the Results section and a diagnosis in the Discussion section.

Additionally to *Parvodinium mixtum* var. *mixtum* (ICN Art. 26), two new varieties can be distinguished:

3a. *Parvodinium mixtum* var. *remotum* Wołosz. ex Kretschmann, Owsianny, Zerdoner &

Table 3. Morphological comparison between the new and formerly described taxa of *Parvodinium* (protologue illustrations and references are provided in the Supplemental online material). Note that the new taxa are characterised by a combination of traits rather than a single autapomorphy (traits, in which already described taxa differ from the new taxa, are in bold). Abbreviation: n.inf., no information.

	nutrition mode	number of spines	cell length [μm]	shape in outline	shape epitheca in outline	cingulum width	cingulum position	sutures	number of intercalary plates	size of antapical plate
Taxa with antapical protuberance(s)										
Parvodinium marciniakii, sp. nov.	phototrophic	1	18–24	ovate	semi-elliptical	regular	sub-median	straight	2	slightly varying
Parvodinium trawinskii, sp. nov.	phototrophic	1	21–26	ovate	semi-elliptical	regular	sub-median	straight	2	similar
Parvodinium africanum	phototrophic	1	24–30	**pentagonal**	**conical**	regular	**median**	straight	2	**unequal**
Parvodinium deflandrei	phototrophic	**2**	**28–35**	**pentagonal**	**conical**	regular	sub-median	straight	2	similar
Parvodinium goslaviense	**heterotrophic**	1	20–25	ovate	**conical**	regular	sub-median	straight	2	equal
Parvodinium inconspicuum	phototrophic	**3**	15	ovate	semi-elliptical	wide	sub-median	straight	n.inf.	n.inf.
"*Peridinium*" *marchicum*	phototrophic	**3**–	22	**pentagonal**	**conical**	regular	**median**	straight	2	**unequal**
"*Peridinium*" *munusculum*	phototrophic	**3**	18–26	**hexagonal**	**conical**	regular	**median**	straight	2	n.inf.
"*Peridinium*" *tatricum*	phototrophic	**2**–	**35–40**	**slender**	**conical**	regular	**median**	straight	2	similar
Taxa without antapical protuberance(s)										
Parvodinium mixtum, sp. nov.	phototrophic	0	14–23	ovate	semi-elliptical	regular	sub-median	straight	2	similar
Parvodinium belizense	phototrophic	0	12–16	**slender**	**conical**	wide	sub-median	straight	2	**unequal**
Parvodinium centenniale	phototrophic	0	**30–44**	**circular**	semi-circular	**conspicuously narrow**	sub-median	straight	2	equal
"*Peridinium*" *dzieduszyckii*	phototrophic	0	**34–40**	ovate	**conical**	regular	**median**	straight	2	**unequal**
"*Peridinium*" *linzium*	phototrophic	0	**32–36**	ovate	semi-circular	regular	**median**	straight	2	equal
Parvodinium lubieniense	phototrophic	0	**35–45**	ovate	**conical**	regular	**median**	straight	2	equal
"*Peridinium*" *minimum*	phototrophic	0	19	**heptagonal**	**concial**	narrow	sub-median	straight	2	equal
Parvodinium morzinense	phototrophic	0	**30–41**	ovate	semi-elliptical	regular	sub-median	**arcuate**	2	similar
"*Peridinium*" *orrei*	phototrophic	0	21–24	**slender**	**conical**	regular	sub-median	straight	n.inf.	n.inf.
"*Glenodinium*" *pusillum*	phototrophic	0	20	ovate	semi-elliptical	regular	sub-median	straight	**0**	n.inf.
Parvodinium umbonatum	phototrophic	0	n.inf.	ovate	**dome-shaped**	**conspicuously narrow**	sub-median	straight	2	n.inf.

Gottschling, var. nov. *Peridinium mixtum* tab. *remotum* Wołosz., not validly published (ICN Art. 38.1.), Acta Societatis Botanicorum Poloniae 21: 315, pl. XII 5–10. 1952.—TYPE [illustration]: Poland. Lesser Poland, Tatra, without exact locality, without date: J. Wołoszyńska s.n. (**holotype, designated here**: pl. XII 6 in Acta Societatis Botanicorum Poloniae 21. 1952) [http://phycobank.org/100121]; [slide with non-fossil specimens]: Poland. Lesser Poland, Tatra, Litworowy Staw Gąsienicowy, 22 Sep 2015: P.M. Owsianny PL018 [J. Kretschmann GeoM*717] (**epitype, designated here**: CEDiT-2018E82!, duplicates: B 40 0042056! M-0289943!) [http://phycobank.org/100122].

Description: Dinphytes small, phototrophic, thecate, the thecal plate pattern distinct. Cells 15–24 μm long, 19–24 μm wide, very widely ovoid, the antapex without protuberance. Tabulation formula: APC (pp, cp, x), 4′, 2a, 7″, 6c, 5s, 5‴, 2⁗; the Plates 3′ and 4″ predominantly separated; the plates Sp and 5‴ adjacent; the epithecal keystone plate: 4″.

Note: A diagnosis is provided in the Discussion section.

3b. *Parvodinium mixtum* var. *conjunctum* Wołosz. ex Kretschmann, Owsianny, Zerdoner & Gottschling, var. nov. *Peridinium mixtum* tab. *conjunctum* Wołosz., not validly published (ICN Art. 38.1.), Acta Societatis Botanicorum Poloniae 21: 315, pl. XII 11. 1952.—TYPE [illustration]: Poland. Lesser Poland, Tatra, Morskie Oko, without date: J. Wołoszyńska s.n. (**holotype, designated here**: pl. XII 11 in Acta Societatis Botanicorum Poloniae 21. 1952) [http://phycobank.org/100123]; [slide with non-fossil specimens]: Poland. Lesser Poland, Tatra, Morkie Oko, 23 Sep 2015: P.M. Owsianny, G. Marciniak & K. Trawiński PL017 [J. Kretschmann GeoM*711] (**epitype, designated here**: CEDiT-2018E83!, duplicates: B 40 0042057! M-0289944!) [http://phycobank.org/100124].

Description: Dinphytes small, phototrophic, thecate. Cells 14–24 μm long, 13–22 μm wide, very widely ovoid, the antapex without protuberance. Tabulation formula: APC (pp, cp, x), 4′, 2a, 7″, 6c, 5s, 5‴, 2⁗; the Plates 3′ and 4″ predominantly adjacent; the plates Sp and 5‴ adjacent; the epithecal keystone plate: 4″.

Note: A diagnosis is provided in the Discussion section.

Methods

Study area: Water tow samples were collected at five lakes in the Tatra Mountains (Tatra National Park, Republic of Poland) – Długi Staw Gąsienicowy, Litworowy Staw Gąsienicowy and Zielony Staw Gąsienicowy on 22 Sep 2015, Morskie Oko on 23 Sep 2015 and Toporowy Staw Niżni on 4 Aug 2016 using a plankton net with a mesh size of 10 μm. Major geographical, morphological and habitat characteristics of the investigated lakes are given in Table 1.

The bedrock of the Tatra Mountains area is heterogeneous and is dominated by granite, gneiss, mica schist and limestone (Hořická et al. 2006; Passendorfer in Mirek 1996; Piotrowska et al. 2013). The majority of lakes is situated in the alpine zone (including Długi Staw Gąsienicowy, Zielony Staw Gąsienicowy and Litworowy Staw Gąsienicowy), and only approximately 30% of lakes in the Tatra Mountains are located in the forest zone, below 1550 m a.s.l. (e.g., Toporowy Staw Niżni). Baumgart-Kotarba et al. (1993), Łajczak in Mirek (1996), Kopáček et al. (2004), Kopáček et al. (2006), Borowiak et al. (2006), Choiński and Strzelczak (2011) and Choiński and Pociask-Karteczka (2014) provide more detailed descriptions of the study area, including the main characteristics of lakes and their catchments (i.e., geographical, geological and hydrochemical data). Hydrobiological, especially physiological and ecological, circumscriptions are summarised in Wołoszyńska (1952), Siemińska (1970), Kawecka in Mirek (1996), Cabała (2005), Hořická et al. (2006), Piątek (2006, 2007), Sacherová et al. (2006) and Lenarczyk and Tsarenko (2013).

Cultivation and morphology: Single motile cells were isolated and placed in 24-well microplates (Zefa; Munich, Germany) containing freshwater WC growth medium (Woods Hole Combo, modified after Guillard and Lorenzen 1972) without silicate. The plates were stored in climate chambers at 12 °C or 18 °C and under 12:12 h light:dark photoperiod. The established monoclonal strains are currently held in the culture collection at the Institute of Systematic Botany and Mycology (University of Munich) and are available upon request. Substrains have been submitted to the Culture Collection of Algae at the University of Cologne: CCAC and the Culture Collection of Baltic Algae: CCBA.

For the preparation of the types, cells of the monoclonal strains were fixed with 2.5% glutaraldehyde (agar scientific; Stansted, Essex, UK). Double-staining was carried out using 0.5% (water-based) astra blue in 2% tartaric acid (Fluka; Buchs, Switzerland) in WC medium and 0.1% (ethanol-based) eosin (Merck; Darmstadt, Germany) during a graded ethanol (Roth; Karlsruhe, Germany) series. Ethanol-based Technovit 7100 (Heraeus; Wehrheim, Germany) was used for embedding, following the manufacturer's instructions. For the final preparation, 30 μl aliquots of the Technovit mixture including the embedded samples were transferred to three slides. The types are deposited at the Centre of Excellence for Dinophyte Taxonomy (CEDiT; Wilhelmshaven, Germany), and duplicates are held in Berlin, B and Munich, M (see section Taxonomic activity). Types and names are registered at PhycoBank [http://phycobank.org].

Cells were observed, documented and measured under a CKX41 inverted microscope (Olympus; Hamburg, Germany) equipped with a phase contrast option and a DP73 digital camera (Olympus). The preparative techniques for light and

scanning electron microscopy (SEM) followed standard protocols (Janofske 2000) and were the same as described in Gottschling et al. (2012). Briefly, cells were fixed in 2.5% glutaraldehyde overnight. Afterwards, specimens were dehydrated in a graded acetone series and critical point dried, followed by sputter-coating with platinum. The Kofoidean system (Fensome et al. 1993; Taylor 1980) was used to designate the plate formula. Image adjustments (such as scaling, cropping, white-balancing, colour management) were carried out in Photoshop® and Illustrator® (Adobe Systems; Munich, Germany), respectively, and images were arranged in QuarkXPress® (Quark Software; Hamburg, Germany). For the statistical analysis of the thecate cell length, R v3.4.2 (R Core Team 2017; freely available at http://www.R-project.org/) and a one-tailed t test (Gosset 1908) with equal variances were used.

Molecular phylogenetics: Genomic DNA was extracted from fresh material using the Nucleo Spin Plant II Kit (Machery-Nagel: Düren, Germany). Various regions of the ribosomal RNA (rRNA) genes including the Internal Transcribed Spacers (ITSs) were amplified using primer pairs specified previously (Gu et al. 2013) and following standard protocols (Gottschling and Plötner 2004; Gottschling et al. 2012). For alignment constitution, we defined three regions of the rRNA: SSU, ITS, LSU and included all Peridiniopsidaceae (Gottschling et al. 2017), whose sequence information of at least two regions were available, along with all rRNA sequences available from *Parvodinium* (the only exception was a short and uninformative EF581380 sequence: Kretschmann et al. 2018; Supplementary Material Table S1). For outgroup comparison, we used all sequences of *Heterocapsa* F.Stein and *Scrippsiella* Balech sensu lato, of which all three rRNA regions were available. Separate matrices were constructed, aligned using 'MAFFT' v6.502a (Katoh and Standley 2013) and concatenated afterwards. The aligned matrices are available as *.nex files upon request. Phylogenetic analyses were carried out using standard procedures described earlier (Kretschmann et al. 2018).

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Appendix A. Supplementary Data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.protis.2018.02.004.

References

Annenkova NV, Hansen G, Moestrup Ø, Rengefors K (2015) Recent radiation in a marine and freshwater dinoflagellate species flock. ISME J **9**:1821–1834

Baumgart-Kotarba M, Kotarba A, Wachniew P (1993) Młodoholoceńskie osady jeziorne Morskiego Oka w tatrach Wysokich oraz ich datowanie radioizotopami ^{210}Pb i ^{14}C. In Kotarba A (ed) Z badań fizyczno-geograficznych w Tatrach. Polska Akademia Nauk, Warszawa, pp 45–61

Below R (1987) Evolution und Systematik von Dinoflagellaten-Zysten aus der Ordnung Peridiniales. I. Allgemeine Grundlagen und Subfamilie Rhaetogonyaulacoideae (Familie Peridiniaceae). Palaeontogr Abt B **205**:1–164

Borowiak D, Polkowska {**Z**, Przyjazny A (2006) The hydrochemistry of high-altitude lakes in selected mountain ranges of Central and Southern Europe. Limnol Rev **6**:21–30

Bourrelly P (1968) Note sur *Peridiniopsis borgei* Lemm. Phykos **7**:1–2

Cabała J (2005) Chrysophycean stomatocysts from Morskee Oko and Żabie Oko lakes in the Tatra National Park, Poland. Acta Soc Bot Pol **74**:305–314

Carty S (2008) *Parvodinium* gen. nov. for the umbonatum group of *Peridinium* (Dinophyceae). Ohio J Sci **108**:103–110

Carty S, Wujek DE (2003) A new species of *Peridinium* and new records of dinoflagellates and silica-scaled chrysophytes from Belize. Carib J Sci **39**:136–139

Choiński A (2006) Katalog jezior Polski. Wydawnictwo Naukowe UAM, Poznań

Choiński A, Pociask-Karteczka J (2014) Morskie Oko. Przyroda i człowiek. Wydawnictwa Tatrzańskiego Parku Narodowego, Zakopane

Choiński A, Strzelczak A (2011) Bathymetric measurements of Morskie Oko Lake. Limnol Rev **11**:89–93

Cichocki W (2015) Ptaki Tatr i Podtatrza. Tatrzański Park Narodowy, Zakopane

Craveiro SC, Calado AJ, Daugbjerg N, Moestrup Ø (2009) Ultrastructure and LSU rDNA-based revision of *Peri-*

dinium group Palatinum (Dinophyceae) with the description of *Palatinus* gen. nov. J Phycol **45**:1175–1194

Dodge JD (1988) An SEM study of thecal division in *Gonyaulax* (Dinophyceae). Phycologia **27**:241–247

Elbrächter M, Meyer B (2001) Plate pattern variability and plate overlap in a clonal culture of the freshwater dinoflagellate *Peridinium umbonatum* Stein species complex (Dinophyceae). Neues Jahrb Geol Paläontol. Abh **219**:21–227

Fensome RA, Taylor FJR, Norris G, Sarjeant WAS, Wharton DI, Williams GL (1993) A classification of living and fossil dinoflagellates. Micropaleontol Spec Publication Number **7**:1–245

Gómez F (2014) Problematic biases in the availability of molecular markers in protists: The example of the dinoflagellates. Acta Protozool **53**:63–75

Gosset WS (1908) The probable error of a mean. Biometrika **6**:1–25

Gottschling M, Kretschmann J, Žerdoner Čalasan A (2017) Description of Peridiniopsidaceae, fam. nov. (Peridiniales, Dinophyceae). Phytotaxa **299**:93–296

Gottschling M, Plötner J (2004) Secondary structure models of the nuclear Internal Transcribed Spacer regions and 5. 8S rRNA in Calciodinelloideae (Peridiniaceae) and other dinoflagellates. Nucleic Acids Res **32**:307–315

Gottschling M, Söhner S, Zinßmeister C, John U, Plötner J, Schweikert M, Aligizaki K, Elbrächter M (2012) Delimitation of the Thoracosphaeraceae (Dinophyceae), including the calcareous dinoflagellates, based on large amounts of ribosomal RNA sequence data. Protist **163**:15–24

Gu H, Kirsch M, Zinßmeister C, Söhner S, Meier KJS, Liu T, Gottschling M (2013) Waking the dead: Morphological and molecular characterization of extant †*Posoniella tricarinelloides* (Thoracosphaeraceae, Dinophyceae). Protist **164**:583–597

Guillard RR, Lorenzen CJ (1972) Yellow-green algae with chlorophyllide c. J Phycol **8**:10–14

Hořická Z, Stuchlík E, Hudec I, Černý M, Fott J (2006) Acidification and the structure of crustacean zooplankton in mountain lakes: The Tatra Mountains (Slovakia, Poland). Biologia **61**:S121–S134

Huitfeldt-Kaas H (1906) Planktonundersøgelser i norske vande. Nationaltrykkeriet, Oslo

Izquierdo López A, Kretschmann J, Žerdoner Čalasan A, Gottschling M (in press) The many faces of *Peridinium cinctum*: Morphological and molecular variability in a common dinophyte. Eur J Phycol

Janofske D (2000) *Scrippsiella trochoidea* and *Scrippsiella regalis*, nov. comb. (Peridiniales, Dinophyceae): A comparison. J Phycol **36**:178–189

Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. Mol Biol Evol **30**:772–780

Kopáček J, Stuchlík E, Hardekopf D (2006) Chemical composition of the Tatra Mountain lakes: Recovery from acidification. Biologia **61**:S21–S33

Kopáček J, Hardekopf D, Majer V, Šenáková P, Stuchlík E, Veselý J (2004) Response of alpine lakes and soils to changes in acid deposition: The MAGIC model applied to the Tatra Mountain region, Slovakia-Poland. J Limnol **63**:143–156

Kremp A, Tahvanainen P, Litaker W, Krock B, Suikkanen S, Leaw CP, Tomas C (2014) Phylogenetic relationships, morphological variation, and toxin patterns in the *Alexandrium ostenfeldii* (Dinophyceae) complex: Implications for species boundaries and identities. J Phycol **50**:81–100

Kretschmann J, Žerdoner Čalasan A, Kusber W-H, Gottschling M (2018) Still curling after all these years: *Glenodinium apiculatum* Ehrenb. (Peridiniales, Dinophyceae) repeatedly found at its type locality in Berlin (Germany). Syst Biodivers **16**:200–209

Kretschmann J, Filipowicz NH, Owsianny PM, Zinßmeister C (2015b) Taxonomic clarification of the unusual dinophyte *Gymnodinium limneticum* Wołosz. (Gymnodiniaceae) from the Tatra Mountains. Protist **166**:621–637

Kretschmann J, Elbrächter M, Zinßmeister C, Söhner S, Kirsch M, Kusber W-H (2015a) Taxonomic clarification of the dinophyte *Peridinium acuminatum* Ehrenb. , ≡ *Scrippsiella acuminata*, comb. nov. (Thoracosphaeraceae, Peridiniales). Phytotaxa **220**:239–256

Lefèvre MM (1925) Contribution à la flore des Pèridiniens de France. Rev Algol **2**:327–342

Lefèvre MM (1927) Sur les variations tabulaires chez les Pèridiniens déeau douce et leur notation. —Diagnoses déesp'ces et de variètès nouvelles. Bull Mus Nat Hist Nat **33**:118–122

Lefèvre MM (1932) Monographie des espèces du genre *Peridinium*. Arch Bot **2**:1–210

Lemmermann EJ (1899) Ergebnisse einer Reise nachi dem Pacific. (H. Schauinsland 1896/97.) Planktonalgen. Abh Naturw Ver Bremen :313–398

Lemmermann EJ (1910) Algen I (Schizophyceen, Flagellaten, Peridineen). Bornträger, Leipzig

Lenarczyk J, Tsarenko PM (2013) Some rare and interesting green algae (Chlorophyta) from subalpine Tatra lakes (High Tatra Mountains, Poland). Oceanol Hydrobiol Stud **42**:225–232

Lindemann EBLW (1918a) Untersuchungen über Süßwasserperidineen und ihre Variationsformen. Arch Protistenkd **39**:209–262

Lindemann EBLW (1918b) Untersuchungen über Süßwasserperidineen und ihre Variationsformen II. Arch Naturgesch **84**:121–194

Lindner L, Gozhik P, Marciniak B, Marks L, Yelovicheva Y (2004) Main climatic changes in the Quaternary of Poland, Belarus and Ukraine. Geol Quart **48**:97–114

Makos M, Nitychoruk J, Zreda M (2013) The Younger Dryas climatic conditions in the Za Mnichem Valley (Polish High Tatra Mountains) based on exposure-age dating and glacier-climate modelling. Boreas **42**:745–761

Marks L (2004) Pleistocene Glacial Limitis in Poland. In Ehlers J, Gibbard PL (eds) Quaternary Glaciations—Extents

and Chronology. Part I: Europe. Elsevier, Amsterdam, pp 295–300

Mendel G (1866) Versuche über Pflanzenhybriden. Verh Naturf Ver Brünn **4**:3–47

Mertens KN, Rengefors K, Moestrup Ø, Ellegaard M (2012) A review of recent freshwater dinoflagellate cysts: Taxonomy, phylogeny, ecology and palaeoecology. Phycologia **51**:612–619

Mirek Z (1996) Przyroda Tatrzańskiego Parku Narodowego. Tatrzański Park Narodowy, Krakow

Mittermeier RA, Gil PR, Hoffman M, Pilgrim J, Brooks T, Mittermeier CG, Lamoreux J, da Fonseca GAB (2005) Hotspots Revisited. Earth's Biologically Richest and Most Endangered Terrestrial Ecoregions. The University of Chicago Press, Chicago

Netzel H, Dürr G (1984) 3. Dinoflagellate Cell Cortex. In Spector DL (ed) Dinoflagellates. Academic Press Inc. (London) Ltd., Orlando (Florida), pp 43–105

Onuma R, Watanabe K, Horiguchi T (2015) *Pellucidodinium psammophilum* gen. & sp. nov. and *Nusuttodinium desymbiontum* sp. nov. (Dinophyceae), two novel heterotrophs closely related to kleptochloroplastidic dinoflagellates. Phycologia **54**:192–209

Padisák J (2009) The Phycogeography of Freshwater Algae. In Likens GE (ed) Encyclopedia of Inland Waters. Academic Press, Oxford, pp 219–223

Pelzer F (1991) Polen: Eine geographische Landeskunde. Wissenschaftliche Buchgesellschaft, Darmstadt

Penard E (1891) Les Péridinacées du Léman. Bull Trav Soc Bot Geneve **6**:1–63

Piątek J (2006) Stomatocysts of the Dolina Gąsienicowa Valley in the Tatra Mts (Poland)1. Czarny Staw Gąsienicowy and Zmarzły Staw Gąsienicowy lakes. Pol Bot J **51**: 61–77

Piątek J (2007) Some silica-scaled chrysophytes from the Tatra Mountains, Poland. Pol Bot J **52**:133–137

Piotrowska K, Michalik M, Rączkowski W, Iwanow A, Wójcik A, Derkacz M, Wasiluk R (2013) Szczegółowa Mapa Geologiczna Polski w skali 1:50 000, arkusz Tatry Wysokie. Narodowe Archiwum Geograficzne. Państwowy Instytut Geologiczny—Państwowy Instytut Badawczy, Warszawa

Playfair GI (1920) Peridinieae of New South Wales. Proc Linn Soc New South Wales **44**:793–818

Popovský J (1968) A contribution to the knowledge of dinoflagellates from Bohemia. Preslia (Prague) **40**:251–263

Popovský J, Pfiester LA (1986) A taxonomical note to the section umbonatum of the genus *Peridinium* Ehrenberg, 1932 (Dinophyceae). Arch Protistenkd **132**:73–77

Popovský J, Pfiester LA (1990) Dinophyceae (Dinoflagellida). In Ettl H, Gärtner G, Gerloff J, Heynig H, Mollenhauer D (eds) Süsswasserflora von Mitteleuropa **Vol 6**. Fischer, Stuttgart, 272p

R Core Team (2017) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna

Sacherová V, Kršková R, Stuchlík E, Hořická Z, Hudec I, Fott J (2006) Long-term change of the littoral *Cladocera* in the Tatra Mountain lakes through a major acidification event. Biologia **61**:S109–S119

Schilling AJ (1891) Die Süsswasser-Peridineen. Flora **74**:220–229

Siemińska J (1970) Some species of Chrysophyceae from Morskie Oko lake in the Tatra Mts. Fragm Flor Geobot **16**:183–186

Stein SFNRv (1883) Der Organismus der Infusionsthiere nach eigenen Forschungen in systematischer Reihenfolge bearbeitet, 3. Abth., 2. Hälfte, Die Naturgeschichte der arthrodelen Flagellaten. Engelmann, Leipzig

Takano Y, Yamaguchi H, Inouye I, Moestrup Ø, Horiguchi T (2014) Phylogeny of five species of *Nusuttodinium* gen. nov. (Dinophyceae), a genus of unarmoured kleptoplastidic dinoflagellates. Protist **165**:759–778

Tardio M, Ellegaard M, Lundholm N, Sangiorgi F, Di Giuseppe G (2009) A hypocystal archeopyle in a freshwater dinoflagellate from the *Peridinium umbonatum* group (Dinophyceae) from Lake Nero di Cornisello, South Eastern Alps, Italy. Eur J Phycol **44**:241–250

Taylor FJR (1980) On dinoflagellate evolution. Biosystems **13**:65–108

Thessen AE, Patterson DJ, Murray SA (2012) The taxonomic significance of species that have only been observed once: The genus *Gymnodinium* (Dinoflagellata) as an example. PLoS One **7**:e44015

Tillmann U, Elbrächter M (2010) Plate Overlap Pattern of *Azadinium spinosum* Elbrächter et Tillmann (Dinophyceae), the Newly Discovered Primary Source of Azaspiracid Toxins. In Ho KC, Zhou MJ, Qi YZ (eds) Proceedings of the 13th International Conference on Harmful Algae. Environmental Publication House, Hong Kong, pp 42–44

Tillmann U, Gottschling M, Nézan E, Krock B, Bilien G (2014) Morphological and molecular characterization of three new *Azadinium* species (Amphidomataceae, Dinophyceae) from the Irminger Sea. Protist **165**:417–444

Tillmann U, Hoppenrath M, Gottschling M, Kusber W-H, Elbrächter M (2017) Plate pattern clarification of the marine dinophyte *Heterocapsa triquetra* sensu Stein (1883) collected at the Kiel Fjord (Germany). J Phycol **53**:1305–1324

Tillmann U, Salas R, Gottschling M, Krock B, O'Driscoll D, Elbrächter M (2012) *Amphidoma languida* sp. nov. (Dinophyceae) reveals a close relationship between *Amphidoma* and *Azadinium*. Protist **163**:701–719

Trafas K (1985) Atlas Tatrzańskiego Parku Narodowego. Polskie Towarzystwo Przyjaciół Nauk o Ziemi/Oddzial Kraków, Zakopane

West GS (1907) Report on the freshwater algae, including phytoplankton, of the Third Tanganyika Expedition, conducted by Dr. W. A. Cunnington, 1904–1905. J Linn Soc Bot **38**: 81–197

Wit-Jóźwik K (1974) Hydrografia Tatr Wysokich: objaśnienia do mapy hydrograficznej Tatry Wysokie 1:50 000. Polska Akademia Nauk, Instytut Geografii, Warsaw

Wołoszyńska J (1916) Polskie Peridineae słodkowodne. —Polnische Süßwasser-Peridineen. Bulletin international de

l'Académie des Sciences de Cracovie, Classe des Sciences Mathématiques et Naturelles. Série B **1915**:260–285

Wołoszyńska J (1919) Glony stawów i młak tatrzańskich. I. — Die Algen der Tatraseen und – Tümpel. I. Bulletin international de l'Académie des Sciences de Cracovie, Classe des Sciences Mathématiques et Naturelles. Série B **1918**: 196–200

Wołoszyńska J (1935) Glony stawłw i mńak tatrzaóskich. II. O dwłch Gymnodinjach z jezior Morskie Oko i Czarny Staw pod Rysami. Bulletin international de l'Académie Polonaise des Sciences et des Lettres, Classe des Sciences Mathématiques et Naturelles. Série B **1935**:1–9

Wołoszyńska J (1936) Die Algen der Tatraseen und Tümpel. III. Peridineen im Winterplankton einiger Tatraseen. Archivum Hydrobiologji i Rybactwa **10**:188–196

Wołoszyńska J (1952) Bruzdnice Tetr i Karpat Wschodnich. Acta Soc Bot Pol **21**:311–316

Zhang Q, Liu G-X, Hu Z-Y (2011) Morphological observation of a freshwater *Peridinium* strain and phylogenetic analysis of *Peridinium*. Plant Sci J **29**:1–10

Publication 2

Molecular phylogenetics of dinophytes harboring diatoms as endosymbionts (Kryptoperidiniaceae, Peridiniales), with evolutionary interpretations and a focus on the identity of *Durinskia oculata* from Prague

KRETSCHMANN, J., ŽERDONER ČALASAN, A. & GOTTSCHLING, M.

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Molecular phylogenetics of dinophytes harboring diatoms as endosymbionts (Kryptoperidiniaceae, Peridiniales), with evolutionary interpretations and a focus on the identity of *Durinskia oculata* from Prague

Juliane Kretschmann, Anže Žerdoner Čalasan, Marc Gottschling*

Department Biologie, Systematische Botanik und Mykologie, GeoBio-Center, Ludwig-Maximilians-Universität München, Menzinger Str. 67, D-80638 München, Germany

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ABSTRACT

Peridinialean dinophytes include a unique evolutionary group of algae harboring a diatom as an endosymbiont (Kryptoperidiniaceae), whose phylogenetic origin and internal relationships are not fully resolved at present. Several interpretations of the thecal plate pattern present in *Durinskia oculata* currently compete and lead to considerable taxonomic confusion. Moreover, it is unclear at present whether the species is restricted to freshwater habitats, or occurs in the marine environment as well. We collected material at the type locality of *D. oculata* in the Czech Republic and established monoclonal strains. Dinophyte cells were studied using light and electron microscopy, and we also determined DNA sequences of several rRNA regions (including the Internal Transcribed Spacers) for molecular characterization and phylogenetics. The morphology of strain GeoM∗662 indicated a plate formula of Po, X, 4′, 2a, 6″, 5c, 5s, 5‴, 2⁗, which was sustained also in form of a microscopic slide serving as an epitype. In the molecular DNA tree based on a matrix composed of concatenated rRNA sequences, strain GeoM∗662 showed a close relationship to other species of *Durinskia*, and the freshwater species clearly differs from the marine members. Two independent colonization events from the marine into the freshwater environment can be inferred within the Kryptoperidiniaceae. We provide a summarizing cladogram of dinophytes harboring a diatom as endosymbiont with evolutionary novelties indicated as well as a morphological key to the 6 species of *Durinskia* that are currently accepted.

1. Introduction

Glenodinium oculatum F.Stein was first described by Stein (1883), who observed the species in water tow samples from the Vltava river near Prague (Czech Republic) collected on an unknown date between 1879 and 1883. No physical specimen linked to the original publication could be found in the course of the present study and therefore, pl. III 5–7 in Stein (1883; Fig. 1) is the only original material of *G. oculatum*. It shows thecate dinophyte cells with a (very) widely ovate outline in ventral view and an epitheca, which is slightly larger than a corresponding hypotheca. Furthermore, chloroplasts are present, and the dinophyte nucleus is located in the epithecal part of the cell. Another subcellular structure is the small, red eyespot situated in the sulcal region of the hypotheca, and the drawing of pl. III 5 (Stein, 1883) shows a distinctive descendent displacement of the cingulum of about its own width. Remarkable is also the life history stage with two daughter cells included in the shell of a coccoid cell (pl. III 7 in Stein, 1883). S.F.N.R. von Stein did not provide scales for his drawings, but the cells' approximate size is in the range of *Heterocapsa steinii* Tillmann, Gottschling, Hoppenrath, Kusber & Elbr. (≡ *Heterocapsa triquetra* sensu Stein, 1883; Tillmann et al., 2017) and *Scrippsiella acuminata* Kretschmann, Elbr., Zinssmeister, S.Soehner, Kirsch, Kusber & Gottschling [= *Scrippsiella trochoidea* (F.Stein) A.R.Loebl.] likewise depicted on the same plate.

The plate pattern of *G. oculatum* is not described in the protologue (Stein, 1883) and was controversial among subsequent authors. Wołoszyńska (1917) was the first to provide drawings with indicated thecal plates and introduced a plate formula with 3 apical, 1 anterior intercalary and 7 precingular plates. The corresponding images have been copied variously or slightly modified in text books (Schiller, 1937; Thompson, 1951; Starmach, 1974; Carty, 2014; Table 1). Jadwiga Wołoszyńska's observations were confirmed, but also challenged by Lindemann (1926), who considered plate arrangement as a variation of patterns and shifting plates (germ., 'bewegliche Hüllenfelderung') present in *G. oculatum*. Among 5 variant types, he described also a plate pattern with 4 apical, 2 anterior intercalary and 6 precingular plates. It was Hansen and Flaim (2007), who first showed the consistency of such a plate pattern using scanning electron microscopy (SEM) and

* Corresponding author.
E-mail address: gottschling@bio.lmu.de (M. Gottschling).

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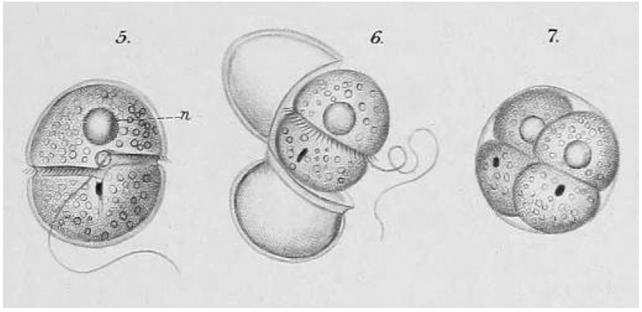

Fig. 1. Original drawings of *Glenodinium oculatum* reproduced from Stein (1883). The leftmost illustration corresponds to the lectotype.

transferred *G. oculatum* taxonomically to *Durinskia* Carty & El.R.Cox. However, the concept of Hansen and Flaim (2007) was rarely adopted since then (Крахмальный, 2011; Darki, 2014) and was not used even in renown textbooks by Lewis and Dodge (2011) and Carty (2014).

Durinskia belongs to a unique group of Dinophyceae (Dinoflagellata under zoological nomenclature) hosting a tertiary endosymbiont derived from a diatom (Tomas et al., 1973; Horiguchi and Pienaar, 1994; Chesnick et al., 1997), named the Kryptoperidiniaceae (Lindemann, 1926). Those so called 'dinotoms' (Imanian et al., 2011) further include *Blixaea* Gottschling, *Galeidinium* Tam. & T.Horig., *Kryptoperidinium* Er.Lindem., and *Unruhdinium* Gottschling (Tamura et al., 2005; Horiguchi and Takano, 2006; Hansen et al., 2007; Zhang et al., 2011b; Gagat et al., 2014; Gottschling et al., 2017; Yamada et al., 2017; Žerdoner Čalasan et al., 2017; the rarely encountered *Dinothrix* Pascher may also belong to this group). Besides 'possessing a diatom endosymbiont' as a highly derived trait, the monophyly of the Kryptoperidiniaceae is also supported by a unique and morphologically conserved type of an eyespot (Dodge, 1984; Horiguchi and Pienaar, 1991; Kreimer, 1999; Pienaar et al., 2007; Takano et al., 2008) that has possibly derived from the original chloroplast (Moestrup and Daugbjerg, 2007). In molecular trees, the Kryptoperidiniaceae constitute a highly supported monophyletic group, but it is not finally resolved at present whether they are embedded in the Thoracosphaeraceae or constitute their sister group (Gottschling and McLean, 2013).

In its current circumscription, *Durinskia* comprises marine (Kofoid and Swezy, 1921; Pienaar et al., 2007), brackish (Levander, 1894), and freshwater species as well (Stein, 1883; Wołoszyńska, 1916). One of the main goals in evolutionary ecology of protists is to understand the processes of marine to freshwater transitions. The osmotic difference between those ecosystems may act as a highly efficient barrier limiting the frequency of transitions (Logares et al., 2007b). However, this scenario is challenged in dinophytes by recent data indicating multiple lineages conquering the barrier in diverse groups such as Thoracosphaeraceae (Moestrup and Daugbjerg, 2007; Craveiro et al., 2013; Gottschling and Söhner, 2013) and Gymnodiniaceae (Kretschmann et al., 2015). Thus, the frequency of dinophyte marine to freshwater transitions, and their impact on diversification, remain to be determined.

The taxonomic confusion of *G. oculatum* is considerable (Table 1), and the name is inconsistently used at present, making meaningful and taxonomically indisputable conclusions about ecology and/or distribution impossible. The problems particularly refer to opposing interpretations of the thecal plate pattern and general ecology, as it has not been worked out whether the species occurs both in the brackish/marine and the freshwater environment, or is restricted to the freshwater habitat only, from which it has been primarily described (Table 1). To track down what S.F.N.R. von Stein had precisely observed more than 130 years ago, we decided to visit the original site and to fish for his species [the approach is discussed in Kretschmann et al. (2017), investigating *Palatinus apiculatus* (Ehrenb.) Craveiro, Calado, Daugbjerg & Moestrup]. In this study, we present a dinophyte collected at the type locality of *G. oculatum*, which is consistent with the protologue of S.F.N.R. von Stein's species. We expanded the investigation with a phylogenetic analysis of the Kryptoperidiniaceae using concatenated sequences of ribosomal RNA (rRNA) to aim at a better understanding of the group's evolutionary origin and internal diversification.

2. Material and methods

2.1. Material collection and processing

During a field trip on Sep 30th, 2015, water tow samples were collected using a plankton net with a mesh size of 20 µm at the Vltava river (50°07′59.8″N, 14°23′37.0″E) near Prague (Czech Republic). Single motile cells were isolated and placed in 24-well microplates (Zefa; Munich, Germany) containing freshwater WC growth medium (Woods Hole Combo modified after Guillard and Lorenzen, 1972) without silicate. The plates were stored in a climate chamber WKS 3200 (Liebherr; Bulle, Switzerland) at 18 °C, 80 µmol photons $m^{-2} s^{-1}$ and a 12:12 h light:dark photoperiod. The established monoclonal strains are currently held in the culture collection at the Institute of Systematic Botany and Mycology (University of Munich) and are available upon request. Substrains have been submitted to the Collection of Algae at the University of Cologne: CCAC, the Culture Collection of Baltic Algae: CCBA, and the Canadian Center for the Culture of Microorganisms: CCCM.

For the preparation of the epitype, cells of the (monoclonal) strain GeoM∗662 were fixed with 2.5% glutaraldehyde (agar scientific; Stansted, Essex, UK). Double-staining was performed using 0.5% (water-based) astra blue in 2% tartaric acid (Fluka; Buchs, Switzerland), followed by two cleaning steps in WC medium for 15 min each and 0.1% (ethanol-based) eosin (Merck; Darmstadt, Germany) during a graded ethanol (Roth; Karlsruhe, Germany) series. Ethanol-based Technovit 7100 (Heraeus; Wehrheim, Germany) was used for embedding, following the manufacturer's instructions. For the final preparation, 40 µl aliquots of the Technovit mixture including the embedded samples were transferred to four glass slides. The epitype is deposited at the Centre of Excellence for Dinophyte Taxonomy (CEDiT; Wilhelmshaven, Germany), and copies are held in Berlin and Munich (see below).

2.2. Morphology

Cells were observed, documented and measured with a CKX41 inverse microscope (Olympus; Hamburg, Germany) equipped with a phase contrast option and a DP73 digital camera (Olympus). The preparative techniques for light and scanning electron microscopy (SEM) followed standard protocols (Janofske, 2000) and were the same as described in Gottschling et al. (2012). Briefly, cells were fixed in 2.5% glutaraldehyde for at least 1 h. Afterwards, specimens were dehydrated in a graded acetone series and critical point dried, followed by sputter-coating with platinum, and analyzed with an LEO 435VP SEM (Oberkochen, Germany).

For nuclear staining, cells (previously fixed in 2.5% glutaraldehyde for 1 h) were treated with 4′-6-diamidino-2-phenylindole (DAPI, 10 µg ml^{-1} final concentration) for 10 min. For visualising of the nuclei and also for observing chloroplasts of motile cells using autofluorescence, a DM 1000 light microscope (Leica; Wetzler, Germany) equipped with a DAPI filter (Leica; excitation: 350/50, dichroic mirror: 400, emission BP 460/50) or GFP ET filter (Leica; excitation: BP 470/40, dichroic mirror: 495, emission BP 525/50), respectively, and a DP73 digital camera (Olympus) were used.

Table 1
Comparison of species, and their names, relevant for the taxonomy discussed in the present study (note that also *Peridinium umbonatum* var. *elpatiewskyi* Ostenf. originally has an epithecal formula 3′ 1a 7″: Ostenfeld, 1907, but this has been superseded with a conserved type: Compère, 1999, and is not discussed further here).

habitat	brackish / marine	freshwater	freshwater	freshwater
epitheca plate formula	4' 2a 6" (asymmetric)	4' 2a 6" (asymmetric)	4' 2a 6" (asymmetric)	3' 1a 7" (symmetric)
cell surface	smooth	smooth	minutely porate	smooth
name to be accepted	*Durinskia baltica* (Levander) Carty & El.R.Cox	*Durinskia oculata* (F.Stein) Gert Hansen & Flaim	*Durinskia dybowskii* (Wołosz.) Carty	*Peridiniopsis lindemannii* (M.Lefèvre) Bourr. or relative
Wołoszyńska (1917)	—	—	—	*Glenodinium oculatum* F.Stein, not validly transferred to *Peridinium* Ehrenb.
Lindemann (1926)	—	*Glenodinium oculatum* F.Stein	*Glenodinium dybowskii* (Wołosz.) Er.Lindem.	*Glenodinium oculatum* F.Stein
Schiller (1937)	*Peridinium balticum* (Levander) Lemmerm.	*Glenodinium oculatum* F.Stein	*Peridinium balticum* (Levander) Lemmerm.	*Glenodinium oculatum* F.Stein
Huber-Pestalozzi (1968)	—	*Glenodinium oculatum* F.Stein	*Glenodinium dybowskii* (Wołosz.) Er.Lindem.	*Glenodinium oculatum* F.Stein
Starmach (1974)	*Peridinium balticum* (Levander) Lemmerm.	*Peridinium balticum* (Levander) Lemmerm.	*Peridinium balticum* (Levander) Lemmerm.	*Clathrocysta aculeata* F.Stein, not validly transferred to *Peridiniopsis* Lemmerm. (name confused for unknown reasons)
Popovský and Pfiester (1990)	*Glenodinium balticum* Levander, not validly transferred to *Peridiniopsis* Lemmerm.	*Glenodinium balticum* Levander, not validly transferred to *Peridiniopsis* Lemmerm.	*Glenodinium balticum* Levander, not validly transferred to *Peridiniopsis* Lemmerm.	*Peridiniopsis oculata* (F.Stein) Bourr.
Hansen and Flaim (2007)	*Durinskia baltica* (Levander) Carty & El.R.Cox	*Durinskia oculata* (F.Stein) Gert Hansen & Flaim	*Durinskia dybowskii* (Wołosz.) Carty	— (not addressed)
Carty (2014)	*Durinskia baltica* (Levander) Carty & El.R.Cox	— (Hansen & Flaim's taxon ignored)	*Durinskia dybowskii* (Wołosz.) Carty	*Peridiniopsis oculata* (F.Stein) Bourr.

2.3. Molecular phylogenetics

Genomic DNA was extracted from fresh material using the Nucleo Spin Plant II Kit (Machery-Nagel: Düren, Germany). Various regions of rRNA including the Internal Transcribed Spacers (ITSs) were amplified using primer pairs specified previously (Gu et al., 2013) and following standard protocols (Gottschling and Plötner, 2004; Gottschling et al., 2012). For amplification of endosymbiont DNA (SSU, ITS, *rbc*L), different primer pairs and PCR protocols were used (Table 2; Medlin et al., 1988; Coolen et al., 2004; Tamura et al., 2005; von Dassow et al., 2006; Brinkmann et al., 2015). Gel electrophoreses yielded single bands that were purified and sequenced. For alignment constitution, we defined three regions of the rRNA: SSU, ITS, LSU, and included all Peridiniales, of which sequence information of all three regions were available, along with all rRNA sequences from the Kryptoperidiniaceae (irrespectively whether they were complete, or not). Separate matrices were constructed, aligned using 'MAFFT' v6.502a (Katoh and Standley, 2013), and concatenated afterwards. The aligned matrices are available as ∗.nex files upon request.

Dinophyte phylogenetic analyses were carried out using Maximum Likelihood and Bayesian approaches, as described in detail previously (Gottschling et al., 2012) using the resources available from the CIPRES Science Gateway (Miller et al., 2010). The Bayesian analysis was performed using 'MrBayes' v3.2.6 (Ronquist et al., 2012, freely available at http://mrbayes.sourceforge.net/download.php) under the GTR + Γ substitution model and the random-addition-sequence method with 10 replicates. We ran two independent analyses of four chains (one cold and three heated) with 20,000,000 cycles, sampled every 1,000th cycle, with an appropriate burn-in (10%) as inferred from the evaluation of the trace files using Tracer v1.5 (http://tree.bio.ed.ac.uk/software/tracer/). For the ML calculation, the MPI version of 'RAxML' v8.2.4 (Stamatakis, 2014, freely available at http://www.exelixis-lab.org/) was applied using the GTR + Γ substitution model. To determine the best fitted ML tree, we executed 10-tree searches from distinct random stepwise addition sequence Maximum Parsimony starting trees and performed 1,000 non-parametric bootstrap replicates. Statistical support values (LBS: ML bootstrap support, BPP: Bayesian posterior probabilities) were drawn on the resulting, best-scoring tree.

3. Results

3.1. Morphology

The monoclonal strain GeoM∗662 exhibited both motile thecate cells (Figs. 2A–B, 3A–E, 45) and immotile coccoid cells (Figs. 2C–E, 3F–J). Motile cells were circular through (very) widely ovate in outline and slightly compressed in dorsiventral direction. The cingular girdle was excavated, and it surrounded the cell with a descendent displacement of its own width (Figs. 3A–E, 4A). The epitheca was hemispherical and slightly larger than the hypotheca, which was hemispherical as well and showed mostly a flattened antapex. Similar to the cingulum, the sulcus was also excavated, widened towards the posterior end of the cell, and reached from the cingulum down nearly to the antapex. Cell length ranged from 19–36 µm (mean: 26 µm; median: 26 µm; sd: 4 µm; n = 75) and width from 18–32 µm (mean: 25 µm; median: 24 µm; sd: 4 µm; n = 75).

The cells were yellowish and hyaline through golden-brown in color and showed numerous irregularly shaped chloroplasts (Figs. 3A–E, 4D). The cytoplasm was filled with numerous granules and frequently contained an orange-red accumulation body without precise position. A rectangular red through dark-red eyespot was clearly visible in the hypotheca in proximity of the sulcus (Fig. 3B–C). The cells contained two different types of nuclei (as inferred from DAPI straining: Fig. 4E): The dinokaryon (with distinctly condensed chromosomes that could be inferred by focussing on different levels) was located centrally or in the epitheca just above the cingulum. The second and smaller eukaryotic

Table 2
Primers and protocols used for the amplification of SSU, ITS, and *rbc*L of the endosymbiont.

Region	Name	Synthesis direction	Sequence	Protocol
18S	EukA	F	5′-ACC TGG TTG ATC CTG CCA GT-3′	Initial denaturation at 95 °C for 3 min; 39 cycles of denaturation at 95 °C for 30 s, primer annealing at 56 °C for 1 min, extension at 68 °C for 1 min; additional extension at 68 °C for 10 min
	Dia-516r	R	5′-CTC ATT CCA ATT GCC AGA CC-3′	
18S	500MGZCF	F	5′-GAC AAT AAA TAA CAA TGC GGG GCC-3′	Initial denaturation at 95 °C for 3 min; 39 cycles of denaturation at 95 °C for 30 s, primer annealing at 55 °C for 1 min, extension at 68 °C for 1 min; additional extension at 68 °C for 10 min
	1263R	R	5′-GTG CCA GCR GCC GCG G-3′	
18S	1122F	F	5′-GGC TGA AAC TTA AAG GAA TTG-3′	Initial denaturation at 95 °C for 3 min; 39 cycles of denaturation at 95 °C for 30 s, primer annealing at 50 °C for 1 min, extension at 68 °C for 1 min; additional extension at 68 °C for 10 min
	D1800R	R	5′-GCT TGA TCC TTC TGC AGG T-3′	
ITS	1645F	F	5′-CTT ATC ATT TAG AGG AAG GTG AAG TCG T-3′	initial denaturation at 95 °C for 3 min; 39 cycles of denaturation at 95 °C for 30 s, primer annealing at 62 °C for 1 min, extension at 68 °C for 1 min; additional extension at 68 °C for 10 min
	28SR	R	5′-CCG CTT CAC TCG CCG TTA CT-3′	
*rbc*L	DiatrbcL1	F	5′-TAT ATA TTG CCT TTT TAT TC-3′	Initial denaturation at 95 °C for 3 min; 39 cycles of denaturation at 95 °C for 30 s, primer annealing at 51 °C for 1 min, extension at 68 °C for 1 min; additional extension at 68 °C for 10 min
	DiatrbcL3	R	5′-AAA CCA CCT TTT AAA CCT TC-3′	
*rbc*L	DiatrbcL2	F	5′-ACA GTA AAA CCW AAA TTA GG-3′	
	DiatrbcL5	R	5′-ATT TGA CCA CAG TGG ATA CC-3′	
*rbc*L	DiatrbcL4	F	5′-TGT AAA TGG ATG CGT ATG T-3′	
	DiatrbcL6	R	5′-GTC TCA CTA TTC AAA TAC TC-3′	

nucleus was positioned left above the dinokaryon in dorsal view (Fig. 4E).

The motile cells were covered by a theca constituted of thin plates (Figs. 4A–C, 5, 6, astra blue staining indicated their cellulosic nature). The cell surface was mostly smooth but irregularly scattered with small circular pores (probably openings of trichocysts) on the thecal plates. Pores on plates ran along the sutures, and some additional pores were irregularly scattered over the plates (Fig. 4A–C). The thecate plate formula was Po, X, 4′, 2a, 6″, 5c, 5s, 5‴, 2⁗ (Figs. 4A–C, 5, 6). The arrangement of the epithecal plates was asymmetric, whereas the apical pore plate (Po) was small and elliptical. The canal (or X or preapical) plate was rectangular in shape and connected Po and 1″. The apical plate 4′ on the right side of the apical pore was twice as large as the apical plates 2′ and 3′, both located on the left side. The first anterior intercalary plate was small and more or less regularly pentagonal in shape, whereas plate 2a was larger, hexagonal, and elongated. The cingulum was composed of 5 plates, whereas the sutures were slightly deviating from those of the pre- and postcingular plates. The first cingular plate was relatively narrow, while the cingular plates 2C through 5C surrounded the rest of the cell approximately one quarter each. In the sulcus consisting of 5 plates, the plates Sa, Ss, and Sm were small and partially covered by the large Sd plate. The left edge of the Sd plate extended towards the middle of the sulcus and covered the flagellar pores. The Sp plate was relatively large and reached the antapex. The arrangement of the hypothecal plates was nearly symmetric. The hypotheca was composed of 5 postcingular and 2 antapical plates of similar size.

Along the boundaries of the thecal plates, the overlap of adjacent plates could be observed. The plate pattern including the overlap pattern is illustrated in Fig. 6C–D. Generally, it followed an imbricate pattern from dorsal through ventral: In the epitheca, the dorsal precingular plate 4″ was the keystone plate, as was the plate 3C in the cingulum. The keystone plate of the hypotheca was postcingular plate 3‴, and the antapical plates laid under the postcingular plates. The large sulcal plate Sp was overlapped by all adjacent plates.

Cell division of thecate cells is normally carried out by eleuteroschisis, whereas the parent organism shed its theca completely. Occasionally, empty epi- and hypothecae (linked and unlinked) were observed at the bottom of the cultivation plates. Thecate cells opened along the upper ridge of the cingulum (i.e., the cingulum was attached to the hypotheca) to release dividing or ecdysing cells. A single coccoid cell developed intrathecately and was released after shedding of the theca (Fig. 3H). Coccoid cells having a color slightly darker than the motile cells were spherical through mostly (very) widely ovoid (Fig. 3F–J). They ranged from 31–48 µm in length (mean: 39 µm; median: 40 µm; SD: 3 µm; n = 50) and 26–41 µm in width (mean: 33 µm; median: 33 µm; SD: 3 µm; n = 50). The cytoplasm of the coccoid cells was filled with numerous brown granules and frequently contained a large, red accumulation body. The mother cell became ovoid and divided into two daughter cells, which were included in a joint shell. The developmental fate of such cells remained elusive.

3.2. Molecular phylogenetics

In total, sequences were generated and deposited as 12 new GenBank entries in the course of the study (Supplementary Material Tab. S1). They include not only rRNA sequences from the hosting dinophyte, but also SSU, ITS, and *rbc*L DNA sequences from the diatom endosymbiont of *G. oculatum*. The phylogenetic analysis of the latter is complex and therefore a matter of a comprehensive, alternate study about the relationships between the endosymbionts and free-living diatoms (Žerdoner Čalasan et al., 2017). The SSU + ITS + LSU alignment of the Peridiniales was 1826 + 1393 + 3013 bp long and comprised 458 + 714 + 673 parsimony informative sites (30%, mean of 12.7 per terminal taxon). Fig. 7 shows the best-scoring Maximum Likelihood (ML) tree (–ln = 59,486.27), with the internal topology not fully

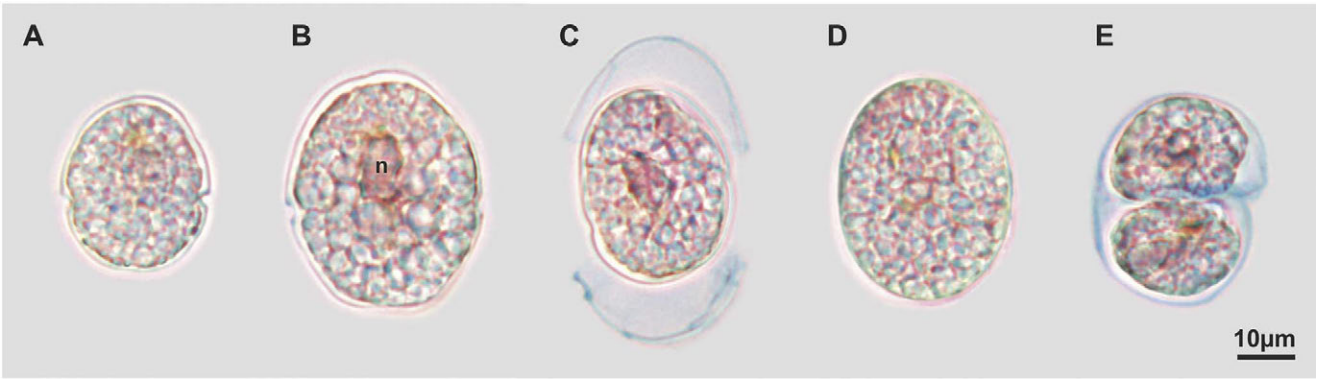

Fig. 2. Motile and immotile stages (stained with astra blue and eosin) prepared as an epitype of *Durinskia oculata* (GeoM*662; light microscopy; all at the same scale). (A–B) Motile thecate cells showing the variation in size. (C–E) Coccoid cells. (C) Coccoid cell with thecal remnant. (D) Coccoid cell. (E) Dividing coccoid cells. Abbreviation: n: dinophyte nucleus.

resolved. However, many nodes were statistically well if not maximally supported, and a number of peridinialean lineages could be recognized such as the Peridiniopsidaceae (99LBS, 1.00BPP) and Peridiniaceae (100LBS, 1.00BPP), *Scrippsiella* Balech *s.l.* (100LBS, 1.00BPP) and a clade including *Pfiesteria* Steid. & J.M.Burkh. and *Thoracosphaera* Kamptner (94LBS, 1.00BPP). The Kryptoperidiniaceae were also monophyletic (97LBS, 1.00BPP) and constituted the sister group (59LBS) of *Blastodinium* Chatton + *Zooxanthella* K.Brandt (73LBS).

Strain GeoM∗662, from which the epitype of *G. oculatum* was prepared, clustered together with other species of *Durinskia* (98LBS, 1.00BPP). They consisted of *D. agilis* (Kof. & Swezy) Saburova, Chomérat & Hoppenrath (100LBS, .96BPP), *D. capensis* Pienaar, Sakai & T.Horig. (96LBS, 1.00BPP), and *Durinskia kwazulunatalensis* Norico Yamada, Sym & T.Horig. (89LBS) as well as a grade rather than a clade comprising dinophytes determined as *Durinskia* cf. *baltica*. *Durinskia* constituted the sister group (87LBS, 1.00BPP) to all other Kryptoperidiniaceae (71LBS), which further segregated into monotypic *Blixaea*, *Galeidinium* (100LBS, 1.00BPP), *Kryptoperidinium* (75LBS, .98BPP), and *Unruhdinium* (100LBS, 1.00BPP). Within Kryptoperidiniaceae, two distinct freshwater lineages could be identified, namely *Unruhdinium* (100LBS, 1.00BPP) and a clade comprising strains GeoM∗662 and GeoM∗663 as well as Chinese dinophytes initially determined as *D. baltica* (100LBS, 1.00BPP). They were only distantly related to each other and constituted sister groups to marine *Blixaea* + *Galeidinium* + *Kryptoperidinium* (87LBS, 1.00BPP) and likewise marine dinophytes determined as *Durinskia* cf. *baltica* (76LBS), respectively.

4. Discussion

4.1. Taxonomic identity of Glenodinium oculatum F.Stein

Reliable species determination is essential for any meaningful application of scientific names in studies of, for example, ecosystem

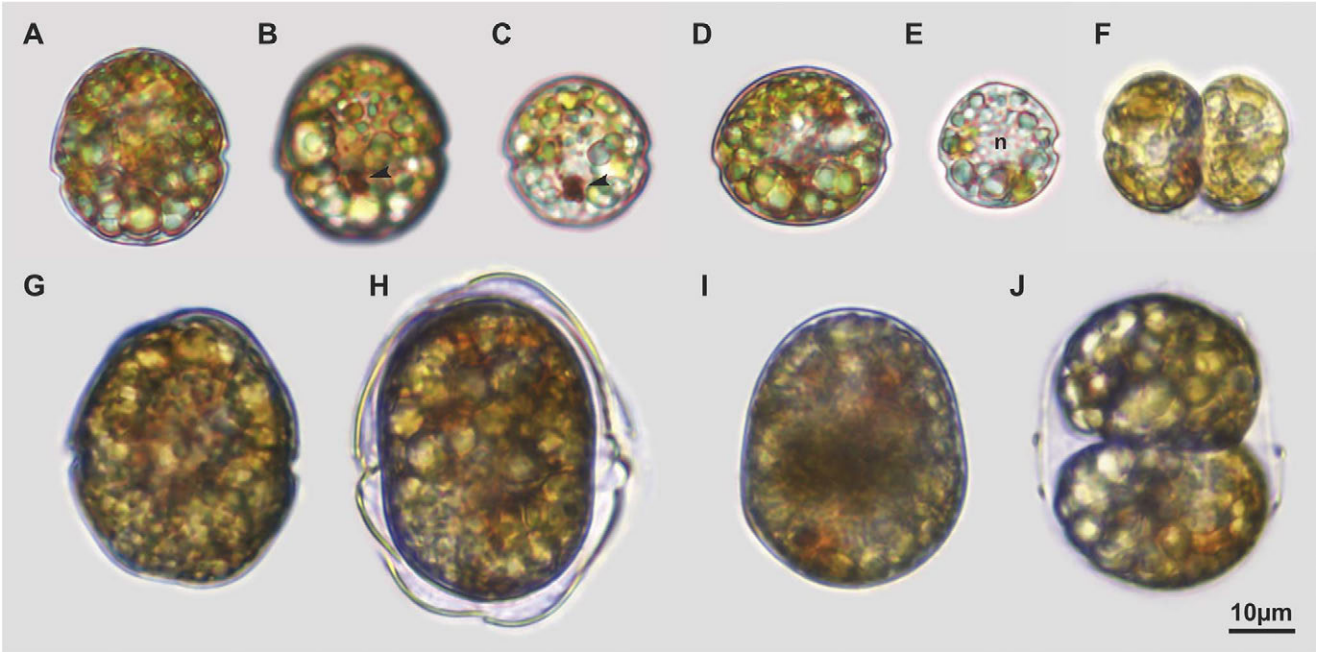

Fig. 3. Motile cells and immotile cells of *Durinskia oculata* (GeoM*662; light microscopy; all at the same scale). (A–E) Motile thecate cells showing the variation in size and shape. (A–B) Same cell at different foci (arrowhead in B indicates an eyespot). (C) Motile cell showing the dark-red, rectangular eyespot (arrowhead). (D–F) Motile thecate cells showing the variation in size and shape. (F–J) Immotile cells. (F–G) Immotile cell laying at the bottom of the cultivation plate. (H) Coccoid cell with thecal remnant. (I) Coccoid cells. (J) Dividing coccoid cell. Abbreviation: n: dinophyte nucleus. (For interpretation of the references to the color in this figure legend, the reader should refer to the web version of this article.)

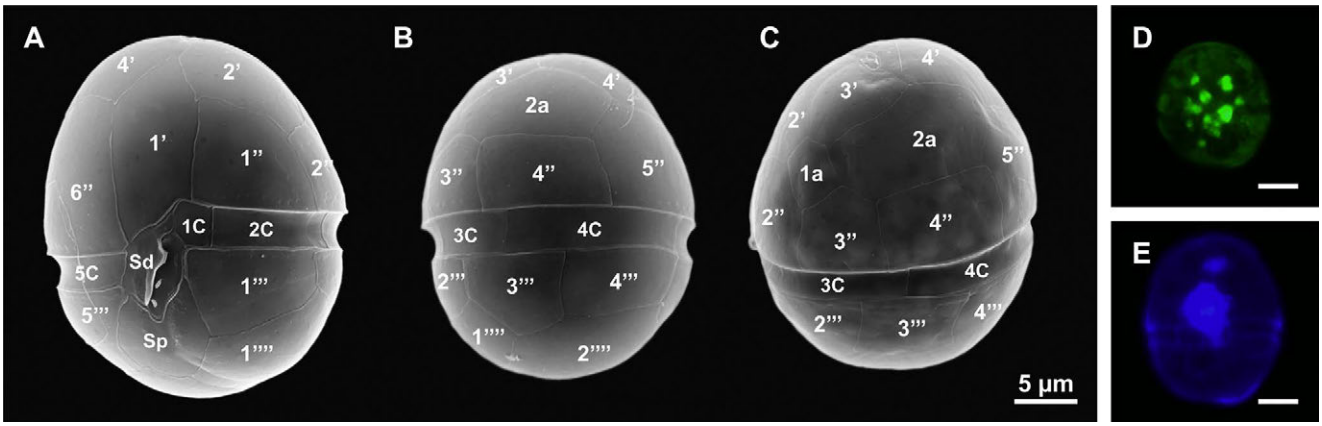

Fig. 4. Motile thecate cells of *Durinskia oculata* [GeoM*662; (A–C) scanning electron microscopy; at the same scale; (D–E) light microscopy; at the same scale]. (A–C) Tabulation pattern of the motile thecate cells. (A) Ventral view. (B) Dorsal view. (C) Dorsal-lateral view. (D) Motile cell under blue light excitation showing chloroplasts of an irregular shape (scale bar: 10µm). (E) DAPI-stained motile cell under UV light showing a large dinophyte nucleus (positioned below) and a small eukaryotic nucleus (positioned above; scale bar: 10µm). Abbreviations: n′: apical plate. n″: precingular plate. n‴: postcingular plate. n⁗: antapical plate. na: anterior intercalary plate. nC: cingular plate. Sd: right sulcal plate. Sp: posterior sulcal plate. (For interpretation of the references to the color in this figure legend, the reader should refer to the web version of this article.)

functioning and community dynamics during seasonal periods, as well as conservation strategies and the impact of invasive species. Until the present study, the taxonomic identity of *G. oculatum* was unclear primarily because of inconsistent interpretations of the thecal plate pattern. Hansen and Flaim (2007) were very cautious with their criticism about the analysis provided by Wołoszyńska (1917), but clearly stated morphological discrepancies between S.F.N.R. von Stein's species and that of J. Wołoszyńska. We agree, and the cells' outline in her drawings is rather obtusely angular at the anterior and posterior end and not as rounded as in *G. oculatum*. Furthermore, the cingulum appears wider in Wołoszyńska (1917) than in Stein (1883), and the displacement is less than half of the (and not as the entire, as Stein, 1883, described) cingulum width. Thus, doubts are allowed that the organisms investigated by S.F.N.R. von Stein and J. Wołoszyńska are conspecific (Hansen and Flaim, 2007; Zhang et al., 2011a; Cavalcante et al., 2017).

Our material collected at the type locality, Vltava river near Prague, is to a great extent consistent with the descriptions and drawings of S.F.N.R. von Stein, including the (very) widely ovate outline of the theca in ventral view, with a slightly larger epitheca. We further confirm the descendent displacement of the cingulum of about its own width (compare Fig. 4A with pl. III 5 of Stein 1883) as well as the eleutherochisis mode of division (pl. III 6). The presence of two daughter cells included in the shell of a coccoid cell (compare Fig. 3F with pl. III 7 of Stein 1883) is likewise crucial for a correct assignment of our material to *G. oculatum*. Such a trait has been rarely illustrated for other freshwater dinophyte species [e.g., in the only distantly related *Gymnodinium rotundatum* Klebs: Klebs, 1912; *Spiniferodinium limneticum* (Wołosz.) Kretschmann & Gottschling: Kretschmann et al., 2015; *P. apiculatus*: Kretschmann et al., 2017], though as the abundance across dinophytes and diagnostic value of this life history stage remains elusive. The consistency between the protologue and our newly established material refers also to subcellular details such as the position of the nucleus in the epitheca and the small, red eyespot positioned in the sulcal area of the hypotheca (whose ultrastructure is much better known today than in the 19th century: Moestrup and Daugbjerg, 2007). The transversal flagellum has been depicted as 'ciliate girdle' of the cingulum, but this (as we today know) wrong determination must be seen in a historical context. Thus, material of strain GeoM*662 is suitable for epitypification of *G. oculatum* in order to remove the taxonomic ambiguity currently linked with that name.

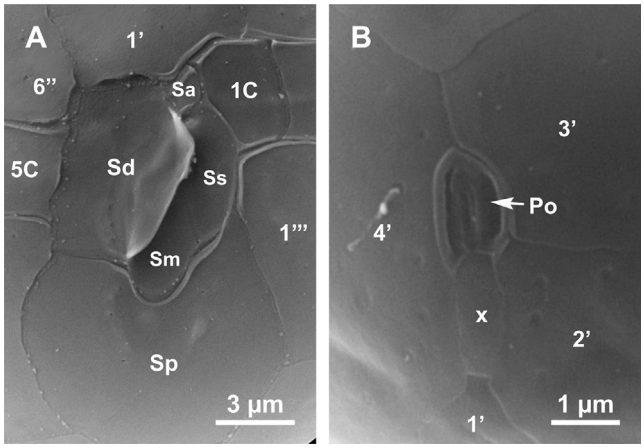

Fig. 5. Details of the tabulation pattern of motile thecate cells of *Durinskia oculata* (GeoM*662; scanning electron microscopy). (A) Ventral view of the sulcal region. (B) Apical view of the apical pore complex. Abbreviations: Po: apical pore plate. n′: apical plate. n″: precingular plate. n‴: postcingular plate. nC: cingular plate. pp: pore plate. Sa: anterior sulcal plate. Sd: right sulcal plate. Sm: median sulcal plate. Sp: posterior sulcal plate. Ss: left sulcal plate. x: canal (preapical) plate.

The epitypification of *G. oculatum* decides the long-lasting debate about the correct interpretation of the epithecal plate pattern. The ongoing morosity of the controversy is illustrated also by Lewis and Dodge (2011) and Carty (2014), who do not cite the transfer of *G. oculatum* to *Durinskia* (Hansen and Flaim, 2007) in their taxonomic headers of *Peridiniopsis oculata* (F.Stein) Bourr. (Table 1). Anyhow, the epitype material of *G. oculatum* clearly shows the existence of 4 apical, 2 anterior intercalary, and 6 precingular plates, as it has already been observed by Lindemann (1926) and later by Hansen and Flaim (2007). In turn, the interpretation of Wołoszyńska (1917), showing 3 apical, 1 anterior intercalary, and 7 precingular plates, has to be rejected hence: The application of the name in a number of textbooks (Thompson, 1951; Starmach, 1974; Popovský and Pfiester, 1990; Lewis and Dodge, 2011; Carty, 2014) does not correspond to S.F.N.R. von Stein's species, and the error must be corrected in future editions and publications.

Irrespectively of the name applied, all dinophytes with epithecae exhibiting the combination of 3 apical, 1 anterior intercalary, and 7 precingular plates are to be removed from *Durinskia*. The question remains, with which species Wołoszyńska (1917) initially confused *G. oculatum*. Hansen and Flaim (2007) did not provide an answer, but the plate pattern comprising 3 apical, 1 anterior intercalary and 7 precingular plates is otherwise found in dinophytes such as *Peridiniopsis lindemannii* (M.Lefèvre) Bourr. from Madagascar (Lefèvre, 1927).

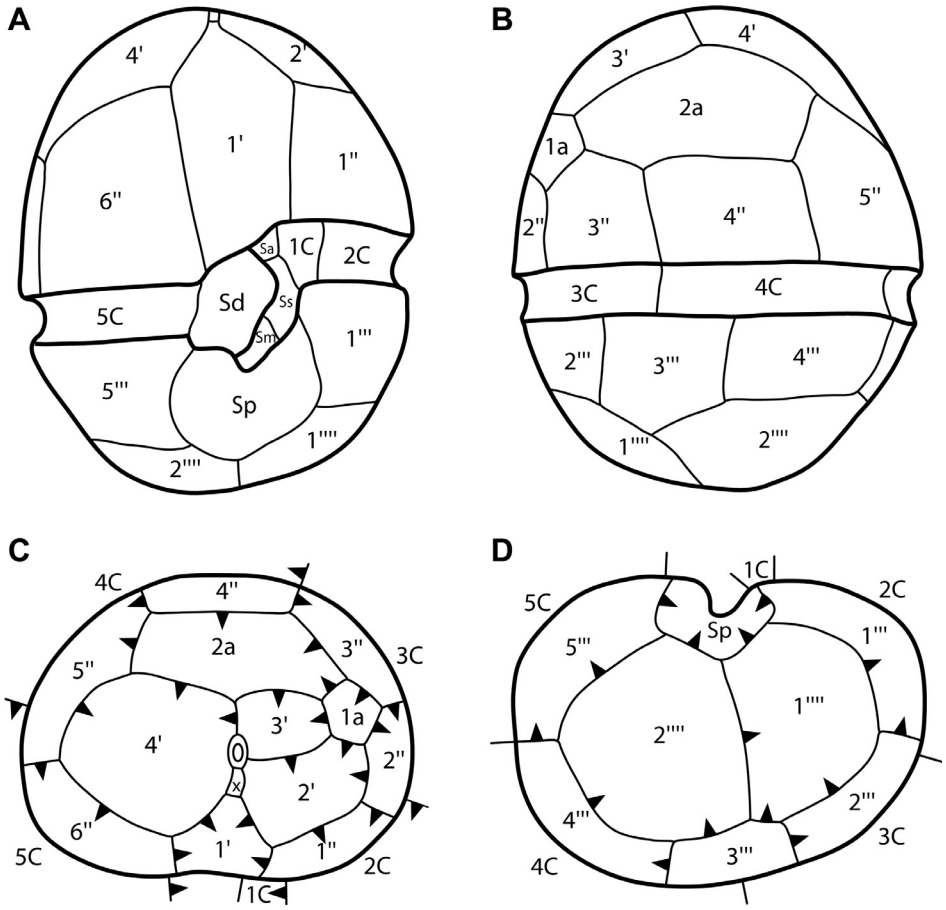

Fig. 6. Schematic drawing of the thecal plates. (A) Ventral view. (B) Dorsal view. (C) Apical view. (D) Antapical view. Abbreviations: n′: apical plate. n″: precingular plate. n‴: postcingular plate. n⁗: antapical plate. na: anterior intercalary plate. nC: cingular plate. Sa: anterior sulcal plate. Sd: right sulcal plate. Sm: median sulcal plate. Sp: posterior sulcal plate. Ss: left sulcal plate. x: canal (preapical) plate. Arrowheads in (C–D) indicate plate overlap pattern.

Jadwiga Wołoszyńska's species, however, differs from *P. lindemannii*, since it is only half of its size, and was not described before her treatment dating to 1917. Thus, the precise name to be applied to the species depicted in Wołoszyńska (1917) remains to be determined.

4.2. Interpretations of the molecular tree

In the molecular tree, *G. oculatum* collected at its type locality is clearly a member of the Kryptoperidiniaceae taxon *Durinskia* and thus, the corresponding taxonomic transfer (Hansen and Flaim, 2007) appears justified. Our strain shares the epitheca plate formula with all species of *Durinskia* (except *D. agilis*, which has 7 instead of 6 precingular plates: Saburova et al., 2012). The correct phylogenetic placement of *Durinskia oculata* (F.Stein) Gert Hansen & Flaim is also corroborated by apomorphic traits of the Kryptoperidiniaceae such as the second endosymbiont nucleus (Fig. 8) shown by DAPI staining in the present study. Furthermore, the Kryptoperidiniaceae are considered to exhibit a unique type of eyespot deriving from a relict plastid (Fig. 8; Dodge, 1984; Horiguchi and Pienaar, 1991; Kreimer, 1999; Moestrup and Daugbjerg, 2007; Pienaar et al., 2007; Takano et al., 2008).

The presence of 3 anterior intercalary plates is the predominant stage found in peridinialean dinophytes (present, for example, in freshwater *Peridinium* and marine *Scrippsiella* and members of the E/Pe-clade: Fensome et al., 1993, but also in *Leonella* Janofske & Karwath from the T/Pf-clade: Janofske and Karwath in Karwath, 2000, *Blastodinium*: Skovgaard et al., 2012, *Zooxanthella*: Probert et al., 2014), indicating the ancestral condition. Subsequently, the reduction to maximally 2 of such plates in Kryptoperidiniaceae (Pienaar et al., 2007; Saburova et al., 2012; You et al., 2015) can be considered the apomorphic state providing further evidence for the monophyly of the Kryptoperidiniaceae (Fig. 8). A comparable though not homologuous development of reduced thecal plate numbers has been shown for pfiesterian dinophytes (Calado et al., 2009). Within the Kryptoperidiniaceae, further reduction in number of epithecal main plates is known from *Unruhdinium* (Liu et al., 2008; Takano et al., 2008; Zhang et al., 2011b, 2014; You et al., 2015), which do not exhibit more than 10 such plates as an apomorphic trait (Fig. 8; Gottschling et al., 2017). *Galeidinium* is extreme in this respect, because it does not show (presumably as secondary loss) any thecal plate pattern (Tamura et al. 2005).

The majority of dinophyte species inhabit marine environments, but there are approximately 350 freshwater species known so far (Mertens et al., 2012). They are scattered over the dinophyte (and peridinialean) phylogenetic tree in mostly small species groups being only distantly related. Such topology is indicative for the ancestral condition of dinophytes (and Peridiniales) living in the marine environment. Nevertheless, marine to freshwater transitions have been considered rare in dinophytes (Logares et al., 2007b), which would be in agreement with other groups of organisms such as the green lineage comprising chlorophytes and land plants (Lewis and McCourt, 2004; Leliaert et al., 2012). The molecular tree, however, indicates that at least two lineages independently colonized the freshwater environment alone in the Kryptoperidiniaceae, namely *D. oculata* (with a single close relative, see below) and *Unruhdinium*. Both lineages are only distantly related to each other and find their closest relatives in dinophytes inhabiting the marine environment (which is true for virtually all freshwater lineages of dinophytes). This challenges once more the ideas of Logares et al. (2007b), whose taxon sample has not been as extensive as it is necessary to draw their general conclusions about the rarity of marine to freshwater transitions.

Freshwater lineages of dinophytes are highly polyphyletic, implying repeated colonization events from the marine into the freshwater

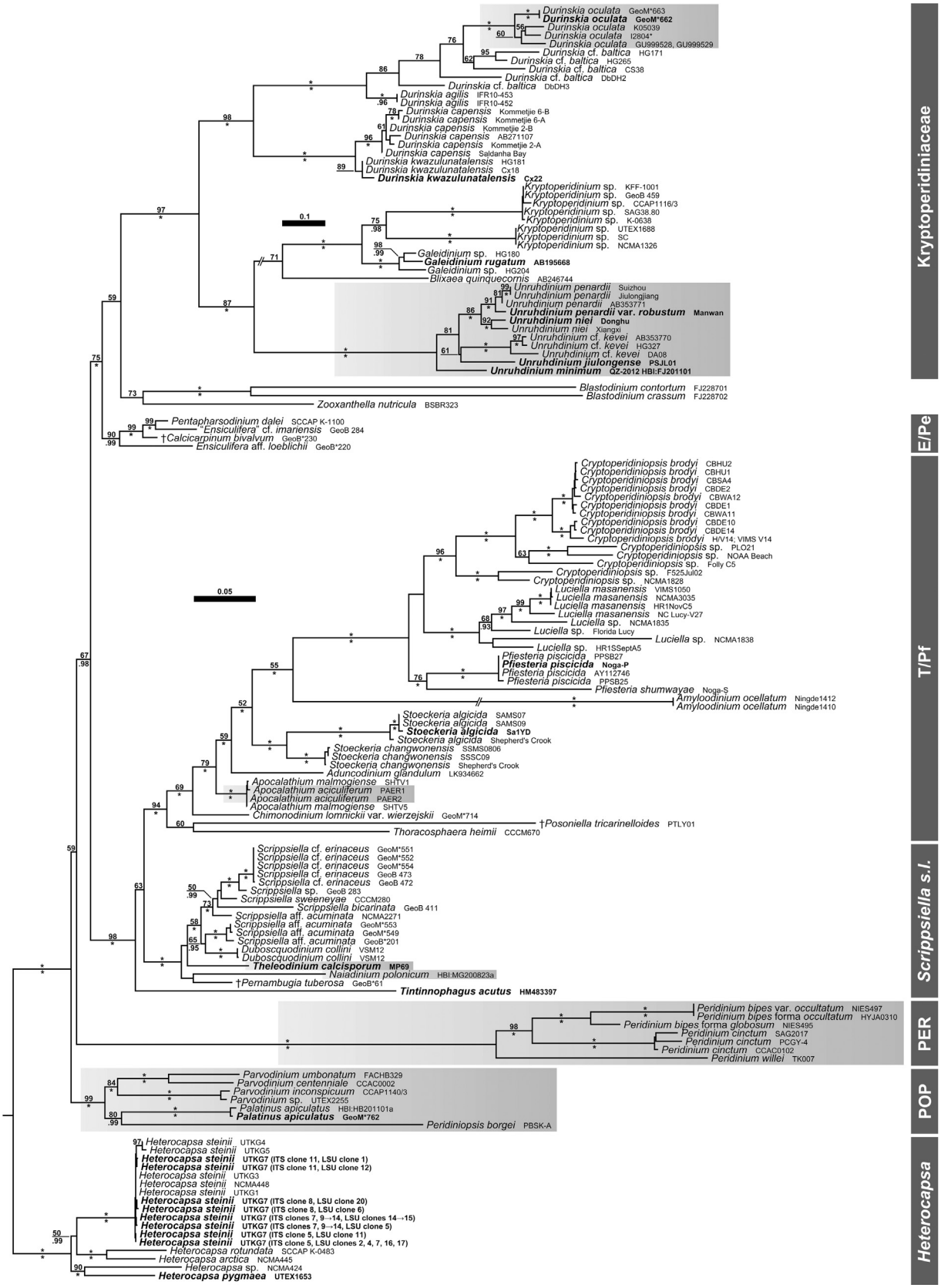

Fig. 7. Maximum Likelihood (ML) tree of 44 Kryptoperidiniaceae operational taxonomic units (OTUs), derived from the comparison of concatenated rRNA sequences. Freshwater taxa are shaded in grey, whereas those taxa are in bold, from which type material has been prepared. Branch lengths are drawn to scale, with the scale bar indicating the number of nt substitutions per site. The numbers on the branches are statistical support values (above: ML bootstrap values, values <50 are not shown; below: Bayesian posterior probabilities, values <.90 are not shown). Asterisks indicate maximal support. Abbreviations: E/Pe: clade including *Ensiculifera* Balech and *Pentapharsodinium* Indel. & A.R.Loebl. PER: Peridiniaceae. POP: Peridiniopsidaceae. T/Pf: clade including *Pfiesteria* and *Thoracosphaera*.

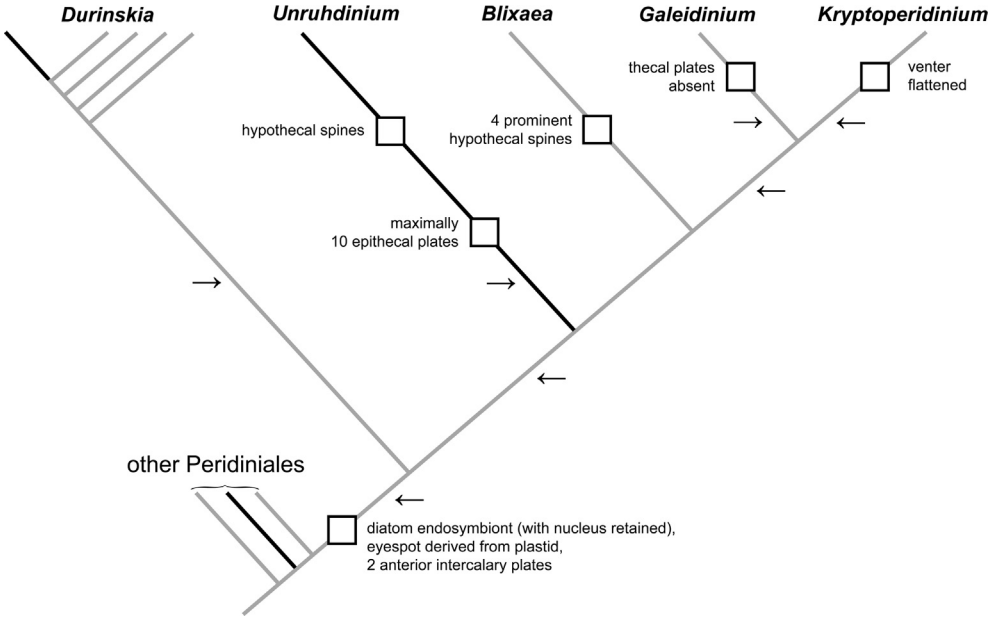

Fig. 8. Annotated cladogram summarizing the results of the study (freshwater lineages are in black, while marine lineages are in grey; apomorphies indicated by squares; arrows indicate high statistical support i.e., LBS > 75 and BPP > .95). Note that it is not clear yet whether antapical spines present in species of *Blixaea* and *Unruhidinium* are homologuous or a result of independent evolution.

environment. This has occurred multiple times even within single lineages such as the Gymnodiniaceae *s.str.* (Kretschmann et al., 2015), the Kryptoperidiniaceae (this study), and the Thoracosphaeraceae (Moestrup and Daugbjerg, 2007; Craveiro et al., 2013; Gottschling and Söhner, 2013). Other examples for a more dynamic evolutionary scenario for dinophytes are the single (though genetically differentiated) species *Alexandrium ostenfeldii* (Paulsen) Balech & Tangen and *Huia caspica* (Ostenf.) H. Gu, K.N. Mertens, T. Liu occurring in both marine and freshwater habitats (Kremp et al., 2014; Gu et al., 2016) as well as the phenotypically differentiated species pair *Apocalathium malmogiense* (G.Sjöstedt) Craveiro, Daugbjerg, Moestrup & Calado (brackish water) and *Apocalathium aciculiferum* (Lemmerm.) Craveiro, Daugbjerg, Moestrup & Calado (freshwater) that share identical rRNA sequences (Gottschling et al., 2005; Logares et al., 2007a; Annenkova et al., 2015). *Durinskia* also includes species living in brackish water (Levander, 1894), and it remains to be worked out whether this trait is a prerequisite for a gradual evolutionary process of marine to freshwater transitions, at least in some cases of the dinophytes.

4.3. Diagnosis of Durinskia oculata

The freshwater species pair *D. oculata* and *Durinskia dybowskii* (Wołosz.) Carty (of which molecular sequence data are not available at present) can be easily distinguished morphologically based on the cell surface being smooth *versus* porate, respectively (Table 1). The intraspecific consistency of such traits, however, must be worked out in future – in the newly established material of GeoM∗662, we have never observed any distinctive pores scattered over the cell surface. It is unclear at present whether this trait really is a diagnostic feature of *D. dybowskii*, and newly collected material from Ukraine (Wołoszyńska, 1916) may clarify this uncertainty. Anyhow, it is more difficult to morphologically distinguish between freshwater *D. oculata* and brackish (or even marine) *D. baltica* due to the lack of diagnostic traits. The molecular tree, however, clearly differentiates between freshwater and marine strains of *Durinskia* (molecular sequence data are not available for cells from brackish habitats at present), irrespectively of the name used: Our strain GeoM∗662 constitutes a monophyletic group together with other freshwater strains (though they have been initially determined as *D. baltica*: Zhang et al., 2011a). We now know the identity of *D. oculata* and have sequence information also from the endosymbiont (Žerdoner Čalasan et al., 2017) with a putative diagnostic importance. However, taxonomic clarification of *D. baltica* based on newly collected material from the type locality (Baltic Sea off Finland i.e., rather brackish than marine environment) is still required. Habitat preference is currently the only diagnostic trait between both species (compared in the key at the end of this study). It is likely that they also differ in terms of DNA sequence data, but this remains elusive until the epitypification of *D. baltica*. Unfortunately, little information is available about the morphology of sequenced *D.* cf. *baltica* strains so as to a more rigorous interpretation of the molecular tree would be possible. Additional work is necessary to enlighten the taxonomic status of the various *D.* cf. *baltica* lineages constituting a grade rather than a clade.

Conclusions about the spatial occurrence and the ecological niche established by species are as good as the quality of the underlying data. In this respect, the situation in *D. oculata* (but also for *D. baltica* and *D. dybowskii*) is currently not advancing due to the taxonomic and nomenclatural debates in the past and the divergent species delimitations in the literature (i.e., different names for the same species, but also different species subsumed under the same name; Table 1). The ostensible data that have been compiled about such species over the past 130 years should be treated with high caution, if they should be used at all. With the DNA sequence information (see also Žerdoner Čalasan et al., 2017) and the consistent plate pattern at hand, a reliable species determination of *D. oculata* is now possible. However, to work out the distribution of the species as well as its specific role in the ecosystem remains a considerable task for the future.

5. Taxonomic activity

***Durinskia oculata* (F.Stein) Gert Hansen & Flaim**, *nom. corr.* (ICN Art. 60.1.), Journal of Limnology 66: 134–136, fig. 31a–g (2007). *Glenodinium oculatum* F.Stein, Der Organismus der arthrodelen Flagellaten nach eigenen Forschungen in systematischer Reihenfolge bearbeitet 3.2: pl. III 5–7 (1883). *Peridiniopsis oculata* (F.Stein) Bourr., *nom. corr.* (ICN Art. 32.2), Protistologica 4: 9 (1968).—**Lectotype, designated here**: [illustration] pl. III 5! in Stein (1883), showing a non-fossil individual from Czech Republic, Vltava river near Prague [exact locality and collecting date unknown].—**Epitype, designated here**: Czech Republic. Hlavní město Praha, Prague, Praha-Sedlec, Vltava river, Sep 30, 2015 [non-fossil]: J. Kretschmann & M. Gottschling D043 [J. Kretschmann GeoM∗662] (CEDiT-2017E72!, duplicates: B 40 0042046!, M-0251367!).

6. Excluded names from *Durinskia*

***Clathrocysta aculeata* F.Stein**, Der Organismus der arthrodelen Flagellaten nach eigenen Forschungen in systematischer Reihenfolge bearbeitet 3.2: pl. IV 6 (1883). *Peridiniopsis aculeata* (F.Stein) Bourr., *nom. corr.* (ICN Art. 32.2.), as used in Starmach & Siemińska, Flora słodkowodna polski 4: 350, fig. 479 (1974). *Glenodinium aculeatum* F.Stein as used in Starmach & Siemińska, Flora słodkowodna polski 4: 350 (1974). The pretended basionym and corresponding combination have never been validly published.

Peridiniopsis baltica (Levander) Bourr., *nom. corr.* (ICN Art. 60.1) as used in Popovský & Pfiester, Süßwasserflora von Mitteleuropa: Dinophyceae (Dinoflagellida): 188 (1990) and subsequent works. The corresponding combination has never been validly published.

Peridinium oculatum (F.Stein) Wołosz., not validly published (ICN Art. 53.1., non *Peridinium oculatum* Dujard., Histoire naturelle des Zoophytes: 374–375. 1841), Rozprawy Wydzialu Matematyczno-Przyrodniczego Akademji Umiej¸etno'sci. Dzial B, Nauki biologiczne 57: 217 (1917).

7. Key

The following key comprises all lineages of the Kryptoperidiniaceae identified at present based on molecular sequence data. The diatom endosymbiont, the eyespot type, and a reduced number of anterior intercalary plates to maximally two are diagnostic and apomorphic traits of the taxon (Fig. 8). In particular, the key includes the 6 species of *Durinskia* that we currently accept.

1a.	Motile cell without thecal plates	*Galeidinium*
1b.	Motile cell exhibiting thecal plates	2
2a.	Motile cells with a strongly flattened venter	*Kryptoperidinium*
2b.	Motile cells globular through variously ovoid	3
3a.	Plate formula: 4′ 0a 6″ or 3′ 1a 6″	*Unruhdinium*
3b.	Number of epithecal main plates > 10	4
4a.	Motile cells exhibiting four long hypothecal spines	*Blixaea*
4b.	Motile cells without spines (*Durinskia*)	5
5a.	precingular plates 7; motile cell with an apical hook	*D. agilis*
5b.	precingular plates 6; motile cell without apical hook	6
6a.	Plates 1a and 1″ adjacent	7
6b.	Plates 1a and 1″ not adjacent	8
7a.	Plates 2a, 3″, and 4″ pentagonal, tetragonal, and pentagonal, respectively	*D. capensis*
7b.	Plates 2a, 3″, and 4″ hexagonal, pentagonal, and tetragonal, respectively	*D. kwazulunatalensis*
8a.	Brackish/marine species	*D. baltica*
8b.	Freshwater species	9
9a.	Thecal plates with porate ornamentation in horizontal rows	*D. dybowskii*
9b.	Thecal plates with few irregularly scattered pores	*D. oculata*

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ympev.2017.10.011.

References

Annenkova, N.V., Hansen, G., Moestrup, Ø., Rengefors, K., 2015. Recent radiation in a marine and freshwater dinoflagellate species flock. ISME J. 9, 1821–1834.
Brinkmann, N., Hodač, L., Mohr, K.I., Hodačová, A., Jahn, R., Ramm, J., Hallmann, C., Arp, G., Friedl, T., 2015. Cyanobacteria and diatoms in biofilms of two karstic streams in Germany and changes of their communities along calcite saturation gradients. Geomicrobiol. J. 32, 255–274.
Calado, A.J., Craveiro, S.C., Daugbjerg, N., Moestrup, Ø., 2009. Description of *Tyrannodinium* gen. nov., a freshwater dinoflagellate closely related to the marine *Pfiesteria*-like species. J. Phycol. 45, 1195–1205.
Carty, S., 2014. Freshwater Dinoflagellates of North America. Cornell University Press, Ithaca.
Cavalcante, K.P., Craveiro, S.C., Calado, A.J., Ludwig, T.A.V., Cardoso, L. de S., 2017. Diversity of freshwater dinoflagellates in the State of Paraná, southern Brazil, with taxonomic and distributional notes. Fottea 17, 240–263.
Chesnick, J.M., Kooistra, W.H.C.F., Wellbrock, U., Medlin, L.K., 1997. Ribosomal RNA analysis indicates a benthic pennate diatom ancestry for the endosymbionts of the dinoflagellates *Peridinium foliaceum* and *Peridinium balticum* (Pyrrhophyta). J. Eukaryot. Microbiol. 44, 314–320.
Compère, P., 1999. Report of the Committee for Algae: 6. Taxon 48, 811–814.
Coolen, M.J.L., Muyzer, G., Rijpstra, W.I.C., Schouten, S., Volkman, J.K., Damste, J.S.S., 2004. Combined DNA and lipid analyses of sediments reveal changes in Holocene haptophyte and diatom populations in an Antarctic lake. Earth Planet. Sci. Lett. 223, 225–239.
Craveiro, C.S., Pandeirada, M.S., Daugbjerg, N., Moestrup, Ø., Calado, A.J., 2013. Ultrastructure and phylogeny of *Theleodinium calcisporum* gen. et sp. nov., a freshwater dinoflagellate that produces calcareous cysts. Phycologia 52, 488–507.
Darki, B.Z., 2014. Recognition of continental dinoflagellates of Iran. Iran. J. Bot. 20, 130–142.
Dodge, J.D., 1984. The functional and phylogenetic significance of dinoflagellate eyespots. Biosystems 16, 259–267.
Fensome, R.A., Taylor, F.J.R., Norris, G., Sarjeant, W.A.S., Wharton, D.I., Williams, G.L., 1993. A classification of living and fossil dinoflagellates. Micropaleontol., Special Pap. 7, 1–245.
Gagat, P., Bodył, A., Mackiewicz, P., Stiller, J.W., 2014. Tertiary plastid endosymbioses in dinoflagellates. In: Löffelhardt, W. (Ed.), Endosymbiosis. Springer, Vienna, pp. 233–290.
Gottschling, M., Keupp, H., Plötner, J., Knop, R., Willems, H., Kirsch, M., 2005. Phylogeny of calcareous dinoflagellates as inferred from ITS and ribosomal sequence data. Mol. Phylogenet. Evol. 36, 444–455.
Gottschling, M., McLean, T.I., 2013. New home for tiny symbionts: Dinophytes determined as *Zooxanthella* are Peridiniales and distantly related to *Symbiodinium*. Mol. Phylogenet. Evol. 67, 217–222.
Gottschling, M., Plötner, J., 2004. Secondary structure models of the nuclear Internal Transcribed Spacer regions and 5.8S rRNA in Calciodinelloideae (Peridiniaceae) and other dinoflagellates. Nucl. Acids Res. 32, 307–315.
Gottschling, M., Söhner, S., 2013. An updated list of generic names in the Thoracosphaeraceae. Microorganisms 1, 122–136.
Gottschling, M., Söhner, S., Zinßmeister, C., John, U., Plötner, J., Schweikert, M., Aligizaki, K., Elbrächter, M., 2012. Delimitation of the Thoracosphaeraceae (Dinophyceae), including the calcareous dinoflagellates, based on large amounts of ribosomal RNA sequence data. Protist 163, 15–24.
Gottschling, M., Žerdoner Čalasan, A., Kretschmann, J., Gu, H., 2017. Two new generic names for dinophytes harbouring a diatom as an endosymbiont, *Blixaea* and *Unruhdinium* (Kryptoperidiniaceae, Peridiniales). Phytotaxa 306, 296–300.
Gu, H., Kirsch, M., Zinßmeister, C., Söhner, S., Meier, K.J.S., Liu, T., Gottschling, M., 2013. Waking the dead: Morphological and molecular characterization of extant †*Posoniella tricarinelloides* (Thoracosphaeraceae, Dinophyceae). Protist 164, 583–597.
Gu, H., Mertens, K.N., Liu, T., 2016. *Huia caspica* gen. & comb. nov., a dinoflagellate species that recently crossed the marine-freshwater boundary. Phycol. Res. 64, 251–258.
Guillard, R.R., Lorenzen, C.J., 1972. Yellow-green algae with chlorophyllide c. J. Phycol. 8, 10–14.
Hansen, G., Daugbjerg, N., Henriksen, P., 2007. *Baldinia anauniensis* gen. et sp nov.: A 'new' dinoflagellate from Lake Tovel, N. Italy. Phycologia 46, 86–108.
Hansen, G., Flaim, G., 2007. Dinoflagellates of the Trentino Province, Italy. J. Limnol. 66, 107–141.
Horiguchi, T., Pienaar, R.N., 1991. Ultrastructure of a marine dinoflagellate, *Peridinium quinquecorne* Abe (Peridiniales) from South-Africa with particular reference to its chrysophyte endosymbiont. Bot. Mar. 34, 123–131.
Horiguchi, T., Pienaar, R.N., 1994. Ultrastructure of a new marine sand-dwelling dinoflagellate, *Gymnodinium quadrilobatum* sp. nov. (Dinophyceae) with special reference to its endosymbiotic alga. Eur. J. Phycol. 29, 237–245.
Horiguchi, T., Takano, Y., 2006. Serial replacement of a diatom endosymbiont in the marine dinoflagellate *Peridinium quinquecorne* (Peridiniales, Dinophyceae). Phycol. Res. 54, 193–200.
Huber-Pestalozzi, G., 1968 Das Phytoplankton des Süßwassers. Systematik und Biologie, second ed. Schweizerbart, Stuttgart.
Imanian, B., Pombert, J.-F., Keeling, P.J., 2011. The complete plastid genomes of the two 'dinotoms' *Durinskia baltica* and *Kryptoperidinium foliaceum*. PLoS ONE 5, e10711.
Janofske, D., 2000. *Scrippsiella trochoidea* and *Scrippsiella regalis*, nov. comb. (Peridiniales, Dinophyceae): A comparison. J. Phycol. 36, 178–189.
Karwath, B., 2000. Ecological studies on living and fossil calcareous dinoflagellates of the equatorial and tropical Atlantic Ocean. Berichte, Fachbereich Geowissenschaften,

Universität Bremen 152, 1–175.
Katoh, K., Standley, D.M., 2013. MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. Mol. Biol. Evol. 30, 772–780.
Klebs, G.A., 1912. Über Flagellaten- und Algen-ähnliche Peridineen. Verh. Naturhist.-Med. Vereins Heidelberg n.s. 11, pp. 369–451.
Kofoid, C.A., Swezy, O., 1921. The Free-Living Unarmored Dinoflagellata. University of California Press, Berkeley.
Крахмальный, А.Ф., 2011. Динофитовые водоросли (Dinophyta) украинского Полесья. In: Сенченко, ГГ, Смаля ІВ (Eds.), Сучасні екологічні проблемн Українського Полісся і суміжнн терторій: зб. наук. працъ / За ред, Lysenko, Nizhyn, pp. 87–97.
Kreimer, G., 1999. Reflective properties of different eyespot types in dinoflagellates. Protist 150, 311–323.
Kremp, A., Tahvanainen, P., Litaker, W., Krock, B., Suikkanen, S., Leaw, C.P., Tomas, C., 2014. Phylogenetic relationships, morphological variation, and toxin patterns in the *Alexandrium ostenfeldii* (Dinophyceae) complex: Implications for species boundaries and identities. J. Phycol. 50, 81–100.
Kretschmann, J., Filipowicz, N.H., Owsianny, P.M., Zinßmeister, C., Gottschling, M., 2015. Taxonomic clarification of the unusual dinophyte *Gymnodinium limneticum* Wołosz. (Gymnodiniaceae) from the Tatra Mountains. Protist 166, 621–637.
Kretschmann, J., Žerdoner Čalasan, A., Kusber, W.-H., Gottschling, M., 2017. Still curling after all these years: *Glenodinium apiculatum* Ehrenb. (Peridiniales, Dinophyceae) repeatedly found at its type locality in Berlin (Germany). Syst. Biodivers (in press).
Lefèvre, M.M., 1927. Sur les variations tabulaires chez les Péridiniens d'eau douce et leur notation. — Diagnoses d'espèces et de variétés nouvelles. Bull. Mus. Natl Hist. Nat. 33, 118–122.
Leliaert, F., Smith, D.R., Moreau, H., Herron, M.D., Verbruggen, H., Delwiche, C.F., De Clerck, O., 2012. Phylogeny and molecular evolution of the green algae. Crit. Rev. Plant Sci. 31, 1–46.
Levander, K.M., 1894. Materialien zur Kenntnis der Wasserfauna in der Umgebung von Helsingfors, mit besonderer Berücksichtigung der Meeresfauna I. Protozoa. Acta Soc. Fauna Flora Fennica 12, 1–115.
Lewis, J., Dodge, J.D., 2011. Phylum Dinophyta (Dinoflagellates). In: John, D.M., Whitton, B.A., Brook, A.J. (Eds.), The Freshwater Algal Flora of the British Isles: An Identification Guide to Freshwater and Terrestrial Algae. Cambridge University Press, Cambridge, pp. 250–274.
Lewis, L.A., McCourt, R.M., 2004. Green algae and the origin of land plants. Am. J. Bot. 91, 1535–1556.
Lindemann, E.B.L.W., 1926. Bewegeliche Hüllenfelderung und ihr Einfluss auf die Frage der Artbildung bei Glenodinien. Archiv Hydrobiol. 16, 437–458.
Liu, G.X., Pei, G.F., Hu, Z.-Y., 2008. *Peridiniopsis niei* sp. nov. (Dinophyceae), a new species of freshwater red tide dinoflagellates from China. Nova Hedwigia 87, 487–499.
Logares, R., Rengefors, K., Kremp, A., Shalchian-Tabrizi, K., Boltovskoy, A., Tengs, T., Shurtleff, A., Klaveness, D., 2007a. Phenotypically different microalgal morphospecies with identical ribosomal DNA: A case of rapid adaptive evolution? Microb. Ecol. 53, 549–561.
Logares, R., Shalchian-Tabrizi, K., Boltovskoy, A., Rengefors, K., 2007b. Extensive dinoflagellate phylogenies indicate infrequent marine-freshwater transitions. Mol. Phylogenet. Evol. 45, 887–903.
Medlin, L., Elwood, H.J., Stickel, S., Sogin, M.L., 1988. The characterization of enzymatically amplified eukaryotic 16S-like rRNA-coding regions. Gene 71, 491–499.
Mertens, K.N., Rengefors, K., Moestrup, Ø., Ellegaard, M., 2012. A review of recent freshwater dinoflagellate cysts: Taxonomy, phylogeny, ecology and palaeocology. Phycologia 51, 612–619.
Miller, M.A., Pfeiffer, W., Schwartz, T., 2010 Creating the CIPRES Science Gateway for inference of large phylogenetic trees. New Orleans LA. In: Proc. Gateway Comp. Env. Worksh., pp. 1–8.
Moestrup, Ø., Daugbjerg, N., 2007. On dinoflagellate phylogeny and classification. In: Brodie, J., Lewis, J. (Eds.), Unravelling the Algae, the Past, Present, and Future of Algal Systematics. CRC Press, Boca Raton, pp. 215–230.
Ostenfeld, C.H., 1907. Beiträge zur Kenntnis der Algenflora des Kossogol-Beekens in der nordwestlichen Mongolei, mit spezieller Berücksichtigung des Phytoplanktons. Hedwigia 46, 365–420.
Pienaar, R.N., Sakai, H., Horiguchi, T., 2007. Description of a new dinoflagellate with a diatom endosymbiont, *Durinskia capensis* sp. nov. (Peridiniales, Dinophyceae) from South Africa. J. Plant Res. 120, 247–258.
Popovský, J., Pfiester, L.A., 1990. Dinophyceae (Dinoflagellida). Fischer, Stuttgart.
Probert, I., Siano, R., Poirier, C., Decelle, J., Biard, T., Tuji, A., Suzuki, N., Not, F., 2014. *Brandtodinium* gen. nov. and *B. nutricula* (Dinophyceae), a dinoflagellate commonly found in symbiosis with polycystine radiolarians. J. Phycol. 50, 388–399.
Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D.L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M.A., Huelsenbeck, J.P., 2012. MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. Syst. Biol. 61, 539–542.
Saburova, M., Chomérat, N., Hoppenrath, M., 2012. Morphology and SSU rDNA phylogeny of *Durinskia agilis* (Kofoid & Swezy) (Peridiniales, Dinophyceae), a thecate, marine, sand-dwelling dinoflagellate formerly classified within *Gymnodinium*. Phycologia 51, 287–302.
Schiller, J., 1937 Flagellatae, in: Kolkwitz R (ed.), Rabenhorst's Kryptogamen-Flora. Zweite Auflage. Band 10, Abt. 3, Teil 2. Alt. t.p.: Dinoflagellatae (Peridineae). Winter, Leipzig.
Skovgaard, A., Karpov, S.A., Guillou, L., 2012. The parasitic dinoflagellates *Blastodinium* spp. inhabiting the gut of marine, planktonic copepods: Morphology, ecology, and unrecognized species diversity. Front. Microbiol. 3 305-305.
Stamatakis, A., 2014. RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30, 1312–1313.
Starmach, K., 1974. Cryptophyceae—Kryptofity, Dinophyceae—Dinofity, Raphidophyceae—Rafidofity. Państwowe Wydawnictwo Naukowe, Warsaw.
Stein, S.F.N.R. von, 1883. Der Organismus Der Infusionsthiere Nach Eigenen Forschungen in Systematischer Reihenfolge Bearbeitet 2. Engelmann, Leipzig.
Takano, Y., Hansen, G., Fujita, D., Horiguchi, T., 2008. Serial replacement of diatom endosymbionts in two freshwater dinoflagellates, *Peridiniopsis* spp. (Peridiniales, Dinophyceae). Phycologia 47, 41–53.
Tamura, M., Shimada, S., Horiguchi, T., 2005. *Galeidinium rugatum* gen. et sp. nov. (Dinophyceae), a new coccoid dinoflagellate with a diatom endosymbiont. J. Phycol. 41, 658–671.
Thompson, R.H., 1951. A new genus and new records of fresh-water Pyrrophyta in the Desmokontae and Dinophyceae. Lloydia 13, 277–299.
Tillmann, U., Hoppenrath, M., Gottschling, M., Kusber, W.-H., Elbrächter, M., 2017. Plate pattern clarification of the marine dinophyte *Heterocapsa triquetra* sensu Stein (1883) collected at the Kiel Fjord (Germany). J. Phycol (in press).
Tomas, R.N., Cox, E.R., Steidinger, K.A., 1973. *Peridinium balticum* (Levander) Lemmermann, an unusual dinoflagellate with a mesocaryotic and an eukaryotic nucleus. J. Phycol. 9, 91–98.
von Dassow, P., Chepurnov, V.A., Armbrust, E.V., 2006. Relationships between growth rate, cell size, and induction of spermatogenesis in the centric diatom *Thalassiosira weissflogii* (Bacillariophyta). J. Phycol. 42, 887–899.
Wołoszyńska, J., 1916. Polskie Peridineae słodkowodne. – Polnische Süßwasser-Peridineen. Bull. Int. Acad. Sci. Cracovie Cl. Sci. Mat. Nat. Sér. B 1915, 260–285.
Wołoszyńska, J., 1917. Budowa okrywy u niektórych Gymno- i Glenodiniów. Rozprawy Wydzialu Matematyczno-Przyrodniczego Akademji Umiejętności. Dzial B, Nauki Biologiczne 57, 185–220.
Yamada, N., Sym, S.D., Horiguchi, T., 2017. Identification of highly divergent diatom-derived chloroplasts in dinoflagellates, including a description of *Durinskia kwazulunatalensis* sp. nov. (Peridiniales, Dinophyceae). Mol. Biol. Evol. 34, 1335–1351.
You, X., Luo, Z., Su, Y., Gu, L., Gu, H., 2015. *Peridiniopsis jiulongensis*, a new freshwater dinoflagellate with a diatom endosymbiont from China. Nova Hedwigia 101, 313–326.
Žerdoner Čalasan, A., Kretschmann, J., Gottschling, M., 2017. Absence of co-phylogeny indicates repeated diatom capture in dinophytes hosting a tertiary endosymbiont. Org. Divers. Evol (in press).
Zhang, Q., Liu, G.-X., Hu, Z.-Y., 2014. Description of a new freshwater bloom-forming dinoflagellate with a diatom endosymbiont, *Peridiniopsis minima* sp. nov. (Peridiniales, Dinophyceae) from China. Algolog. Stud. 145, 119–133.
Zhang, Q., Liu, G.-X., Hu, Z.-Y., 2011a. *Durinskia baltica* (Dinophyceae), a newly recorded species and genus from China, and its systematics. J. Syst. Evol. 49, 476–485.
Zhang, Q., Liu, G.-X., Hu, Z.-Y., 2011b. Morphological differences and molecular phylogeny of freshwater blooming species, *Peridiniopsis* spp. (Dinophyceae) from China. Eur. J. Protistol. 47, 149–160.

Publication 3

Still curling after all these years: *Glenodinium apiculatum* Ehrenb. (Peridiniales, Dinophyceae) repeatedly found at its type locality in Berlin (Germany)

KRETSCHMANN, J., ŽERDONER ČALASAN, A., KUSBER, W.-H. & GOTTSCHLING, M.

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Research Article

Still curling after all these years: *Glenodinium apiculatum* Ehrenb. (Peridiniales, Dinophyceae) repeatedly found at its type locality in Berlin (Germany)

JULIANE KRETSCHMANN[1], ANŽE ŽERDONER ČALASAN[1], WOLF-HENNING KUSBER[2] & MARC GOTTSCHLING[1]

[1]Department Biologie, Systematische Botanik und Mykologie, GeoBio-Center, Ludwig-Maximilians-Universität München, Menzinger Str. 67, D – 80 638 Munich, Germany
[2]Botanischer Garten und Botanisches Museum Berlin-Dahlem, Freie Universität Berlin, Königin-Luise-Straße 6-8, D – 14195 Berlin, Germany

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The contemporary occurrence of dinophytes at their type localities has not been intensely studied so far, despite the type locality's crucial importance for any reliable scientific name application. The microscopist and phycologist Ch.G. Ehrenberg described a number of dinophyte species more than 150 years ago, many of which are currently taxonomically ambiguous. We collected water tow and sediment samples at those same localities in Berlin that Ch.G. Ehrenberg may have visited as well. We isolated and established several strains of *Glenodinium apiculatum* that we investigated by applying contemporary microscopic and molecular methods. The plate formula of the species was 4′, 2a, 7″, 6c, 5s, 5‴, 2⁗, without an apical pore complex, and the most distinctive morphological trait of *Glenodinium apiculatum* was the spiny hypotheca. The spines were irregularly scattered over hypothecal plate surface and arranged in raised edges between thecal plates. As inferred from molecular phylogenetics, *Glenodinium apiculatum* is assigned to *Palatinus*, which is an element of the Peridiniopsidaceae as a part of the Peridiniales. For taxonomic purposes, we epitypified Ch.G. Ehrenberg's taxon with newly collected material to ensure a reliable determination in the future. *Palatinus apiculatus* is not a fleeting star, and a number of dinophytes show a remarkably high fidelity to the sites from which they were originally described, even if the description was carried out a long time ago.

Key words: biogeography, dinoflagellates, Ehrenberg, niche, protists, *Palatinus*, taxonomy

Introduction

Almost 180 years ago, Ch.G. Ehrenberg (1795–1876) described *Glenodinium apiculatum* Ehrenb. in his seminal work 'Infusionsthierchen' (Ehrenberg, 1838). The published figures (corresponding to sheet 671: Fig. 1, deposited in the Ehrenberg collection curated at the Museum for Natural History, Berlin: BHUPM) show dinophyte cells with a length of ~50 μm. They exhibit distinct thecal plates showing an asymmetrical plate pattern of the epitheca, multiple chloroplast lobes, and an eyespot in the sulcal region. The cells are spherical through ovate and elliptic in outline, with a more or less distinct twist of the epitheca to the left in relation to the hypotheca. However, the most distinctive trait of the species is the presence of multiple minute spines at the antapex. Ehrenberg (1838) observed the species regularly over several years in the spring (e.g., 2 Apr 1835, noted on sheet 671) 'near Berlin', but the exact locality is unknown. During the 'Infusionsthierchen'-period, Berlin metropolis was much smaller than nowadays, and Ch.G. Ehrenberg appears to have been predominantly collecting outside the gates of the city in the 'Thiergarten' (corresponding to today's inner-city park 'Großer Tiergarten' as part of the Tiergarten district: Mollenhauer, 2002). For aquatic habitats near Berlin in the 1830s, a lower nutrient load and a rather muddy substrate have been inferred, but today, significant changes are encountered towards demarcated water bodies with sinking groundwater levels and a drier climate (Geissler, Kusber, & Jahn, 2004).

Correspondence to: Marc Gottschling. E-mail: gottschling@bio.lmu.de

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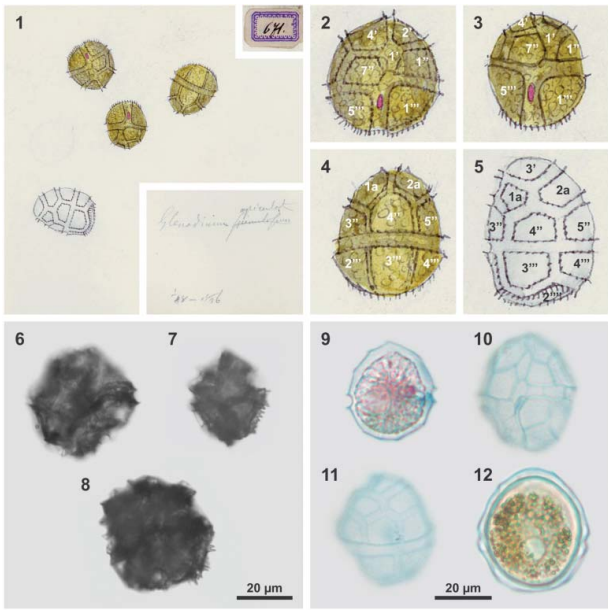


Figs 1–12. *Glenodinium apiculatus*. 1–5. Ch.G. Ehrenberg's original material of *Glenodinium apiculatus.* 1. water-coloured drawings (sheet 671, deposited in the Ehrenberg collection curated at the Museum for Natural History, Berlin: BHUPM; http://download.naturkundemuseum-berlin.de/Ehrenberg/Ec%20Drawings/Ec%20draw%20001-999/Ec%20draw%20600-699/ECdraw671.jpg). 2–5. enlarged original water-coloured drawings of sheet 671 with thecal plate labelled using the Kofoidean system. 6–8. dried motile cells on a mica mounted with Canada balsam prepared by Ch.G.Ehrenberg (LM). 9–12. epitype of *Glenodinium apiculatus* (GeoM*762; stained with astra blue and eosin; LM). 9. motile cell. 10. empty theca in ventral view. 11. empty theca in lateral-dorsal view. 12. coccoid cell. Abbreviations: n′: apical plate. n″: precingular plate. n‴: postcingular plate. n⁗: antapical plate. na: anterior intercalary plate.

It seems as though neither Lauterborn (1896) nor Lemmermann (1900) were aware of Ch.G. Ehrenberg's species and described *Peridinium palatinum* Lauterborn (Fig. S1) and *Peridinium marssonii* Lemmerm., respectively, exhibiting spinulose antapical plates as well. Shortly after, Huitfeldt-Kaas (1900) was again unaware of *G. apiculatum* and the work of Lauterborn (1896) and Lemmermann (1900) and described another species with 'spines in the corners of polar plates', namely *Peridinium laeve* Huitf.-Kaas. Notably, V.V.H. Huitfeldt-Kaas (1867–1941) was the first, who also illustrated the epithecal plate pattern of his species exhibiting a symmetrical arrangement (Fig. S4). Ten years later, Lemmermann (1910) provided the first illustrations of *P. marssonii* having a distinctly asymmetrical arrangement of epithecal plates (Fig. S2). Lindemann (1925, 1928) considered the difference between the symmetrical and asymmetrical conformation rather as expression of two forms present in a single species, and it was thus left to Lefèvre (1925) in a summarizing approach to recognize the distinctiveness of the trait corresponding to the uniqueness of two species. However, he described a species new to science, *Peridinium pseudolaeve* M.Lefèvre (Fig. S5), relying on E. Lindemann's interpretation of, instead of relying directly on, V.V.H. Huitfeldt-Kaas' species.

Today, Ch.G. Ehrenberg's and M. Lefèvre's species are assigned to *Palatinus* Craveiro, Calado, Daugbjerg & Moestrup, from which two species are currently recognized (Craveiro, Calado, Daugbjerg, & Moestrup, 2009), namely *P. apiculatus* (Ehrenb.) Craveiro, Calado, Daugbjerg & Moestrup from Berlin (type species) and *P. pseudolaevis* (M.Lefèvre) Craveiro, Calado, Daugbjerg & Moestrup from Upper Savoy in France. They share the presence of variously spinose ridges, particularly between hypothecal plates, and absence of an apical pore complex (APC). The two species can be distinguished based on the conformation of the epitheca having a plate formula 4′ 2a 7″: the plates are arranged symmetrically in *P. pseudolaevis* (Huitfeldt-Kaas, 1900; Lefèvre, 1925), but are distinctively displaced in *P. apiculatus* leading to a characteristically elongated second intercalary plate (Craveiro et al., 2009; Ehrenberg, 1838). *Palatinus apiculatus* is a well-known species, whose ultrastructure (Craveiro et al., 2009) shows a large central pyrenoid, which is penetrated by cytoplasmic tubes and radiates into chloroplast lobes. The presence of a peduncle differentiates the species from Peridiniaceae s.str. (Calado, Hansen, & Moestrup, 1999; Craveiro et al., 2009).

Despite *Palatinus apiculatus* being a distinct and easily recognizable dinophyte species, it should be linked taxonomically to contemporary material. There is an on-going discussion (also in terms of ecological restoration), whether such material should be chosen from originally local populations or rather via an ecology-based approach (Jones, 2013; McKay, Christian, Harrison, & Rice, 2005; Seddon, 2010). To the best of our knowledge, the presence of dinophyte species at their type localities has only been investigated by a handful of studies, and our own experience indicates that they in fact show remarkably high site fidelity. They are thus still present in localities, where they were originally described from (even if this is a century or more years ago). We here report on established strains that have been collected in the Tiergarten (Berlin, Germany) and correspond to the protologue and Ch.G. Ehrenberg's illustrations of *G. apiculatum*. By explicit investigations of such material, we thus aim at contributing to a reliable and consistent taxonomy of freshwater dinophytes and promoting the importance of the type locality in such cases.

Materials and methods

Cultivation and morphology

Water tow samples were collected using a plankton net with a mesh size of 20 μm at different localities in the

Tiergarten district (Berlin, Germany) on 25 June 2015 (52°30.831′N, 13°20.770′E) and 28 March 2016 (52°30.746′N, 13°20.508′E). Single motile cells were isolated and placed in 24-well microplates (Zefa; Munich, Germany) containing freshwater WC growth medium (Woods Hole Combo, modified after Guillard & Lorenzen, 1972) without silicate. The plates were stored in climate chambers at 12°C or 18°C and a 12:12 h L:D photoperiod. The established monoclonal strains are currently held in the culture collection at the Institute of Systematic Botany and Mycology (University of Munich) and are available upon request. Substrains have been submitted to the Collection of Algae at the University of Cologne (CCAC) and the Culture Collection of Baltic Algae (CCBA).

For the preparation of the epitype, cells of the monoclonal strain GeoM*762 (collected on 28 March 2016) were fixed with 2.5% glutaraldehyde (agar scientific; Stansted, Essex, UK). Double-staining was performed using 0.5% (water-based) astra blue in 2% tartaric acid (Fluka; Buchs, Switzerland) in WC medium and 0.1% (ethanol-based) eosin (Merck; Darmstadt, Germany) during a graded ethanol (Roth; Karlsruhe, Germany) series. Ethanol-based Technovit 7100 (Heraeus; Wehrheim, Germany) was used for embedding, following the manufacturer's instructions. For the final preparation, 40 μl aliquots of the Technovit mixture including the embedded samples were transferred to three slides. The epitype is deposited at the Centre of Excellence for Dinophyte Taxonomy (CEDiT; Wilhelmshaven, Germany), and duplicates are held in Berlin, B and Munich, M (see section on Taxonomic evaluation). Types and names are registered at PhycoBank [http://phycobank.org].

Cells were observed, documented and measured with a CKX41 inverted microscope (Olympus; Hamburg, Germany) equipped with a phase contrast option and a DP73 digital camera (Olympus). The preparative techniques for light and scanning electron microscopy (SEM) followed standard protocols (Janofske, 2000) and were the same as described in Gottschling et al. (2012). Briefly, cells were fixed in 2.5% glutaraldehyde overnight. Afterwards, specimens were dehydrated in a graded acetone series and critical point dried, followed by sputter-coating with platinum. The Kofoidean system (Fensome et al., 1993; Taylor, 1980) was used to designate the plate formula. Image adjustments (such as scaling, cropping, white-balancing, colour management) were done in Photoshop® and Illustrator® (Adobe Systems; Munich, Germany), respectively, and images were arranged with QuarkXPress® (Quark Software; Hamburg, Germany).

Molecular phylogenetics

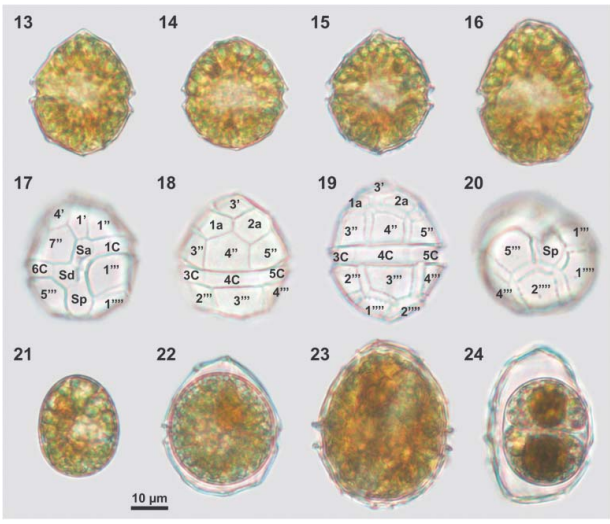
Genomic DNA was extracted from fresh material using the Nucleo Spin Plant II Kit (Machery-Nagel; Düren, Germany). Various regions of the ribosomal RNA (rRNA) genes including the Internal Transcribed Spacers (ITSs) were amplified using primer pairs specified previously (Gu et al., 2013) and following standard protocols (Gottschling & Plötner, 2004; Gottschling et al., 2012). For alignment constitution, we defined three regions of the rRNA: SSU, ITS, LSU, and included all Peridiniales, of which sequence information of all three regions were available, along with all rRNA sequences available from Peridiniopsidaceae (including multiple strains from *P. apiculatus* collected at different localities across Central Europe; Table S1, see online supplemental material, which is available from the article's Taylor & Francis Online page at http://doi.org/10.1080/14772000.2017.1375045). Separate matrices were constructed, aligned using 'MAFFT' v6.502a (Katoh & Standley, 2013) and concatenated afterwards. The aligned matrices are available as *.nex files upon request.

Phylogenetic analyses were carried out using Maximum Likelihood (ML) and Bayesian approaches, as described in detail previously (Gottschling et al., 2012) using the resources available from the CIPRES Science Gateway (Miller, Pfeiffer, & Schwartz, 2010). The Bayesian analysis was performed using 'MrBayes' v3.2.6 (Ronquist et al., 2012; freely available at http://mrbayes.sourceforge.net/download.php) under the GTR+Γ substitution model and the random-addition-sequence method with 10 replicates. We ran two independent analyses of four chains (one cold and three heated) with 20,000,000 cycles, sampled every 1,000th cycle, with an appropriate burn-in (10%) as inferred from the evaluation of the trace files using Tracer v1.5 (http://tree.bio.ed.ac.uk/software/tracer/). For the ML calculation, the MPI version of 'RAxML' v8.2.4 (Stamatakis, 2014; freely available at http://www.exelixis-lab.org/) was applied using the GTR+Γ substitution model. To determine the best fitted ML tree, we executed 10-tree searches from distinct random stepwise addition sequence Maximum Parsimony starting trees and performed 1,000 non-parametric bootstrap replicates. Statistical support values (LBS: ML bootstrap support, BPP: Bayesian posterior probabilities) were drawn on the resulting, best-scoring tree.

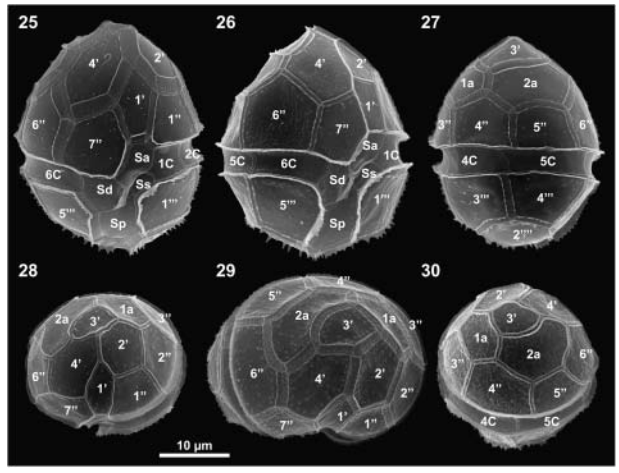
Results

Contemporary material consistent with an old protologue

The monoclonal strains of *P. apiculatus*, collected at different dates and localities, were morphologically indistinguishable. The strains exhibited both motile, thecate cells (Figs 9, 13–16, 25–30, 32, 34) and immotile, coccoid cells (Figs 12, 21–24, 33, 35), but the motile cells were predominant. The motile cells were golden-brown in colour and densely filled with numerous granules. Frequently, an orange-red accumulation body was observed within the



Figs 13–24. Motile thecate and immotile cells of *Palatinus apiculatus* (GeoM*762; LM; all at the same scale). 13–16. motile thecate cells showing variation in size and shape. 17–20. empty thecae showing the tabulation pattern. 17. ventral view. 18–20. dorsal view. 20. antapical view. 21–24. immotile coccoid cells. 21. coccoid cell. 22–23. coccoid cell of different size with thecal remnant. 24. dividing coccoid cell with thecal remnant. Abbreviations: n′: apical plate. n″: precingular plate. n‴: postcingular plate. n⁗: antapical plate. na: anterior intercalary plate. nC: cingular plate. Sa: anterior sulcal plate. Sd: right sulcal plate. Sp: posterior sulcal plate.



Figs 25–30. Motile thecate cells of *Palatinus apiculatus* showing the tabulation pattern (GeoM*762; SEM; all at the same scale). 25. ventral view. 26. ventral view of a motile cell with an epitheca strongly twisted to the left. 27. dorsal view. 28–29. apical view from the ventral side. 30. apical view from the dorsal side. Abbreviations: n′: apical plate. n″: precingular plate. n‴: postcingular plate. n⁗: antapical plate. na: anterior intercalary plate. nC: cingular plate. Sa: anterior sulcal plate. Sd: right sulcal plate. Sp: posterior sulcal plate. Ss: left sulcal plate.

epitheca slightly above the cingulum. In the sulcal region, a red area (interpreted as eyespot) could be observed. The dinokaryon with distinctly condensed chromosomes was located medial in the dorsal part of the cell. Additionally, numerous empty thecae were observed either at the bottom of the cultivation plates or floating in the medium (Figs 10–11, 17–20).

Thecate cells were ovate in outline and slightly compressed in dorsiventral direction. In ventral view, motile cells showed an epithecal twisting to the left of varying degrees in relation to the hypotheca (Figs 9–10, 17, 25–26, 31, 34, S6). The epitheca was hemispherical and occasionally slightly acuminate at the apex showing small tips, caused by thickened thecal plate edges, in the cells' outline (Figs 9–11, 13–16, 32). The hypotheca was occasionally slightly smaller than the epitheca and likewise hemispherical. The cingular girdle was excavated, and it surrounded the motile cell with a descendent displacement approximately of its own width (Figs 10, 17, 25–26, 31, 34, S6). The sulcus was likewise excavated and extended from the cingulum down to the antapex. The size of thecate cells ranged from 29–41 μm (GeoM*762; mean: 35 μm; median: 35 μm; SD: 3 μm; n = 50) in length and from 27–35 μm (mean: 31 μm; median: 20 μm; SD: 2 μm; n = 50) in width. We also measured thecate cells from the original field samples, which were 37–46 μm length and 33–40 μm in width (n = 11).

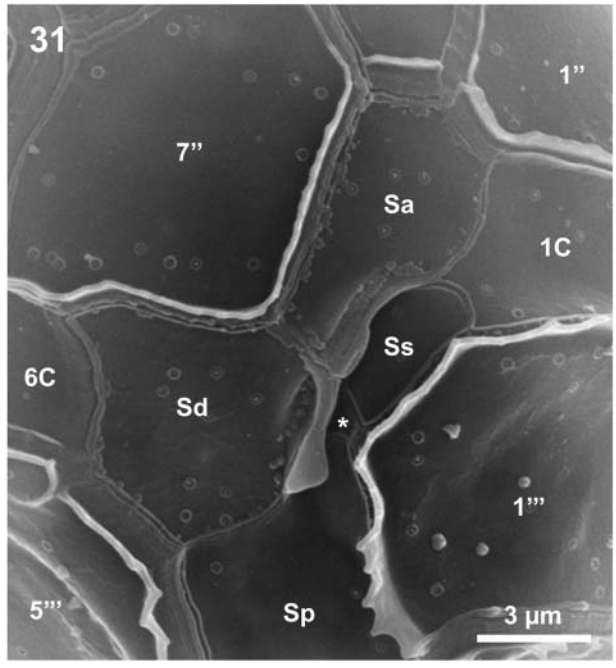
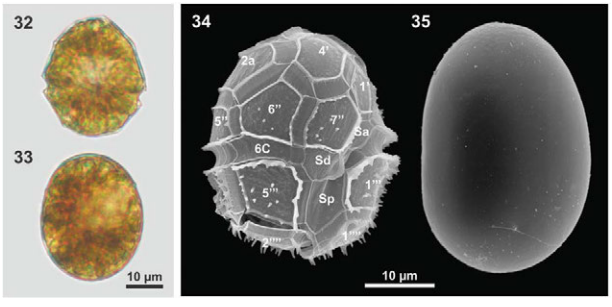


Fig. 31. Enlarged sulcal region of *Palatinus apiculatus* (GeoM*762; SEM). Asterisk indicates the sulcal plate Sm. Abbreviations: n″: precingular plate. n‴: postcingular plate. nC: cingular plate. Sa: anterior sulcal plate. Sd: right sulcal plate. Sm: median sulcal plate. Sp: posterior sulcal plate. Ss: left sulcal plate.

The motile cells were covered by a theca built of cellulosic plates (Figs 9–12, 17–20, 25–31, 34, S6–S18; astra blue staining indicated their cellulosic composition). The surface of the cultivated cells was smooth but in some cases slightly ornamented (Figs 26, S8). Small, circular pores (probably openings of trichocysts) were mostly irregularly arranged near the plate boundaries or randomly scattered over the thecal plate (Figs 25–31, 34, S6–S18). The sutures between the thecal plates varied from small lines through wide bands (Figs 25–31, 34, S6–S18) showing cross striations.

The thecate plate formula was 4′, 2a, 7″, 6c, 5s, 5‴, 2⁗ (Figs 17–20, 25–31, 34, S6–S18). Both anterior intercalary plates were hexagonal in shape, but the intercalary plate 1a was smaller than the elongated plate 2a, leading to an asymmetrical arrangement of the epitheca. Located on the dorsal side of the cell, the apical plate 3′ was tetragonal with a convex plate boundary towards the intercalary plate 2a. The cingulum was composed of six plates, whereas the sutures were slightly deviating from those of the pre- and postcingular plates. The sulcus consisted of five plates, whereas the plates Sm and Ss were small and partially covered by the large plates Sa and Sd. The left edge of the Sd plate, and the posterior end of the Sa plate, extended towards the middle of the sulcus and covered the flagellar pores. The Sp plate was relatively large and reached the antapex. The arrangement of the hypothecal plates was nearly symmetrical. The hypotheca was composed of five postcingular and two antapical plates of similar size. The edges of the hypothecal plates, especially of plates 1‴ and 5‴ adjacent to the sulcal plate Sp, showed raised bands with numerous notches and blunt spines. Additionally, single blunt spines were irregularly scattered over the surface of the postcingular and antapical plates.



Figs 32–35. Motile thecate and immotile cells of *Palatinus apiculatus* (GeoM*743; collected on 25 Jun 2015; 32–33: light microscopy, at the same scale; 34–35: scanning electron microscopy, at the same scale). 32. motile thecate cell. 33. immotile coccoid cells. 34. motile thecate cells in ventral-lateral view. 35. immotile coccoid cell showing a smooth surface. Abbreviations: n′: apical plate. n″: precingular plate. n‴: postcingular plate. n⁗: antapical plate. na: anterior intercalary plate. nC: cingular plate. Sa: anterior sulcal plate. Sd: right sulcal plate. Sp: posterior sulcal plate.

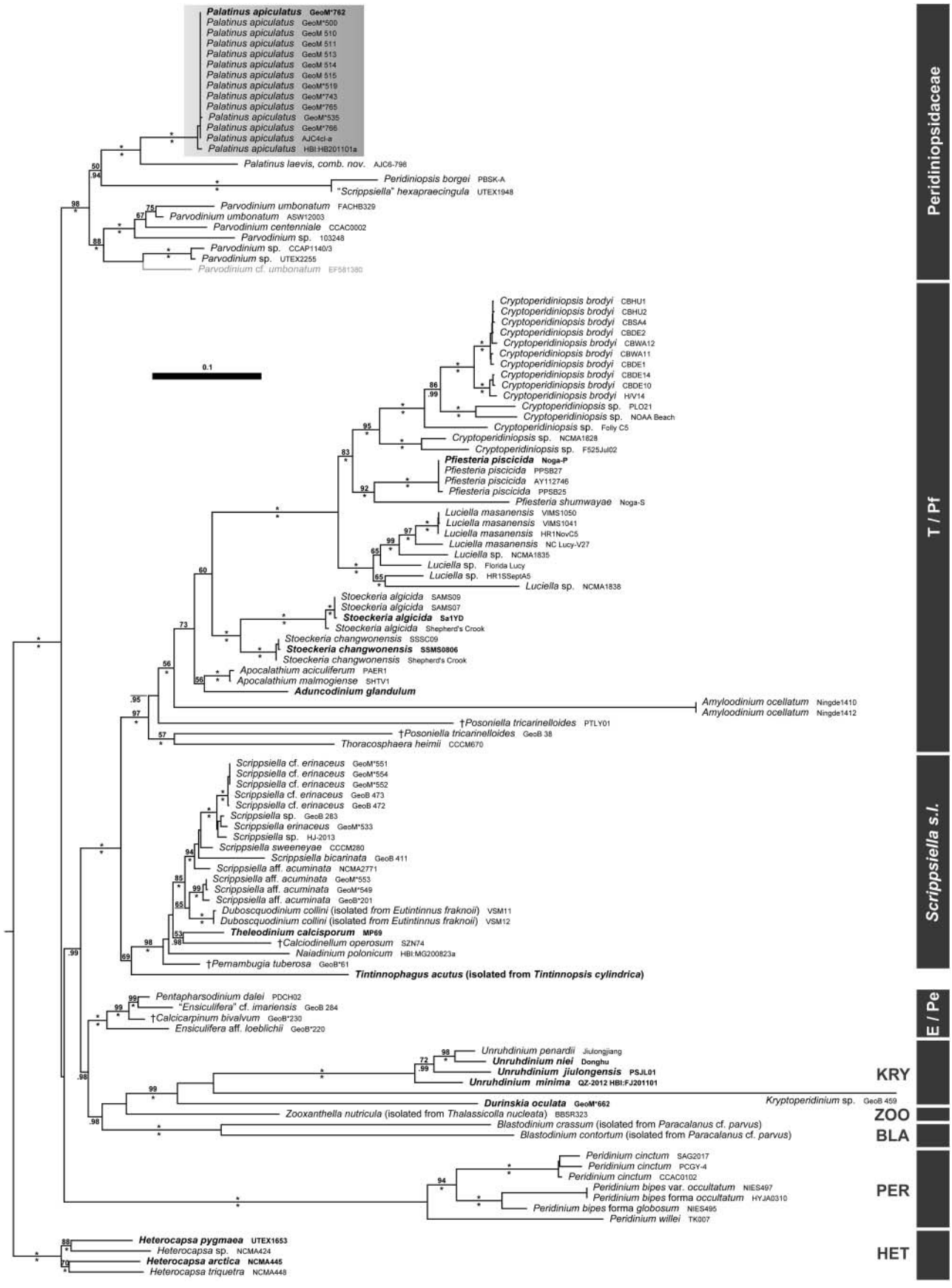
In the cultivated strain, deviations from the typical plate pattern were observed regarding epithecal plates (Figs S10–S18). The variations consisted mainly of additional sutures or plates. Occasionally, an additional precingular plate led to a heptagonal anterior intercalary plate 1a or 2a, respectively (Figs S10–S14). Figure S15 shows a subdivision of the apical plate 3′, but both plates together showed the typical plate shape of the apical plate 3′. In few cases, fusion of two precingular plates or of a precingular plate with an apical plate were observed (Figs S16–S18).

Cell division of thecate cells is normally carried out by eleuteroschisis. Dividing or ecdysing cells exited thecate cells through an opening on the hypotheca, provided by missing antapical but also postcingular plates (not shown). A single coccoid cell developed intrathecately and was released after shedding of the theca (Figs 22–24). Coccoid cells were coloured slightly darker than the motile cells and were spherical through mostly widely ovoid (Figs 12, 21–24, 33, 35). They ranged from 28–45 μm in length (mean: 34 μm; median: 34 μm; SD: 3 μm; n = 50) and 21–36 μm in width (mean: 29 μm; median: 29 μm; SD: 3 μm; n = 50) and showed a smooth surface (Fig. 35). The cytoplasm of the coccoid cells was filled with numerous brown granules and contained frequently a large, red accumulation body (Figs 21–22). A coccoid mother cell became ovoid and divided into two daughter cells, which were included in a joint shell (Fig. 24). The developmental fate of such cells remained elusive.

Low sequence variability within *Palatinus apiculatus*

In total, sequences were generated and deposited as 10 new GenBank entries in the course of the study (Table S1, see supplemental material online). The SSU+ITS+LSU alignment of the Peridiniales was 1775+1309+3603 bp long and comprised 383+737+611 parsimony informative sites (26%, mean of 15.1 per terminal taxon). Figure 36 shows the best-scoring Maximum Likelihood (ML) tree (−ln = 58745.02), with the internal topology not fully resolved. However, many nodes were statistically well if not maximally supported, and a number of peridinialean lineages could be distinguished such as *Heterocapsa* F.Stein (100LBS, 1.00BPP), Kryptoperidiniaceae (99LBS, 1.00BPP), Peridiniaceae (100LBS, 1.00BPP), *Scrippsiella* Balech *s.l.* (98LBS, 1.00BPP), and a clade including *Pfiesteria* Steid. & J.M.Burkh. and *Thoracosphaera* Kamptner (97LBS, 1.00BPP). The Peridiniopsidaceae were also monophyletic (98LBS, 1.00BPP), but their sister group could not be determined reliably.

Strain GeoM*762, from which the epitype of *G. apiculatum* was prepared, clustered together with other accessions of *P. apiculatus* (100LBS, 1.00BPP), and sequence


Palatinus apiculatus GeoM*762
Palatinus apiculatus GeoM*500
Palatinus apiculatus GeoM 510
Palatinus apiculatus GeoM 511
Palatinus apiculatus GeoM 513
Palatinus apiculatus GeoM 514
Palatinus apiculatus GeoM 515
Palatinus apiculatus GeoM*519
Palatinus apiculatus GeoM*743
Palatinus apiculatus GeoM*765
Palatinus apiculatus GeoM*535
Palatinus apiculatus GeoM*766
Palatinus apiculatus AJC4cl-a
Palatinus apiculatus HBI:HB201101a
Palatinus laevis, comb. nov. AJC6-796
Peridiniopsis borgei PBSK-A
"Scrippsiella" hexapraecingula UTEX1948
Parvodinium umbonatum FACHB329
Parvodinium umbonatum ASW12003
Parvodinium centenniale CCAC0002
Parvodinium sp. 103248
Parvodinium sp. CCAP1140/3
Parvodinium sp. UTEX2255
Parvodinium cf. umbonatum EF581380
0.1
Cryptoperidiniopsis brodyi CBHU1
Cryptoperidiniopsis brodyi CBHU2
Cryptoperidiniopsis brodyi CBSA4
Cryptoperidiniopsis brodyi CBDE2
Cryptoperidiniopsis brodyi CBWA12
Cryptoperidiniopsis brodyi CBWA11
Cryptoperidiniopsis brodyi CBDE1
Cryptoperidiniopsis brodyi CBDE14
Cryptoperidiniopsis brodyi CBDE10
Cryptoperidiniopsis brodyi HV14
Cryptoperidiniopsis sp. PLO21
Cryptoperidiniopsis sp. NOAA Beach
Cryptoperidiniopsis sp. Foliy C5
Cryptoperidiniopsis sp. NCMA1828
Cryptoperidiniopsis sp. F525Jul02
Pfiesteria piscicida Noga-P
Pfiesteria piscicida PPSB27
Pfiesteria piscicida AY112746
Pfiesteria piscicida PPSB25
Pfiesteria shumwayae Noga-S
Luciella masanensis VIMS1050
Luciella masanensis VIMS1041
Luciella masanensis HR1NovC5
Luciella masanensis NC Lucy-V27
Luciella sp. NCMA1835
Luciella sp. Florida Lucy
Luciella sp. HR15SeptA5
Luciella sp. NCMA1838
Stoeckeria algicida SAMS09
Stoeckeria algicida SAMS07
Stoeckeria algicida Sa1YD
Stoeckeria algicida Shepherd's Crook
Stoeckeria changwonensis SSSC09
Stoeckeria changwonensis SSMS0806
Stoeckeria changwonensis Shepherd's Crook
Apocalathium aciculiferum PAER1
Apocalathium malmogiense SHTV1
Aduncodinium glandulum
Amyloodinium ocellatum Ningde1410
Amyloodinium ocellatum Ningde1412
†Posoniella tricarinelloides PTLY01
†Posoniella tricarinelloides GeoB 38
Thoracosphaera heimii CCCM670
Scrippsiella cf. erinaceus GeoM*551
Scrippsiella cf. erinaceus GeoM*554
Scrippsiella cf. erinaceus GeoM*552
Scrippsiella cf. erinaceus GeoB 473
Scrippsiella cf. erinaceus GeoB 472
Scrippsiella sp. GeoB 283
Scrippsiella erinaceus GeoM*533
Scrippsiella sp. HJ-2013
Scrippsiella sweeneyae CCCM280
Scrippsiella bicarinata GeoB 411
Scrippsiella aff. acuminata NCMA2771
Scrippsiella aff. acuminata GeoM*553
Scrippsiella aff. acuminata GeoM*549
Scrippsiella aff. acuminata GeoB*201
Dubosquodinium collini (isolated from Eutintinnus fraknoii) VSM11
Dubosquodinium collini (isolated from Eutintinnus fraknoii) VSM12
Theleodinium calcisporum MP69
†Calciodinellum operosum SZN74
Naiadinium polonicum HBI:MG200823a
†Pernambugia tuberosa GeoB*61
Tintinnophagus acutus (isolated from Tintinnopsis cylindrica)
Pentapharsodinium dalei PDCH02
"Ensiculifera" cf. imariensis GeoB 284
†Calciarpinum bivalvum GeoB*230
Ensiculifera aff. loeblichii GeoB*220
Unruhdinium penardii Jiulongjiang
Unruhdinium niei Donghu
Unruhdinium jiulongensis PSJL01
Unruhdinium minima QZ-2012 HBI:FJ201101
Durinskia oculata GeoM*662
Kryptoperidinium sp. GeoB 459
Zooxanthella nutricula (isolated from Thalassicolla nucleata) BBSR323
Blastodinium crassum (isolated from Paracalanus cf. parvus)
Blastodinium contortum (isolated from Paracalanus cf. parvus)
Peridinium cinctum SAG2017
Peridinium cinctum PCGY-4
Peridinium cinctum CCAC0102
Peridinium bipes var. occultatum NIES497
Peridinium bipes forma occultatum HYJA0310
Peridinium bipes forma globosum NIES495
Peridinium willei TK007
Heterocapsa pygmaea UTEX1653
Heterocapsa sp. NCMA424
Heterocapsa arctica NCMA445
Heterocapsa triquetra NCMA448
Peridiniopsidaceae
T / Pf
Scrippsiella s.l.
E / Pe
KRY
ZOO
BLA
PER
HET

variability was notably low within this clade. It constituted the sister species of *Palatinus laevis*, comb. nov. (100LBS, 1.00BPP), and taxa with asymmetrical and symmetrical epithecae were thus clearly distinct in the molecular tree. The other two lineages of Peridiniopsidaceae included species of *Parvodinium* (88LBS, 1.00BPP) and *Peridiniopsis borgei* Lemmerm. (i.e., type species of *Peridiniopsis*), showing a close relationship to "*Scrippsiella*" *hexapraecingula* T.Horig. & Chihara (100LBS, 1.00BPP). Peridiniopsidaceae were almost exclusively restricted to freshwater habitats, with the only exception of "*Scrippsiella*" *hexapraecingula* collected in the Pacific Ocean. DNA-based records of *P.apiculatus* originated from various localities across Central Europe including those in Germany and Ukraine.

Discussion

The morphology of our cultivated material is to a great extent consistent with the description and drawings of the species Ch.G. Ehrenberg introduced as *G. apiculatum* (Ehrenberg, 1838). This refers to the general shape of the motile cells, the position of the eyespot in the sulcal region ('Auge' in Ehrenberg, 1838), as well as to the presence of spiny plate boundaries and irregularly scattered spines of the hypothecal plates. Amazingly, the original drawings exhibit a distinct plate pattern in such detail, that the thecal plates can be designated using the Kofoidean system (though it was unknown to Ch.G. Ehrenberg: compare, e.g., Fig. 5 with Fig. 11). Furthermore, it is evident from Ch.G. Ehrenberg's drawings of an empty thecate cell (Figs 1, 5) that *G. apiculatum* possess two anterior intercalary plates of different sizes leading to an asymmetrical plate arrangement of the epitheca. This is an important trait to delimit species of *Palatinus*, as it is discussed below, and we have thus used material of strain GeoM*762 for epitypification of *G. apiculatum*.

Cells of our cultivated material are slightly smaller than the measurements given by Ch.G. Ehrenberg on the drawings and using the Paris line of 2.5 mm (Jahn, 1995). However, the largest cells of the original material preserved by Ch.G. Ehrenberg himself and presented here for the first time (Figs 6–8) are likewise smaller than his noted maximal measurements. Moreover, it is well known that thecate cells in the field are frequently larger than those in cultivation, which has also been observed for *P. apiculatus* (Craveiro et al., 2009). Thus, the slight differences in size are not in conflict with the protologue. Moreover, Ehrenberg (1838) has specified *Chara* L. as co-occurring with *G. apiculatum*, which was not confirmed in the present study. Nevertheless, *Chara* appears more susceptible to habitat loss and/or eutrophication and has thus disappeared from the inner city of Berlin (Kusber, Jahn, & Korsch, 2017).

In the molecular DNA tree, there is a clear distinction between organisms assigned to *Palatinus* exhibiting either symmetrical or asymmetrical conformation of the epitheca, which can be considered a diagnostic feature to distinguish between two species. Lefèvre (1925) was first to acknowledge this morphological distinction, and it remains unjustified why Craveiro et al. (2009) treated *P. laeve*, with symmetrical conformation (Huitfeldt-Kaas, 1900; Lindemann, 1917; Fig. S4), as a variety under a species with asymmetrical conformation (i.e., *P. apiculatus*; Figs 1–8) in parallel to a taxon at the species level, which likewise exhibits the symmetrical conformation of the epitheca, namely *P. pseudolaevis* (Fig. S5). Unfortunately, no DNA sequence data are available for their strain under investigation (NIES1405) and determined as *P. apiculatus* var. *laevis* (Huitf.-Kaas) Craveiro, Calado, Daugbjerg & Moestrup, which would help to explain the authors' concept in *Palatinus*. In comparison, it appears more likely that Huitfeldt-Kaas (1900) discovered the first species of *Palatinus* distinct from *P. apiculatus* (see the corresponding new combination in the Appendix, see supplemental material online).

The DNA tree further indicates the correct systematic placement of *P. apiculatus* in the Peridiniopsidaceae (Gottschling, Kretschmann, & Žerdoner Čalasan, 2017), and not to taxa today assigned to the Protoperidiniaceae as occasionally suggested by Meunier (1919) or to the Peridiniaceae, under which species of *Palatinus* have been initially described. The reduced number of not more than two intercalary plates might be discussed as morphological apomorphy of this monophyletic group composed of *Palatinus*, *Parvodinium*, and *Peridiniopsis*. Presence and absence of an APC have been considered taxonomically important early in history (Lemmermann, 1910), and the concept has been used continuously in the 20th century (Bourrelly, 1970; Huber-Pestalozzi, 1968; Popovský & Pfiester, 1990; Starmach, 1974). However,

Fig. 36. Maximum Likelihood (ML) tree (–ln = 58690.00) of 116 peridinialean operational taxonomic units (OTUs) under the GTR +Γ substitution model. Typified OTUs are in bold, and branch lengths are drawn to scale, with the scale bar indicating the number of nucleotide substitutions per site. The numbers on the branches are statistical support values (above: ML bootstrap values, values <50 are not shown; below: Bayesian posterior probabilities, values <0.90 are not shown; asterisks indicate maximal support; note that statistical support values were derived from analyses without EF581380 being a short SSU sequence). *Palatinus apiculatus* diagnostically showing the asymmetrical epitheca conformation is highlighted by a grey box. Abbreviations: BLA: Blastodiniaceae. E/Pe: clade including *Ensiculifera* Balech and *Pentapharsodinium* Indel. & A.R.Loebl. HET: *Heterocapsa*. KRY: Kryptoperidiniaceae. PER: Peridiniaceae. T/Pf: clade including *Pfiesteria* and *Thoracosphaera*. ZOO: *Zooxanthella* K.Brandt.

molecular data now show that this trait is evolutionarily homoplastic, and that taxa with (*Parvodinium*, *Peridiniopsis*) and without APC (*Palatinus*) can be found in a given group such as the Peridiniopsidaceae. The absence of an APC in *Palatinus* is nowadays considered a reduction, yet the reason behind it is currently unknown.

Dinophyte cultivation enables us to investigate the species in more detail than was possible in the time of Ch.G. Ehrenberg. We confirm that motile thecate cells do not represent the only ontogenetic stage, and that coccoid cells are also an integral part of the present species' life-history. Such cells are morphologically indistinguishable from coccoid cells firstly described as *Peridinium anglicum* West (West, 1909). Little is known about the precise function of such cells beyond the general assumption of being dormant zygotes (Dale, 1983; Fensome et al., 1993; Mertens, Rengefors, Moestrup, & Ellegaard, 2012; Pfiester & Anderson, 1987). However, we have never observed any fusion of cells (i.e., karyogamy) or four-cell aggregations (i.e., indication for meiosis) which would indicate towards sexuality in *P. apiculatus*. Another important investigable feature based on cultivated material is intraspecific (or even -strain) variability. Spines along plate boundaries and ridges considerably vary in number, size and thickness amongst individual cells of *P. apiculatus* (Figs 25–27, 34). A comparison between specimens collected in the field has shown that thecal plate surface of cultivated motile cells is smoother, and the hypothecal spines are shorter and less developed than in wild specimens (Craveiro et al., 2009).

There is little doubt that we have re-collected a species from its type locality, which was described almost 180 years ago. In the era of heavy anthropogenic influence causing tremendous ecological alterations, it is remarkable that dinophytes are found as such in the sites, from which they were originally recorded a long time ago (Höll, 1928, provides some more Berlin morphology-based records from the 1920s). Taxonomically, this fact enables us to clarify the identity of species for reliable determination, which is a continuous challenge in the microbial world. Before our present study on *P. apiculatus*, this approach has been successfully applied to, for example, *Spiniferodinium limneticum* (Wołosz.) Kretschmann & Gottschling (Kretschmann, Filipowicz, Owsianny, Zinßmeister, & Gottschling, 2015) and even to such species discovered earlier in the 19th century as *Exuviaella marina* Cienk. (McLachlan, Boalch, & Jahn, 1997) and *Scrippsiella acuminata* (Ehrenb.) Kretschmann, Zinssmeister, S.Soehner, Elbr., Kusber & Gottschling (Kretschmann et al., 2015). This is the reason why the ecology-based approach for taxonomic clarification (John et al., 2014; Saburova, Chomerat, & Hoppenrath, 2012) does not appear as a first choice. It should therefore be followed only in exceptional cases, after an exhaustive though ultimately unsuccessful search of an organism at its type locality has already taken place.

The question remains, which dinophyte taxa may also belong to *Palatinus*? Variously spin(ul)ose plate boundaries, and similar conformations of the epitheca, are reported from *Glenodinium alpinum* Perty collected at Lake Lugano (Perty, 1852), *P. palatinum* from Ludwigshafen (Lauterborn, 1896; Fig. S1), *P. marssonii* from Berlin (Lemmermann, 1900; Fig. S2), British *P. anglicum* (West, 1909; Fig. S3), and *Peridinium godlewskii* Wołosz. from the Ukrainian lake Białogórski (Wołoszyńska, 1916). Furthermore, Lindemann (1918a, b) distinguished a number of subspecies and varieties from Germany and Poland. The time has come to clarify the taxonomic identity of all such names, including the synonymization of, for example, *P. palatinus*, *P. marssonii*, and *P. apiculatus*, using contemporary molecular and morphological methods. *Palatinus apiculatus* appears as a widely distributed species, but its precise conservation status cannot be evaluated because of insufficient occurrence data (Geissler & Kies, 2003; Täuscher, 2013). Our epitypification approach in *P. apiculatus* and other species will help to disentangle the complex and confusing taxonomy and nomenclature of unicellular organisms such as the dinophytes.

Taxonomy

1. *Palatinus apiculatus* (Ehrenb.) Craveiro, Calado, Daugbjerg & Moestrup, Journal of Phycology 45: 1178, figs 1–13. 2009. *Glenodinium apiculatum* Ehrenb., Infusionsthierchen: 258, pl. XXII 24. 1838. *Peridinium apiculatum* (Ehrenb.) Clap. & J.Lachm., Mémoires de l'Institut National Genevois 5: 404. 1859. *Properidinium apiculatum* (Ehrenb.) Meunier, Mémoires du Musée Royal d'Histoire Naturelle de Belgique 8: 60, pl. XVIII 47–52. 1919.–**Lectotype, designated here**: [illustration] original drawings sub No. 671 at BHUPM!, showing a non-fossil individual from Germany. Berlin, Berlin [exact locality unknown], 2 Apr 1835. [http://phycobank.org/100023]–**Epitype, designated here**: [slide with non-fossil specimens] Federal Republic of Germany. Berlin, Berlin (52°31′N, 13°21′E), 28 Mar 2016: M. Gottschling D047 [J. Kretschmann GeoM*762] (CEDiT-2017E68!, duplicates: B 40 0042045! [http://herbarium.bgbm.org/object/B400042045] M-0289351!) [http://phycobank.org/100024].–Other original elements: dried specimen (mica) mounted with Canada balsam comprising several non-fossil individuals from Germany. Berlin, Berlin [exact locality unknown, most likely Tiergarten], 2 Apr 1835, collected by Ch.G. Ehrenberg (BHUPM Infusionsthierchen XCIX: 1!)

2. *Palatinus laevis* (Huitf.-Kaas) Gottschling, Kretschmann & Zerdoner, comb. nov., basionym: *Peridinium laeve* Huitf.-Kaas, Skrifter / Videnskabsselskapet i Kristiania, Matematisk-Naturvidenskapelig Klasse 1900: 4,

figs 1–5. 1900. *Peridinium palatinum* forma *laeve* (Huitf.-Kaas) Er.Lindem., Botanisches Archiv 11: 478. 1925. *Peridinium apiculatum* forma *laeve* (Huitf.-Kaas) Er.Lindem., Archiv für Protistenkunde 63: 260. 1928. *Peridinium palatinum* tab. *betadeltabitravectum* forma *laeve* (Huitf.-Kaas) M.Lefèvre, Archives de Botanique 6: 105, fig. 321. 1932. *Peridinium palatinum* forma *laeve* (Huitf.-Kaas) M.Lefèvre, Archives de Botanique 6: 105, 107, figs 327–334. 1932. *Palatinus apiculatus* var. *laevis* (Huitf.-Kaas) Craveiro, Calado, Daugbjerg & Moestrup, Journal of Phycology 45: 1178, fig. 13a–c. 2009.–Type: Kingdom of Norway. Østlandet, Oslo, Padderudvandet and Sognsvandet [date unknown]: V.V.H. Huitfeldt-Kaas s.n. [disposition of original material other than the illustrations unknown]. [http://phycobank.org/100025]

= ***Peridinium pseudolaeve*** **M.Lefèvre** (*nom. nov. pro Peridinium laeve* sensu Lindemann, 1918a), nom. corr. (ICN Art. 60.9.), **syn. nov.**, Revue Algologique 2: 341, pl. XI 6–9. 1925. *Palatinus pseudolaevis* (M.Lefévre) Craveiro, Calado, Daugbjerg & Moestrup, Journal of Phycology 45: 1178, fig. 13d–i. 2009.–Type: French Republic. Auvergne-Rhône-Alpes, Upper Savoy, without exact locality [Aug–Sep 1924]: G.-V. Deflandre s.n. [disposition of original material other than the illustrations unknown].

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Disclosure statement

No potential conflict of interest was reported by the authors.

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Supplemental data

Supplemental data for this article can be accessed here: https://doi.org/10.1080/14772000.2017.1375045.

References

Bourrelly, P. (1970). *Les algues bleues et rouges les euglénides, peridiniens et cryptomandines*. Paris: Boubée.

Calado, A. J., Hansen, G., & Moestrup, Ø (1999). Architecture of the flagellar appartus and related structures in the type species of *Peridinium*, *P. cinctum* (Dinophyceae). *European Journal of Phycology*, *34*, 179–191.

Craveiro, S. C., Calado, A. J., Daugbjerg, N., & Moestrup, Ø (2009). Ultrastructure and LSU rDNA-based revision of *Peridinium* group Palatinum (Dinophyceae) with the description of *Palatinus* gen. nov. *Journal of Phycology*, *45*, 1175–1194.

Dale, B. (1983). Dinoflagellate resting cysts: "Benthic plankton." In G. A. Fryxell (Ed.), *Survival strategies of the algae* (pp. 69–136). Cambridge: Cambridge University Press.

Ehrenberg, Ch. G. (1838). Zwölfte Familie: Kranzthierchen. In Ch.G. Ehrenberg (Ed.), *Die Infusionsthierchen als vollkommene Organismen* (pp. 249–259). Leipzig: Voss.

Fensome, R. A., Taylor, F. J. R., Norris, G., Sarjeant, W. A. S., Wharton, D. I., & Williams, G. L. (1993). A classification of living and fossil dinoflagellates. *Micropaleontology* Special Publication, *7*, 1–245.

Geissler, U., & Kies, L. (2003). Artendiversität und Veränderungen in der Algenflora zweier städtischer Ballungsgebiete Deutschlands: Berlin und Hamburg. *Nova Hedwigia, Beihefte*, *126*, 1–777.

Geissler, U., Kusber, W.-H., & Jahn, R. (2004). The diatom flora of Berlin (Germany): a spotlight on some documented taxa as a case study on historical biodiversity. In A. Witkowski (Ed.), *Eighteenth international diatom symposium 2004* (pp. 91–105). Miedzyzdroje: Biopress.

Gottschling, M., Kretschmann, J., & Žerdoner Čalasan, A. (2017). Description of Peridiniopsidaceae, fam. nov. (Peridiniales, Dinophyceae). *Phytotaxa*, *299*, 293–296.

Gottschling, M., & Plötner, J. (2004). Secondary structure models of the nuclear Internal Transcribed Spacer regions and 5.8S rRNA in Calciodinelloideae (Peridiniaceae) and other dinoflagellates. *Nucleic Acids Research*, *32*, 307–315.

Gottschling, M., Söhner, S., Zinßmeister, C., John, U., Plötner, J., Schweikert, M., … Elbrächter, M. (2012). Delimitation of the Thoracosphaeraceae (Dinophyceae), including the calcareous dinoflagellates, based on large amounts of ribosomal RNA sequence data. *Protist*, *163*, 15–24.

Gu, H., Kirsch, M., Zinßmeister, C., Söhner, S., Meier, K. J. S., Liu, T., & Gottschling, M. (2013). Waking the dead: morphological and molecular characterization of extant †*Posoniella tricarinelloides* (Thoracosphaeraceae, Dinophyceae). *Protist*, *164*, 583–597.

Guillard, R. R., & Lorenzen, C. J. (1972). Yellow-green algae with chlorophyllide c. *Journal of Phycology*, *8*, 10–14.

Höll, K. (1928). *Oekologie der Peridineen. Studien über den Einfluß chemischer und physikalischer Faktoren auf die Verbreitung der Dinoflagellaten im Süßwasser*. Jena: Fischer.

Huber-Pestalozzi, G. (1968). Dinophyceae. In G. Huber-Pestalozzi (Ed.), *Das Phytoplankton des Süßwassers. Systematik und Biologie* (2. ed.) (pp. 94–303). Stuttgart: Schweizerbart.

Huitfeldt-Kaas, H. (1900). Die limnetischen Peridineen in norwegischen Binnenseen. *Skrifter / Videnskabsselskapet i Kristiania, Matematisk-Naturvidenskapelig Klasse*, *1900*, 3–7.

Janofske, D. (2000). *Scrippsiella trochoidea* and *Scrippsiella regalis*, nov. comb. (Peridiniales, Dinophyceae): a comparison. *Journal of Phycology*, *36*, 178–189.

Jahn, R. (1995). C. G. Ehrenberg's concept of the diatoms. *Archiv für Protistenkunde*, *146*, 109–116.

John, U., Litaker, R. W., Montresor, M., Murray, S. A., Brosnahan, M. L., & Anderson, D. M. (2014). Formal revision of the *Alexandrium tamarense* species complex (Dinophyceae) taxonomy: the introduction of five species with emphasis on molecular-based (rDNA) classification. *Protist*, *165*, 779–804.

Jones, T. A. (2013). When local isn't best. *Evolutionary Applications*, *6*, 1109–1118.

Katoh, K., & Standley, D. M. (2013). MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution*, *30*, 772–780.

Kretschmann, J., Elbrächter, M., Zinßmeister, C., Söhner, S., Kirsch, M., Kusber, W.-H., & Gottschling, M. (2015). Taxonomic clarification of the dinophyte *Peridinium acuminatum* Ehrenb., ≡ *Scrippsiella acuminata*, comb. nov. (Thoracosphaeraceae, Peridiniales). *Phytotaxa*, *220*, 239–256.

Kretschmann, J., Filipowicz, N. H., Owsianny, P. M., Zinßmeister, C., & Gottschling, M. (2015). Taxonomic clarification of the unusual dinophyte *Gymnodinium limneticum* Wołosz. (Gymnodiniaceae) from the Tatra Mountains. *Protist*, *166*, 621–637.

Kusber, W.-H., Jahn, R., & Korsch, H. (2017). Rote Liste und Gesamtartenliste der Armleuchteralgen (Characeae) von Berlin. In Der Landesbeauftragte für Naturschutz und Landschaftspflege / Senatsverwaltung für Umwelt, Verkehr und Klimaschutz (Ed.), *Rote Listen der gefährdeten Pflanzen, Pilze und Tiere von Berlin*. Berlin: Universitätsverlag der TU Berlin.

Lauterborn, R. (1896). Diagnosen neuer Protozoen aus dem Gebiete des Oberrheins. *Zoologischer Anzeiger*, *19*, 14–18.

Lefèvre, M. M. (1925). Contribution à la flore des Pèridiniens de France. *Revue Algologique*, *2*, 327–342.

Lemmermann, E. J. (1900). Beiträge zur Kenntnis der Planktonalgen. III. Neue Schwebealgen aus der Umgebung Berlins. *Berichte der Deutschen Botanischen Gesellschaft*, *18*, 24–32.

Lemmermann, E. J. (1910). *Algen I (Schizophyceen, Flagellaten, Peridineen)*. Leipzig: Bornträger.

Lindemann, E. B. L.W. (1917). Beiträge zur Kenntnis des Seenplanktons der Provinz Posen. (Südwestposener Seengrupe.) II. *Zeitschrift der Naturwissenschaftlichen Abteilung der Deutschen Gesellschaft für Kunst und Wissenschaft in Posen*, *24*, 2–41.

Lindemann, E. B. L.W. (1918a). Untersuchungen über Süßwasserperidineen und ihre Variationsformen. *Archiv für Protistenkunde*, *39*, 209–262.

Lindemann, E. B. L.W. (1918b). Untersuchungen über Süßwasserperidineen und ihre Variationsformen II. *Archiv für Naturgeschichte*, *84*, 121–194.

Lindemann, E. B. L.W. (1925). Peridineen des Oberrheins und seiner Altwässer. *Botanisches Archiv*, *11*, 474–481.

Lindemann, E. B. L.W. (1928). Vorläufige Mitteilung. *Archiv für Protistenkunde*, *63*, 259–260.

McKay, J. K., Christian, C. E., Harrison, S., & Rice, K. J. (2005). "How local is local?" – a review of practical and conceptual issues in the genetics of restoration. *Restoration Ecology*, *13*, 432–440.

McLachlan, J. L., Boalch, G. T., & Jahn, R. (1997). Reinstatement of the genus *Exuviaella* (Dinophyceae, Prorocentrophycidae) and an assessment of *Prorocentrum lima*. *Phycologia*, *36*, 38–46.

Mertens, K. N., Rengefors, K., Moestrup, Ø., & Ellegaard, M. (2012). A review of recent freshwater dinoflagellate cysts: taxonomy, phylogeny, ecology and palaeocology. *Phycologia*, *51*, 612–619.

Meunier, A. (1919). Microplancton de la mer Flamande. 3. Les péridiniens. *Mémoires du Musée Royal d'Histoire Naturelle de Belgique*, *8*, 1–116.

Miller, M. A., Pfeiffer, W., & Schwartz, T. (2010). *Creating the CIPRES Science Gateway for inference of large phylogenetic trees*. Proceedings of the Gateway Computing Environments Workshop (GCE, pp. 1–8), New Orleans.

Mollenhauer, D. (2002). Studies on algae in Berlin-Dahlem between 1930 and 1990 – A glimpse into the servants' room of research – The phycologist Johannes Gerloff. Protist, *153*, 311–323.

Perty, M. (1852). *Zur Kenntniss kleinster Lebensformen nach Bau, Funktionen, Systematik, mit Specialverzeichniss der in der Schweiz beobachteten*. Bern: Jent & Reinert.

Pfiester, L. A., & Anderson, D. M. (1987). Dinoflagellate reproduction. In: F. J. R. Taylor (Ed.), *The biology of dinoflagellates* (pp. 611–648). Oxford: Blackwell.

Popovský, J., & Pfiester, L. A. (1990). *Dinophyceae (Dinoflagellida)*. Stuttgart: Fischer.

Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D. L., Darling, A., Höhna, S., … Huelsenbeck, J. P. (2012). MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology*, *61*, 539–542.

Saburova, M., Chomerat, N., & Hoppenrath, M. (2012). Morphology and SSU rDNA phylogeny of *Durinskia agilis* (Kofoid & Swezy) comb. nov. (Peridiniales, Dinophyceae), a thecate, marine, sand-dwelling dinoflagellate formerly classified within *Gymnodinium. Phycologia*, *51*, 287–302.

Seddon, P. J. (2010). From reintroduction to assisted colonization: moving along the conservation translocation spectrum. *Restoration Ecology 18*, 796–802.

Starmach, K. (1974). *Cryptophyceae—Kryptofity*, *Dinophyceae—Dinofity*, *Raphidophyceae—Rafidofity*. Warsaw: Państwowe Wydawnictwo Naukowe.

Täuscher, L. (2013). Checklisten und Gefährdungsgrade der Algen des Landes Brandenburg. III. Checklisten und Gefährdungsgrade der Raphidophyceae/Chloromonadophyceae, Haptophyta (Haptophyceae/Prymnesiophyceae), Cryptophyta (Cryptophyceae), Dinophyta (Dinophyceae) und Euglenophyta (Euglenophyceae). *Verhandlungen des Botanischen Vereins Berlin Brandenburg*, *146*, 109–128.

Taylor, F. J. R. (1980). On dinoflagellate evolution. *BioSystems*, *13*, 65–108.

West, G. S. (1909). A biological investigation of the Peridinieæ of Sutton Park, Warwickshire. *New Phytologist*, *8*, 181–196.

Wołoszyńska, J. (1916). Polskie Peridineae słodkowodne. – Polnische Süßwasser-Peridineen. *Bulletin international de l'Académie des Sciences de Cracovie, Classe des Sciences Mathématiques et Naturelles. Série B*, *1915*, 260–285.

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Publication 4

Zero intercalary plates in *Parvodinium* (Peridiniopsidaceae, Peridiniales) and phylogenetics of *P. elpatiewskyi*, comb.nov.

KRETSCHMANN, J., ŽERDONER ČALASAN, A., MEYER, B. & GOTTSCHLING, M.

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ORIGINAL PAPER

Zero Intercalary Plates in *Parvodinium* (Peridiniopsidaceae, Peridiniales) and Phylogenetics of *P. elpatiewskyi*, comb. nov.

Juliane Kretschmann[a], Anže Žerdoner Čalasan[a,b], Barbara Meyer[a], and Marc Gottschling[a,1]

[a]Department Biologie, Systematische Botanik und Mykologie, GeoBio-Center, Ludwig-Maximilians-Universität München, Menzinger Str. 67, D-80 638 München, Germany
[b]Department of Botany, School of Biology and Chemistry, University of Osnabrück, Barbarastr. 11, D-49076 Osnabrück, Germany

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***Parvodinium elpatiewskyi*, comb. nov., is a common freshwater dinophyte without intercalary plates and with various spines on hypothecal sutures. However, the taxonomy of the species has had a complex history, and its systematic placement remained unclear. The conserved type of *P. elpatiewskyi*, comb. nov., illustrated here for the first time using electron microscopy, is an environmental sample. Based on the newly collected material from Berlin (Germany) we provide a morphological description using light and electron microscopy as well as new molecular rRNA sequence data to specify the phylogenetic position of *P. elpatiewskyi*, comb. nov. This species belongs to Peridiniopsidaceae, more precisely to *Parvodinium*, which usually possesses two intercalary plates. However, evolutionary inference indicates the loss of such plates in *P. elpatiewskyi*, comb. nov. Other traits that are of taxonomic importance and have not received enough attention in the past are the large Sd plate converging the second antapical plate and the presence of cellular hypocystal opening during replication.**

Key words: Biodiversity; dinoflagellates; Germany; molecular phylogenetics; morphology; taxonomy; type material.

Introduction

Parvodinium elpatiewskyi (Ostenf.) Kretschmann, Zerdoner & Gottschling, comb. nov. (Peridiniopsidaceae, Peridiniales), is a common dinophyte occurring in eutrophic lakes and peat bogs across Europe, Asia and the Americas (Ascencio et al. 2015; Carty 2014; Cavalcante et al. 2017; Höll 1928; Lindemann 1919; Moestrup and Calado 2018; Popovský and Pfiester 1990). It is one of a few peridinialean freshwater species without intercalary plates, which are otherwise present in, for example, *Tyrannodinium* Calado, Craveiro, Daugbjerg & Moestrup from the Thoracosphaeraceae (Calado et al. 2009) and *Unruhdinium* Gottschling from the Kryptoperidiniaceae (Liu et al. 2008; Takano et al. 2008). Another important trait of this species is

[1]Corresponding author;
e-mail gottschling@bio.lmu.de (M. Gottschling).

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the presence of various spines on the hypothecal sutures.

The taxonomy of *P. elpatiewskyi*, comb. nov., has had a turbulent history and has not been completely resolved until today. Initially, it was described from Mongolia as a variety of *Peridinium umbonatum* F.Stein having three apical and one intercalary plate (Ostenfeld 1907). A few years later, Lindemann (1919) misapplied the name to a species from Germany that exhibits four apical and no intercalary plates. To the best of our knowledge, this concept was adopted by all subsequent authors, but the change has been taxonomically unfixed for a long period of time. It was Meyer and Elbrächter (1996) who proposed to conserve the type of *P. elpatiewskyi*, comb. nov., with German material consistent with E. Lindemann's interpretation, but not with C. Ostenfeld's original intent. The proposal was accepted (Compère 1999), but no illustrations of original material have been available until today. This is particularly unfortunate as the conserved type is in fact an environmental sample.

The precise phylogenetic placement of *P. elpatiewskyi*, comb. nov., in the dinophyte tree has remained unclear as well. After the elevation from a variety to the species level, it was firstly placed in *Peridinium* Ehrenb. (Lemmermann 1910), which was taxonomically treated very broadly during that time. Later, the species was assigned to *Glenodinium* Ehrenb., which Schiller (1937) himself considered a rather artificial group of peridinialean dinophytes. Until recently (Moestrup and Calado 2018), *P. elpatiewskyi*, comb. nov., was placed in *Peridiniopsis* Lemmerm., since Bourrelly (1968) used it as a taxonomic (likewise heterogeneous) substitute name for *Glenodinium*. It is worthy to note that the taxon was never considered to be related to species today assigned to *Parvodinium* Carty, mainly because of the two intercalary plates that are commonly present in this taxon (versus no intercalary plate in *P. elpatiewskyi*, comb. nov.).

In this study, we show that *P. elpatiewskyi*, comb. nov., belongs to the Peridiniopsidaceae, whose elements' life-histories are only scarcely known at present. *Parvodinium inconspicuum* (Lemmerm.) Carty is a homothallic species, whose thecate gametes fuse and form a new cell in the intervening space between them (strain UTEX LB2255: Pfiester et al. 1984). The diploid, smooth cell first develops its own theca, sequentially forms a new cell inside the theca and sheds the old theca after a period of time (similar to matryoshka doll principle). The last step is the development of an immotile (though thecate) sporocyte that grows large, and the two consequent meiotic divisions may or may not directly follow. The resulting four motile meiospores develop into the haploid and vegetative thecate stage. Intrathecately formed coccoid cells ('cysts') are occasionally reported for members of the Peridiniopsidaceae such as *Palatinus apiculatus* (Ehrenb.) Craveiro, Calado, Daugbjerg & Moestrup (= *Peridinium anglicum* G.S.West: West 1909) and various species of *Parvodinium* (Kretschmann et al. 2018a; Lefèvre 1927; Lindemann, 1919; Schilling 1891; Thompson 1947). The reverse formation of a thecate cell inside a coccoid cell is reported from *P. apiculatus* (West 1909) and *Peridiniopsis borgei* Lemmerm. (Entz 1926). Furthermore, a second type of immotile cell with the shape of a thecate cell is reported from *P. inconspicuum* and *Parvodinium umbonatum* (F.Stein) Carty (Chu et al. 2008; Tardio et al. 2009), and this cell is characterised by a hypocystal archaeopyle. This highly unusual opening of a hypothecal equivalent, together with observations of peculiar cellular stages during replication, underline the morphological diversity and complexity of peridinialean metagenesis. Furthermore, our knowledge on ploidy levels of the various cell types is extremely scarce at present.

In this study, we present the first DNA sequences gained from cultivated material determined as *P. elpatiewskyi*, comb. nov. We infer the phylogenetic placement of the species in the dinophyte tree and show that the number of intercalary plates varies considerably in the Peridiniopsidaceae, to which group it is assigned. We also provide illustrations of type material, which is an important step forward in the taxonomic disentanglement of frequently encountered freshwater dinophytes.

Results

Morphologies Found in the Isotype of *Parvodinium elpatiewskyi*, comb. nov.

The isotype of *P. elpatiewskyi*, comb. nov., is an environmental sample comprising a large number of different organisms, including cyanobacteria, fungi, brachionid rotifers, *Ceratium* sp. and peridinialean dinophytes (Supplementary Material Figs S1, S3). Among the latter, at least two taxa can be differentiated, namely *Peridiniopsis cunningtonii* var. *pseudoquadridens* Er.Lindem. (Supplementary Material Fig. S3) and *P. elpatiewskyi*, comb. nov. (Figs 1 and 2). *Parvodinium elpatiewskyi*, comb. nov. (n = 148 cells), was more frequent than *P. cunningtonii* var. *pseudoquadridens* (n = 36 cells) in the material used for SEM investigation. The morphology of *P. elpatiewskyi*, comb. nov., in the concept

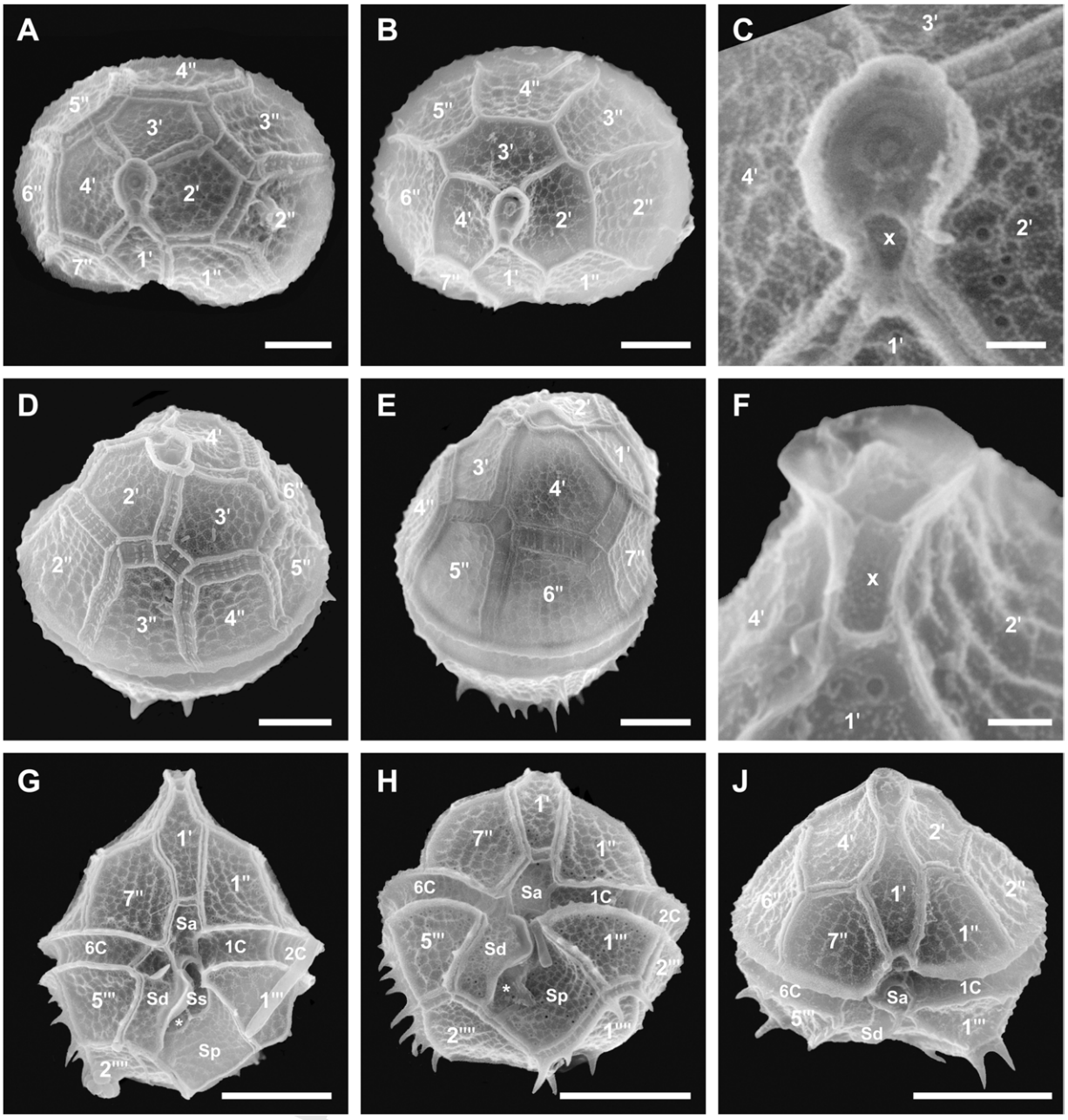

Figure 1. Motile thecate cells assigned to *Parvodinium elpatiewskyi*, comb. nov., present in the SEM-preparation of the isotype (B 40 0043809; scale bars: 1 μm in C, F, otherwise: 10 μm). **A**. apical view showing symmetric epithecal plate pattern with pentagonal plate 3′. **B**. apical view showing asymmetric epithecal plate pattern with hexagonal plate 3′. **C**. apical pore complex in apical view. **D**. dorsal-lateral view showing symmetric epithecal plate pattern with pentagonal apical plate 3′ and hypothecal spines. **E**. right lateral view showing symmetric epithecal plate pattern with pentagonal apical plate 3′ and hypothecal spines. **F**. apical pore complex in ventral view. **G**. ventral view showing the large plate Sp, hypothecal spines and contact between plates Sd and 2′′′′. **H**. ventral view showing the large plate Sp, hypothecal spines and a contact point/line between plates Sd and 2′′′′. **J**. ventral view. Abbreviations: n′: apical plate. n′′: precingular plate. n′′′: postcingular plate. n′′′′: antapical plate. nC: cingular plate. Sa: anterior sulcal plate. Sd: right sulcal plate. Sp: posterior sulcal plate. Ss: left sulcal plate. *: median sulcal plate.

of the typifying authors, is further illustrated in Supplementary Material Figure S2 based on material collected at the type locality a year after the type material was gathered (i.e., in 1977).

The shape of the epitheca in cells assigned to *P. elpatiewskyi*, comb. nov., was conical and had a slightly acuminate apex (Fig. 1G, Supplementary Material Fig. S2D–E). The hypotheca was semi-circular to pentagonal in outline (Fig. 1G–H, Supplementary Material Fig. S2D–E) showing several antapical spines along the plate sutures (Figs 1D–E, G–J, 2, Supplementary Material Fig. S2D–F). The size of the motile cells ranged from 22–33 μm (mean: 27 μm; median: 26 μm; sd: 3 μm; n = 44) in length (with spines) and from 14–30 μm (mean: 23 μm; median: 23 μm; sd: 3 μm; n = 113) in width. The cingulum was excavated, and it surrounded the motile cell with a descendent displacement by approximately half of its own width (Fig. 1G–J, Supplementary Material Fig. S2E). The sulcus was likewise excavated, extending slightly into the epitheca. It widened towards the posterior end of the cell and reached down to the antapex (Figs 1G–H, 2 G–J, Supplementary Material Fig. S2E).

The cell surface of the thecal plates showed an inconspicuously reticulate ornamentation and was irregularly scattered with small circular pores (probably openings of trichocysts). The sutures between the plates varied in their thickness and were cross-striated (Figs 1A, C–E, 2 A–B, F–H, Supplementary Material Fig. S2C). The thecate plate formula was pp, cp, x, 4′, 0a, 7″, 6c, 5s, 5‴, 2⁗ (Figs 1, 2, Supplementary Material Fig. S2). The arrangement of the epithecal plates was mostly symmetric, exhibiting a pentagonal apical plate 3′ (Figs 1A, D–E, 2 B, Suppementary Material Fig. S2A–C; n = 33) or sometimes a hexagonal apical plate 3′, which was in contact with plate 6″ (Figs 1B, 2 C, Supplementary Material Fig. S2D; n = 28). Among the sulcal plates, the Sd plate was notably large, reaching down to the antapex, and was in contact with antapical plate 2⁗ (Figs 1G–H, 2 G–J, Supplementary Material S2E).

The shape of the epitheca in cells assigned to *P. cunningtonii* var. *pseudoquadridens* was conical and had a slightly acuminate apex, whereas the hypotheca was hemispheric (Supplementary Material Fig. S3E–G). The size of the motile cells ranged from 21–37 μm (mean: 24 μm; median: 24 μm; sd: 2 μm; n = 10) in length (with spines) and from 15–25 μm (mean: 19 μm; median: 19 μm; sd: 2 μm; n = 35) in width. The cingulum was excavated, and it surrounded the motile cell with a descendent displacement by approximately half of its own width (Supplementary Material Fig. S3E–F, H). The sulcus was likewise excavated, extending slightly into the epitheca. It widened towards the posterior end of the cell and reached down to the antapex (Supplementary Material Fig. S3F, H–J).

The cell surface of the thecal plates showed an inconspicuously reticulate ornamentation and was irregularly scattered with small circular pores (probably openings of trichocysts). The sutures between the plates varied in their thickness and were cross-striated (Supplementary Material Fig. S3B, E–F, J). The thecate plate formula was pp, cp, x, 4′, 1a, 6″, 6c, 5s, 5‴, 2⁗ (Supplementary Material Fig. S3). The intercalary plate was on the right side of the epitheca (Supplementary Material Fig. S3A, G) and occasionally very close to the apical pore complex, seeming the existence of five apical and no intercalary plates. The epithecal plate formula was 4′, 1a, 7″ in one exceptional case only (Supplementary Material Fig. S3C) combining traits of *P. cunningtonii* var. *pseudoquadridens* and *P. elpatiewskyi*, comb. nov., respectively. Among the sulcal plates, the Sd plate was notably large and reached down to the antapex, but was never in contact with antapical plate 2⁗ (Supplementary Material Fig. S3F–J). All hypothecal plates, but particularly plates 1‴, 5‴, 1⁗ and 2⁗, exhibited distinct spines or larger protuberances in their centres (Supplementary Material Fig. S3E–J).

Morphology of *Parvodinium elpatiewskyi*, comb. nov. (clonal strain)

The strain GeoM 735 and the monoclonal substrains GeoM*833, GeoM*834, GeoM*835 and GeoM*836 were morphologically indistinguishable. They exhibited at least three different stages during life-history, namely motile thecate cells (Figs 3A–B, G–P, 4A–G) and two different morphologies of immotile cells (Figs 3C–F, 4H–J). The coloration of the motile cells was golden-yellow and showed a small, red area in the sulcal region (Fig. 3A–B). Numerous empty thecae were observed in the cultivation plates, indicating cell division by eleuteroschisis. Empty thecate cells showed an opening on the hypotheca due to divergence of plates along the sutures (Figs 3K, 4 G) or even lost sulcal (Fig. 3M), postcingular (Fig. 3O) as well as antapical plates (Fig. 3N, P).

The shape of the epitheca was conical and had a slightly acuminate apex. The hypotheca was semi-circular to pentagonal in outline and showed several antapical spines along the plate sutures. The size of the motile cells ranged from 22–32 μm (GeoM*836; mean: 28 μm; median: 28 μm; sd: 2 μm; n = 50)

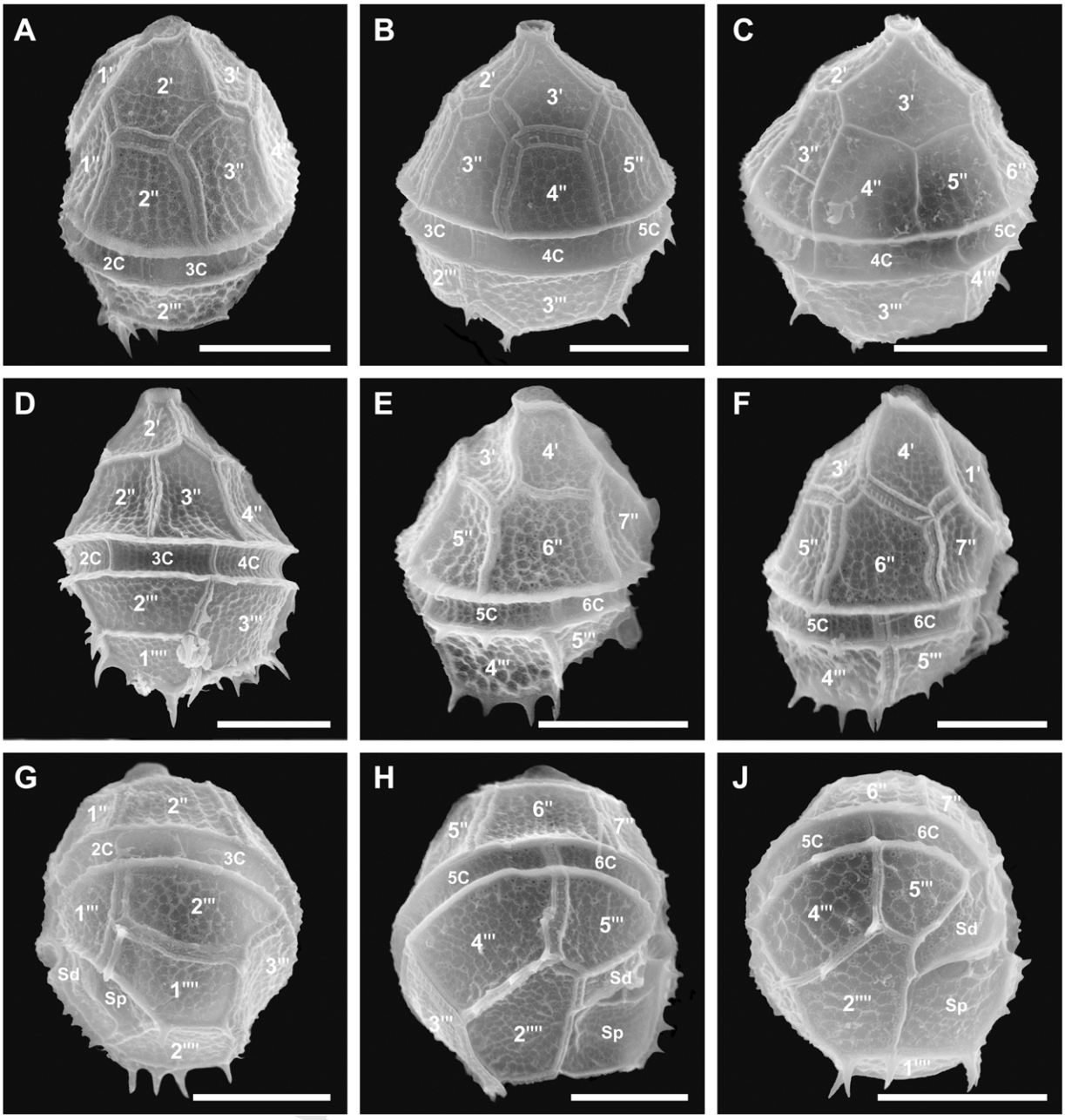

Figure 2. Motile thecate cells assigned to *Parvodinium elpatiewskyi*, comb. nov., present in the SEM-preparation of the isotype (B 40 0043809; scale bars: 10 μm). **A**. lateral view showing hypothecal spines. **B**. dorsal view showing symmetric epithecal plate pattern with pentagonal plate 3′ and hypothecal spines. **C**. dorsal view showing asymmetric epithecal plate pattern with hexagonal plate 3′ and hypothecal spines. **D**. left dorsal-lateral view showing hypothecal spines. **E**. right dorsal-lateral view showing hypothecal spines. **F**. right dorsal-lateral view showing hypothecal spines. **G–J**. lateral views showing the large plate Sp, a contact point/line between plates Sd and 2′′′′ and hypothecal spines. Abbreviations: n′: apical plate. n′′: precingular plate. n′′′: postcingular plate. n′′′′: antapical plate. nC: cingular plate. Sd: right sulcal plate. Sp: posterior sulcal plate.

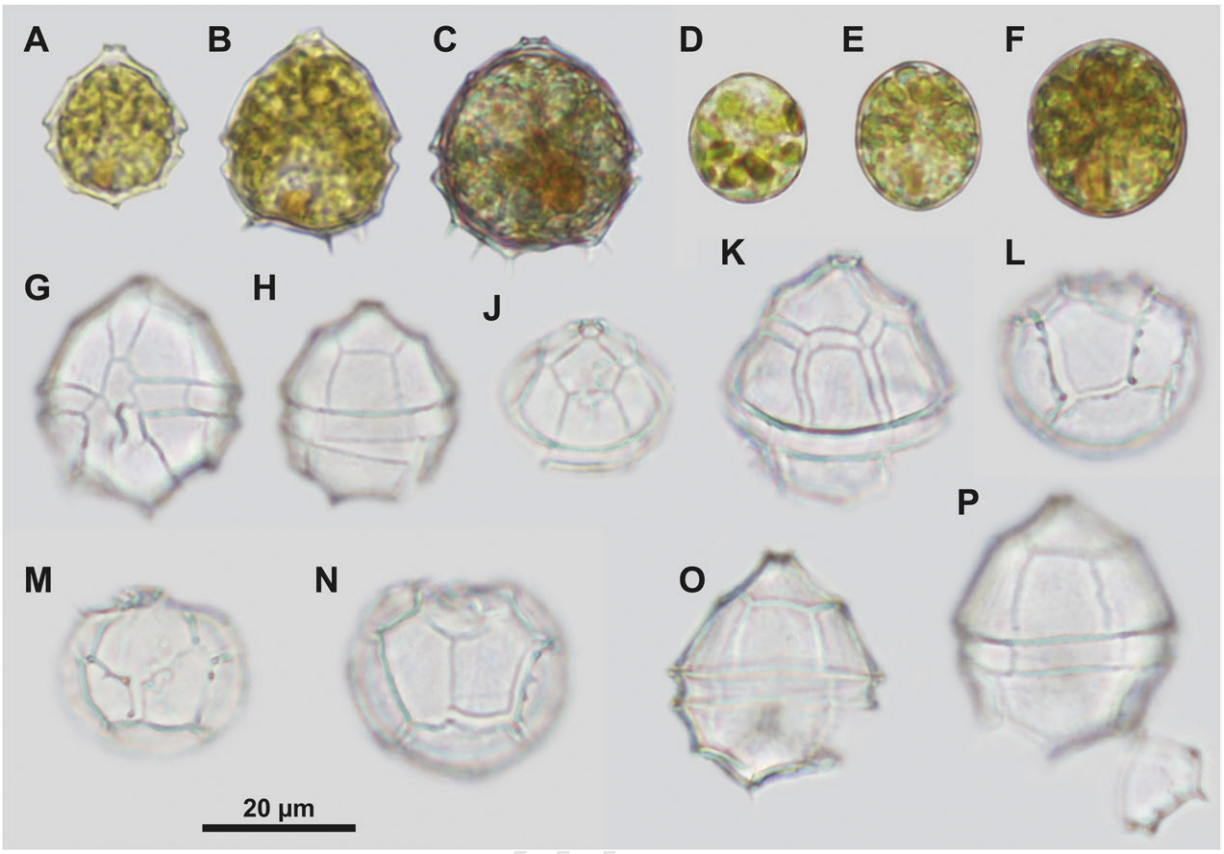

Figure 3. Motile thecate and immotile cells of *Parvodinium elpatiewskyi*, comb. nov. (A–J, M. GeoM*836; K–L, N–O. GeoM 735; P. GeoM*833; LM; all at the same scale). **A–B**. motile thecate cells of different sizes. **C**. immotile thecate cell. **D–F**. immotile coccoid cells of different sizes. **G–P**. empty thecae. **G**. ventral view. **H**. dorsal view showing symmetric epithecal plate pattern with pentagonal apical plate 3′. **J**. dorsal-apical view showing symmetric epithecal plate pattern with pentagonal apical plate 3′. **K**. dorsal-lateral view showing asymmetric epithecal plate pattern with hexagonal apical plate 3′. **L**. antapical view showing hypothecal spines and a contact between plates Sd and 2′′′′. **M**. antapical view showing an opening on the hypotheca resulting from the loss of a posterior sulcal plate. **N**. antapical view showing an opening on the hypotheca after loss of an antapical plate. **O**. dorsal view showing an opening on the hypotheca due to loss of a postcingular plate. **P**. dorsal view showing an opening on the hypotheca, with the antapical plate still attached.

in length and from 17–26 µm (GeoM*836; mean: 23 µm; median: 23 µm; sd: 2 µm; n = 50) in width. The cingulum was excavated, and it surrounded the motile cell with a descendent displacement by approximately half of its own width (Figs 3G, 4 A). The sulcus was likewise excavated, extending slightly into the epitheca. It widened towards the posterior end of the cell and reached down to the antapex (Figs 3G, 4 A, G).

The cell surface of the thecal plates showed an inconspicuously reticulate ornamentation and was irregularly scattered with small circular pores on the thecal plates (probably openings of trichocysts). The sutures between the plates varied in their thickness and were cross-striated (Figs 3G–P, 4 A–H). The thecate plate formula was pp, cp, x, 4′, 0a, 7′′, 6c, 5 s, 5′′′, 2′′′′ (Figs 3G–P, 4 A–H). The arrangement of the epithecal plates was mostly symmetric, showing a pentagonal apical plate 3′ (Figs 3H–J, 4 B–C, H), or sometimes a hexagonal apical plate 3′ that was in contact with plate 6′′ (Figs 3J, 4 E–F). Among the sulcal plates, the Sd plate was notably large, reached down to the antapex and was in contact with antapical plate 2′′′′ (Figs. 3K, 4 A, G).

The first morphotype of immotile cells developed intrathecately. The cells appeared similar to motile cells (Figs 3C, 4 H), but were golden-brown in colour, and their size ranged from 24–35 µm in length (GeoM*836; mean: 30 µm; median: 30 µm; SD: 2 µm; n = 50) and 21–30 µm in width (GeoM*836; mean: 27 µm; median: 27 µm; SD: 2 µm; n = 50). The cytoplasm of such immotile

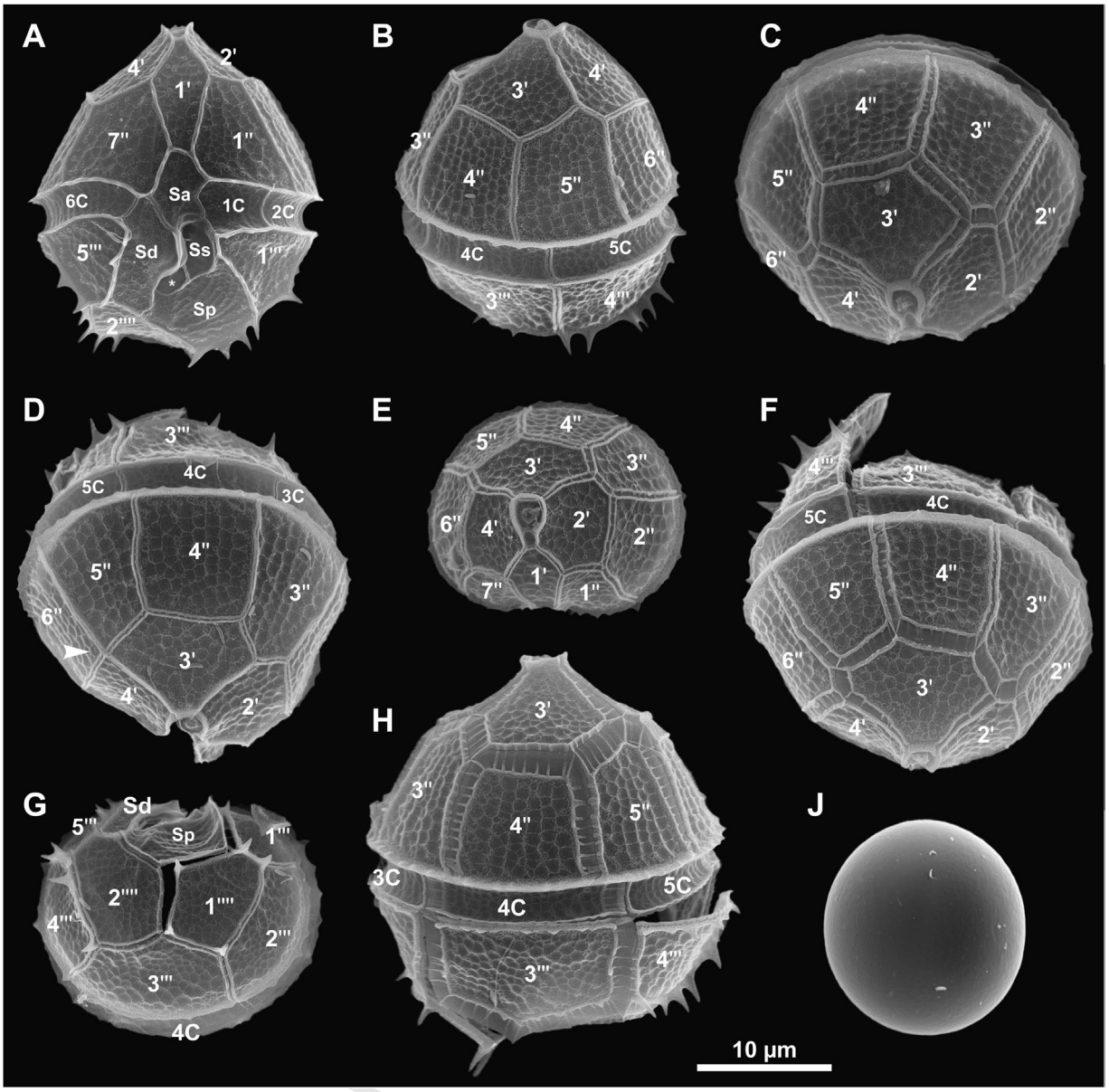

Figure 4. Motile thecate and immotile cells of *Parvodinium elpatiewskyi*, comb. nov. (A–E, J. GeoM 735; B–D, F–H. GeoM*836; SEM; all at the same scale). **A**. motile thecate cells in ventral view (asterisk indicates the sulcal plate Sm). **B**. motile thecate cell in right dorsal-lateral view showing symmetric epithecal plate pattern with pentagonal apical plate 3′. **C**. epitheca in dorsal-apical view showing a pentagonal apical plate 3′. **D**. dorsal view showing asymmetric epithecal plate pattern with pentagonal apical plate 3′; note unusual sutures (arrow) leading to an epithecal pattern that was described as *P. elpatiewskyi* var. *collineatum* Er.Lindem. from Northern Germany (Lindemann 1919). **E**. epitheca in apical view showing asymmetric epithecal plate pattern with hexagonal apical plate 3′. **F**. theca in apical view showing asymmetric epithecal plate pattern with hexagonal apical plate 3′. **G**. antapical view showing hypothecal spines and a contact between plates Sd and 2′′′′. **H**. immotile thecate cell. **J**. immotile coccoid cell. Abbreviations: n′: apical plate. n′′: precingular plate. n′′′: postcingular plate. n′′′′: antapical plate. na: anterior intercalary plate. nC: cingular plate. Sa: anterior sulcal plate. Sd: right sulcal plate. Sm: median sulcal plate. Sp: posterior sulcal plate. Ss: left sulcal plate.

cells was filled with numerous brown granules and frequently contained a large, red accumulation body in the hypotheca (Fig. 3C). The cells of the second immotile morphotype were smaller and ranged from 17–26 μm in length (GeoM*836; mean: 20 μm; median: 20 μm; SD: 2 μm; n = 50) and 14–23 μm in width (GeoM*836; mean: 18 μm; median: 18 μm; SD: 2 μm; n = 50), and the shell had a smooth surface (Figs 3D–F, 4 J). The cytoplasm of those coccoid cells was filled with numerous golden through brown granules and contained frequently a large, red accumulation body (Fig. 3F). The fate of such cells could not be determined.

Molecular Phylogeny

The SSU + ITS + LSU alignment was 1,800 + 652 + 2,478 bp long and comprised 116 + 377 + 321 parsimony informative sites (17%, mean of 23.26 per terminal taxon) as well as 1,467 distinct alignment patterns. Figure 5 shows the best-scoring Bayesian tree (−ln = 18,746), which recovered the Peridiniopsidaceae as monophyletic (100LBS, 1.00BPP), comprising the three major lineages: *Palatinus* (100LBS, 1.00BPP), *Peridiniopsis* (100LBS, 1.00BPP) and *Parvodinium*. The latter showed two highly supported clades (each 100LBS, 1.00BPP), which included either *P. elpatiewskyii*, comb. nov., or accessions determined as *P. umbonatum*, the type species of *Parvodinium*. All accessions determined as *P. elpatiewskyi*, comb. nov., constituted a monophylum (100LBS, 1.00BPP) and showed a close relationship to *P. inconspicuum* (88LBS, 1.00BPP), *Parvodinium mixtum* Wołosz. ex Kretschmann, Owsianny, Zerdoner & Gottschling, *Parvodinium travinskii* Kretschmann, Owsianny, Zerdoner & Gottschling (100LBS, 1.00BPP) and the earliest branching *Parvodinium marciniakii* Kretschmann, Owsianny, Zerdoner & Gottschling (100LBS, 1.00BPP). Peridiniopsidaceae are predominantly of freshwater origin, but two marine taxa were included in the *Peridiniopsis* lineage. Notably, the marine taxa did not constitute a monophyletic group, but "*Scrippsiella*" *hexapraecingula* T.Horig. & Chihara was closely related to the freshwater *Peridiniopsis borgei*.

Discussion

Traits

Parvodinium elpatiewskyi, comb. nov., is a conspicuous freshwater dinophyte that is already documented in a number of previous SEM studies (Ascencio et al. 2015; Cavalcante et al. 2017; Hansen and Flaim 2007; Крахмальный, 2008). It does not appear to be morphologically very variable. The outline shape can vary slightly from stockily pentagonal through broadly ovate with an acuminate apex. Position, length and shape of hypothecal spines and protuberances may also differ but only to a certain extent. These conclusions are drawn from both the first SEM inspection of type material as well as from the study of cultivated material established from a single cell, which is consistent with this type material.

Size and arrangement of the sulcal plate Sd have not received enough attention in the past. In *P. elpatiewskyi*, comb. nov., this sulcal plate is relatively large and is always connected to the antapical plate 2′′′′. This conformation is rare in Peridiniopsidaceae (and Peridiniales as well) and has been otherwise observed in *P. marciniakii* (Kretschmann et al. 2018a) and *Peridinium pygmaeum* Er.Lindem. (Lindemann 1920) only (*Peridinium tatricum* Wołosz. may also exhibit this trait: Wołoszyńska 1916). Moreover, the converging plates Sd and 2′′′′ help to differentiate *P. elpatiewskyi*, comb. nov., from the otherwise similar *P. cunningtonii* var. *pseudoquadridens* that is also present in the type material. In the molecular tree, taxa with the Sd plate connected to the second antapical plate (i.e., *P. elpatiewskyi*, comb. nov., and *P. marciniakii*) do not constitute a monophyletic group, thus an independent evolution should be considered.

Despite its relatively large size in comparison to its closely related taxa, molecular phylogenetics undoubtedly place *P. elpatiewskyi*, comb. nov., in the Peridiniopsidaceae and in *Parvodinium*. The species is thus embedded in a group that predominantly exhibits two intercalary plates (Gottschling et al. 2017; exceptionally three in "*Scrippsiella*" *hexapraecingula*: Horiguchi and Chihara 1983; Loeblich et al. 1979; or one in *P. borgei*: Lemmermann 1904), and inference of character evolution indicates a loss of two intercalary plates in *P. elpatiewskyi*, comb. nov. Molecular phylogenetics (Gottschling et al. 2017; Žerdoner Čalasan et al. 2019) further shows that reduction of (intercalary) plates has taken place several times independently in Peridiniales, namely at least three times in *Parvodinium* (Peridiniopsidaceae), *Tyrannodinium* (Thoracosphaeraceae) and *Unruhdinium* (Kryptoperidiniaceae). Therefore, the generic circumscription of *Parvodinium*, and the familial circumscription of Peridiniopsidaceae, have to be adjusted accordingly. Peridiniopsidaceae show a

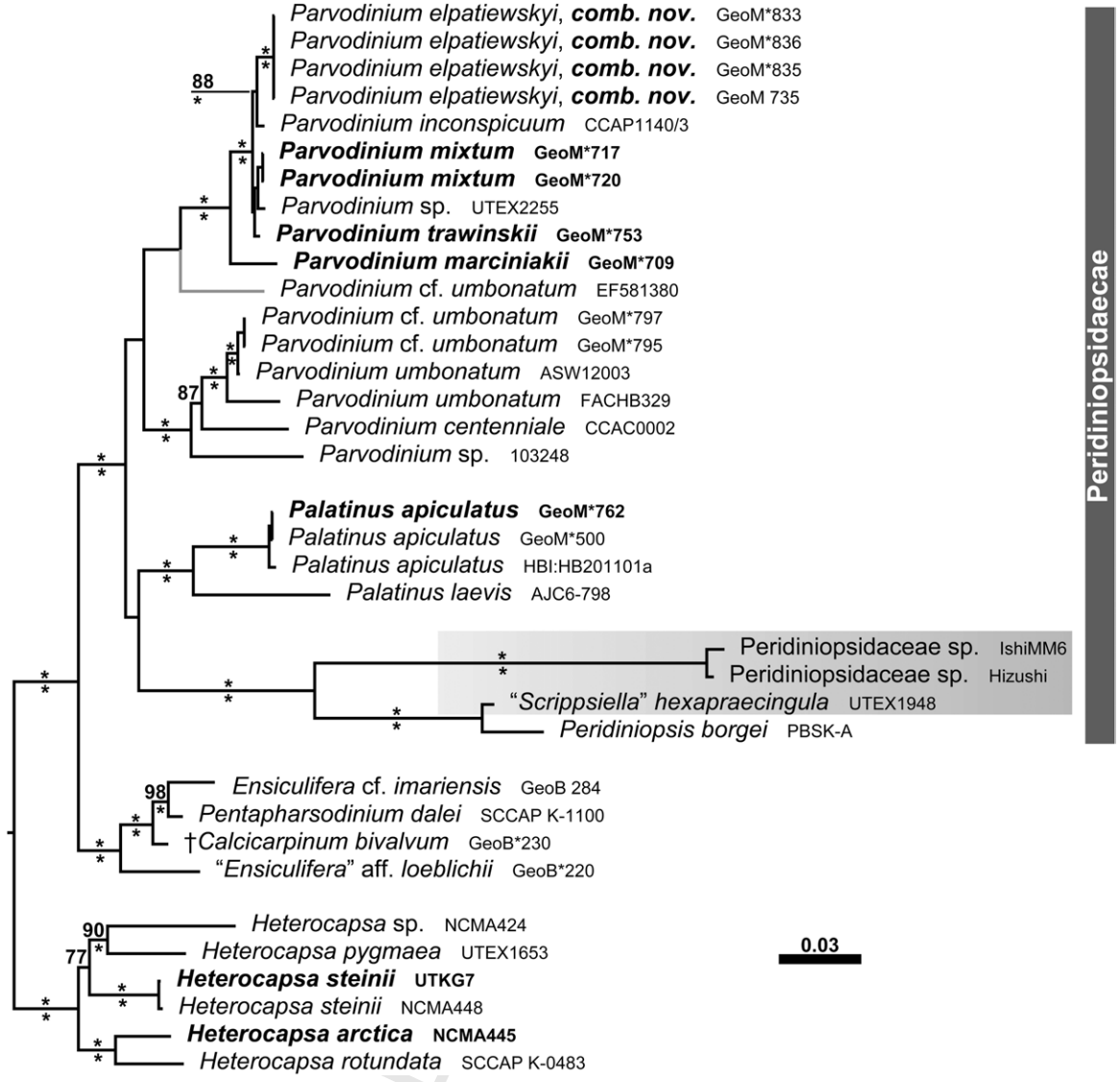

Figure 5. Bayesian tree of 25 peridiniopsidacean operational taxonomic units (OTUs) under the GTR+Γ substitution model. Typified OTUs are highlighted in bold, and branch lengths are drawn to scale, with the scale bar indicating the number of nucleotide substitutions per site. The numbers on the branches are statistical support values (above: ML bootstrap values, values <50 are not shown; below: Bayesian posterior probabilities, values <.90 are not shown; asterisks indicate maximal support; note that statistical support values were derived from analyses without EF581380, the corresponding branch is, therefore, shaded in grey). Marine Peridiniopsidaceae are shaded grey.

similarly large diversity of epithecal conformation as Thoracosphaeraceae (incl. Pfiesteriaceae: Calado et al. 2009), and this underlines the remarkably dynamic evolution of this trait. Moreover, Žerdoner Čalasan et al. (2019) have noted that evolutionary transitions have taken place from the marine into the freshwater environment exclusively, and originally freshwater Periodiniopsidaceae may account for the first dinophyte example of a reversal transition in this respect.

Life-history of Peridiniopsidaceae is complex and largely unexplored at present. The formation of a coccoid cell ('cyst') within a thecate cell is a phenomenon that has been described numerously for different members of this taxon (Kretschmann et al. 2018a; Lefèvre 1927; Lindemann 1919; Schilling 1891; Thompson 1947; West 1909) and therefore, the presence of such cells in *P. elpatiewskyi*, comb. nov., does not come as a surprise. These are, however, firstly documented here, as the drawings

provided by Ostenfeld (1907) refer to a now different species. Those coccoid cells that are formed intrathecately are a part of the diploid stage during metagenesis and may thus become thecate cells later, which leads to the production of four meiospores (Pfiester et al. 1984).

Lindemann (1919, 1928) described vegetative replication for cells of *P. elpatiewskyi*, comb. nov. ('valvate division'), that we could not confirm in the cultivated material. Regardless, the numerous empty thecate cells with openings on the hypotheca indicate a highly unusual division mode that has been also shown for *P. apiculatus* (Kretschmann et al. 2018c) and other species of *Parvodinium* (Kretschmann et al. 2018a). Future research is necessary to answer the question whether this division mode is an apomorphy of entire Peridiniopsidaceae. Hypocystal opening of immotile cells, corresponding to the release of antapical plate equivalents, has been documented for *P. inconspicuum* and *P. umbonatum* (Chu et al. 2008; Tardio et al. 2009). A similar archaeopyle has been described for Danian †*Caligodinium amiculum* Drugg (Manum and Williams 1995), and whether this fossil can be assigned to Peridiniopsidaceae must be worked out in future research. The link of the immotile cells to the corresponding thecate cells as well as the integration of all such cells into metagenesis remain obscure.

Taxonomy

Despite the morphological distinctiveness of *P. elpatiewskyi*, comb. nov., its taxonomy remains unresolved at present. Conservation of the type (Compère 1999; Meyer and Elbrächter 1996) has institutionalised another severe misapplication of an old dinophyte name (and an alternative name for C. Ostenfeld's species has not been allocated so far: Kretschmann et al. 2018b). From our contemporary point of view, we probably would have resolved the case of *P. elpatiewskyi*, comb. nov., differently, as we already did for other names such as *Peridinium acuminatum* Ehrenb. (Kretschmann et al. 2015) and *Glenodinium triquetrum* Ehrenb. (Gottschling et al. 2018). Nevertheless, the conservation has not made the name *P. elpatiewskyi*, comb. nov., unambiguous, but has provided new taxonomic problems: The conserved 'type' is an environmental sample with some cells of *P. elpatiewskyi*, comb. nov., that have never been documented in a publication before. We show that a different, though morphologically very similar *P. cunningtonii* var. *pseudoquadridens* is present in this environmental sample as well. In addition, numerous other organisms that can be easily told apart from *P. elpatiewskyi*, comb. nov., are also present in the same environmental sample (ICN Arts 8.2., 9.14). The types of this ecologically important species, exhibiting the currently published morphologies, should be substantiated with an epitype in the future research (preferably prepared from material collected at Plön, see below) to assure the unambiguous application of the name *P. elpatiewskyi*, comb. nov.

Reliable delimitation of *P. elpatiewskyi*, comb. nov., from other, similar dinophyte species remains to be worked out in the future as well. *Peridinium pygmaeum* shares the epithecal conformation (i.e., 4′, 0a, 7″) and the large Sd plate that is in contact with the second antapical plate (Lindemann 1920). The only trait that separates this taxon from *P. elpatiewskyi*, comb. nov., would be the cell size (and possibly a smaller plate 1⁗), but this feature has been shown to vary, at least under cultivation conditions. The material presented here originates from a site close to the type locality of *P. pygmaeum* (i.e., lakes of river Havel near Fürstenberg in Brandenburg, Germany). This collection site also shows a comparable ecology and includes cells of a size (i.e., 22 μm length) that would, according to Lindemann (1920), correspond to *P. pygmaeum*. We thus agree with Popovský and Pfiester (1990) and Cavalcante et al. (2017) that *P. pygmaeum* is most likely synonymous with *P. elpatiewskyi*, comb. nov., and not distinct from it as treated in Moestrup and Calado (2018). Furthermore, the unusual epithecal conformation (i.e., 4′, 0a, 7″) is found in Ukrainian *Peridinium charkowiense* Matv. (Матвиенко 1938) and Austrian *Peridinium hiemale* J.Schiller (Schiller 1955). Neither of these taxa exhibit a hypothecal spine or protuberance, as it is characteristic for *P. elpatiewskyi*, comb. nov., and have a more spherical shape. Thus, they appear to be distinct from *P. elpatiewskyi*, comb. nov. Nevertheless, their possible assignment to *Parvodinium* should be elaborated based on newly collected material at the corresponding type localities.

Nomenclature

Parvodinium elpatiewskyi (Ostenf.) Kretschmann, Zerdoner & Gottschling, comb. nov., basionym: *Peridinium umbonatum* var. *elpatiewskyi* Ostenf., Hedwigia 46: 391, pl. IX 9–12. 1907. *Peridinium elpatiewskyi* (Ostenf.) Lemmerm., Kryptogamenflora der Mark Brandenburg. Dritter Band [Algen I (Schizophyceen, Flagellaten, Peridineen)]: 670, figs 20–24. 1910.

Glenodinium elpatiewskyi (Ostenf.) J.Schiller, Rabenhorst's Kryptogamen-Flora. Zweite Auflage. Band 10, Abt. 3, Teil 2. Alt. t.p.: Dinoflagellatae (Peridineae): 115, fig. 113. 1937. *Peridiniopsis elpatiewskyi* (Ostenf.) Bourr., Protistologica 4: 9. 1968.—Type (cons., Compère, 1999): Germany. Schleswig-Holstein, Plön, Plußsee [non-fossil environmental sample], Aug 2, 1976: B. Meyer 244 [B 40 0036959!, holotype; CEDiT2019I99!, isotype).

Notes: The act is registered at phycobank http://phycobank.org/102076. From the isotype, two SEM-stubs (B 40 0043809!, CEDiT2019RM98!) were prepared. For the correct application of the name, all elements in the environmental sample that do not show the traits depicted in Figs. 1, 2 and Supplementary Material Fig. S2 are disregarded (ICN Arts 8.2, 9.14).

Lindemann (1919) identified the wrong application of *Peridinium marchicum* Lemmerm. (with 2 intercalary plates) by Wołoszyńska (1916), as she introduced the new variety *P. marchicum* var. *simplex* Wołosz. (without any intercalary plate). The latter is probably a synonym of *P. elpatiewskyi*, comb. nov., typ. cons. (Lindemann 1919; Moestrup and Calado 2018), but none of such names refer to the original intent of Ostenfeld (1907). The Mongolian taxon of the latter author, thus, remains unnamed until today (Kretschmann et al. 2018b).

With the unusual state of zero anterior intercalary plates, *P. elpatiewskyi*, comb. nov., is morphologically distinct from other species of *Parvodinium*, thus the introduction of a new taxon at the generic level appears feasible at a first glance. However, this proceeding would render the remainders of *Parvodinium* paraphyletic. An alternative solution to this problem would be an inclusion of further species of *Parvodinium* in the newly generated taxon. Nevertheless, if species such as *P. mixtum* were to be included in the new taxon, it would be hard to delimit it against *Parvodinium* in a strict sense. The strongest argument against the erection of a new generic name is the unknown identity of *P. umbonatum* (see also survey in Moestrup and Calado 2018), the type species of *Parvodinium*. There are a number of sequences associated with that name available in GenBank (Fig. 5), but none of which is from the type locality or show the distinct morphology of the historical taxon. If *P. elpatiewskyi* and *P. umbonatum* are to be placed on the same phylogenetic branch in the future, the new generic name would immediately become a later synonym of *Parvodinium*. Elevating this taxon on the generic level would thus become superfluous and therefore, we do not see any significant advantages of a new generic name. Further, similar examples of taxa with slightly deviating plate patterns are *Durinskia* Carty & El.R.Cox (Kretschmann et al. 2018b) and *Heterocapsa* F.Stein (Tillmann et al. 2017). Surely, nobody would elevate *Durinskia agilis* (Kof. & Swezy) Saburova, Chomérat & Hoppenrath, or other species of *Heterocapsa* different from *Heterocapsa steinii* Tillmann, Gottschling, Hoppenrath, Kusber & Elbr., to new taxa at the generic level based on minor diagnostic traits. For all the above reasons, we think that *P. elpatiewskyi*, comb. nov., is best placed in *Parvodinium* at this moment in time, at least as long as we do not uncover the entire diversity of the taxon in question.

Methods

Material collection, cultivation and morphology: The investigated material was obtained from three different sources: 1) The type material was collected at the Plußsee in Germany (Schleswig-Holstein, Plön) on 2 Aug 1976 and was fixed in formalin. It is stored in the Berlin herbarium and at the Centre of Excellence for Dinophyte Taxonomy (CEDiT, see Nomenclature section). 2) Further material was collected at the Plußsee as well on 1 Aug 1977. 3) During a field trip on 26 Jun 2015, water tow samples were collected in a pond (52°29.522′N, 13°14.066′E) from the area of the Nature Conservation Centre Ökowerk (Germany, Berlin) using a plankton net with a mesh size of 20 μm. Motile cells were isolated and placed in 24-well microplates (Zefa; Munich, Germany) containing freshwater WC growth medium (Woods Hole Combo, modified after Guillard and Lorenzen 1972) without silicate. The plates were stored in climate chambers at 12 °C or 18 °C, respectively, and under 12:12 h light:dark photoperiod. One strain (GeoM 735) as well as four monoclonal substrains (GeoM*833, GeoM*834, GeoM*835 and GeoM*836) were established and are kept at the Institute of Systematic Botany and Mycology (University of Munich).

Cells were observed, documented and measured under a CKX41 inverted microscope (Olympus; Hamburg, Germany) equipped with a phase contrast option and a DP73 digital camera (Olympus). The preparative techniques for light and scanning electron microscopy (SEM) followed standard protocols (Janofske 2000) and were the same as described in Gottschling et al. (2012). Briefly, cells were fixed in 2.5% glutaraldehyde overnight. Afterwards, specimens were dehydrated in a graded acetone series and critical point dried, followed by sputter-coating with platinum. The Kofoidean system (Fensome et al. 1993; Taylor 1980) was used to designate the plate formula. Image adjustments (such as scaling, cropping, white-balancing, colour management) were carried out in Photoshop® and Illustrator® (Adobe Systems; Munich, Germany), respectively, and images were arranged in QuarkXPress® (Quark Software; Hamburg, Germany).

Molecular phylogenetics: Genomic DNA was extracted from fresh material using the Nucleo Spin Plant II Kit (Machery-Nagel; Düren, Germany). Various regions of the ribosomal RNA (rRNA) genes including the Internal Transcribed Spacers (ITSs) were amplified using primer pairs specified previously (Gu et al. 2013) and following standard protocols (Gottschling

and Plötner 2004; Gottschling et al. 2012). For alignment constitution, we defined three regions of the rRNA: SSU, ITS, LSU and included a systematically representative set of Peridiniopsidaceae (Gottschling et al. 2017; Supporting Information Tab. S1). For outgroup comparison, we used all sequences of *Heterocapsa*, *Ensiculifera* Balech and close relatives, for which we had genetic data for all three regions SSU, ITS and LSU at hand. Separate matrices were constructed, aligned using 'MAFFT' v6.502a (Katoh and Standley 2013) and concatenated afterwards. The aligned matrices are available as *.nex files upon request. Phylogenetic analyses were carried out using standard procedures (Kretschmann et al. 2018c).

Declarations of Interest

None.

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Appendix A. Supplementary Data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.protis.2019.125700.

References

Ascencio E, Rivera P, Cruces F (2015) Morfología de *Peridiniopsis elpatiewskyi* (Ostenfeld) Bourrelly (Dinophyceae) encontrada por primera vez en aguas continentales de Chile. Gayana Botanica **72**:42–46

Bourrelly P (1968) Notes sur les péridiniens d'eau douce. Protistologica **4**:5–13

Calado AJ, Craveiro SC, Daugbjerg N, Moestrup Ø (2009) Description of *Tyrannodinium* gen. nov., a freshwater dinoflagellate closely related to the marine *Pfiesteria*-like species. J Phycol **45**:1195–1205

Carty S (2014) Freshwater Dinoflagellates of North America. Cornell University Press, Ithaca, 280 p

Cavalcante KP, Craveiro SC, Calado AJ, Ludwig TAV, Cardoso LdS (2017) Diversity of freshwater dinoflagellates in the State of Paraná, southern Brazil, with taxonomic and distributional notes. Fottea **17**:240–263

Chu G, Sun Q, Rioual P, Boltovskoy A, Liu Q, Sun P, Han J, Liu J (2008) Dinocyst microlaminations and freshwater "red tides" recorded in Lake Xiaolongwan, northeastern China. J Paleolimnol **39**:319–333

Compère P (1999) Report of the Committee for Algae: 6. Taxon **48**:811–814

Entz G (1926) Beiträge zur Kenntnis der Peridineen. I. Zur Morphologie und Biologie von *Peridinium borgei* Lemmermann. Arch Protistenkd **56**:397–416

Fensome RA, Taylor FJR, Norris G, Sarjeant WAS, Wharton DI, Williams GL (1993) A Classification of Living and Fossil Dinoflagellates. Micropaleontology Spec Publ **7**:1–245

Gottschling M, Plötner J (2004) Secondary structure models of the nuclear Internal Transcribed Spacer regions and 5. 8S rRNA in Calciodinelloideae (Peridiniaceae) and other dinoflagellates. Nucleic Acids Res **32**:307–315

Gottschling M, Kretschmann J, Žerdoner Čalasan A (2017) Description of Peridiniopsidaceae, fam. nov. (Peridiniales, Dinophyceae). Phytotaxa **299**:293–296

Gottschling M, Tillmann U, Kusber W-H, Hoppenrath M, Elbrächter M (2018) A Gordian knot: Nomenclature and taxonomy of *Heterocapsa triquetra* (Peridiniales: Heterocapsaceae). Taxon **67**:179–185

Gottschling M, Söhner S, Zinßmeister C, John U, Plötner J, Schweikert M, Aligizaki K, Elbrächter M (2012) Delimitation of the Thoracosphaeraceae (Dinophyceae), including the calcareous dinoflagellates, based on large amounts of ribosomal RNA sequence data. Protist **163**:15–24

Gu H, Kirsch M, Zinßmeister C, Söhner S, Meier KJS, Liu T, Gottschling M (2013) Waking the dead: Morphological and molecular characterization of extant †*Posoniella tricarinelloides* (Thoracosphaeraceae, Dinophyceae). Protist **164**:583–597

Guillard RR, Lorenzen CJ (1972) Yellow-green algae with chlorophyllide c. J Phycol **8**:10–14

Hansen G, Flaim G (2007) Dinoflagellates of the Trentino Province, Italy. J Limnol **66**:107–141

Höll K (1928) Oekologie der Peridineen. Studien über den Einfluß chemischer und physikalischer Faktoren auf die Verbreitung der Dinoflagellaten im Süßwasser. Fischer, Jena, 105 p

Horiguchi T, Chihara M (1983) *Scrippsiella hexapraecingula* sp. nov. (Dinophyceae), a tide pool dinoflagellate from the Northwest Pacific. Bot Mag, Tokyo **96**:351–358

Janofske D (2000) *Scrippsiella trochoidea* and *Scrippsiella regalis*, nov. comb. (Peridiniales, Dinophyceae): A comparison. J Phycol **36**:178–189

Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. Mol Biol Evol **30**:772–780

Крахмальный **АФ** (2008) Морфология теки *Peridiniopsis elpatiewskyi* (Ostenf.) Bourr. (Dinophyta). Альгология **18**:366–374

Kretschmann J, Žerdoner Čalasan A, Gottschling M (2018b) Molecular phylogenetics of dinophytes harbouring diatoms as endosymbionts (Kryptoperidiniaceae, Peridiniales), with evolu-

tionary interpretations and a focus on the identity of *Durinskia oculata* from Prague. Mol Phylogenet Evol **118**:392–402

Kretschmann J, Owsianny PM, Žerdoner Čalasan A, Gottschling M (2018a) The hot spot in a cold environment: Puzzling *Parvodinium* (Peridiniopsidaceae, Peridiniales) from the Polish Tatra Mountains. Protist **169**:206–230

Kretschmann J, Žerdoner Čalasan A, Kusber W-H, Gottschling M (2018c) Still curling after all these years: *Glenodinium apiculatum* Ehrenb. (Peridiniales, Dinophyceae) repeatedly found at its type locality in Berlin (Germany). Syst Biodivers **16**:200–209

Kretschmann J, Elbrächter M, Zinßmeister C, Söhner S, Kirsch M, Kusber W-H, Gottschling M (2015) Taxonomic clarification of the dinophyte *Peridinium acuminatum* Ehrenb. , ≡ *Scrippsiella acuminata*, comb. nov. (Thoracosphaeraceae, Peridiniales). Phytotaxa **220**:239–256

Lefèvre MM (1927) Sur les variations tabulaires chez les Péridiniens d'eau douce et leur notation. — Diagnoses d'espèces et de variétés nouvelles. Bull Mus Nat d'Histoïre Naturelle **33**:118–122

Lemmermann EJ (1904) Das Plankton schwedischer Gewässer. Arkiv Bot **2**:1–209

Lemmermann EJ (1910) Algen I (Schizophyceen, Flagellaten, Peridineen). Bornträger, Leipzig, 712 p

Lindemann EBLW (1919) Untersuchungen über Süßwasserperidineen und ihre Variationsformen. Arch Protistenkd **39**:209–262

Lindemann EBLW (1920) Untersuchungen über Süßwasserperidineen und ihre Variationsformen II. Arch Naturgesch **84**:121–194

Lindemann EBLW (1928) Abteilung Peridineae (Dinoflagellatae). In Engler A, Prantl K (eds) Die Natürlichen Pflanzenfamilien. 2. edn Engelmann, Leipzig, pp 3–104

Liu G-X, Pei GF, Hu Z-Y (2008) *Peridiniopsis niei* sp. nov. (Dinophyceae), a new species of freshwater red tide dinoflagellates from China. Nova Hedw **87**:487–499

Loeblich AR III, Sherley JL, Schmidt RJ (1979) Redescription of the thecal tabulation of *Scrippsiella gregaria* (Lombard and Capon) comb. nov. (Pyrrhophyta) with light and scanning electron microscopy. Proc Biol Soc Wash **92**:45–50

Manum SB, Williams GL (1995) Hypocystal archeopyles in the dinoflagellate cyst genus *Caligodinium* Drugg. Palynology **19**:183–190

Матвієнко ОМ (1938) Матеріали до вивчення водоростей УРСР. І. Водорості Клюквеного болота. Ученi записки Харківського державного університету ім. О.М. Горького. Книга 14 Труди Н.-д. інституту ботаніки **3**:29–70

Meyer B, Elbrächter M (1996) (1235) Proposal to conserve the name *Peridinium elpatiewskyi* (Dinophyceae) with a conserved type. Taxon **45**:531–532

Moestrup Ø, Calado AJ (2018) Dinophyceae. Springer, Berlin, 560 p

Ostenfeld CH (1907) Beiträge zur Kenntnis der Algenflora des Kossogol-Beekens in der nordwestlichen Mongolei, mit spezieller Berücksichtigung des Phytoplanktons. Hedwigia **46**:365–420

Pfiester LA, Timpano P, Skvarla JJ, Holt JR (1984) Sexual reproduction and meiosis in *Peridinium inconspicuum* Lemmermann (Dinophyceae). Am J Bot **71**:1121–1127

Popovský J, Pfiester LA (1990) Dinophyceae (Dinoflagellida). Fischer, Stuttgart, 272 p

Schiller J (1937) Dinoflagellatae (Peridineae) in monographischer Behandlung. 2. Teil, Lief. 4. In Dr. L. Rabenhorst's Kryptogamen-Flora von Deutschland, Österreich und der Schweiz. Akad. Verlagsges. Leipzig , 589 p

Schiller J (1955) Untersuchungen an den planktischen Protophyten des Neusiedlersees 1950-1954, I. Teil. Wissenschaftliche Arbeiten aus dem Burgenland **9**:1–66

Schilling AJ (1891) Die Süsswasser-Peridineen. Flora **74**:220–229

Takano Y, Hansen G, Fujita D, Horiguchi T (2008) Serial replacement of diatom endosymbionts in two freshwater dinoflagellates, *Peridiniopsis* spp. (Peridiniales, Dinophyceae). Phycologia **47**:41–53

Tardio M, Ellegaard M, Lundholm N, Sangiorgi F, Di Giuseppe G (2009) A hypocystal archeopyle in a freshwater dinoflagellate from the *Peridinium umbonatum* group (Dinophyceae) from Lake Nero di Cornisello, South Eastern Alps, Italy. Eur J Phycol **44**:241–250

Taylor FJR (1980) On dinoflagellate evolution. Biosystems **13**:65–108

Thompson RH (1947) Fresh-water dinoflagellates of Maryland. Contribution / Maryland Department of Research and Education. Chesapeake Biological **67**:3–24

Tillmann U, Hoppenrath M, Gottschling M, Kusber W-H, Elbrächter M (2017) Plate pattern clarification of the marine dinophyte *Heterocapsa triquetra* sensu Stein (1883) collected at the Kiel Fjord (Germany). J Phycol **53**:1305–1324

West GS (1909) A biological investigation of the Peridinieæ of Sutton Park, Warwickshire. New Phytol **8**:181–196

Wołoszyńska J (1916) Polskie Peridineae słodkowodne. – Polnische Süßwasser-Peridineen. Bulletin international de l'Académie des Sciences de Cracovie, Classe des Sciences Mathématiques et Naturelles. Série B **1915**: 260–285

Žerdoner Čalasan A, Kretschmann J, Gottschling M (2019) They are young, and they are many: Dating freshwater lineages in unicellular dinophytes. Environ Microbiol **21**: 4125–4135

Publication 5

Description of Peridiniopsidaceae, fam. nov. (Peridiniales, Dinophyceae)

GOTTSCHLING, M., **KRETSCHMANN, J.** & ŽERDONER ČALASAN, A.

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Description of Peridiniopsidaceae, fam. nov. (Peridiniales, Dinophyceae)

MARC GOTTSCHLING*, JULIANE KRETSCHMANN & ANŽE ŽERDONER ČALASAN
Department Biologie, Systematische Botanik und Mykologie, GeoBio-Center, Ludwig-Maximilians-Universität München, Menzinger Str. 67, D – 80 638 Munich, Germany
** Corresponding author, e-mail: gottschling@bio.lmu.de*

The vast majority not only of dinophytes, but also of Peridiniales, live in the marine environment, and some 350 (out of ca 2.500) species are present in freshwater habitats (Mertens *et al.* 2012). Many freshwater Peridiniales constitute small and only distantly related species groups embedded in predominantly marine clades (e.g., *Apocalathium*, *Chimonodinium*, *Naiadinium* in the Thoracosphaeraceae; species assigned to "*Peridiniopsis*" in the Kryptoperidiniaceae; Zhang *et al.* 2011b, Gottschling & Söhner 2013, Annenkova *et al.* 2015; Fig. 1). However, the most distinguished freshwater group of the Peridiniales are the Peridiniaceae including the type species, *Peridinium cinctum*, and other frequently encountered species such as *Peridinium bipes*, *Peridinium volzii* and *Peridinium willei*.

Molecular sequence data have greatly elucidated the phylogenetic relationships of dinophytes in the past two decades. We now know that not all freshwater species formerly assigned to *Peridinium* constitute a monophyletic group, which is also reflected in the establishment of numerous new scientific names (at, e.g., the generic level). A rigorous classification of the Peridiniales is still pending but in several publications (Tillmann *et al.* 2012, 2014, Gottschling & Söhner 2013, Gottschling & McLean 2013, Gu *et al.* 2013), we aimed at an improved knowledge of phylogenetic systematics for dinophytes in general and for the Peridiniales in particular by concatenating ribosomal RNA sequences (Fig. 1). As a result, a number of monophyletic and statistically well supported lineages can be recognised in the Peridiniales, more or less corresponding to established taxonomic units based on morphology. Probably not less than 90% of peridinialean species (known from molecular sequence data) can now be reliably placed into one of the following taxa at the family level: Blastodiniaceae, Heterocapsaceae, Kryptoperdiniaceae, Peridiniaceae, Protoperidiniaceae, Thoracosphaeraceae and Zooxanthellaceae.

A predominant freshwater clade being distantly related to *Peridinium* (Craveiro *et al.* 2009, Zhang *et al.* 2011a, Gottschling & Söhner 2013) remains without a name at the family level. It consists of *Palatinus*, *Parvodinium* and *Peridiniopsis*. The latter was segregated from *Peridinium* early in history (Lemmermann 1904), but has not received broad attention until Bourrelly (1968a) transferred all species formerly assigned to *Glenodinium* to *Peridiniopsis*. This rather simplistic taxonomic rearrangement was not justified as we know today, because many species Bourrelly (1968a) placed in *Peridiniopsis* are today identified members of other, already well-established peridinialean lineages such as the Kryptoperidiniaceae and Thoracosphaeraceae (Moestrup & Daugbjerg 2007, Takano *et al.* 2008, Calado *et al.* 2009, Zhang *et al.* 2011b, Gu *et al.* 2013).

The Peridiniaceae are currently treated to include *Peridiniopsis*, species of *Peridinium* (in a broad sense) with the formation of an apical pore complex (APC) and those without such a structure (Popovský & Pfiester 1990, who included also *Thompsodinium* here in an appendix). At first sight, the clade comprising *Palatinus*, *Parvodinium* and *Peridiniopsis* (in a strict sense) may appear morphologically heterogeneous, as it includes elements of all three taxonomic units of the Peridiniaceae. However, the triumvirate can be distinguished from the Peridiniaceae (in a strict sense) because of the consistently reduced number of intercalary plates: Peridiniaceae (in a strict sense) usually have three intercalary plates, while members of Peridiniopsidaceae, fam. nov., as treated here possess not more than two such plates. The presence of predominantly five cingular plates in Peridiniaceae (in a strict sense) may also be supportive to distinguish it from Peridiniopsidaceae, fam. nov., as treated here having six of such plates (Bourrelly 1968b, Carty 2008, Craveiro *et al.* 2009).

Utrastructural studies have improved our knowledge of dinophytes at least as much as the sequencing approach. A feeding organelle coined peduncle is reported from many Peridiniales (Calado & Moestrup 2002, Craveiro *et al.* 2009, Craveiro *et al.* 2015, Kang *et al.* 2015), but has been explicitly found absent from *Peridinium cinctum* (Calado *et al.* 1999). Knowledge of other Peridiniaceae (in a strict sense) is scarce, but presence/absence of peduncle microtubules may argue as a diagnostic trait between the two freshwater clades as well. Further differences may be uncovered referring, for example, to the connection of peripheral lobes to a central pyrenoid in Peridiniopsidaceae, fam. nov. (Calado & Moestrup 2002, Craveiro *et al.* 2009), *versus* a system with numerous chloroplasts distributed peripherally in *Peridinium cinctum* (Calado *et al.* 1999).

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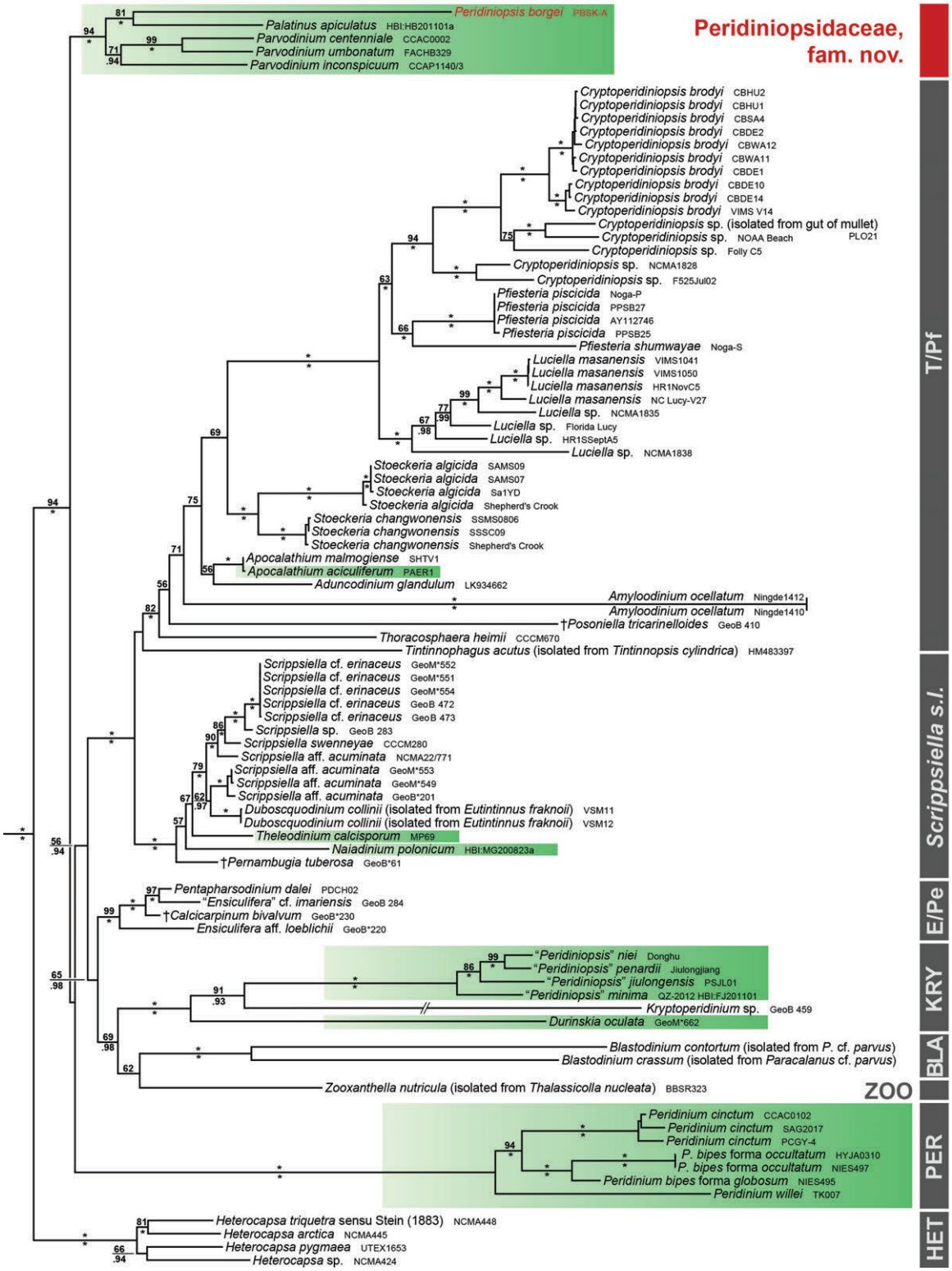

FIGURE 1. Peridiniaceae and Peridiniopsidaceae, fam. nov., have distinct systematic positions in the peridinialean molecular tree. Maximum Likelihood (ML) tree (–ln=62.863,31) of 89 Peridiniales operational taxonomic units (OTUs; plus 42 Amphidomataceae as outgroup, not shown) under the GTR+Γ substitution model. For alignment constitution, we defined three regions of the rRNA: SSU, ITS, LSU, and included all Peridiniales, of which sequence information in all three regions were available. Freshwater lineages are shaded in green, and the Peridiniopsidaceae, fam. nov., with its type species, *Peridiniopsis borgei*, are highlighted. Branch lengths are drawn to scale, with the scale bar indicating the number of nucleotide substitutions per site. The numbers on the branches are statistical support values (above: ML bootstrap values derived from 1.000 non-parametric replicates, values <50 are not shown; below: Bayesian posterior probabilities derived from two independent analyses of four chains with 20.000.000 cycles, sampled every 1.000th cycle, values <.90 are not shown). Asterisks indicate maximal support. Abbreviations: BLA: Blastodiniaceae. E/Pe: clade including *Ensiculifera* and *Pentapharsodinium*. HET: Heterocapsaceae. KRY: Kryptoperidiniaceae. PER: Peridiniaceae. T/Pf: clade including *Pfiesteria* and *Thoracosphaera*. ZOO: Zooxanthellaceae.

For all the above reasons, we here introduce a new peridinialean taxon at the family level:

Peridiniopsidaceae Gottschling, Kretschmann & Zerdoner, **fam. nov.**—Type genus: *Peridiniopsis* Lemmerm. (with its type species, *P. borgei* Lemmerm.), Ark. Bot. 2 (1904): 134.

Description: Thecate, phototrophic, free-living primarily freshwater dinophytes. Kofoidian plate formula: ≤4', ≤2a, ≤7'', 6c, 5s, 5''', 2'''', apical pore complex present (*Parvodinium*, *Peridiniopsis*) or absent (*Palatinus*). Plate surface smooth through granulate, but never with ridges forming areolae, hypotheca variously spinose through smooth. Chloroplast lobes radiating from a central pyrenoid; eyespot and peduncle microtubules present. Dividing or ecdysing cells exiting the theca through the antapical area. n≈40 (Holt & Pfiester 1982).

The Peridiniopsidaceae, fam. nov., are taxonomically distinct and may include some 15 species occurring in temperate through tropical freshwater habitats around the world (frequently encountered species are *Palatinus apiculatus*, *Parvodinium africanum*, *P. centenniale*, *P. deflandrei*, *P. goslaviense*, *P. inconspicuum*, *P. lubieniense*, *P. umbonatum* and *Peridiniopsis borgei*). They can be delimited from other peridinialean families based on a combination of traits (Tab. 1) including the preferred freshwater *versus* the otherwise primarily marine environment. They are further distinct from the other genuine freshwater family, Peridiniaceae, because of the presence of not more than two (*versus* three) intercalary plates and six (*versus* five) cingular plates. Ultrastructural traits such as the presence of peduncle microtubules and chloroplast lobes radiating from a central pyrenoid may provide further evidence for the distinctiveness of the new family. Last but not least, the Peridiniopsidaceae, fam. nov., constitute a monophyletic lineage of the Peridiniales distinct from the Peridiniaceae and other peridinialean lineages in molecular phylogenetics (Fig. 1). Future research will enlighten the systematic positions of *Glochidinium*, *Staszicella*, *Thompsodinium* and many other dinophytes in either of Peridiniaceae, Peridiniopsidaeceae, fam. nov., or other (established or even more new) families in the Peridiniales. Family circumscriptions would have to be adjusted if applicable.

TABLE 1. Comparison between the new family, the Peridiniaceae and other peridinialean dinophytes. Note that it is a combination of traits that makes the Peridiniopsidaceae, fam. nov., a distinct lineage of the Peridiniales deserving its own name.

trait	Peridiniaceae	Peridiniopsidaceae, fam. nov.	other peridinialean dinophytes	main literature source
habitat	freshwater	freshwater	mostly marine	original descriptions
molecular phylogenetics	distinct from Peridiniopsidaceae, fam. nov., and other peridinialean dinophytes	distinct from Peridiniaceae and other peridinialean dinophytes	distinct from Peridiniaceae and Peridiniopsidaceae, fam. nov.,	Gottschling & McLean (2013), Gottschling & Söhner (2013), Gu *et al.* (2013)
number of intercalary plates	3	≤2	varying	original descriptions and floras, Bourrelly (1968b)
number of cingular plates	5	6	varying, but predominantly 5 or 6	original descriptions and floras, Bourrelly (1968b)
peduncle	absent in *Peridinium*	present in *Palatinus* and *Peridiniopsis*	frequently present, but trait not densely sampled yet	Calado et al. (1999), Calado & Moestrup (2002), Craveiro *et al.* (2009)

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References

Annenkova, N.V., Hansen, G., Moestrup, Ø. & Rengefors, K. (2015) Recent radiation in a marine and freshwater dinoflagellate species flock. *ISME Journal* 9: 1821–1834.
https://doi.org/10.1038/ismej.2014.267

Bourrelly, P. (1968a) Notes sur les péridiniens d'eau douce. *Protistologica* 4: 5–13.

Bourrelly, P. (1968b) Note sur *Peridiniopsis borgei* Lemm. *Phykos* 7: 1–2.

Calado, A.J. & Moestrup, Ø. (2002) Ultrastructural study of the type species of *Peridiniopsis*, *Peridiniopsis borgei* (Dinophyceae), with special reference to the peduncle and flagellar apparatus. *Phycologia* 41: 567–584. https://doi.org/10.2216/i0031-8884-41-6-567.1

Calado, A.J., Hansen, G. & Moestrup, Ø. (1999) Architecture of the flagellar appartus and related structures in the type species of *Peridinium*, *P. cinctum* (Dinophyceae). *European Journal of Phycology* 34: 179–191. https://doi.org/10.1080/09670269910001736232

Calado, A.J., Craveiro, S.C., Daugbjerg, N. & Moestrup, Ø. (2009) Description of *Tyrannodinium* gen. nov., a freshwater dinoflagellate closely related to the marine *Pfiesteria*-like species. *Journal of Phycology* 45: 1195–1205. https://doi.org/10.1111/j.1529-8817.2009.00735.x

Carty, S. (2008) *Parvodinium* gen. nov. for the umbonatum group of *Peridinium* (Dinophyceae). *Ohio Journal of Science* 108: 103–107.

Craveiro, S.C., Calado, A.J., Daugbjerg, N. & Moestrup, Ø. (2009) Ultrastructure and LSU rDNA-based revision of *Peridinium* group Palatinum (Dinophyceae) with the description of *Palatinus* gen. nov. *Journal of Phycology* 45: 1175–1194. https://doi.org/10.1111/j.1529-8817.2009.00739.x

Craveiro, S.C., Daugbjerg, N., Moestrup, Ø. & Calado, A.J. (2015) Fine-structural characterization and phylogeny of *Peridinium polonicum*, type species of the recently described genus *Naiadinium* (Dinophyceae). *European Journal of Protistology* 51: 259–279. https://doi.org/10.1016/j.ejop.2015.05.001

Gottschling, M. & Söhner, S. (2013) An updated list of generic names in the Thoracosphaeraceae. *Microorganisms* 1: 122–136. https://doi.org/10.3390/microorganisms1010122

Gottschling, M. & McLean, T.I. (2013) New home for tiny symbionts: Dinophytes determined as *Zooxanthella* are Peridiniales and distantly related to *Symbiodinium*. *Molecular Phylogenetics and Evolution* 67: 217–222. https://doi.org/10.1016/j.ympev.2013.01.003

Gu, H., Kirsch, M., Zinßmeister, C., Söhner, S., Meier, K.J.S., Liu, T. & Gottschling, M. (2013) Waking the dead: Morphological and molecular characterization of extant †*Posoniella tricarinelloides* (Thoracosphaeraceae, Dinophyceae). *Protist* 164: 583–597. https://doi.org/10.1016/j.protis.2013.06.001

Holt, J.R. & Pfiester, L.A. (1982) A technique for counting chromosomes of armored dinoflagellates, and chromosome numbers of 6 freshwater dinoflagellate species. *American Journal of Botany* 69: 1165–1168. https://doi.org/10.2307/2443090

Kang, N.S., Jeong, H.J., Moestrup, Ø., Jang, T.Y., Lee, S.Y. & Lee, M.J. (2015) *Aduncodinium* gen. nov and *A. glandula* comb. nov (Dinophyceae, Pfiesteriaceae), from coastal waters off Korea: Morphology and molecular characterization. *Harmful Algae* 41: 25–37. https://doi.org/10.1016/j.hal.2014.11.002

Lemmermann, E.J. (1904) Das Plankton schwedischer Gewässer. *Arkiv för Botanik* 2: 1–209.

Mertens, K.N., Rengefors, K., Moestrup, Ø. & Ellegaard, M. (2012) A review of recent freshwater dinoflagellate cysts: Taxonomy, phylogeny, ecology and palaeocology. *Phycologia* 51: 612–619. https://doi.org/10.2216/11-89.1

Moestrup, Ø. & Daugbjerg, N. (2007) On dinoflagellate phylogeny and classification. *In:* Brodie, J. & Lewis, J. (Eds) *Unravelling the algae, the past, present, and future of algal systematics*. CRC Press, Boca Raton, pp. 215–230. https://doi.org/10.1201/9780849379901.ch12

Popovský, J. & Pfiester, L.A. (1990) *Dinophyceae (Dinoflagellida)*. Fischer, Stuttgart.

Takano, Y., Hansen, G., Fujita, D. & Horiguchi, T. (2008) Serial replacement of diatom endosymbionts in two freshwater dinoflagenates, *Peridiniopsis* spp. (Peridiniales, Dinophyceae). *Phycologia* 47: 41–53. https://doi.org/10.2216/07-36.1

Tillmann, U., Gottschling, M., Nézan, E., Krock, B. & Bilien, G. (2014) Morphological and molecular characterization of three new *Azadinium* species (Amphidomataceae, Dinophyceae) from the Irminger Sea. *Protist* 165: 417–444. https://doi.org/10.1016/j.protis.2014.04.004

Tillmann, U., Salas, R., Gottschling, M., Krock, B., O'Driscoll, D. & Elbrächter, M. (2012) *Amphidoma languida* sp. nov. (Dinophyceae) reveals a close relationship between *Amphidoma* and *Azadinium*. *Protist* 163: 701–719. https://doi.org/10.1016/j.protis.2011.10.005

Zhang, Q., Liu, G.-X. & Hu, Z.-Y. (2011a) Morphological observation of a freshwater *Peridinium* strain and phylogenetic analysis of *Peridinium*. *Plant Science Journal* 29: 1–10. https://doi.org/10.3724/SP.J.1142.2011.10001

Zhang, Q., Liu, G.-X. & Hu, Z.-Y. (2011b) Morphological differences and molecular phylogeny of freshwater blooming species, *Peridiniopsis* spp. (Dinophyceae) from China. *European Journal of Protistology* 47: 149–160. https://doi.org/10.1016/j.ejop.2011.03.001

Publication 6

Two new generic names for dinophytes harbouring a diatom as an endosymbiont, *Blixaea* and *Unruhdinium* (Kryptoperidiniaceae, Peridiniales)

GOTTSCHLING, M., ŽERDONER ČALASAN, A., **KRETSCHMANN, J.** & GU, H.

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Correspondence

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Two new generic names for dinophytes harbouring a diatom as an endosymbiont, *Blixaea* and *Unruhdinium* (Kryptoperidiniaceae, Peridiniales)

MARC GOTTSCHLING[1,*], ANŽE ŽERDONER ČALASAN[1], JULIANE KRETSCHMANN[1] & HAIFENG GU[2]
[1] *Department Biologie, Systematische Botanik und Mykologie, GeoBio-Center, Ludwig-Maximilians-Universität München, Menzinger Str. 67, D–80638 München, Germany*
[2] *Third Institute of Oceanography, State Oceanic Administration, 361005 Xiamen, China*
[*] *corresponding author, e-mail: gottschling@bio.lmu.de*

Kryptoperidiniaceae are a small group of dinophytes hosting a tertiary endosymbiont derived from a diatom (Tomas *et al.* 1973, Horiguchi & Pienaar 1994). Those so called 'dinotoms' (Imanian *et al.* 2011) include *Dinothrix*, *Durinskia*, *Galeidinium*, *Kryptoperidinium* (= *Phyllodinium*) and some species currently assigned to "*Peridiniopsis*" and "*Peridinium*" (Tamura *et al.* 2005, Horiguchi & Takano 2006, Hansen *et al.* 2007, Zhang *et al.* 2011). Besides 'possessing a diatom endosymbiont' as a highly derived trait, the monophyly of Kryptoperidiniaceae is also supported by a unique type of eyespot that has possibly derived from the original chloroplast (Moestrup & Daugbjerg 2007). In molecular trees, Kryptoperidiniaceae constitute a well supported monophyletic group (Kretschmann *et al.* unpubl.), but it is not finally resolved at present, whether they are embedded in the Thoracosphaeraceae or constitute their sister group (Gottschling & McLean 2013). Regarding habitat preference, molecular trees further indicate at least two independent marine→freshwater transitions in the Kryptoperidiniaceae during the late Paleogene at the latest (Žerdoner Čalasan *et al.* unpubl.).

As currently treated, "*Peridiniopsis*" and "*Peridinium*" are highly polyphyletic assemblages, and a major effort during the past years was put into the disentanglement of the present taxonomic confusion and inconsistency (Carty 2008, Calado 2011, Craveiro *et al.* 2011, 2016, Kretschmann *et al.* 2015, to mention only a few studies). A rigorous classification of peridinialean dinophytes is still pending but in several publications (Tillmann *et al.* 2012, 2014, Gottschling & McLean 2013, Gottschling & Söhner 2013, Gottschling *et al.* 2017, Gu *et al.* 2013), we aimed at an improved knowledge about phylogenetic systematics of dinophytes by concatenating ribosomal RNA sequences. As a result, some species currently assigned to "*Peridiniopsis*" and "*Peridinium*" (Horiguchi & Takano 2006, Liu *et al.* 2008, Takano *et al.* 2008, Zhang *et al.* 2011, 2014, Yamada *et al.* 2015, You *et al.* 2015) clearly belong to Kryptoperidiniaceae, but not to the taxa, under which they were initially described. Based on molecular and morphological data they represent two distinct evolutionary lineages that are described here as new, namely *Blixaea*, gen. nov., and *Unruhdinium*, gen. nov. New combinations are provided for those species names only, of which morphological and molecular data are available for critical examination.

The specificity of the diatom endosymbiont for their host is not rigorously worked out at present. Some dinophyte species appear to harbour genetically different endosymbionts (Yamada *et al.* in press, Žerdoner Čalasan *et al.* unpubl.), while other species maintain endosymbionts with very similar DNA sequences (e.g., *Unruhdinium* cf. *kevei*, comb. nov., and *Unruhdinium jiulongense*, comb. nov.: Takano *et al.* 2008, You *et al.* 2015). Nevertheless, Kryptoperidiniaceae are highly selective towards specific groups of diatoms and do not recruit them arbitrarily: The endosymbiont of marine *Blixaea quinquecornis*, comb. nov., for example, is part of a well resolved group within *Chaetoceros*—probably the largest taxon of marine centric diatoms (Horiguchi & Takano 2006)—, and endosymbionts of freshwater *Unruhdinium*, gen. nov., cluster within freshwater *Cyclotella*, but neither within other freshwater species of *Nitzschia* as in the case of *Durinskia*, *Galeidinium* and *Kryptoperidinium*.

Taxonomic activity

Blixaea Gottschling, *gen. nov.*—Type: *Blixaea quinquecornis* (T.H.Abé) Gottschling, *comb. nov.*

Description:—Thecate, phototrophic, free-living, primarily marine dinophytes harbouring a *Chaetoceros*-like diatom as endosymbiont, which is separated from the host by a single unit membrane. Kofoidian plate formula: 3', 2a, 7'', 5c, 5''', 2'''', apical pore complex present. Plate surface smooth through granulate, hypotheca with (three through five) predominantly

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four distinct spines of varying length. Chloroplasts numerous and belonging to the endosymbiotic alga; eyespot surrounded by three membranes.

Etymology:—The name honours Blixa Bargeld (*1959), who is singer, musician and founder of the Berlin music group Einstürzende Neubauten. The generic name *Blixaea* is sufficiently distinct from malvalean *Bixa* (Linné 1753), because of both the differential auditory phonetics and the diverging taxonomic assignments, that ICN Art. 53 does not apply.

Blixaea, gen. nov., is currently monotypic, but may include more taxa such as "*Peridinium*" *quinquecorne* var. *trispiniferum* from Mexico (Aké-Castillo & Vázquez 2011). The diagnostic feature of *Blixaea*, gen. nov., is the presence of predominantly four distinctive hypothecal spines (Abé & Saitō 1981), and a *Chaetoceros*-like diatom as endosymbiont (Horiguchi & Pienaar 1991) has not been reported from any other Kryptoperidiniaceae. In molecular phylogenetics, *Blixaea*, gen. nov., is distinct from all Kryptoperidiniaceae, of which DNA sequence data are available (Horiguchi & Takano 2006, Yamada *et al.* in press, Kretschmann *et al.* unpubl.), and it does not show any filamentous or palmelloid growth as *Dinothrix* (Pascher 1927), of which no DNA sequence data are available at present. It further differs from *Peridinium*, under which it was initially described, in both habitat preference (marine *versus* freshwater) and the presence of not more than two (*versus* three) intercalary plates.

Blixaea quinquecornis (T.H.Abé) Gottschling, *comb. nov.*, basionym: *Peridinium quinquecorne* T.H.Abé, Science Reports of the Tohoku Imperial University. Series 4, Biology 2: 410, fig. 30. 1927. *Protoperidinium quinquecorne* (T.H.Abé) Balech, Revista del Museo Argentino de Ciencias Naturales Bernardino Rivadavia e Instituto Nacional de Investigación de las Ciencias Naturales / Hidrobiología 4: 59. 1974.—Type: Japan. Honshū, Tōhoku, Mutsu Bay (collection date unknown).

Peridinium quinquecorne has been synonymised with *Heterocapsa quadridentata* (Hansen 1995, Okolodkov *et al.* 2016), which would have taxonomic priority over the type species selected here. We hesitate to designate the older but largely unused name as type species as long as the diatom endosymbiont has not been verified based on material investigated under that name.

Unruhdinium Gottschling, *gen. nov.*—Type: *Unruhdinium jiulongense* (H.Gu) Gottschling, *comb. nov.*

Description:—Thecate, phototrophic, free-living, primarily freshwater dinophytes usually harbouring a *Cyclotella*-like diatom as endosymbiont, which is separated from the host by a single unit membrane. Kofoidian plate formula with maximally ten epithecal plates (including 6'') and 5c, 5''', 2'''', apical pore complex present. Plate surface smooth through granulate though never ornamented by a network of minute ridges, hypotheca with a varying number of more or less distinctive spines. Chloroplasts numerous and belonging to the endosymbiotic alga; eyespot surrounded by three membranes.

Etymology:—The name honours Andrew Chudy (*1957, a.k.a. N.U.Unruh), who is musician, experimental percussionist and instruments inventor. He is best known for his work with the Berlin music group Einstürzende Neubauten, of which he also is a founder.

Unruhdinium, gen. nov., currently comprise 5–10 species with the presence of *Cyclotella*-like diatoms as endosymbionts and a reduced number of epithecal plates as diagnostic traits. The general plate formula of the epitheca should be 4' 0a 6'' (Bourrelly 1968, Zhang *et al.* 2011), but *Unruhdinium jiulongense*, comb. nov., and *Unruhdinium minimum*, comb. nov., are described as having 3' 1a 6'' (Zhang *et al.* 2014, You *et al.* 2015). This inconsistency makes it difficult to separate the entirety of *Unruhdinium* from *Peridiniopsis* (under which many of its constituent species were initially described) likewise having 3' 1a 6'' (Calado & Moestrup 2002), although both taxa are only distantly related in molecular phylogenetics (Gottschling *et al.* 2017). Nevertheless, the differing number of cingular plates (6c in *Peridiniopsis versus* 5c in *Unruhdinium*, gen. nov.), the presence of longer through shorter spines on the hypotheca in *Unruhdinium*, gen. nov. (absent in *Peridiniopsis borgei*), and the cell surface (never ornamented by a network of ridges in *Unruhdinium*, gen. nov., but in *P. borgei*) may further argue for the uniqueness of *Unruhdinium*, gen. nov.

In molecular phylogenetics, *Unruhdinium*, gen. nov., is distinct from all Kryptoperidiniaceae, of which DNA sequence data are available (Liu *et al.* 2008, Takano *et al.* 2008, Zhang *et al.* 2011, 2014, You *et al.* 2015, Yamada *et al.* in press, Kretschmann *et al.* unpubl.), and it does not show any filamentous or palmelloid growth as *Dinothrix* (Pascher 1927), of which no DNA sequence data are available at present. It is one of the two freshwater lineages identified in the Kryptoperidiniaceae, and it differs from *Durinskia oculata*, which has a regular formula of 4' 2a 6'' in the conformation of the epitheca (Kretschmann *et al.* unpubl.). The endosymbiont's nucleus could not be confirmed yet for *Unruhdinium niei*, comb. nov., in light microscopy (Liu *et al.* 2008), but the numerous chloroplasts are being also part of, and therefore evidence for, the engulfed alga. Its presence also in this species is further corroborated by sequencing of endosymbiont loci (Zhang *et al.* 2014).

Unruhdinium jiulongense (H.Gu) Gottschling, *comb. nov.*, basionym: *Peridiniopsis jiulongensis* H.Gu in X.You, Z.Luo, Y.Su, L.Gu & H.Gu, Nova Hedwigia 101: 316–318, figs 1–3. 2015.—Type: People's Republic of China. Fujian, Zhangzhou, Jiulongjiang River, Xipi reservoir (December, 2012).

Unruhdinium kevei (Grigorszky & Vagas) Gottschling, *comb. nov.*, basionym: *Peridinopsis kevei* Grigorszky & Vagas in Grigorszky, Vagas, Borics, Klee, Ant.Schmidt & Borbély, Acta Botanica Hungaria 43: 168–172, figs 2–21. 2001.—Type: Hungary. Jász-Nagykun-Szolnok, Mezőtúr, Peresi Holt-Körös (collection date unknown).

Unruhdinium minimum (Qi Zhang, G.X.Liu & Z.Y.Hu) Gottschling, *comb. nov.*, basionym: *Peridiniopsis minima* Qi Zhang, G.X.Liu & Z.Y.Hu, Algological Studies 145/146: 122, figs 1–3. 2014.—Type: People's Republic of China. Fujian, Zhangzhou, Jiulongjiang River (August, 2011).

Unruhdinium niei (G.X.Liu & Z.Y.Hu) Gottschling, *comb. nov.*, basionym: *Peridiniopsis niei*, G.X.Liu & Z.Y.Hu, Nova Hedwigia 87: 490–496, figs 3–6. 2008.—Type: People's Republic of China. Hubei, Wuhan, East Lake (Donghu) (March 18, 2004).

Unruhdinium penardii (Lemmerm.) Gottschling, *comb. nov.*, basionym: *Glenodinium penardii* Lemmerm., Hedwigia 39 Beiblatt: 117. 1900. *Peridiniopsis penardii* (Lemmerm.) Bourr., Protistologica 4: 9. 1968.—Type: Swiss Confederation. Geneva, Lake Geneva (collection date unknown). Note: Lemmermann (1910) used the same epithet for a new species of *Peridinium* and included the name *Glenodinium penardii* in his taxonomic header. Thus, it remains unclear whether Lemmermann (1910) considered it as distinct from the present species (then, it was not validly published because of ICN Art. 52.1.), or a combination of it (then, the indicated 'spec. nov.' is confusing). Anyhow, the combination of Lindemann (1925) back to *Glenodinium* is not validly published.

Unruhdinium penardii var. ***robustum*** (Qi Zhang, G.X.Liu & Z.Y.Hu) Gottschling, *comb. nov.*, basionym: *Peridiniopsis penardii* var. *robusta* Qi Zhang, G.X.Liu & Z.Y.Hu, European Journal of Protistology 47: 151, figs 2–3, 4C. 2011.—Type: People's Republic of China. Yunnan, Manwan, Luodi River (Apr, 2008).

There is some connection between the productivity of the Berlin music group Einstürzende Neubauten and the promotion of issues in natural science ('Newtons Gravitätlichkeit', 'Total eclipse of the sun'), biology ('DNS Wasserturm') and also botany ('Blume', 'In the garden', 'Sag' mir, wo die Blumen sind'). However, our present choice for new generic names in the dinophytes may stimulate a discussion about the contemporariness and usefulness of Recommendation 20A (h) in the *International Code of Nomenclature for algae, fungi, and plants* not to 'dedicate genera to persons quite unconnected with botany, mycology, phycology, or natural science in general.' Historically, the recommendation goes back to the *Vienna Rules* at the beginning of the 20th century when (phanerogam) botanists may have considered themselves rather at the end of the biodiversity assessment in terms of taxa at the generic level. We are aware today, however, that myriads of lineages remain to be named, particularly in the microbial world of algae and fungi, arguing against an unnecessary limitation and for a more liberal and open-minded application of *The Code* in this respect. This would be in tradition of, for example, Linné (1753), who dedicated many of his plant names to mythological figures, and has been recently readopted with naming the fern *Gaga* dedicated to one of the contemporary heroes (Li *et al.* 2012). The approach has also found broad application in zoology and thus, our proposal comes as a small but perceivable step forward towards the harmonisation (if not even unification) of *The Codes*, being an important motivational drive for the work on such ambiregnal protists as the dinophytes.

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References

Abé, T.H. & Saitō, M. (1981) *Studies on the family Peridinidae: An unfinished monograph of the armoured Dinoflagellata*. Abe Tōru Ikō Shuppankai.

Aké-Castillo, J.A. & Vázquez, G. (2011) *Peridinium quinquecorne* var. *trispiniferum* var. nov. (Dinophyceae) from a brackish environment. *Acta Botanica Mexicana* 94: 125–140.
https://doi.org/10.21829/abm94.2011.273

Bourrelly, P. (1968) Notes sur les péridiniens d'eau douce. *Protistologica* 4: 5–13.

Calado, A.J. (2011) On the identity of the freshwater dinoflagellate *Glenodinium edax*, with a discussion on the genera *Tyrannodinium* and *Katodinium*, and the description of *Opisthoaulax* gen. nov. *Phycologia* 50: 641–649.
https://doi.org/10.2216/11-21.1

Calado, A.J. & Moestrup, Ø. (2002) Ultrastructural study of the type species of *Peridiniopsis*, *Peridiniopsis borgei* (Dinophyceae), with

special reference to the peduncle and flagellar apparatus. *Phycologia* 41: 567–584.
https://doi.org/10.2216/i0031-8884-41-6-567.1

Carty, S. (2008) *Parvodinium* gen. nov. for the umbonatum group of *Peridinium* (Dinophyceae). *Ohio Journal of Science* 108: 103–107.

Craveiro, S.C., Daugbjerg, N., Moestrup, Ø. & Calado, A.J. (2016) Studies on *Peridinium aciculiferum* and *Peridinium malmogiense* (=*Scrippsiella hangoei*): Comparison with *Chimonodinium lomnickii* and description of *Apocalathium* gen. nov. (Dinophyceae). *Phycologia* 56: 21–35.
https://doi.org/10.2216/16-20.1

Craveiro, S.C., Calado, A.J., Daugbjerg, N., Hansen, G. & Moestrup, Ø. (2011) Ultrastructure and LSU rDNA-based phylogeny of *Peridinium lomnickii* and description of *Chimonodinium* gen. nov. (Dinophyceae). *Protist* 162: 590–615.
https://doi.org/10.1016/j.protis.2011.03.003

Gottschling, M., Kretschmann, J. & Žerdoner Čalasan, A. (2017) Description of Peridiniopsidaceae, fam. nov. (Peridiniales, Dinophyceae). *Phytotaxa* 299: 293–296.
https://doi.org/10.11646/phytotaxa.299.2.16

Gottschling, M. & McLean, T.I. (2013) New home for tiny symbionts: Dinophytes determined as *Zooxanthella* are Peridiniales and distantly related to *Symbiodinium*. *Molecular Phylogenetics and Evolution* 67: 217–222.
https://doi.org/10.1016/j.ympev.2013.01.003

Gottschling, M. & Söhner, S. (2013) An updated list of generic names in the Thoracosphaeraceae. *Microorganisms* 1: 122–136.
https://doi.org/10.3390/microorganisms1010122

Gu, H., Kirsch, M., Zinßmeister, C., Söhner, S., Meier, K.J.S., Liu, T. & Gottschling, M. (2013) Waking the dead: Morphological and molecular characterization of extant †*Posoniella tricarinelloides* (Thoracosphaeraceae, Dinophyceae). *Protist* 164: 583–597.
https://doi.org/10.1016/j.protis.2013.06.001

Hansen, G. (1995) Analysis of the thecal plate pattern in the dinoflagellate *Heterocapsa rotundata* (Lohmann) comb. nov. (= *Katodinium rotundatum* (Lohmann) Loeblich). *Phycologia* 34: 166–170.

Hansen, G., Daugbjerg, N. & Henriksen, P. (2007) *Baldinia anauniensis* gen. et sp nov.: A 'new' dinoflagellate from Lake Tovel, N. Italy. *Phycologia* 46: 86–108.
https://doi.org/10.2216/0031-8884(2007)46[86:BAGESN]2.0.CO;2

Horiguchi, T. & Pienaar, R.N. (1991) Ultrastructure of a marine dinoflagellate, *Peridinium quinquecorne* Abe (Peridiniales) from South-Africa with particular reference to its chrysophyte endosymbiont. *Botanica Marina* 34: 123–131.
https://doi.org/10.1515/botm.1991.34.2.123

Horiguchi, T. & Pienaar, R.N. (1994) Ultrastructure of a new marine sand-dwelling dinoflagellate, *Gymnodinium quadrilobatum* sp. nov. (Dinophyceae) with special reference to its endosymbiotic alga. *European Journal of Phycology* 29: 237–245.
https://doi.org/10.1080/09670269400650691

Horiguchi, T. & Takano, Y. (2006) Serial replacement of a diatom endosymbiont in the marine dinoflagellate *Peridinium quinquecorne* (Peridiniales, Dinophyceae). *Phycological Research* 54: 193–200.
https://doi.org/10.1111/j.1440-1835.2006.00426.x

Imanian, B., Pombert, J.-F. & Keeling, P.J. (2011) The complete plastid genomes of the two 'dinotoms' *Durinskia baltica* and *Kryptoperidinium foliaceum*. *PLoS One* 5: e10711.
https://doi.org/10.1371/journal.pone.0010711

Kretschmann, J., Elbrächter, M., Zinßmeister, C., Söhner, S., Kirsch, M., Kusber, W.-H. & Gottschling, M. (2015) Taxonomic clarification of the dinophyte *Peridinium acuminatum* Ehrenb., ≡ *Scrippsiella acuminata*, comb. nov. (Thoracosphaeraceae, Peridiniales). *Phytotaxa* 220: 239–256.
https://doi.org/10.11646/phytotaxa.220.3.3

Lemmermann, E.J. (1910) *Algen I (Schizophyceen, Flagellaten, Peridineen)*. Bornträger, Leipzig.
https://doi.org/10.5962/bhl.title.4953

Li, F.-W., Pryer, K.M. & Windham, M.D. (2012) *Gaga*, a new fern genus segregated from *Cheilanthes* (Pteridaceae). *Systematic Botany* 37: 845–860.
https://doi.org/10.1600/036364412X656626

Lindemann, E.B.L.W. (1925) III. Klasse: Dinoflagellatae (Peridineae). *In:* Schoenichen, W. (Ed.) *Einfachste Lebensformen des Tier- und Pflanzenreiches. Naturgeschichte der mikroskopischen Süßwasserbewohner*. Bermühler, Berlin, pp. 144–195.

Linné, C.v. (1753) *Species plantarum, exhibentes plantas rite cognitas, ad genera relatas, cum differentiis speciíicis, nominibus trivialibus, synonymis selectis, locis natalibus, secundum systema sexuale digestas*. Salvius, Stockholm.

Liu, G.-X., Pei, G.F. & Hu, Z.-Y. (2008) *Peridiniopsis niei* sp. nov. (Dinophyceae), a new species of freshwater red tide dinoflagellates from China. *Nova Hedwigia* 87: 487–499.
https://doi.org/10.1127/0029-5035/2008/0087-0487

Moestrup, Ø. & Daugbjerg, N. (2007) On dinoflagellate phylogeny and classification. *In:* Brodie, J. & Lewis, J. (Eds.) *Unravelling the algae, the past, present, and future of algal systematics*. CRC, Boca Raton, pp. 215–230. https://doi.org/10.1201/9780849379901.ch12

Okolodkov, Y.B., Campos-Bautista, G. & Gárate-Lizárraga, I. (2016) Circadian rhythm of a red-tide dinoflagellate *Peridinium quadridentatum* in the port of Veracruz, Gulf of Mexico, its thecal morphology, nomenclature and geographical distribution. *Marine Pollution Bulletin* 108: 289–296.

Pascher, A. (1927) Die braunen Algenreihen aus der Verwandtschaft der Dinoflagellaten (Dinophyceen). *Archiv für Protistenkunde* 58: 1–54.

Takano, Y., Hansen, G., Fujita, D. & Horiguchi, T. (2008) Serial replacement of diatom endosymbionts in two freshwater dinoflagellates, *Peridiniopsis* spp. (Peridiniales, Dinophyceae). *Phycologia* 47: 41–53. https://doi.org/10.2216/07-36.1

Tamura, M., Shimada, S. & Horiguchi, T. (2005) *Galeidinium rugatum* gen. et sp. nov. (Dinophyceae), a new coccoid dinoflagellate with a diatom endosymbiont. *Journal of Phycology* 41: 658–671. https://doi.org/10.1111/j.1529-8817.2005.00085.x

Tillmann, U., Gottschling, M., Nézan, E., Krock, B. & Bilien, G. (2014) Morphological and molecular characterization of three new *Azadinium* species (Amphidomataceae, Dinophyceae) from the Irminger Sea. *Protist* 165: 417–444. https://doi.org/10.1016/j.protis.2014.04.004

Tillmann, U., Salas, R., Gottschling, M., Krock, B., O'Driscoll, D. & Elbrächter, M. (2012) *Amphidoma languida* sp. nov. (Dinophyceae) reveals a close relationship between *Amphidoma* and *Azadinium*. *Protist* 163: 701–719. https://doi.org/10.1016/j.protis.2011.10.005

Tomas, R.N., Cox, E.R. & Steidinger, K.A. (1973) *Peridinium balticum* (Levander) Lemmermann, an unusual dinoflagellate with a mesocaryotic and an eukaryotic nucleus. *Journal of Phycology* 9: 91–98.

Yamada, N., Sym, S.D. & Horiguchi, T. (in press) Identification of highly divergent diatom-derived chloroplasts in dinoflagellates, including a description of *Durinskia kwazulunatalensis* sp. nov. (Peridiniales, Dinophyceae). *Molecular Biology and Evolution*. https://doi.org/10.1093/molbev/msx054

Yamada, N., Tanaka, A. & Horiguchi, T. (2015) Pigment compositions are linked to the habitat types in dinoflagellates. *Journal of Plant Research* 128: 923–932. https://doi.org/10.1007/s10265-015-0745-4

You, X., Luo, Z., Su, Y., Gu, L. & Gu, H. (2015) *Peridiniopsis jiulongensis*, a new freshwater dinoflagellate with a diatom endosymbiont from China. *Nova Hedwigia* 101: 313–326. https://doi.org/10.1127/nova_hedwigia/2015/0272

Zhang, Q., Liu, G.-X. & Hu, Z.-Y. (2011) Morphological differences and molecular phylogeny of freshwater blooming species, *Peridiniopsis* spp. (Dinophyceae) from China. *European Journal of Protistology* 47: 149–160. https://doi.org/10.1016/j.ejop.2011.03.001

Zhang, Q., Liu, G.-X. & Hu, Z.-Y. (2014) Description of a new freshwater bloom-forming dinoflagellate with a diatom endosymbiont, *Peridiniopsis minima* sp. nov. (Peridiniales, Dinophyceae) from China. *Algological Studies* 145: 119–133. https://doi.org/10.1127/1864-1318/2014/0159

Publication 7

The many faces of *Peridinium cinctum* (Peridiniaceae, Peridiniales): Morphological and molecular variability in a common dinophyte

IZQUIERDO LÓPEZ, A., **KRETSCHMANN, J.**, ŽERDONER ČALASAN, A. & GOTTSCHLING, M.

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The many faces of *Peridinium cinctum* (Peridiniaceae, Peridiniales): morphological and molecular variability in a common dinophyte

Alejandro Izquierdo López, Juliane Kretschmann, Anže Žerdoner Čalasan and Marc Gottschling

Department Biologie, Systematische Botanik und Mykologie, GeoBio-Center, Ludwig-Maximilians-Universität München, Menzinger Str. 67, D – 80638 München, Germany

ABSTRACT

Peridinium cinctum is a common freshwater dinophyte with a long history of research. Erich Lindemann was the first to assess intraspecific variability in this species focusing on plate pattern variation. Since then, this issue has been neglected but with the application of DNA sequence diagnostics, a combination of morphological and molecular characters may enable taxonomic delimitations. Our aim was to identify distinct morphotypes using plate pattern as the main characteristic and then compare them to the geographic occurrence of particular ribotypes (as inferred from sequences of the Internal Transcribed Spacer: ITS) in samples from Central Europe. Approximately 200 observations were carried out under the inverse light microscope for each of a total of 15 strains. We observed two main variations from the abundant plate pattern in *P. cinctum*, namely an unusual position of the 2a plate and the irregular shape of the 1a plate. In 88 (predominantly clonal) strains, we identified five different ribotypes (submitted as 71 new GenBank entries) which had no clear correlation to the defined morphotypes and/or spatial occurrences. In four cases, we detected two distinct ribotypes at the same locality. However, samples collected south of the Danube River presented a different predominant morphotype from the rest of the samples, thus implying a potential biogeographic signal as inferred from morphology. In general, there is morphological and molecular variability in *P. cinctum*, which is under-studied and which may uncover geographic or ecological correlations or even the existence of cryptic species.

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KEYWORDS Central Europe; diagnosis; microscopy; ribotype; sequence data

Introduction

Peridinium cinctum (O.F.Müller) Ehrenberg is a thecate dinophyte and the type of *Peridinium* Ehrenberg and the Peridiniaceae. It is considered a common and generalist species (i.e. tolerant to highly diverse conditions) in freshwater ecosystems (Höll, 1928; Boltovskoy, 1975). Ecologically, the species inhabits shallow mesotrophic to eutrophic water bodies (Calado *et al.*, 1999) with pH values ranging from 4 to 8 (Boltovskoy, 1975), and is further characterized as oxyphilic and eurytrophic (Höll, 1928). *Peridinium cinctum* is widely distributed, ranging over Eurasia and Africa (Smith & Smith, 2015) to Australia (Day *et al.*, 1995). In North America, where *Peridinium gatunense* Nygaard is the predominant species of the corresponding group, the presence of *P. cinctum* is questionable (Carty, 2014). Despite its ubiquity and a long research history that dates back to the 19th century (Müller, 1796; Ehrenberg, 1832; Stein, 1883), many questions regarding *P. cinctum* are still unanswered, including the magnitude of intraspecific genetic and morphological variability and the possible presence of cryptic species. Studies on *P. cinctum*, as well as delimitation of related species described under *Peridinium*, may contribute to resolving this issue.

Traditionally, morphology is an important and reliable source of classification in dinophyte taxonomy (Hoppenrath, 2017). Amongst morphological diagnostic features, plate pattern (or tabulation) plays an important role, be it the number, arrangement and/or shape of the plates in thecate dinophytes. It has been used as a key feature to describe and delimitate species (Balech, 1980; Abé, 1981), as well as to complement molecular data (Kremp *et al.*, 2014; Kretzschmar *et al.*, 2017). However, plate pattern has been found to present intraspecific variability (Gu *et al.*, 2013*b*; Yeo & Shin, 2013; Tillmann *et al.*, 2014), and phenomena such as plate shifting or fusion are common in some species (Elbrächter & Meyer, 2001; Gottschling *et al.*, 2005*b*; Tillmann *et al.*, 2014). Based on the interpretations provided by Stein (1883: pl. XII 9–19) the epithecal tabulation pattern of *P. cinctum* is circumscribed by Kofoid's (1909) formula and is defined as 4′ 3a 7″. Deviations from the basal formula include additional plates, restructuring of plate positions or lack of plates. Plate shape, plate size or the position of the sutures between the plates is not reflected in the Kofoidean formula, but is still important when assessing morphological variability.

CONTACT Marc Gottschling gottschling@bio.lmu.de

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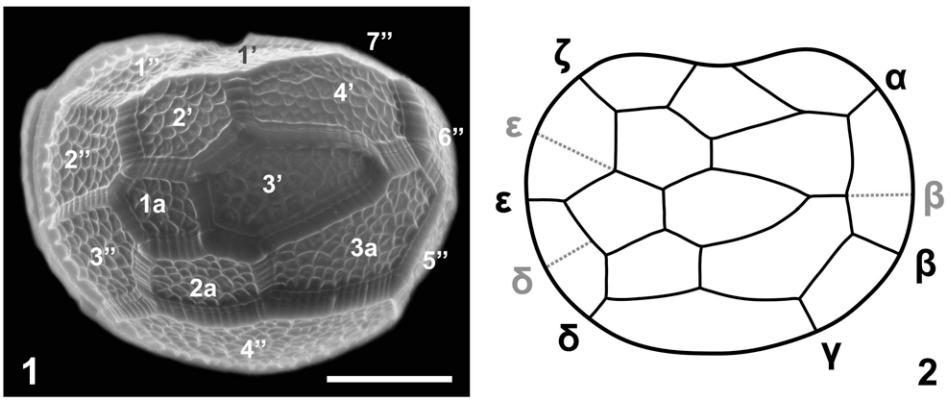
Erich Lindemann (1888–1945) was the first to assess morphological variability in *P. cinctum*. He was aware of the difficulties in distinguishing between individuals with an abnormal plate pattern and a new variety. Thus, the 14 new subordinate taxa of *P. cinctum* that he described validly were not based on the morphological characteristics of single cells, but on regular observations of various samples (Lindemann 1917, 1918*b*). These were referred to as 'forms' when the overall shape of the cell was what differed (e.g. presence/absence of spines, colour, dorso-ventral depressions), or 'varieties' when the tabulation pattern differed. Varieties included morphotypes with different plate sizes (*P. cinctum* var. *regulatum* Er.Lindemann, *P. cinctum* var. *irregulatum* Er.Lindemann), plate fusions (*P. cinctum* var. *laesum* Er.Lindemann) and plate shapes (*P. cinctum* var. *dissimile* Er.Lindemann). In addition, Lindemann (1918*b*) also differentiated between changes in the position of the sutures between the precingular plates, eventually affecting which plates are connected to each other. Thus, he invented a labelling system to describe such changes of the suture position using Greek letters for each of the six sutures between the precingular plates (Figs 1 and 2).

Based on the Greek alphabet the term <*collineatum*> (used as epithets) describes a suture present in a usual position, connecting to another suture forming a continuation (Figs 1 and 2). Similar usage of the term <*travectum*> is applied, when a suture is at its usual position and connected to a plate different from those in the regular epithecal conformation of *P. cinctum*. This terminology serves as a way to record the variations in *P. cinctum*. Out of these taxa, only *P. cinctum* forma *ovoplanum* Er.Lindemann seems to have been widely recognized (Pfiester, 1975; Spector *et al.*, 1981), while other taxa are mostly ignored. Lindemann (1918*a*, *b*) also made use of the above-mentioned traits to differentiate species from *P. cinctum*, mainly *P. eximium* Er.Lindemann, *P. germanicum* Er.Lindemann and *P. rhenanum* Er.Lindemann, all of which are considered synonyms in the current taxonomy of the species (Popovský & Pfiester, 1990).

With the arrival of genetic data in dinophytes, the traditional morphological taxonomy has been left behind (Zinßmeister *et al.*, 2011; Tillmann *et al.*, 2014). Regions such as the Internal Transcribed Spacers (ITSs) of the ribosomal RNA (rRNA) have already been used in several phylogenetic analyses down to the species level (Gottschling *et al.*, 2005*b*; Litaker *et al.*, 2003). Differences in this or similar regions, also referred to as ribotypes in case of rRNA sequences, have been used to differentiate between different species of, for example, *Alexandrium* Halim (John *et al.*, 2014; Kremp *et al.*, 2014), parasitic *Blastodinium* Chatton (Skovgaard *et al.*, 2012) or endosymbionts of corals such as *Symbiodinium* LaJeunesse (Thornhill *et al.*, 2007). In the case of *P. cinctum*, a characterization of its genetic variability is scarce, but two different ribotypes were observed in previous studies (Gottschling *et al.*, 2005*a*; Logares *et al.*, 2009).

In this study, we aim to depict consistent deviations from the common plate pattern in the epitheca of *P. cinctum*, which we dub morphotypes. We expect these morphotypes to represent some of the morphological variation that this dinophyte potentially entails (Lindemann, 1918*b*). We also differentiate between distinct ribotypes based on the ITS region. Hence, an association between distinct morphotypes and ribotypes could set the basis for reliable determination of (putatively cryptic) species in *P. cinctum*, such as those found in other dinophyte species complexes including *Gambierdiscus* Adachi & Fukuyo (Richlen *et al.*, 2008) and *Scrippsiella* Balech (Montresor *et al.*, 2003; Söhner *et al.*, 2012).



Figs 1 & 2. Kofoidean plate designation in *Peridinium cinctum* and indication of the suture positions. Fig. 1: Kofoidean notation for apical plate pattern in morphotype M1 (SEM image of strain GeoM*685). Fig. 2: Scheme representing morphotype M1 and the position of the sutures. Usage of the Greek letters goes back to Lindemann (1918*a*), and the scheme is based on an illustration of the basic plate pattern (Lindemann, 1917, 1918*b*). Abbreviations: n′: apical plate, n″: precingular plate, na: anterior intercalary plate. Scale bar: 10 µm.

We are aware that a certain phenotype cannot always correlate with a specific genotype *a priori*. Environmental factors, for example, can play an important role in shaping the morphology of a dinophyte (i.e. modification). Night/day cycles in *Ceratium ranipes* Cleve (Pizay *et al.*, 2009) or fluid mobility in *Ceratocorys horrida* F.Stein (Zirbel *et al.*, 2000), for instance, have been found to modify their original plate pattern. Even when cultivated *in vitro*, where conditions are consistent over time and equal for all strains, differences in environmental conditions prior to cell isolation have been found to influence morphology (Kim *et al.*, 2004).

Overall, this project aims to give an insight into the morphological variability of *P. cinctum*, a subject that has not been questioned for almost a century. This variability could correlate, for example, with different ribotypes or environmental conditions. Given the reported abundance of this species in freshwater systems across Europe and around the world, we expect that unveiling morphological and genetic variation in this species is of great interest for taxonomy and ecology. We will not know about the conservation status of this species until its delimitation is resolved and precise occurrence data are available. Therefore, variability in *P. cinctum* is an interesting subject worth considering, and this variability may consist of both morphological and genetic intraspecific variability.

Materials and methods

The study is a part of ongoing research on morphology, evolution and taxonomy of dinophytes. We used 72 monoclonal strains of P. *cinctum* from our culture collection (Supplementary table S1) as well as 16 strains with sequence information deposited in GenBank (Gottschling *et al.*, 2005*a*; Logares *et al.*, 2007, 2009; Stern *et al.*, 2012; Zinßmeister *et al.*, 2012; Gottschling & Söhner, 2013). Our monoclonal strains originated from samples collected across different freshwater reservoirs in central Europe (in compliance with the Convention on Biological Diversity: CBD). Individual cells of *P. cinctum* were isolated from the original field samples under an inverse light microscope (LM) CKK41 (Olympus; Tokyo, Japan). Cells were cultivated under sterile conditions and kept in a WC medium (Guillard & Lorenzen, 1972) in six-well microplates (Zefa; Munich, Germany). Samples underwent a regimen of 12 hours of light/day at 80 µmol photons $m^{-2}s^{-1}$ and 18°C in a climate chamber WKS 3200 (Liebherr; Bulle, Switzerland) for 2–4 months.

For the study of epithecal morphology, we focused on 15 strains covering the variation across different ribotypes or different localities (Czech Republic, Germany, Poland) or both. Observations were carried out at different points in time but did not take more than 2 weeks per strain. The observations of the different cells were carried out under the inverse LM. We took between 100 and 120 images of individual cells per strain with a CellSens Olympus DP73 camera (Olympus; Tokyo, Japan) attached to the inverse LM. We focused mainly on the epitheca, although some dorsal, ventral and antapical views were also documented. These figures were complemented by images taken under the scanning electron microscope (SEM). Preparations for the SEM followed standard protocols (Janofske, 2000). Generally, these involved dehydrating samples with increasing concentrations of acetone, critical point drying and final covering with platinum. Contrary to the standard procedure, dinophyte samples were positioned between double layers of filters instead of single filters, which is a common practice that has proven successful (Kretschmann *et al.*, 2015).

Analysis of plate pattern and the defining of morphotypes were performed using LM and SEM images as references. Plate pattern was used as the main defining characteristic. The process of analysing and grouping of the images into different forms was repeated several times until a practical classification, conclusive for all strains, was obtained. Our classification aimed to obtain stable, common and easily identifiable morphotypes.

Morphotypes were defined from our observations of different compositions of plate pattern in the epitheca. Special attention was given to features such as plate shape, connections between plates and position of the sutures. Only forms with a consistent occurrence of more than 10% of the total observations (from the analysed images) were included into the final delimitation of the morphotypes. If an identified form did not occur in at least 10% of the observations of a particular strain, it was classified under an additional group described under the name ‘others’, which included different, rare morphotypes. Once a morphotype was established, all samples were observed a second time, classifying approximately 100 individual cells per strain into the defined morphotypes, which we added to the 100 previously classified images for our final results. Some images taken from the interior of the theca were mirrored digitally for an easier morphological comparison.

Genomic DNA was extracted from fresh material using the Nucleo Spin Plant II Kit (Machery-Nagel; Düren, Germany). Various regions of the ribosomal RNA genes including the ITSs were amplified using previously specified primer pairs (Gu *et al.*, 2013*a*) following standard protocols (Gottschling & Plötner, 2004; Gottschling *et al.*, 2012). Gel electrophoreses yielded single bands that were purified and sequenced. Sequences were edited and assembled

using Sequencher™ v5.1 (Gene Codes; Ann Arbor, Michigan, USA). For visual comparison, the alignment editor 'Se-Al' (Rambaut, 2001) was used. For comparative purposes, the secondary structure of the ITS molecules (Gottschling & Plötner, 2004) were taken into account.

For drawing pie charts of ribotype distribution and morphotype prevalence, the package 'plotly' (Sievert *et al.*, 2016; freely available at https://CRAN.R-project.org/package=plotly) of the software 'R' v3.2.5 (R Core Team, 2016; freely available at https://www.R-project.org/) was used. The map was created using R and the package 'raster' (Hijmans, 2016; freely available at https://CRAN.R-project.org/package=raster) and edited with imaging software 'GIMP' v2.8 (The GIMP Team, 2017; freely available at https://www.gimp.org/). Image adjustments (such as scaling, cropping, white-balancing, colour management) were made in Photoshop® (Adobe Systems; Munich, Germany) and image arrangements in QuarkXPress® (Quark Software; Hamburg, Germany).

Results

Our analysis of ITS sequence data showed five distinct ribotypes (Supplementary fig. S1), establishing classes of sequence similarities without intermediates. Sequences from ribotype 1 (r1: GeoM*777) and ribotype 2 (r2: GeoM*679) were identical, except for the two positions in the ITS1 and the presence of a 12-base-pair-long insertion/deletion at the beginning of the ITS2. Ribotypes 3 to 5 (r3: GeoM*670, r4: CCAC0102, r5: GeoM*672) did not include the ITS2 deletion found in ribotype 1, but the sequences exhibited several base substitutions across the 5.8S region when compared with ribotypes r1 and r2. Moreover, ribotypes r3, r4 and r5 showed considerable differences in their ITS1 and ITS2 primary sequences, particularly in the unpaired segment between pairing region III and the 5.8S rRNA. A geographic correlation to the ribotypes could not be inferred by geographic mapping (Fig. 3). However, we found two different ribotypes present at the same

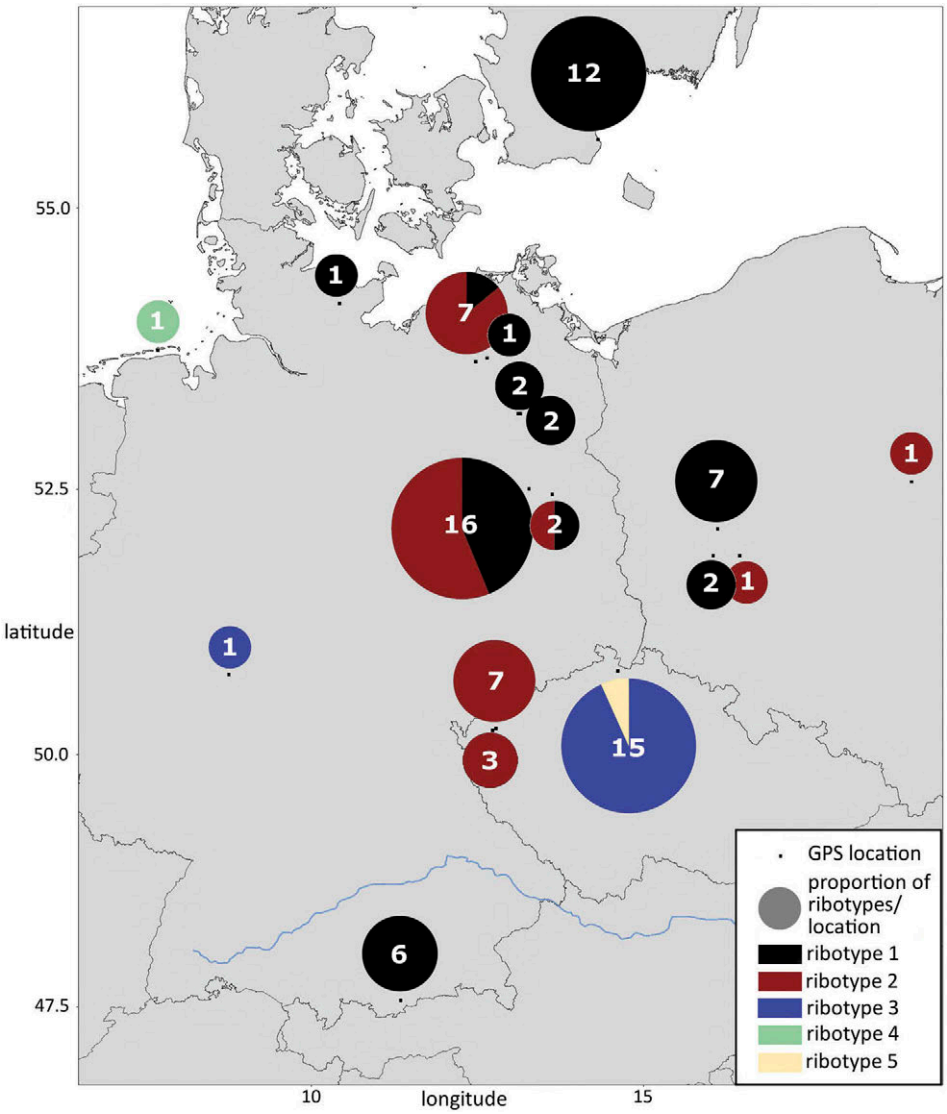


Fig. 3. Distribution of ribotypes across Central Europe. Circle size corresponds to the number of strains investigated from a certain locality (also specified in the circles). The Danube River is indicated.

locality in four cases, namely Lake Máchův, Halensee, Krakower See and Müggelsee (Supplementary table S1).

We identified three morphological types, which appeared consistently across all the samples. These morphotypes are referred to as M1, M2 and M3 and illustrated in Figs 1 and 4–15. A fourth group (M4) was created to gather all morphological variations, which could not be classified into one of the three distinct morphotypes (Supplementary fig. S2). These forms were rare and did not represent more than 10% of the observations performed per sample. All morphotypes presented the usual Kofoidean thecal formula for the epitheca of *P. cinctum*, specified as 4′ 3a 7″. Some variations of this pattern, including plate splitting and plate fusion, were found, but were infrequent and could not be arranged into one of the main three morphotypes (Supplementary fig. S2). Of the three main morphotypes, M1 was in correspondence with the established plate pattern for *P. cinctum*, whereas M2 and M3 presented deviations from this structure. Morphotypes M1 and M2 could be easily differentiated, while M3 presented a transitional morphology between these two. Therefore, morphotype M3 constituted a more heterogeneous group.

Morphotypes M2 and M3 (Figs 4–15) differed from the basic tabulation found in *P. cinctum* in three main morphological characteristics: shape of the 1a plate, position of the ε-suture and position of the 2a plate. In M1, the shape of the 1a plate was regularly pentagonal, and the ε-suture was in its anticipated conformation (Fig. 1). In the other morphotypes (Figs 4–15), the 1a plate was strongly reduced on one of its sides, modifying its shape to irregularly pentagonal or even tetragonal. This also affected the position of the ε-suture (Figs 4–15, black arrowheads), whilst the suture between plates 2″ and 1a was distinctly shortened. In some cases, the ε-suture was even connected to the suture lying between plates 1a and 2′, appearing as a prolongation of this suture.

The apical plate 2a was usually connected to the plate 3″ through a broad lateral extension in M1 (Fig. 1). In M2, this connection did not exist, and the 2a plate was connected to the plate 4″ only (e.g. Fig. 4, white arrowhead). In M3, a connection existed, but it was reduced in comparison to M1 (e.g. Fig. 10, white arrowhead). In this case, the 2a plate was only connected to plate 3″ through one of its vertexes. These conformations also affected the position of the suture between plates 1a and 2a. This suture was normally broad and connected to the middle part of the 3″ plate. In M2, this suture was displaced and reached either the lower part of the 3′ plate or was connected to the plate 4″ (Fig. 4). In the latter case, this created an almost lateral separation between plates 1a and 2a. In M3, the suture between plates 1a and 2a was displaced to the bottom of the 3″ plate but never reached other precingular plates (Fig. 10). This position was sometimes followed by a singular curvature of the suture, giving rise to a cochleariform 2a plate.

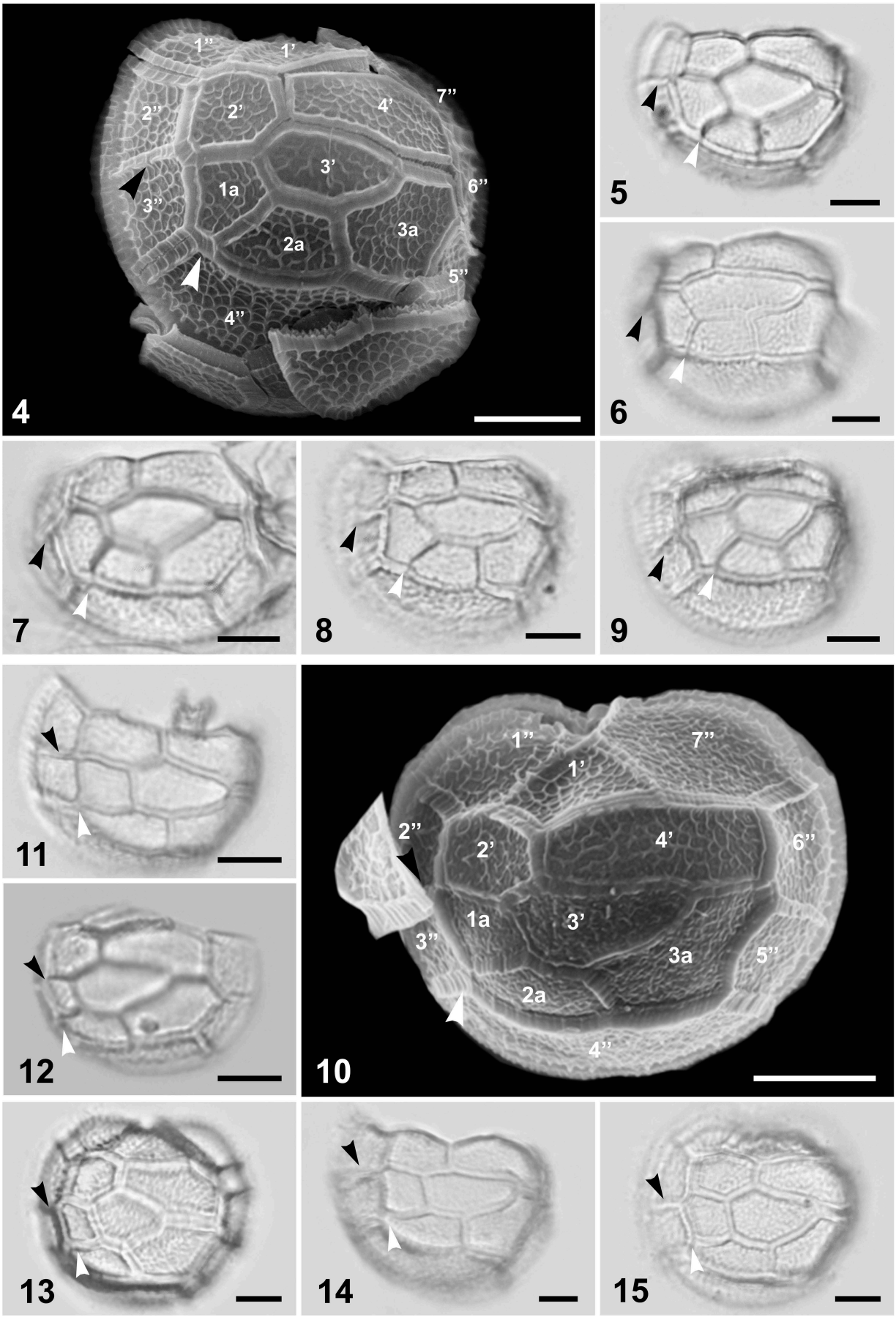
In respect to the source locality, all morphotypes were found in all strains, regardless of their origin or ribotype. Thus, each strain was represented by all morphotypes. However, the frequency of morphotypes differed between strains or localities (Fig. 16). Overall, these frequencies were not distributed randomly and could be classified into two main patterns. In the first pattern (P1), most of the cells displayed the usual thecal constitution of *P. cinctum*. Therefore, 50% (in GeoM*640, Krakower See) to almost 80% of all individuals (in GeoM*737, also from Krakower See) exhibited M1 (Fig. 16, black shading). For the first pattern (P1), M3 (Fig. 16, light grey shading) ranged from lower than 10% (GeoM*596) to a maximum of 30% (in GeoM*640). Regarding M2 (Fig. 16, dark grey shading), this morphotype appeared at extremely low frequencies, with the exception of GeoM*776, in which it did not exceed more than 15% of all observations.

The second pattern (P2) was found only in the samples from Walchensee (Bavaria) – the only ones collected south of the Danube (Fig. 16). In this case, most of the cells presented a plate pattern that deviated from regular *P. cinctum* (but notably not in the same way as in *P. cinctum* var. *regulatum*, which E. Lindemann found in Walchensee). The morphotype M1 appeared at a lower frequency, ranging from 15% (in GeoM*644) to less than 30% (in GeoM*645). On the other hand, M3 was present to a higher extent than in P1, ranging from 30% (GeoM*645) to almost 70% (GeoM*644). In addition, the frequency of M2 was also higher than in the samples from outside the Walchensee, ranging from 15% (GeoM*653) to 30% (GeoM*652).

Discussion

Correlation of data and taxonomic delimitation

Species delimitation in unicellular organisms is challenging and nowadays usually uses a combination of morphological and molecular data. Considerable morphological variability had already been uncovered in *P. cinctum* a century ago (Lindemann, 1917, 1918*a*, *b*), and our study contributes information on DNA sequence variation. The existence of three new (plus two known: Gottschling *et al.*, 2005*a*; Logares *et al.*, 2009) ITS ribotypes is documented here, and the question arises whether they can be correlated with other traits. Genetic differentiation of ITS might be congruent with spatial occurrence in other dinophytes (Finney *et al.*, 2010; Al-Kandari *et al.*, 2011),



Figs 4–15. Examples for epithecal morphotypes M2 (Figs 4–9) and M3 (Figs 10–15). Fig. 4: GeoM*645 from Walchensee. Fig. 5: GeoM*640 from Krakower See. Fig. 6: GeoM*644 from Walchensee. Fig. 7: GeoM*776 from Lake Wolsztyńskie. Fig. 8: GeoM*652 from Walchensee. Fig. 9: GeoM*646 from Walchensee. Fig. 10: GeoM*644 from Walchensee. Fig. 11: GeoM*646 from Walchensee. Fig. 12: GeoM*685 from Müggelsee. Fig. 13: GeoM*645 from Walchensee. Fig. 14: GeoM*652 from Walchensee. Fig. 15: GeoM*645 from Walchensee. Black arrows indicate the position of the ε-suture, note the <*collineatum*> conformation (Fig. 2). White arrows indicate the connection between the 2a plate and the precingular plates, note how it reaches the 3″ plate only from a vertex in fig. 10. Scale bar: 10 µm.

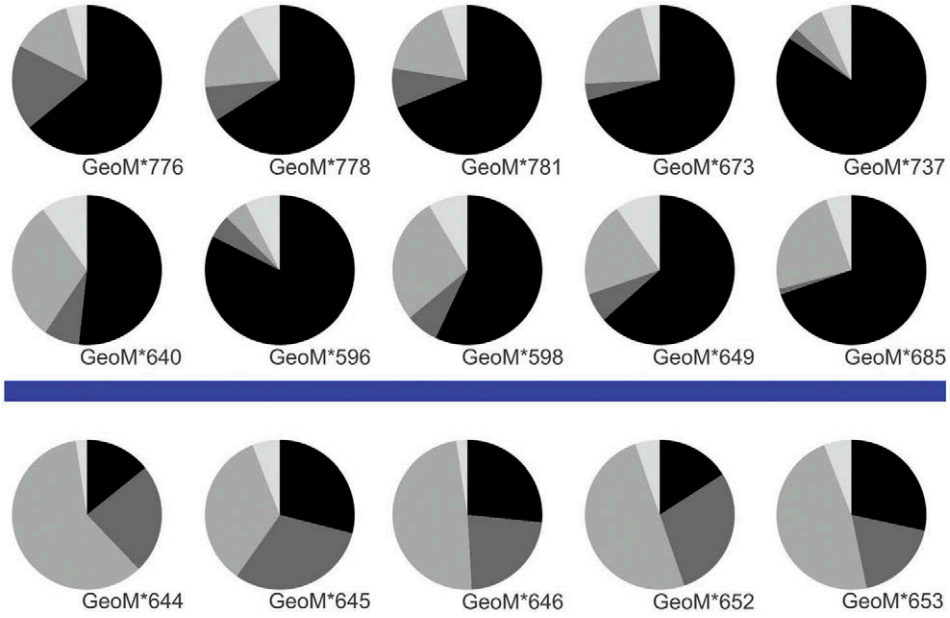


Fig. 16 . Prevalence of morphotypes at the localities across Central Europe. A total of 15 strains, in which morphological analysis was performed, are presented. Each strain (corresponding to a pie chart) presents a different proportion of M1 (black), M2 (dark grey), M3 (light grey) and M4 (white). Note the frequency differences between strains established from north and south of the Danube River (thick bar between 2nd and 3rd row of pie charts).

but this can be excluded for *P. cinctum*. Particular ribotypes are widely distributed and occasionally, more than one ribotype occurs at the same locality.

All investigated cultivated material contained intra-strain morphological variability, which included all morphotypes identified. Moreover, neither of the two patterns of intra-strain variability corresponded to a specific ribotype. Samples with different plate patterns presented the same ribotype (e.g. GeoM*644 and GeoM*776), while strains with different ribotypes occasionally exhibited the same pattern (e.g. GeoM*649 and GeoM*685). A correlation may exist for other characteristics, although ribosomal sequences do not always have a diagnostic potential (e.g. strains of *Karenia* Gert Hansen & Moestrup exhibiting differentiated physiologies: Loret *et al.*, 2002; strains of *Gambierdiscus* showing diverse morphological features: Richlen *et al.*, 2008). Thus, ITS sequence data are not indicative for speciation in *P. cinctum*, and their variation corresponds rather to intraspecific variability, as it has also been shown in, for example, *Alexandrium ostenfeldii* (Paulsen) Balech & Tangen (Kremp *et al.*, 2014).

Nevertheless, a relationship between intra-strain variability and locality may exist. Samples from the same locality were mainly consistent in their morphotypes and variability patterns, despite ribotypes being different (GeoM*596 and GeoM*598 from Halensee) or even identical (GeoM*652 and GeoM*653 from Walchensee). Moreover, two main patterns of morphotype frequencies were found in regard to intra-strain variability, namely P1: where M1 (i.e. the general plate pattern of *P. cinctum*) is dominant, while the other morphotypes are infrequent and P2: where M3 is dominant and M2 more frequent than in P1. It is striking that P2 was found in all five strains from Walchensee, located south of the Danube. The ribotype of the Walchensee strains (r1) is also present in other strains, but those samples show P1 (e.g. GeoM*685 and GeoM*776). Thus, frequencies of morphotypes rather than distinct morphotypes appear indicative for divergence in *P. cinctum*.

Consistency of morphotypes

Peridinium cinctum presents notable variability in its plate pattern, not only between different strains but also within strains. This morphological variability has been mostly ignored, despite the common presence of this dinophyte in freshwater systems. The works of Lindemann (1917, 1918*a*) were pioneering, defining the first alterations in plate pattern for this species. Our morphotypes add to his work and further strengthen the idea that phenotypic variation in this species should be considered in future taxonomic studies, as it has been in, for example, species of *Azadinium* Elbrächter & Tillmann (Tillmann *et al.*, 2014). Most strains under investigation were established at more or less the same time and based on material which was collected at more or less at the same time. Thus, we do not expect the variability to be a result of, for example, different strain age.

From the three morphotypes, M1 (Figs 1 and 2) represents the typical plate pattern for *P. cinctum* as depicted by Lindemann (1917, 1918*b*) and observed through other studies (Boltovskoy, 1975). M2 and M3 present deviations from this structure, mainly as differences in plate connection, plate shape and position of the sutures, and are chosen for their consistent presence across strains and for their easily identifiable traits. Their classification is, therefore, generally straightforward. In some cases, though, it has been difficult to decide whether an individual would be classified as either morphotype M1 or M3. We consider morphotype M3 a transitional morphology and an intermediate form between morphotypes M1 and M2. While morphotype M2 has well-defined characteristics, such as the absence of connection between the plates 2a and 3″, the differences between morphotypes M1 and M3 are more open to interpretation. In some cases, the ε-suture is even connected to the suture between plates 1a and 2′, a phenomenon described as <*epsiloncollineatum*> by Lindemann (1918*b*). Regardless, these cases have not been observed so frequently, and further analysis of the images of the Walchensee strains, where the more heterogeneous M3 is the most dominant, has not altered the initial classification.

We did not use the morphotypes for their taxonomic value (but maybe their frequencies do have some, see above) but to separate the morphological variability found in our samples in a comprehensible way. Despite our objective, we are aware that these morphologies do not represent the overall variability found in our samples of *P. cinctum*. Plates with slightly different morphologies: lengths, or sizes, as well as fusions or fissions (Supplementary fig. S2) were also present, but were more sporadic and difficult to differentiate. In addition, our morphotypes only cover feature changes from the epithecal side, but do not consider the hypotheca. This is partially due to the number of hypothecal plates being smaller, hence a lower degree of variation is expected in this hemisphere.

The original environmental conditions of the sampling site may have influenced the morphotypes expressed by the Walchensee samples, even after being grown in cultivation for a longer period of time, which has also been found in other dinophytes (Kim *et al.*, 2004). Several environmental factors are known to influence the phenotype of dinophyte species. Viscosity and fluid motility, for example, have been found to be of great importance in the motility of some dinophytes (Zirbel *et al.*, 2000; Orchard *et al.*, 2016) and thus, we may expect plate pattern to differ with viscosity. The sampled lakes also differ geographically and ecologically in terms of altitude and/or physiochemical conditions, all of which may have affected the morphology of the Walchensee samples.

In conclusion, the common dinophyte *P. cinctum* exhibits morphological and molecular variability that can be recognized easily. From samples collected in the Czech Republic, Germany and Poland, three main morphotypes are recognized: one regarding the basal form of *P. cinctum*, while the others present changes in the shape of the 1a plate and the connection between the 2a plate and the precingular plates. Neither the distinct morphotypes nor the two frequency patterns of variability correlate with distinct ribotypes. The ribosomal ITS region has been proposed as a species-specific barcode marker in dinophytes (Gottschling *et al.*, 2005*b*; Litaker *et al.*, 2007; Stern *et al.*, 2012), but its potential use may differ across subordinate groups. The situation in *Peridinium* rather resembles gonyaulacalean *Alexandrium* (Kremp *et al.*, 2014), in fact showing more intraspecific variability than peridinialean *Apocalathium* (Gottschling *et al.*, 2005*a*; Annenkova *et al.*, 2015), in which morphologically and ecologically differentiated species share the same ITS sequence. If different morphotype frequencies north and south of the Danube River are indicative of separation and isolation in *P. cinctum*, then ITS sequence data are definitely not. Other traits unrelated to plate pattern may uncover alternative associations between ribotypes and morphologies in future research.

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Author contributions

A. Izquierdo López, J. Kretschmann, M. Gottschling: original concept; A. Izquierdo López, J. Kretschmann, A. Žerdoner Čalasan, M. Gottschling: drafting and editing manuscript; J. Kretschmann: strain cultivation; A. Izquierdo López, J. Kretschmann: microscopy; A. Žerdoner Čalasan, M. Gottschling: analysis of molecular sequence data; A. Izquierdo López: statistical analyses.

Supplementary information

The following supplementary material is accessible via the Supplementary Content tab on the article's online page at http://doi.org/10.1080/09670262.2017.1397198
Supplementary table 1. Voucher list.

Supplementary fig. 1. Alignment of the full ITS sequences comprising five distinct ribotypes, exemplified by indicated strains.
Supplementary fig. 2. Examples of aberrant forms classified into type M4.

ORCID

Marc Gottschling http://orcid.org/0000-0002-4381-8051

References

Abé, T. (1981). *Studies on the Family Peridinidae: An Unfinished Monograph of the Armoured Dinoflagellata*. Abe Tōru Ikō Shuppankai, Tokyo.

Al-Kandari, M.A., Highfield, A.C., Hall, M.J., Hayes, P. & Schroeder, D.C. (2011). Molecular tools separate harmful algal bloom species, *Karenia mikimotoi*, from different geographical regions into distinct sub-groups. *Harmful Algae*, **10**: 636–643.

Annenkova, N.V., Hansen, G., Moestrup, Ø. & Rengefors, K. (2015). Recent radiation in a marine and freshwater dinoflagellate species flock. *ISME Journal*, **9**: 1821–1834.

Balech, E. (1980). On thecal morphology of dinoflagellates with special emphasis on circular and sulcal plates. *Anales del Centro de Ciencas del Mar y Limnología, Universidad Nacional Autónoma de México*, **7**: 57–68.

Boltovskoy, A. (1975). Estructura y estereoultrastructura tecal de dinoflagelados. II. *Peridinium cinctum* (Müller) Ehrenberg. *Physis Sección B*, **34**: 73–84.

Calado, A.J., Hansen, G. & Moestrup, Ø. (1999). Architecture of the flagellar apparatus and related structures in the type species of *Peridinium, P. cinctum* (Dinophyceae). *European Journal of Phycology*, **34**: 179–191.

Carty, S. (2014). *Freshwater Dinoflagellates of North America*. Cornell University Press, Ithaca.

Day, S.A., Wickham, R.P., Entwisle, T.J. & Tyler, P.A. (1995). *Bibliographic Checklist of Non-marine Algae in Australia*. Australian Biological Resources Study, Canberra.

Ehrenberg, C.G. (1832). Über die Entwickelung und Lebensdauer der Infusionsthiere; nebst ferneren Beiträgen zu einer Vergleichung ihrer organischen Systeme. *Abhandlungen der Königlichen Akademie der Wissenschaften in Berlin*, **1831**: 1–154.

Elbrächter, M. & Meyer, B. (2001). Plate pattern variability and plate overlap in a clonal culture of the freshwater dinoflagellate *Peridinium umbonatum* Stein species complex (Dinophyceae). *Neues Jahrbuch für Geologie und Paläontologie, Abhandlungen*, **219**: 221–227.

Finney, J.C., Pettay, D.T., Sampayo, E.M., Warner, M.E., Oxenford, H.A. & LaJeunesse, T.C. (2010). The relative significance of host–habitat, depth, and geography on the ecology, endemism, and speciation of coral endosymbionts in the genus *Symbiodinium*. *Microbial Ecology*, **60**: 250–263.

Gottschling, M. & Plötner, J. (2004). Secondary structure models of the nuclear Internal Transcribed Spacer regions and 5.8S rRNA in Calciodinelloideae (Peridiniaceae) and other dinoflagellates. *Nucleic Acids Research*, **32**: 307–315.

Gottschling, M. & Söhner, S. (2013). An updated list of generic names in the Thoracosphaeraceae. *Microorganisms*, **1**: 122–136.

Gottschling, M., Keupp, H., Plötner, J., Knop, R., Willems, H. & Kirsch, M. (2005a). Phylogeny of calcareous dinoflagellates as inferred from ITS and ribosomal sequence data. *Molecular Phylogenetics and Evolution*, **36**: 444–455.

Gottschling, M., Knop, R., Plötner, J., Kirsch, M., Willems, H. & Keupp, H. (2005b). A molecular phylogeny of *Scrippsiella sensu lato* (Calciodinellaceae, Dinophyta) with interpretations on morphology and distribution. *European Journal of Phycology*, **40**: 207–220.

Gottschling, M., Söhner, S., Zinßmeister, C., John, U., Plötner, J., Schweikert, M., Aligizaki, K. & Elbrächter, M. (2012). Delimitation of the Thoracosphaeraceae (Dinophyceae), including the calcareous dinoflagellates, based on large amounts of ribosomal RNA sequence data. *Protist*, **163**: 15–24.

Gu, H., Kirsch, M., Zinßmeister, C., Söhner, S., Meier, K.J. S., Liu, T. & Gottschling, M. (2013a). Waking the dead: morphological and molecular characterization of extant †*Posoniella tricarinelloides* (Thoracosphaeraceae, Dinophyceae). *Protist*, **164**: 583–597.

Gu, H., Luo, Z., Krock, B., Witt, M. & Tillmann, U. (2013b). Morphology, phylogeny and azaspiracid profile of *Azadinium poporum* (Dinophyceae) from the China Sea. *Harmful Algae*, **21-22**: 64–75.

Guillard, R.R. & Lorenzen, C.J. (1972). Yellow-green algae with chlorophyllide c. *Journal of Phycology*, **8**: 10–14.

Hijmans, R.J. (2016). raster: geographic data analysis and modeling. R package version 2.5.8.

Höll, K. (1928). *Oekologie der Peridineen. Studien über den Einfluß chemischer und physikalischer Faktoren auf die Verbreitung der Dinoflagellaten im Süßwasser*. Fischer, Jena.

Hoppenrath, M. (2017). Dinoflagellate taxonomy – a review and proposal of a revised classification. *Marine Biodiversity*, **2**: 381–403.

Janofske, D. (2000). *Scrippsiella trochoidea* and *Scrippsiella regalis*, nov. comb. (Peridiniales, Dinophyceae): a comparison. *Journal of Phycology*, **36**: 178–189.

John, U., Litaker, R.W., Montresor, M., Murray, S.A., Brosnahan, M.L. & Anderson, D.M. (2014). Formal revision of the *Alexandrium tamarense* species complex (Dinophyceae) taxonomy: the introduction of five species with emphasis on molecular-based (rDNA) classification. *Protist*, **165**: 779–804.

Kim, C.-J., Aritsune, U., Kim, C.-H. & Yoshihiko, S. (2004). Molecular phylogenetic relationships within the genus *Alexandrium* (Dinophyceae) based on the nuclear-encoded SSU and LSU rDNA D1-D2 sequences. *Ocean Science Journal*, **39**: 172–185.

Kofoid, C.A. (1909). On *Peridinium steinii* Jörgensen, with a note on the nomenclature of the skeleton of the Peridinidae. *Archiv für Protistenkunde*, **16**: 25–47.

Kremp, A., Tahvanainen, P., Litaker, W., Krock, B., Suikkanen, S., Leaw, C.P. & Tomas, C. (2014). Phylogenetic relationships, morphological variation, and toxin patterns in the *Alexandrium ostenfeldii* (Dinophyceae) complex: implications for species boundaries and identities. *Journal of Phycology*, **50**: 81–100.

Kretschmann, J., Elbrächter, M., Zinßmeister, C., Söhner, S., Kirsch, M., Kusber, W.-H. & Gottschling, M. (2015). Taxonomic clarification of the dinophyte *Peridinium acuminatum* Ehrenb., ≡ *Scrippsiella acuminata*, comb. nov. (Thoracosphaeraceae, Peridiniales). *Phytotaxa*, **220**: 239–256.

Kretzschmar, A.L., Verma, A., Harwood, D.T., Hoppenrath, M. & Murray, S. (2017). Characterization of *Gambierdiscus*

lapillus sp. nov. (Gonyaulacales, Dinophyceae): a new toxic dinoflagellate from the Great Barrier Reef (Australia). *Journal of Phycology*, **53**: 283–297.

Lindemann, E.B.L.W. (1917). Beiträge zur Kenntnis des Seenplanktons der Provinz Posen. (Südwestposener Seengrupe.) II. *Zeitschrift der Naturwissenschaftlichen Abteilung der Deutschen Gesellschaft für Kunst und Wissenschaft in Posen*, **24**: 2–41.

Lindemann, E.B.L.W. (1918a). Untersuchungen über Süßwasserperidineen und ihre Variationsformen. *Archiv für Protistenkunde*, **39**: 209–262.

Lindemann, E.B.L.W. (1918b). Untersuchungen über Süßwasserperidineen und ihre Variationsformen II. *Archiv für Naturgeschichte*, **84**: 121–194.

Litaker, R.W., Vandersea, M.W., Kibler, S.R., Reece, K.S., Stokes, N.A., Steidinger, K.A., Millie, D.F., Bendis, B.J., Pigg, R.J. & Tester, P.A. (2003). Identification of *Pfiesteria piscida* (Dinophyceae) and *Pfiesteria*-like organisms using Internal Transcribed Spacer-specific PCR assays. *Journal of Phycology*, **39**: 754–761.

Litaker, R.W., Vandersea, M.W., Kibler, S.R., Reece, K.S., Stokes, N.A., Lutzoni, F.M., Yonish, B.A., West, M.A., Black, M.N.D. & Tester, P.A. (2007). Recognizing dinoflagellate species using ITS rDNA sequences. *Journal of Phycology*, **43**: 344–355.

Logares, R., Shalchian-Tabrizi, K., Boltovskoy, A. & Rengefors, K. (2007). Extensive dinoflagellate phylogenies indicate infrequent marine-freshwater transitions. *Molecular Phylogenetics and Evolution*, **45**: 887–903.

Logares, R., Boltovskoy, A., Bensch, S., Laybourn-Parry, J. & Rengefors, K. (2009). Genetic diversity patterns in five protist species occurring in lakes. *Protist*, **160**: 301–317.

Loret, P., Tengs, T., Villareal, T.A., Singler, H., Richardson, B., McGuire, P., Morton, S., Busman, M. & Campbell, L. (2002). No difference found in ribosomal DNA sequences from physiologically diverse clones of *Karenia brevis* (Dinophyceae) from the Gulf of Mexico. *Journal of Plankton Research*, **24**: 735–739.

Montresor, M., Sgrosso, S., Procaccini, G. & Kooistra, W. H.C.F. (2003). Intraspecific diversity in *Scrippsiella trochoidea* (Dinophyceae): evidence for cryptic species. *Phycologia*, **42**: 56–70.

Müller, O.F. (1796). *Animalcula infusoria fluviatilia et marina, quæ detecit, systematice descripsit et ad vivum delineari curavit*. Möller, Copenhagen.

Orchard, M.J., Humphries, S., Schuech, R. & Menden-Deuer, S. (2016). The influence of viscosity on the motility and sensory ability of the dinoflagellate *Heterocapsa triquetra*. *Journal of Plankton Research*, **38**: 1062–1076.

Pfiester, L.A. (1975). Sexual reproduction of *Peridinium cinctum* f. *ovoplanum* (Dinophyceae). *Journal of Phycology*, **11**: 259–265.

Pizay, M.-D., Lemée, R., Simon, N., Cras, A.-L., Laugier, J.-P. & Dolan, J.R. (2009). Night and day morphologies in a planktonic dinoflagellate. *Protist*, **160**: 565–575.

Popovský, J. & Pfiester, L.A. (1990). *Dinophyceae (Dinoflagellida)*. Fischer, Stuttgart.

R Core Team. (2016). *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna.

Rambaut, A. (2001). Se-Al. Sequence alignment program v2.0a72. Oxford.

Richlen, M.L., Morton, S.L., Barber, P.H. & Lobel, P.S. (2008). Phylogeography, morphological variation and taxonomy of the toxic dinoflagellate *Gambierdiscus toxicus* (Dinophyceae). *Harmful Algae*, **7**: 614–629.

Sievert, C., Parmer, C., Hocking, T., Chamberlain, S., Ram, K., Corvellec, M. & Despouy, P. (2016). plotly: Create interactive web graphics via 'plotly.js'. R package version 4.5.6.

Skovgaard, A., Karpov, S.A. & Guillou, L. (2012). The parasitic dinoflagellates *Blastodinium* spp. inhabiting the gut of marine, planktonic copepods: morphology, ecology, and unrecognized species diversity. *Frontiers in Microbiology*, **3**: 305.

Smith, T. & Smith, C. (2015). *Taxonomic Catalogue of Algae from Ghana (Africa) and New Additions*. Algae Press, Ave Maria.

Söhner, S., Zinßmeister, C., Kirsch, M. & Gottschling, M. (2012). Who am I – and if so, how many? Species diversity of calcareous dinophytes (Thoracosphaeraceae, Peridiniales) in the Mediterranean Sea. *Organisms Diversity and Evolution*, **12**: 339–348.

Spector, D.L., Pfiester, L.A. & Triemer, R.E. (1981). Ultrastructure of the dinoflagellate *Peridinium cinctum* f. *ovoplanum*. II. Light and electron microscopic observations on fertilization. *American Journal of Botany*, **68**: 34–43.

Stein, S.F.N.R. von. (1883). *Der Organismus der Infusionsthiere nach eigenen Forschungen in systematischer Reihenfolge bearbeitet* **3**(**2**). Engelmann, Leipzig.

Stern, R.F., Andersen, R.A., Jameson, I., Küpper, F.C., Coffroth, M.-A., Vaulot, D., Le Gall, F., Veron, B., Brand, J.J., Skelton, H., Kasai, F., Lilly, E.L. & Keeling, P.J. (2012). Evaluating the ribosomal Internal Transcribed Spacer (ITS) as a candidate dinoflagellate barcode marker. *PLoS ONE* **7**: e42780.

Thornhill, D.J., LaJeunesse, T.C. & Santos, S.R. (2007). Measuring rDNA diversity in eukaryotic microbial systems: how intragenomic variation, pseudogenes, and PCR artifacts confound biodiversity estimates. *Molecular Ecology*, **16**: 5326–5340.

Tillmann, U., Gottschling, M., Nézan, E., Krock, B. & Bilien, G. (2014). Morphological and molecular characterization of three new *Azadinium* species (Amphidomataceae, Dinophyceae) from the Irminger Sea. *Protist*, **165**: 417–444.

Yeo, H.G. & Shin, E.Y. (2013). Plate patterns of *Protoperidinium* spp. in Korean coastal waters. *International Journal of Bio-Science and Bio-Technology*, **5**: 91–98.

Zinßmeister, C., Söhner, S., Facher, E., Kirsch, M., Meier, K.J.S. & Gottschling, M. (2011). Catch me if you can: the taxonomic identity of *Scrippsiella trochoidea* (F.Stein) A. R.Loebl. (Thoracosphaeraceae, Dinophyceae). *Systematics and Biodiversity*, **9**: 145–157.

Zinßmeister, C., Söhner, S., Kirsch, M., Facher, E., Meier, K.J.S., Keupp, H. & Gottschling, M. (2012). Same but different: two novel bicarinate species of extant calcareous dinophytes (Thoracosphaeraceae, Peridiniales) from the Mediterranean Sea. *Journal of Phycology*, **48**: 1107–1118.

Zirbel, M.J., Veron, F. & Latz, M.I. (2000). The reversible effect of flow on the morphology of *Ceratocorys horrida* (Peridiniales, Dinophyta). *Journal of Phycology*, **36**: 46–58.

Publication 8

Typification for reliable application of subspecific names within *Peridinium cinctum* (Peridiniales, Dinophyceae)

ROMEIKAT, C., IZQUIERDO LÓPEZ, A., TIETZE, C., **KRETSCHMANN, J.** & GOTTSCHLING, M.

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Typification for reliable application of subspecific names within *Peridinium cinctum* (Peridiniales, Dinophyceae)

CORINNA ROMEIKAT[1], ALEJANDRO IZQUIERDO LÓPEZ[1], CHRISTINE TIETZE[1], JULIANE KRETSCHMANN[1] & MARC GOTTSCHLING[1,*]

[1] *Department Biologie, Systematische Botanik und Mykologie, GeoBio-Center, Ludwig-Maximilians-Universität München, Menzinger Str. 67, D - 80638 München, Germany*

[*] *corresponding author, e-mail: gottschling@bio.lmu.de*

Abstract

The application of scientific names is determined by means of nomenclatural types, and every name has to be typified properly. The concept has limitations for unicellular organisms, because original material frequently consists of drawings and/or inadequately preserved physical material. *Peridinium cinctum* is an abundant freshwater microalga and variable in both morphology and genotype. Morphological variation is mainly expressed in its epithecal conformation: shape deviations of plates, plate rearrangements, plate fusion and plate additions. Different epithecal conformations were traditionally described as either varieties of *P. cinctum* or were established as closely related species. Despite this, relations between varieties, ribotypes and geographic locations were oversighted, and the full spectrum of plate variation in *P. cinctum* is still not well represented. For this reason, we sampled localities in Germany and Poland, from which varieties of *P. cinctum* were described a century ago. We cultivated monoclonal strains, exhibiting two distinct ITS ribotypes, and assessed their epithecal variation of morphology. Based on ca 2,500 observations of individual cells we report a plethora of both plate and suture deviations from the archetypical epithecal conformation of *P. cinctum*. Morphologies corresponding to previously described varieties were rare, even at their type localities. Nevertheless, we found morphologies consistent with protologues in four cases and use this material for epitypification. These varieties are now linked to specific DNA sequences, allowing reliable application of scientific names for future studies.

keywords: Central Europe, epithecal conformation, epitype; microscopy, morphology, ribotype, sequence data, species, variety

Introduction

Principle II of the International Code of Nomenclature for algae, fungi, and plants (ICN: Turland *et al.* 2018) describes how the application of scientific names is determined by means of nomenclatural types. Likewise, type material provides the objective standard of reference for the application of the name it bears (Hitchcock 1921, Daston 2004, Jarvis 2007, Renner 2016). Unicellular organisms may account for more than three quarters of all eukaryotic species, but only a tiny fraction has been taxonomically inventoried so far (Norton *et al.* 1996, Pawlowski *et al.* 2012). Type material, particularly of older names in the microscopic realm, often consists of specimens permanently mounted on glass slides or more frequently of illustrations only (Lazarus 1998, Padial *et al.* 2010). This makes direct, unambiguous application of names based on such types problematic if not impossible, because the type material lacks sufficient characters to definitively connect the name to a modern species delimitation. Molecular methods have become an important tool to identify unicellular organisms (Blaxter 2004, Miller 2007, Keck *et al.* 2018) but without new taxonomic activity, they cannot be applied to historical names (Padial *et al.* 2010). Virtually, all scientific names introduced in the time prior to DNA sequencing are prone to taxonomic confusion but ideally, every name should be unambiguous and clarified particularly in the microbial world.

Peridinium cinctum (O.F.Müller 1773: 98–99) Ehrenberg (1832a: 38) is a historically and ecologically important freshwater dinophyte species that is abundant in meso- through eutrophic habitats worldwide (Boltovskoy 1975, Moestrup & Calado 2018). Furthermore, it is the type species of *Peridinium* Ehrenberg (1832b: 74) and the

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Peridiniaceae. The Peridiniaceae constitute a monophyletic group in molecular phylogenetics (Gottschling *et al.* 2017, in press, Kretschmann *et al.* 2018b) that originated in the Cretaceous, diversifying not earlier than the K/Pg-boundary (Žerdoner Čalasan *et al.* 2019) to multiple species. They include several, widely common freshwater species found in Central Europe besides *Peridinium cinctum*, such as *Peridinium bipes* F.Stein (1883: pl. VIII 6–8) and *Peridinium willei* Huitfeldt-Kaas (1900: 5–6, figs 6–9).

Since its description, multiple scientific names at the species level and below have been associated with and/or synonymised under *P. cinctum* based on morphological variation (Moestrup & Calado 2018). Particularly, Lindemann (1917, 1919, 1920) recognised multiple variations in epithecal conformation of *P. cinctum* from Central Europe, which were described as either new varieties of *P. cinctum* or new species of *Peridinium*. These distinctions were made under a specific working method: Once a particular morphology was documented from more than one locality, a distinct taxon was then recognised, even if it was rare (Lindemann 1920: 123). Taxa introduced at the rank of variety were considered to retain their 'typical' plate arrangement of *P. cinctum* to some extent, whereas taxa at the rank of species were considered to distinctly differ from the regular pattern (Lindemann 1920: 123, 173).

These varieties and species similar to *P. cinctum* were mainly established based on different epithecal plate conformations. The regular plate formula of the epitheca exhibits a Kofoidean formula of 4′ 3a 7″ in *P. cinctum*, but numerous deviations were reported (e.g., plate modifications, fusion or splitting of plates) both by Lindemann (1920) and other authors (see synonymy in Moestrup & Calado 2018). Four such taxa exhibit fused plates (plates 4′+3a in *P. cinctum* var. *curvatum* Er.Lindemann 1920: 167, figs 160–162; plates 2′+3′ in *P. cinctum* var. *dissimile* Er.Lindemann 1920: 166, figs 158–159; plates 1a+2a in *Peridinium germanicum* Er.Lindemann 1919: 250–251, figs 116–117; plates 3′+3a in *P. cinctum* var. *laesum* Er.Lindemann 1920: 165–166, figs 156–157), whereas three of them have split plates (plate 3′ in *Peridinium eximium* Er.Lindemann 1920: 167–168, figs 163–166; plate 3a in *Peridinium rhenanum* Er.Lindemann 1919: 249–250, figs 114–115; plate 3a and precingular plates in *Peridinium scallense* Er.Lindemann 1920: 170–171, figs. 175–177).

The morphological and genetic variability of *P. cinctum* has been similarly assessed within monoclonal strains obtained from various localities across Central Europe (Izquierdo López *et al.* 2018). Three basic morphotypes were readily distinguished: Morphotype M1 corresponds to the established plate pattern for *P. cinctum* (Stein 1883: pl. XII 11), whereas morphotypes M2 and M3 differ in three main morphological characteristics, namely the shape of the 1a plate, position of the ε-suture and position of the 2a plate. Moreover, uncommon modifications of the epithecal pattern were also reported. Some morphotypes appeared more abundantly in certain locations than others and although a certain latitudinal differentiation was inferred, the limited samples did not allow for a definite conclusion. The variability within *P. cinctum* regarding DNA sequences of the Internal Transcribed Spacers (ITSs) was found in five distinct ribotypes, but none of them could be associated with specific epithecal conformations or established morphotypes (Izquierdo López *et al.* 2018). Occasionally, more than one ribotype was reported present at the same locality, but their distribution could not be correlated with any particular geographical pattern.

The taxonomic identity of ambiguous scientific names can be established with the tool of epitypification (Turland *et al.* 2018), which consists in designating new types based on material that reflects the original author's intentions. The significant difference in relation to the historical types is that current epitypes can be linked to living material enabling DNA sequencing, an approach previously implemented for other dinophyte species such as *Durinskia oculata* (F.Stein 1883: pl. III 5–7) Gert Hansen & Flaim (2007: 134–136, fig. 31a–g; Kretschmann *et al.* 2018a), *Palatinus apiculatus* (Ehrenberg 1838: 258, pl. XXII 24) Craveiro, Calado, Daugbjerg & Moestrup (2009: 1178, figs 1–13; Kretschmann *et al.* 2018b) and *Prorocentrum micans* Ehrenberg (1835: Physikalische Klasse: 307–308; Tillmann *et al.* 2019). Therefore, our study aims to continue the work of Izquierdo López *et al.* (2018), by increasing taxon sample and reporting encountered E. Lindemann's varieties and species, while recovering their names for further epitypifications and assigning them, if possible, to living material. We target these historical names and re-collect contemporary material at type localities in order to establish monoclonal strains. If these strains display morphologies consistent with corresponding, previously recognised morphotypes of varieties of *P. cinctum*, then designation of interpretative epitypes is possible. As in any other species, synonymised names at the species level and below are taxonomically obscure and not in use by contemporary authors. However, some of these names could relate to unsuspected morphotypes and potentially cryptic species and therefore, recognising this variety and subsequently performing epitypifications in common freshwater species such as *P. cinctum* can be of future use to taxonomical and ecological studies on this species.

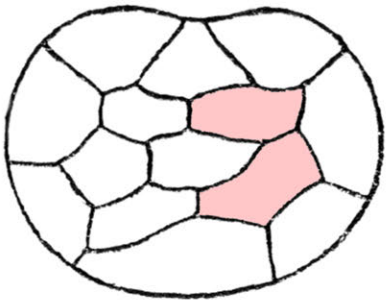


Abb. 12

P. cinctum var. regulatum mihi (= *P. cinctum* Schilling) aus dem Kainoweteich bei Trachenberg in Schlesien. September 1912. Das abgebildete Exemplar zeigt eine kleine Abweichung der linken hinteren Apikalplatte.

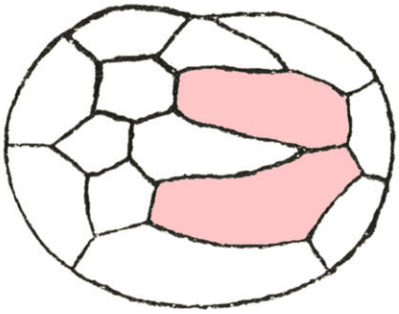


Abb. 14

P. cinctum var. irregulatum mihi aus dem Wollsteiner See. Juni 1916.

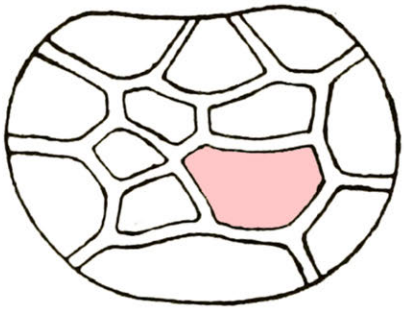


Fig. 156.
Peridinium cinctum var. *laesum* n. var. Epivalvatäfelung.
(56 μ lang; 50 μ breit.)
(Wollsteiner See 11. 7. 1916.)

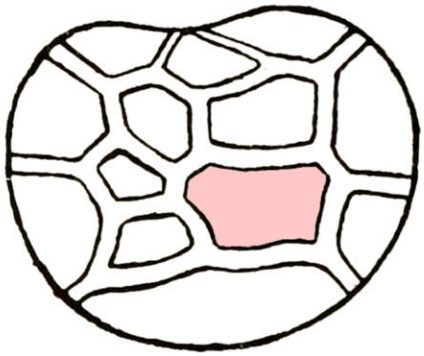


Fig. 157.
Peridinium cinctum var. *laesum* n. var. (Schöhsee 12. 7. 1918.)

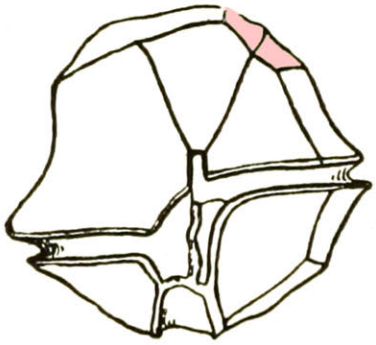


Fig. 158.
Peridinium cinctum var. *dissimile* n. var. Ventral. (Lindensee 13. 5. 1916.) (56 μ lang; 50 μ breit. Rechte at größer als die linke.)

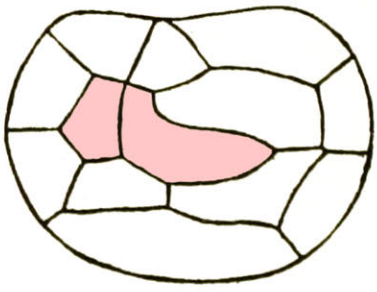


Fig. 159.
Peridinium cinctum var. *dissimile* n. var. Epivalvatäfelung.

FIGURE 1. Original material (drawings) of Erich Lindemann, referring to varieties of *Peridinium cinctum*. Diagnostic traits are highlighted by red shading, and locality and date are given in the original legends. Note that Figs 12, 14 are turned around 180° for better comparability and that Fig. 14 is the **lectotype** of *P. cinctum* var. *irregulatum* (Lindemann 1917).

Material & Methods

This study is a follow-up of Izquierdo López *et al.* (2018), providing more references to the biology of *P. cinctum* and detailed method descriptions regarding sequencing and microscopy. Figures 1–2 show original material of taxa associated with *P. cinctum* from localities in Germany and Poland inspected in the present study (Tab. 1). Figures 1.14, 2.166, 2.191 and 2.193 were used for lectotypifications (see Taxonomic Appendix).

Pragmatic approaches to overcome taxonomic ambiguity

The strains established from material collected at selected localities in Germany and Poland (Tab. 1) showed two different ribotypes. At German Walchensee and Lake Röblin / Lake Schwedt and Polish Lake Wolsztyn, ribotype 1 was present exclusively, whereas Polish Lake Krzycko exhibited ribotype 2 only. At German Lake Krakow, Halensee and Müggelsee, ribotypes 1 and 2 were found as well, which confirms previous data (Izquierdo López *et al.* 2018). No correlation between molecular sequence data and geographic distribution appears to be present.

Our morphological assessment showed that all cells investigated presented the regular epithecal formula of 4′ 3a 7″ (Figs 3–13, S13–S21), with the only exception of those few cells comprising either fusion of plates (Figs S7–S12) or split of plates (Figs 14–16, S1–S6). In all strains under investigation, morphotype M1 (Izquierdo López *et al.* 2018), namely the archetypical epithecal conformation, was predominant (Figs 3–4), ranging from 50% (GeoM*640) to 95% in strains. The other morphotypes were present to lesser extent, ranging from nearly absent to 25% for morphotype M2 (GeoM*640: Fig. 9) and 20% for morphotype M3 (GeoM*778), respectively. Also this is in accordance with observations made earlier (Izquierdo López *et al.* 2018).

Morphologies present in strains collected at type localities and consistent with corresponding protologues were markedly rare (Tab. 1), as documented previously (Lindemann 1917, 1920). *Peridinium cinctum* var. *betacollineatum* Er.Lindemann (1920: 180, fig. 191) had originally been described from Lake Krakow and is confirmed in this study (Figs 5–8) as well as present in Walchensee (Izquierdo López *et al.* 2018). However, the conformation was rare and was encountered not more than ten (out of ca 600) cells at the type locality, and similar numbers applied to cells assignable to *P. cinctum* var. *epsiloncollineatum* Er.Lindemann (1920: 180, fig. 193). The latter variety had originally been described from Polish Lake Wolsztyn and is confirmed here (Fig. 10), but showed some wider occurrences in Lake Krzycko, Lake Krakow (Fig. 9), Müggelsee and Walchensee (Izquierdo López *et al.* 2018: figs 4–15 therein, particularly fig. 11).

Peridinium cinctum var. *irregulatum* Er.Lindemann (1917: 31) had been described from Polish Lake Wolsztyn and is confirmed from there in this study (Fig. 13), and a similar morphology was present also in material from Lake Röblin / Lake Schwedt (Fig. 11) and from Walchensee (Fig. 12). However, the variety was overall rare and is delimited as presenting both plates 4′ and 3a three times longer than plates 2′ and 2a (Lindemann 1917), respectively and thus, this morphology may be difficult to assess, though. We did not sample the type locality at lake Koniowo in Poland, but found *P. cinctum* var. *regulatum* Er.Lindemann (1917: 29–30, fig. 12) in Walchensee (Figs S13–14), which was listed later also by Lindemann (1920). In the course of the present study, it was the most frequently encountered variety of *P. cinctum* and was similarly present in other lakes such as Halensee (Fig. S15) and Müggelsee in Berlin. Though well represented, we refrain from the epitypification of *P. cinctum* var. *regulatum* as long as we do not have material from the type locality (i.e., Lake Koniowo) but in the meantime, the corresponding strains (Tab. 1) may serve as reference material for this variety.

Peridinium eximium had been described from two localities (one in North Rhine-Westphalia), of which Polish Lake Wolsztyn is confirmed in the present study (Figs 15–16). Similar morphologies occurred occasionally and were found in Halensee, Müggelsee (Fig. 14) and Walchensee. We could not identify more taxa from their corresponding type localities in Germany and Poland, but found a single morphology similar to each of three other varieties (*P. cinctum* var. *curvatum* from Walchensee: Fig. S10; *P. cinctum* var. *dissimile* from Lake Wolsztyn: Fig. S11; *P. cinctum* var. *deltatravectum* Er.Lindemann 1920: 178, fig. 194, from Lake Wolsztyn: Fig. S19). Occasionally, we found morphologies similar to *P. germanicum* (Fig. S7) and *P. rhenanum* (Figs S1–S3), but such forms remain to be recollected at their type localities as well.

Most varieties described by Lindemann (1917, 1919, 1920) have not been identified at their type (or other) localities after their initial description. A frequently encountered explanation is that freshwater localities have experienced huge ecological alterations in the past century, by pollution or otherwise (Ptacnik *et al.* 2008, Moestrup & Calado 2018).

TABLE 1: Target taxa associated with *Peridinium cinctum* (arranged with respect to their type localities in Germany and Poland) and their retrieval. Author of all names is Erich Lindemann. Note the scarcity of the varieties and presumable species similar to *P. cinctum*. Strains currently not active, and hence terminated, are indicated by a dagger, although fixed material is stored in our collections.

locality (number)	expected taxa (diagnostic trait)	strain No [investigator]	GenBank accession number(s) [ribotype]	n [observation/ LM / SEM]	presence / absence expected taxa (Figure)
Germany. Mecklenburg-Western Pomerania, Lake Krakow (D010)	*P. cinctum* var. *betacollineatum* (Fig. 2.191)	†GeoM*582 [MG]	KY554676 (ITS) [RB2]	100 / 50 / 50	no <*betacollineatum*>
		GeoM*640 (≡ CCAC6703B) [AIL]	KY554691 (ITS+LSU) [RB2]	121 / 49 / 30	no <*betacollineatum*>
		GeoM*688 [CT]	KY554718 (ITS) [RB2]	93 / 40 / 0	Fig. 7
		GeoM*689 [CT]	KY554719 (ITS) [RB2]	100 / 40 / 0	Fig. 8
		†GeoM*737 [AIL]	KY554724 (ITS) [RB1]	100 / 100 / 0	no <*betacollineatum*>
		GeoM*738 (≡ CCAC9043B) [CT]	KY554725 (ITS) [RB2]	96 / 60 / 0	Figs 5–6
Germany. Brandenburg, Oberhavel, Lake Röblin / Lake Schwedt (D012)	*P. cinctum* var. *epsiloncollineatum*, *nom. illeg.* (Fig. 2.192)	GeoM*721 (≡ CCAC9042B) [CT]	KY554722 (ITS) [RB1]	100 / 100 / 0	no <*epsiloncollineatum*>
		GeoM*722 [CT]	KY554723 (ITS) [RB1]	100 / 100 / 0	no <*epsiloncollineatum*>
Germany. Berlin, Halensee (D020)	——	†GeoM*596 [AIL]	KY554686 (ITS) [RB1]	100 / 100 / 0	——
		†GeoM*598 [AIL]	KY554688 (ITS) [RB2]	100 / 100 / 0	——
Germany. Berlin, N Müggelsee (D015)	——	†GeoM*649 [AIL]	KY554697 (ITS) [RB2]	119 / 56 / 8	——
		†GeoM*685 [AIL]	KY554717 (ITS) [RB1]	92 / 73 / 11	——
Germany, Bavaria, Bad Tölz-Wolfratshausen, Walchensee (D035)	——	†GeoM*644 [AIL]	KY554692 (ITS) [RB1]	129 / 40 / 7	——
		†GeoM*645 [AIL]	KY554693 (ITS) [RB1]	108 / 61 / 22	——
		†GeoM*646 [AIL]	KY554694 (ITS+LSU) [RB1]	96 / 73 / 0	——
		†GeoM*652 [AIL]	KY554698 (ITS) [RB1]	86 / 84 / 2	——
		†GeoM*653 [AIL]	KY554699 (ITS) [RB1]	99 / 70 / 0	——
Poland. Greater Poland, Lake Wolsztyn (PL051)	*P. cinctum* var. *curvatum* (Fig. 2.162)	GeoM*773 [CR]	KY554728 (ITS) [RB1]	160 / 122 / 25	<*eximium*> (Fig. 15), <*irregulatum*> (Fig. 13), but no other taxa
	P. cinctum var. *epsiloncollineatum* (Fig. 2.193)	†GeoM*774 [CR]	KY554729 (ITS) [RB1]	100 / 11 / 0	no such taxa
	P. cinctum var. *irregulatum* (Fig. 1.14)	†GeoM*776 [AIL]	KY554731 (ITS) [RB1]	100 / 100 / 0	<*epsiloncollineatum*> (Fig. 10), but no other taxa
	P. cinctum var. *laesum* (Fig. 1.156–1.157)	†GeoM*777 [CR]	KY554732 (ITS) [RB1]	100 / 10 / 0	no such taxa
		†GeoM*782 [CR]	KY554736 (ITS) [RB1]	100 / 8 / 0	no such taxa
	P. eximium (Fig. 2.165)	GeoM*783 [CR]	KY554737 (ITS) [RB1]	100 / 6 / 0	<*eximium*> (Fig. 16)
Poland. Greater Poland, Leszno, Lake Krzycko (PL042)	*P. cinctum* var. *dissimile* (Fig. 1.158–1.159)	†GeoM*778 [AIL]	MK405485 (SSU), KY554733 (ITS), MK405486 (LSU) [RB2]	100 / 99 / 0	no <*dissimile*>

Fig. 160.
Peridinium cinctum var. *curvatum* n. var. Ventral (Wollsteiner See.)

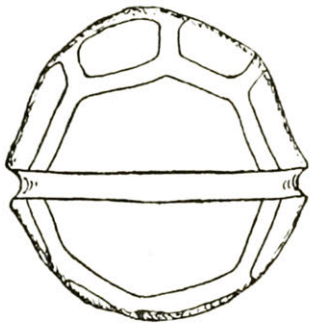


Fig. 161.
Peridinium cinctum var. *curvatum* n. var. Dorsal.

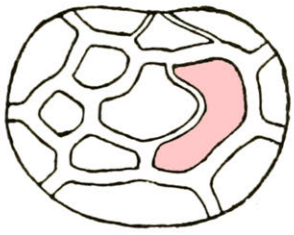


Fig. 162.
Peridinium cinctum var. *curvatum* n. var. Epivalvatäfelung.

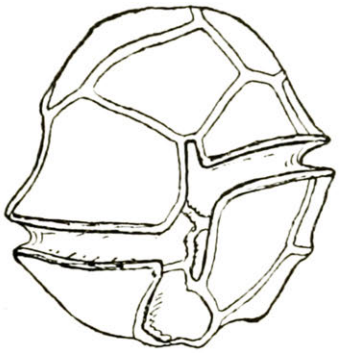


Fig. 163.
Peridinium eximium n. sp. Ventral. (Eschbachtalsperre 13. 12. 1904.) (leicht dorsoventral abgeplattet.)

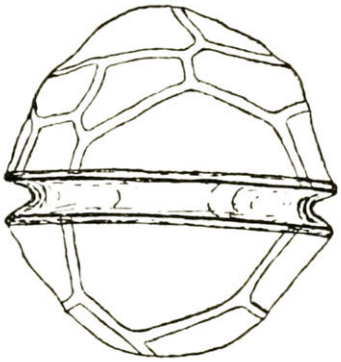


Fig. 164.
Peridinium eximium n. sp. Dorsal.

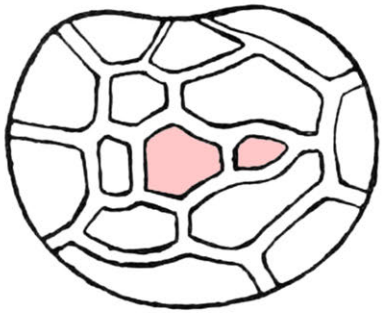


Fig. 166.
Peridinium eximium n. sp. Epivalvatäfelung. (Wollsteiner See 11. 7. 1916.) (weniger dorsoventral abgeplattet.)

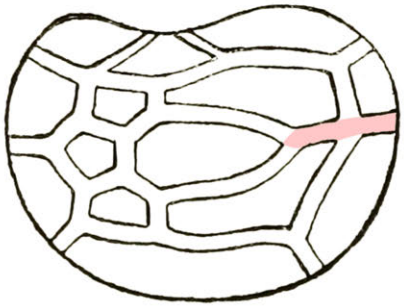


Fig. 191.
Peridinium cinctum var. *β-collineatum* n. var.

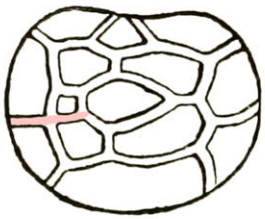


Fig. 192.
Peridinium cinctum var. *ε-collineatum* n. var.

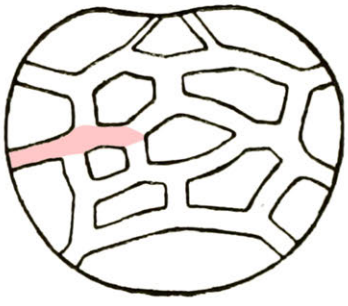
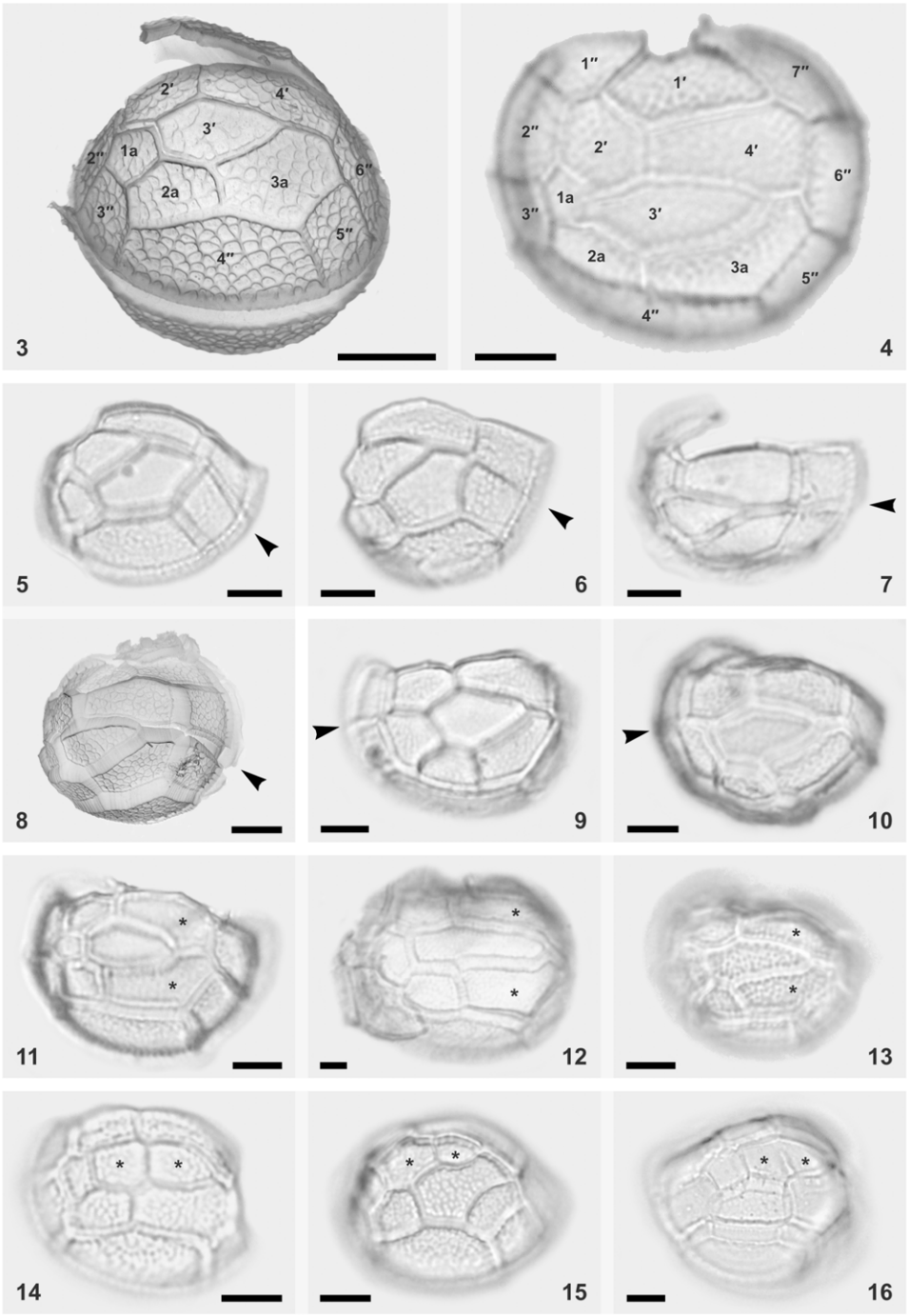


Fig. 193.
Peridinium cinctum var. *ε-collineatum* n. var.

FIGURE 2. Original material (drawings) of Erich Lindemann, referring to *Peridinium eximium* and varieties of *Peridinium cinctum*. Diagnostic traits are highlighted by red shading, and locality and date are given in the original legends. Note that Fig. 166 is the **lectotype** of *P. eximium*, Fig. 191 the **lectotype** of *P. cinctum* var. *betacollineatum* and Fig. 193 the **lectotype** of *P. cinctum* var. *epsiloncollineatum* (Lindemann 1920), respectively.



FIGURES 3–16. Morphological variability within *Peridinium cinctum*. Figs 3–4: Regular epithecal conformation (plates labelled using the Kofoidean system). Fig. 3: GeoM*738 from Lake Krakow. Fig. 4: GeoM*596 from Halensee. Figs 5–8: Irregular position of the β-suture (black arrows), note the <*collineatum*> conformation (Izquierdo López *et al.*, 2018). Figs 5–6: GeoM*738 from Lake Krakow (both mirrored). Fig. 7: GeoM*689 from Lake Krakow (mirrored; **epitype** of *P. cinctum* var. *betacollineatum*). Fig. 8: GeoM*688 from Lake Krakow. Figs 9–10: Irregular position of the ε-suture (black arrows), note the <*collineatum*> conformation (Izquierdo López *et al.* 2018). Fig. 9. GeoM*640 from Lake Krakow (mirrored). Fig. 10. GeoM*776 from Lake Wolsztyn (**epitype** of *P. cinctum* var. *epsiloncollineatum*). Figs 11–13: Epithecal conformation with elongated plates 4′ and 3a (indicated by asterisks), leading to a morphology of *P. cinctum* var. *irregulatum* (Lindemann, 1917). Fig. 11: GeoM*721 from Röblinsee. Fig. 12: GeoM*644 from Walchensee (mirrored). Fig. 13: GeoM*773 from Lake Wolsztyn (mirrored; **epitype** of *P. cinctum* var. *irregulatum*). Figs 14–16: Epithecal conformation with plate 3′ divided into two parts (indicated by asterisks). Fig. 14: GeoM*685 from Müggelsee (mirrored). Fig. 15: GeoM*773 from Lake Wolsztyn (mirrored). Fig. 16: GeoM*783 from Lake Wolsztyn (mirrored, **epitype** of *P. eximium*). Scale bars: 10 µm.

Despite this, epitypification has been specially successful at localities that have been heavily affected by human impact such as Berlin ponds (Kretschmann *et al.* 2018b), the Kiel Fjord (Kretschmann *et al.* 2015, Tillmann *et al.* 2019) or the Vltava River (Kretschmann *et al.* 2018a). The lack of previously described varieties could also be explained by their factual scarcity. It may not be enough to count ca 100 cells in each strain to record some uncommon morphologies, a taxonomic bias widely known in biodiversity assessments.

The lack of previously described protologues is contrasted by the observation of additional morphologies that were not assessed by E. Lindemann or other researchers. The study of clonal strains enables us to reconsider the taxonomic value of such (and previous) observations. The presence of few cells with deviating epithecal plate formulas (because of, e.g., split or fused plates) within otherwise homogeneous cultivated material is indicative that such morphologies have no diagnostic potential (at least within *P. cinctum*) and should not be used for delimitation of reproductively isolated units (i.e., species). This procedure, though, was extensively used by Lindemann (1920) as exemplified by *P. eximium*. From our results, we encountered morphologies similar to that of *P. eximium* (i.e., split of plate 3′), but these were sporadically that we still consider the name a synonym of *P. cinctum*. We encourage further taxonomical assessment of previously synonymised names such as Polish *P. germanicum* and German *P. rhenanum* and *P. scallense*, whose morphologies were occasionally found in the material inspected here. Anyhow, we consider *P. cinctum* a distinct species showing much morphological and some genetic intraspecific variability (Izquierdo López *et al.* 2018), similarly to its latest comprehensive treatment (Moestrup & Calado 2018).

Overall, morphological variation in *P. cinctum* has historically been recognised through the delimitation of varieties under *P. cinctum* and sometimes species with unique epithecal conformation (plate shape, plate fusion, plate addition). We recover some of these morphologies, recognised 100 years ago, from observations in monoclonal strains and select corresponding images for epitypification (see below). Such names may prove useful once, for example, cryptic diversity within a *P. cinctum* species complex is uncovered. These morphologies were further encountered in other localities, and we also document new epithecal conformations, as well as the lack of previously described varieties from their type localities. With that, we continue our assessment on morphological variation of *P. cinctum*. The origin of this variation, besides its relation with particular genotypes or geographic distribution though, is still a work in progress.

Nomenclature and taxonomic activity

Peridinium cinctum (O.F.Müll.) Ehrenb., Abhandlungen der Königlichen Akademie der Wissenschaften in Berlin 1830: 38. 1832. *Vorticella cincta* O.F.Müll., Vermium terrestrium et fluviatilium, seu animalium infusoriorum, helminthicorum et testaceorum, non marinorum, succincta historia 1.1: 98–99. 1773. *Urceolaria cincta* (O.F.Müll.) Lam., Histoire naturelle des animaux sans vertèbres 2: 41. 1816. Type [non-fossil]:—DENMARK, without precise locality, Nov 1780–1781, *O.F. Müller s.n.*

= *Peridinium cinctum* var. *betacollineatum* Er.Lindem., Archiv für Naturgeschichte 84.8: 180, fig. 191. 1920. Type [illustration of non-fossil specimen]:—GERMANY. Mecklenburg-Western Pomerania: Krakower See, Oct 1917, *E. Lindemann s.n.* (**lectotype**, **designated here**: Fig. 191! in Lindemann, 1920, here reproduced as Fig. 2.191); [illustration of non-fossil specimen]:—GERMANY. Mecklenburg-Western Pomerania: Krakower See, 24 Jun 2015, *J. Kretschmann & M. Gottschling [J. Kretschmann GeoM*688] D010* (**epitype**, **designated here**: Fig. 7!) [http://phycobank.org/101910].—Lefèvre (1932: 85) used the taxon for a combination that was not validly published (ICN Arts 6.10, 24.2), though.

= *Peridinium cinctum* var. *epsiloncollineatum* Er.Lindem., ***nomen illegitimum*** (**designated here** according to Turland *et al.*, 2018: Art. 53.5), Archiv für Naturgeschichte 84.8: 180, fig. 192. 1920. Original material [illustration of non-fossil specimen]:—GERMANY. Brandenburg: Fürstenberg, Baalen-See, Aug 1919; Schleswig-Holstein: Ostholstein, Malente, Kellersee, 27 Aug 1917: *E. Lindemann s.n.* (Fig. 192! in Lindemann, 1920, here reproduced as Fig. 2.192) [http://phycobank.org/101902].—Lefèvre (1932: 85) used the taxon for a combination that was not validly published (ICN Arts 6.10, 24.2), though.

= *Peridinium cinctum* var. *epsiloncollineatum* Er.Lindem., Archiv für Naturgeschichte 84.8: 180, fig. 193. 1920. Type [illustration of non-fossil specimen]:—POLAND. Greater Poland: Wolsztyn, Jezioro Wolsztyńskie, 11 Jul 1916, *E. Lindemann s.n.* (**lectotype**, **designated here**: Fig. 193 in Lindemann, 1920, here reproduced as Fig. 2.193); [illustration of non-fossil specimen]:—POLAND. Greater Poland. Wolsztyn, Jezioro Wolsztyńskie 3 Jun 2016, *J. Kretschmann, M. Gottschling & P.M. Owsianny [J. Kretschmann GeoM*776] PL050* (**epitype**, **designated here**: Fig. 10!) [http://phycobank.org/101911].

= *Peridinium cinctum* var. *irregulatum* Er.Lindem., Deutsche Gesellschaft für Kunst und Wissenschaft in Posen. Zeitschrift der Naturwissenschaftlichen Abteilung (des Naturwissenschaftlichen Vereins). Posen 24: 31[, 33], fig. 14. 1917. *Peridinium cinctum*

forma *irregulatum* (Er.Lindem.) M.Lefèvre, Archives de Botanique 2 Mém. 5: 89. 1932. Type [illustration of non-fossil specimen]:—POLAND. Greater Poland: Wolsztyn, Jezioro Wolsztyńskie, Jun 1916, *E. Lindemann s.n.* (**lectotype**, **designated here**: Fig. 14! in Lindemann, 1917, here reproduced as Fig. 1.14); [illustration of non-fossil specimen]:—POLAND. Greater Poland: Wolsztyn, Jezioro Wolsztyńskie, 3 Jun 2016, *J. Kretschmann, M. Gottschling & P.M. Owsianny [J. Kretschmann GeoM*773] PL051* (**epitype**, **designated here**: Fig. 13!) [http://phycobank.org/101912].

= *Peridinium eximium* Er.Lindem., Archiv für Naturgeschichte 84.8: 167–168, figs 163–166. 1920. Type [illustration of non-fossil specimen]:—POLAND. Greater Poland: Wolsztyn, Jezioro Wolsztyńskie, 11 Jul 1916, *E. Lindemann s.n.* (**lectotype**, **designated here**: Fig. 166! in Lindemann, 1920, here reproduced as Fig. 2.166); [illustration of non-fossil specimen]:—POLAND. Greater Poland: Wolsztyn, Jezioro Wolsztyńskie, 3 Jun 2016, *J. Kretschmann, M. Gottschling & P.M. Owsianny [J. Kretschmann GeoM*783] PL050* (**epitype**, **designated here**: Fig. 16!) [http://phycobank.org/101913].—Other original material: non fossil specimens from Germany. NRW, Remscheid, Eschbachtalsperre, 13 Dec 1904, collected by E. Lindemann and illustrated as Figs 163–165 in Lindemann (1920). Lefèvre (1932: 88) used the taxon for a combination that was not validly published (ICN Arts 24.2, 52.1), though.

Note: The scarcity of the taxa here typified disables the preparation of slides for light microscopy. Exceptionally and differently from our previous approaches, we therefore decided to use illustrations here not only for lectotypification but also for the designation of epitypes (ICN Art. 40.5). Pictures were taken from cells or their remnants, which were cultivated as monoclonal strains (i.e., established from a single cell). Thus, the epitypes do not exhibit DNA intrinsically, but are linked to material with corresponding genetic information.

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References

Blaxter, M.L. (2004) The promise of a DNA taxonomy. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences* 359: 669–679.
https://doi.org/10.1098/rstb.2003.1447

Boltovskoy, A. (1975) Estructura y estereoultrastructura tecal de dinoflagelados. II. *Peridinium cinctum* (Müller) Ehrenberg. *Physis Sección B* 34: 73–84.

Craveiro, S.C., Calado, A.J., Daugbjerg, N. & Moestrup, Ø. (2009) Ultrastructure and LSU rDNA-based revision of *Peridinium* group *Palatinum* (Dinophyceae) with the description of *Palatinus* gen. nov. *Journal of Phycology* 45: 1175–1194.
https://doi.org/10.1111/j.1529-8817.2009.00739.x

Daston, L. (2004) Type specimens and scientific memory. *Critical Inquiry* 31: 153–182.
https://doi.org/10.1086/427306

Ehrenberg, Ch.G. (1832a) Beiträge zur Kenntnis der Organisation der Infusorien und ihrer geographischen Verbreitung. *Abhandlungen der Königlichen Akademie der Wissenschaften in Berlin* 1830: 1–88.
https://doi.org/10.5962/bhl.title.143632

Ehrenberg, Ch.G. (1832b) Über die Entwickelung und Lebensdauer der Infusionsthiere; nebst ferneren Beiträgen zu einer Vergleichung ihrer organischen Systeme. *Abhandlungen der Königlichen Akademie der Wissenschaften in Berlin* 1831: 1–154.

Ehrenberg, Ch.G. (1835) Dritter Beitrag zur Erkenntnis großer Organisation in der Richtung des kleinsten Raumes. *Abhandlungen der Königlichen Akademie der Wissenschaften in Berlin* 1833, *Physikalische Klasse*: 145–336.

Ehrenberg, Ch.G. (1838) Zwölfte Familie: Kranzthierchen. *Die Infusionsthierchen als vollkommene Organismen*. Voss, Leipzig, pp. 249–259.

Gottschling, M., Chacón, J., Žerdoner Čalasan, A., Neuhaus, S., Kretschmann, J., Stibor, H. & John, U. (In press) Phylogenetic placement of environmental sequences using taxonomically reliable databases helps to rigorously assess dinophyte biodiversity in Bavarian lakes (Germany). *Freshwater Biology*.

Gottschling, M., Kretschmann, J. & Žerdoner Čalasan, A. (2017) Description of Peridiniopsidaceae, fam. nov. (Peridiniales, Dinophyceae). *Phytotaxa* 299 (2): 293–296.
https://doi.org/10.11646/phytotaxa.299.2.16

Hansen, G. & Flaim, G. (2007) Dinoflagellates of the Trentino Province, Italy. *Journal of Limnology* 66: 107–141. https://doi.org/10.4081/jlimnol.2007.107

Hitchcock, A.S. (1921) The type concept in systematic botany. *American Journal of Botany* 8: 251–255. https://doi.org/10.1002/j.1537-2197.1921.tb05622.x

Huitfeldt-Kaas, H. (1900) Die limnetischen Peridineen in norwegischen Binnenseen. *Skrifter / Videnskabsselskapet i Kristiania, Matematisk-Naturvidenskapelig Klasse*: 3–7.

Izquierdo López, A., Kretschmann, J., Žerdoner Čalasan, A. & Gottschling, M. (2018) The many faces of *Peridinium cinctum*: Morphological and molecular variability in a common dinophyte. *European Journal of Phycology* 53: 156–165. https://doi.org/10.1080/09670262.2017.1397198

Jarvis, C. (2007) *Order out of chaos. Linnaean plant names and their types.* The Linnean Society, London.

Keck, F., Vasselon, V., Rimet, F., Bouchez, A. & Kahlert, M. (2018) Boosting DNA metabarcoding for biomonitoring with phylogenetic estimation of operational taxonomic units' ecological profiles. *Molecular Ecology Resources* 18: 1299–1309. https://doi.org/10.1111/1755-0998.12919

Kretschmann, J., Elbrächter, M., Zinßmeister, C., Söhner, S., Kirsch, M., Kusber, W.-H. & Gottschling, M. (2015) Taxonomic clarification of the dinophyte *Peridinium acuminatum* Ehrenb., ≡ *Scrippsiella acuminata*, *comb. nov.* (Thoracosphaeraceae, Peridiniales). *Phytotaxa* 220 (3): 239–256. https://doi.org/10.11646/phytotaxa.220.3.3

Kretschmann, J., Žerdoner Čalasan, A. & Gottschling, M. (2018a) Molecular phylogenetics of dinophytes harbouring diatoms as endosymbionts (Kryptoperidiniaceae, Peridiniales), with evolutionary interpretations and a focus on the identity of *Durinskia oculata* from Prague. *Molecular Phylogenetics and Evolution* 118: 392–402. https://doi.org/10.1016/j.ympev.2017.10.011

Kretschmann, J., Žerdoner Čalasan, A., Kusber, W.-H. & Gottschling, M. (2018b) Still curling after all these years: *Glenodinium apiculatum* Ehrenb. (Peridiniales, Dinophyceae) repeatedly found at its type locality in Berlin (Germany). *Systematics and Biodiversity* 16: 200–209. https://doi.org/10.1080/14772000.2017.1375045

Lazarus, D.B. (1998) The Ehrenberg Collection and its curation. *In*: Williams, D.M. & Huxley, R. (Eds.) *Christian Gottfried Ehrenberg (1795-1876): The man and his legacy*. The Linnean Society, London, pp. 31–48.

Lefèvre, M.M. (1932) Monographie des espèces du genre *Peridinium*. *Archives de Botanique* 2: 1–210.

Lindemann, E.B.L.W. (1917) Beiträge zur Kenntnis des Seenplanktons der Provinz Posen. (Südwestposener Seengrupe.) II. *Zeitschrift der Naturwissenschaftlichen Abteilung der Deutschen Gesellschaft für Kunst und Wissenschaft in Posen* 24: 2–41.

Lindemann, E.B.L.W. (1919) Untersuchungen über Süßwasserperidineen und ihre Variationsformen. *Archiv für Protistenkunde* 39: 209–262.

Lindemann, E.B.L.W. (1920) Untersuchungen über Süßwasserperidineen und ihre Variationsformen II. *Archiv für Naturgeschichte* 84.8: 121–194.

Miller, S.E. (2007) DNA barcoding and the renaissance of taxonomy. *Proceedings of the National Academy of Sciences of the United States of America* 104: 4775–4776. https://doi.org/10.1073/pnas.0700466104

Moestrup, Ø. & Calado, A.J. (2018) *Dinophyceae*. Springer, Berlin, pp 57–451. https://doi.org/10.1007/978-3-662-56269-7_7

Norton, T.A., Melkonian, M. & Andersen, R.A. (1996) Algal biodiversity. *Phycologia* 35: 308–326. https://doi.org/10.2216/i0031-8884-35-4-308.1

Müller, O.F. (1773) *Vermium terrestrium et fluviatilium, seu animalium infusoriorum, helminthicorum et testaceorum, non marinorum, succincta historia 1.1*. Heineck & Faber, Leipzig, 72 pp. https://doi.org/10.5962/bhl.title.46299

Padial, J.M., Miralles, A., De la Riva, I. & Vences, M. (2010) The integrative future of taxonomy. *Frontiers in Zoology* 7: 16. https://doi.org/10.1186/1742-9994-7-16

Pawlowski, J., Audic, S., Adl, S., Bass, D., Belbahri, L., Berney, C., Bowser, S.S., Cepicka, I., Decelle, J., Dunthorn, M., Fiore-Donno, A.M., Gile, G.H., Holzmann, M., Jahn, R., Jirků, M., Keeling, P.J., Kostka, M., Kudryavtsev, A., Lara, E., Lukeš, J., Mann, D.G., Mitchell, E.A.D., Nitsche, F., Romeralo, M., Saunders, G.W., Simpson, A.G.B., Smirnov, A.V., Spouge, J.L., Stern, R.F., Stoeck, T., Zimmermann, J., Schindel, D. & de Vargas, C. (2012) C BOL Protist Working Group: Barcoding eukaryotic richness beyond the animal, plant, and fungal kingdoms. *PLoS Biology* 10: e1001419. https://doi.org/10.1371/journal.pbio.1001419

Ptacnik, R., Solimini, A.G., Andersen, T., Tamminen, T., Brettum, P., Lepistö, L., Willén, E. & Rekolainen, S. (2008) Diversity predicts stability and resource use efficiency in natural phytoplankton communities. *Proceedings of the National Academy of Sciences of the*

United States of America 105: 5134–5138.
https://doi.org/10.1073/pnas.0708328105

Renner, S.S. (2016) A return to Linnaeus's focus on diagnosis, not description: The use of DNA characters in the formal naming of species. *Systematic Biology* 65: 1085–1095.
https://doi.org/10.1093/sysbio/syw032

Stein, S.F.N.R.v. (1883) *Der Organismus der Infusionsthiere nach eigenen Forschungen in systematischer Reihenfolge bearbeitet* 3.2. Engelmann, Leipzig.

Tillmann, U., Hoppenrath, M. & Gottschling, M. (2019) Reliable determination of *Prorocentrum micans* Ehrenb. (Prorocentrales, Dinophyceae) based on newly collected material from the type locality. *European Journal of Phycology* 54: 417–431.
https://doi.org/10.1080/09670262.2019.1579925

Turland, N.J., Wiersema, J.H., Barrie, F.R., Greuter, W., Hawksworth, D.L., Herendeen, P.S., Knapp, S., Kusber, W.-H., Li, D.-Z., Marhold, K., May, T.W., McNeill, J., Monro, A.M., Prado, J., Price, M.J. & Smith, G.F. (2018) *International Code of Nomenclature for algae, fungi, and plants (Shenzhen Code) adopted by the Nineteenth International Botanical Congress Shenzhen, China, July 2017*. Koeltz, Glashütten.
https://doi.org/10.12705/Code.2018

Žerdoner Čalasan, A., Kretschmann, J. & Gottschling, M. (2019) They are young, and they are many: Dating freshwater lineages in unicellular dinophytes. *Environmental Microbiology* 21: 4125–4135.

Publication 9

Towards global distribution maps of unicellular organisms such as calcareous dinophytes based on DNA sequence information

ŽERDONER ČALASAN, A., **KRETSCHMANN, J.**, FILIPOWICZ, N.H., IRIMIA, R.-E., KIRSCH, M. & GOTTSCHLING, M.

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Towards global distribution maps of unicellular organisms such as calcareous dinophytes based on DNA sequence information

Anže Žerdoner Čalasan[1,2] · Juliane Kretschmann[1] · Natalia H. Filipowicz[3] · Ramona-Elena Irimia[4,5] · Monika Kirsch[6] · Marc Gottschling[1]

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Abstract

Despite recent fruitful attempts to elucidate microbial biogeography in more detail, knowledge of distribution still lags behind for dinophytes. Evolutionary phenomena, such as cryptic speciation and modification due to the environment, hamper reliable conclusions about the distribution of this important plankton group. We combined newly collected samples from the Black Sea (ten new strains from three localities) with occurrence data, which have been gathered extensively over the past decade, in order to provide the first global distribution maps of four specific ribotypes assigned to the *Scrippsiella* lineage (Thoracosphaeraceae, Peridiniales) collected at a total of 39 sites. They showed a wide, partly overlapping distribution and shared the presence primarily at the coastal localities. Differences in abundance of specific ribotypes were observed, but the ribotype corresponding to the globally most frequently encountered species *Scrippsiella acuminata* has not yet been found in the Black Sea. We discuss the significance of DNA-based records for distribution maps particularly of unicellular organisms such as dinophytes. Based on a collective approach as exemplified in our study, we may start to understand in detail the ecological basis and the dynamics of the individual colonisation/invasion events, species establishment and consequent distribution in the microbiome, all of which have been changing drastically due to the ongoing climate change.

Keywords Biogeography · Black Sea · Dinoflagellates · Dispersal · Niche

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✉ Marc Gottschling
gottschling@bio.lmu.de

[1] Department Biologie, Systematische Botanik und Mykologie, GeoBio-Center, Ludwig-Maximilians-Universität München, Menzinger Str. 67, D – 80 638 Munich, Germany

[2] Department of Botany, School of Biology and Chemistry, University of Osnabrück, Barbarastr. 11, D – 49076, Osnabrück, Germany

[3] Department of Biology and Pharmaceutical Botany, Medical University of Gdańsk, Hallera 107, PL – 80-416 Gdańsk, Poland

[4] Faculty of Biology, Alexandru Ioan Cuza University of Iași, Carol I, 20A, RO – 700 505 Iași, Romania

[5] NIRDBS/Stejarul Research Centre for Biological Sciences, Alexandru cel Bun St. 6, RO – 610 004 Piatra Neamț, Romania

[6] Universität Bremen, Fachbereich Geowissenschaften – Fachrichtung Historische Geologie/Paläontologie, Klagenfurter Straße, D – 28 359 Bremen, Germany

Introduction

Closely integrated biotic and abiotic interactions characterise living conditions of biological species and shape their current spatial occurrences worldwide. Distribution of living beings is influenced mostly by vicariance, (short to long distance) dispersal, selection and drift (Vellend 2010). There is an insignificant number of cases in the microbial world that document the origin of sister species (or groups) due to spatial fragmentation, namely allopatric speciation in silty lakes (Evans et al. 2009). This does not necessarily reflect the rare occurrence, since it is highly plausible that effects of allopatric speciation are superimposed by dispersal. Speciation along depth ('depth–parapatric speciation') may occur in vertically structured plankton populations, leading to diversification without spatial fragmentation (Weiner et al. 2012). Dispersal by any means might be of great importance for protists (Foissner 2007; Martiny et al. 2006), but their dispersal ability does not necessarily lead to the establishment of stable populations at new localities (Renner 2004; Table 1).

Table 1 Possible combinations between altitude of ecological niche and dispersal potential of biological species, with consequences for their distribution. Note that dispersal does not equal establishment of new populations (Gillespie et al. 2012), as it greatly depends on the impact of ecological drivers forcing selection/environmental filtering. Furthermore, a clear correlation between body size and dispersal capacity has not been observed (Martiny et al. 2006)

Species' traits	Dispersal potential low	Dispersal potential high
Ecological niche narrow	Endemism	Wide distribution at ecologically specific localities/habitats
Ecological niche broad	Regionally restricted distribution	'Everything is everywhere'

In this respect, the concept of an 'ecological licence' is a key, describing a 'previously not utilised unit of the environment that is suitable for becoming an ecological dimension of an organism's niche' (Osche 1966). As a result, the pattern of ecologically more tolerant species being more widely distributed than ecologically more selective species should also be inferred among unicellular microorganisms (Fritz et al. 2013). Irrefutable conclusions about the spatial occurrence, and the ecological niche realised by species, are as good as the quality of the underlying data. The distribution of flowering plants and larger animals is relatively easy to assess, as they are well represented in extensive collections (Krupnick and Kress 2005; Mayer et al. 2013; Rocha et al. 2014). Many such specimens have been digitised in the past decade, providing a set of easily accessible data on morphology, with a potential link to their DNA sequence. Furthermore, permanently available and very precise occurrence records can also be obtained from specimens registered in various online platforms and databases (e.g., GBIF, GBOL, JSTOR, Tropicos®, WoRMS). However, such powerful and continuously curated online repositories are currently almost nonexistent for microorganisms, which partially explains why distribution maps of protists are still lacking (Soininen 2012). Some floras and other studies include morphology-based maps for selected species or species groups on a larger scale (Tsarenko et al. 2006; Rintala et al. 2010), while DNA-based occurrence surveys include geographically much smaller regions (Cuvelier et al. 2010; Kohli et al. 2014; Massana et al. 2015; Elferink et al. 2017).

Both biological phenomena and technical challenges are the reasons behind data scarcity resulting in insufficient knowledge on distributions in the microbiome (Caron 2009). Complex determination procedures and inconsistent nomenclature lead to misidentifications, which severely hamper the understanding of protist distribution. Moreover, we lack a generally accepted basis for species delimitation (Boenigk et al. 2012), which is also a common hindering factor in unicellular dinophytes. The intraspecific morphological variability in this group can be extensive, which has already been shown in studies of easily cultivatable species such as *Alexandrium ostenfeldii* (Paulsen) Balech & Tangen (Kremp et al. 2014), "*Gymnodinium*" *aureolum* Hulburt (Tang et al. 2008) and *Polykrikos kofoidii* Chatton (Matsuoka and Cho 2000). In contrast, some dinophyte lineages build species complexes as a result of cryptic speciation (John et al. 2014; LaJeunesse and Thornhill 2011). This feature again prevents the understanding of the phylogeography and distribution of unicellular organisms in detail.

A cryptic species complex has also been identified in the *Scrippsiella* Balech s.l. lineage of the calcareous dinophytes (Thoracosphaeraceae, Peridiniales). The group is an integral part of the phytoplankton communities worldwide and has an extensive fossil record (Vink 2004; Elbrächter et al. 2008; Gottschling et al. 2012). A wide sequence variety of molecular ribotypes has been discovered in the *Scrippsiella* lineage (Montresor et al. 2003; Gottschling et al. 2005; Söhner et al. 2012). This sequence variety can be found particularly in the Internal Transcribed Spacers (ITSs), which are part of the ribosomal RNA (rRNA) operon. Most members of the *Scrippsiella acuminata* (Ehrenb.) Kretschmann, Zinssmeister, S.Soehner, Elbr., Kusber & Gottschling [=*Scrippsiella trochoidea* (F.Stein) A.R.Loebl.] species complex share both the consistent tabulation pattern in the motile thecate cells and the characteristic coccoid cells of ovoid shape, with numerous styliform spines developed on the cell surface. Thus, species of the *Scrippsiella* complex cannot be determined based on morphology but solely on molecular sequence data. Sequences of the small and large rRNA subunits (SSU and LSU, respectively) are not indicative for species delimitation in this lineage, as they are too conserved. However, those of the ITS can be used (Montresor et al. 2003; Gottschling and Kirsch 2009; Söhner et al. 2012) as they can indicate genetic distances of $p < 0.04$ (Litaker et al. 2007). These features make the *Scrippsiella* group particularly prone to false identification, which furthermore hinders any reliable conclusions (including biogeography) of this cryptic species group. Successful endeavours have been undertaken to clarify the taxonomy in this group (Zinßmeister et al. 2011; Kretschmann et al. 2014, 2015a), which was another reason to exemplify our approach using this group. Preliminary phylogeographic distribution assessment indicates that the wide distribution of *Scrippsiella* species is mostly limited to continental shelves, and that it at least partially overlaps (Gottschling et al. 2005).

The easily accessible, yet unreliable data problem is illustrated by the application of online repositories such as the Global Biodiversity Information Facility (GBIF; http://www.gbif.org/) that has the vision 'to enable public access on data of all types of life on Earth including occurrence data, shared across national boundaries via the internet'. However, the situation is far from this vision when it comes to unicellular organisms, and the dinophyte species complex *Scrippsiella* s.l. serves as an illustrative example. On December 19, 2017, we found 3.442 noted occurrences (2.778 of which were georeferenced) while searching for the string '*Scrippsiella trochoidea*' (i.e., the formerly accepted name used for *S. acuminata*; Kretschmann et al. 2015a), but it was not clear which records were based on DNA sequence information. Namely, many records referred to 'human observation', without a clear indication whether such data were identified based on morphology or genetics, and without the option to critically verify the observer, who determined a species as such. Furthermore, GBIF also offered an alternative search result under string 'Scrippsiella troichoidea'—610 occurrences, 605 of which were georeferenced—recognising it as an accepted species. This human error illustrates the importance of taxonomy in every aspect of biological research and challenges the reliability of online data at this particular repository. Precise knowledge about the occurrence of particular species assigned to the *Scrippsiella* complex obtained from the available data on GBIF is therefore very limited.

The aim of the research was a global distribution assessment of selected *Scrippsiella* species based on DNA sequence information. We also present the first corresponding records of *Scrippsiella* collected in the Black Sea. Nearly all dinophyte taxa have been considered cosmopolitan with no species endemic in this inland sea (Gómez and Boicenco 2004), yet this statement lacks any indisputable data based on DNA sequences. The formerly elusive taxonomic identity of 'true' *S. acuminata* has recently been clarified, and two species names have been epitypified (Zinßmeister et al. 2011; Kretschmann et al. 2015a), which gives our study an even stronger credibility. Precise determination is of great importance for the *Scrippsiella* lineage, as some of its morphologically indistinguishable members are not only one of the most abundant calcareous dinophytes worldwide but are also considered responsible [under the name of *Scrippsiella* cf. *erinaceus* (Kamptner) Kretschmann, Zinssmeister & Gottschling] for harmful algal blooms (Tang and Gobler 2012). Our conclusions may encourage the use of similar approaches of combining morphology with DNA barcoding for other unicellular species, which is of great importance for any rigorous statements about conservation strategies, the impacts of invasive species and the effects of climate change on biodiversity.

Materials and methods

Water tow and sediment samples (Söhner et al. 2012) were collected in the Black Sea off Romania (Tab. S1), a semi-enclosed basin, whose only connection to the Earth's oceans is through narrow straits (<110 m depth; Dardanelles Straits, Sea of Marmara; Ozsoy et al. 2001). Rivers supply the Black Sea with phosphorus and nitrogen, causing it to be a very fertile land-locked inland sea (Bakan and Büyükgüngör 2000), and keep the salinity in the surface layer relatively low (Murray et al. 2006). Average surface salinity of the Black Sea varies between 17‰ and 19‰ (Milchakova and Phillips 2003), making it approximately only half as salty as the Mediterranean Sea, where salinity increases approximately from 36‰ on the east side to 38‰ on the west side (Said et al. 2011).

Predominantly monoclonal strains were established from the samples, as previously described in detail (Kretschmann et al. 2014). Ten strains were cultivated in a climate chamber WKS 3200 (Liebherr, Bulle, Switzerland) at 18 °C, 80 µmol photons m^{-2} s^{-1} and a 12:12h light:dark photoperiod. The strains are currently held in the culture collections at the Institute of Systematic Botany and Mycology (University of Munich) or at the Institute of Historical Geology/Palaeontology (University of Bremen, Germany) and are available upon request. Cells were observed, documented and measured with a CKX41 inverse microscope (Olympus, Hamburg, Germany) equipped with a DP73 digital camera (Olympus). The preparative techniques for light (LM) and scanning electron microscopy (SEM) followed predominantly standard protocols (Janofske 2000), previously described in Gottschling et al. (2012). The Kofoidean system (Fensome et al. 1993; Taylor 1980) was used to designate the plate formulae.

Genomic DNA was extracted from fresh strain material, using the Nucleo Spin Plant II Kit (Machery-Nagel; Düren, Germany). Various loci of the ribosomal RNA (rRNA) including the ITSs were amplified, using the primer pairs specified previously and following standard protocols (Gottschling et al. 2012; Gu et al. 2013). Gel electrophoreses yielded single bands that were purified and sequenced. Similarities between sequences were inferred using NCBI Blast Search (Altschul et al. 1997) and with a sequence similarity matrix provided by BioEdit Sequence Alignment Editor (Hall 2011).

Over the past two decades, approximately 120 coastal and 100 pelagial marine localities around the globe have been sampled for the presence of dinophytes (not all of them included individuals of the *Scrippsiella* lineage). When preparing the distribution maps, we used only algae showing distinct ITS ribotypes (Gottschling and Kirsch 2009; Söhner et al. 2012) of *S. acuminata*, *S.* aff. *acuminata* and *S.* cf. *erinaceus*, respectively (for species determination, see the Results

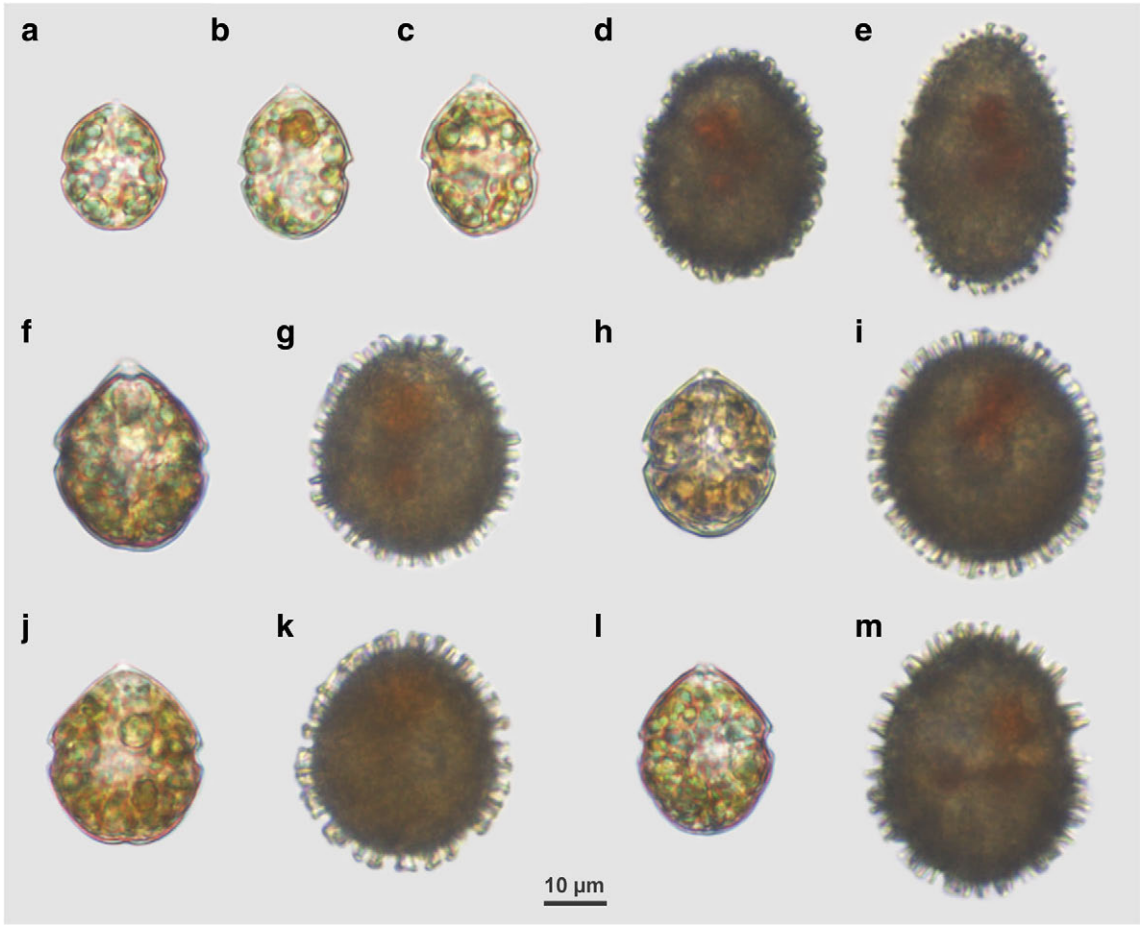

Fig. 1 Motile and coccoid stages of *Scrippsiella lachrymosa*, *S.* aff. *acuminata* and *S.* cf. *erinaceus* (LM; all images at the same scale). **a** Motile cell of *S. lachrymosa* (GeoM*575). **b**–**e** Motile and coccoid cells of *S.* aff. *acuminata*. **b**, **c** Motile cells of GeoM*549 and GeoM*553. **d**, **e** Coccoid cells of GeoM*553 showing numerous spines developed at the cell surface. **f**–**m** Motile and coccoid cells with a characteristic spiny surface of *S.* cf. *erinaceus*. **f**, **g** Motile and coccoid cells of GeoM*550. **h**, **i** Motile and coccoid cells of GeoM*551. **j**, **k** Motile and coccoid cells of GeoM*552. **l**, **m** Motile and coccoid cells of GeoM*554

section). Our own occurrence data were based on strains that were subsequently established in the laboratory from geo-referenced material collected on various field trips (Vink 2004; Gottschling et al. 2005; Gottschling and Kirsch 2009; Söhner et al. 2012). Sequencing methodology (to obtain not only ITS but also SSU and LSU for future phylogenetic studies) was the same as described above. Additionally, ITS sequence data downloaded from GenBank were taken into account (corresponding to a total of 39 localities; Table S1). Data from the Tara Oceans project (Sunagawa et al. 2015; Vargas et al. 2015) or amplicon-sequencing surveys (Cuvelier et al. 2010; Kohli et al. 2014; Massana et al. 2015; Elferink et al. 2017) could not be acknowledged, as the SSU and/or LSU loci are not indicative for species. All the coordinates for particular ribotypes were gathered in a spreadsheet, converted to *.xml format in Earth Point (under Earth Point academic free licence) and exported as a shapefile (SHP) in Zonum converter (Zonum Solution, Free Software Tools). Final maps were drawn using DIVA-GIS 7.5.0 (http://www.diva-gis.org).

Results and discussion

Cryptic species of *Scrippsiella* in the Black Sea

Any irrefutable statements on invasion potential, conservation status of particular species or distribution changes due to altered climate rely on precise occurrence data. Only for a few notable dinophyte exceptions such as *Alexandrium minutum* Halim (McCauley et al. 2009) and *A. ostenfeldii* (Kremp et al. 2014) have these data been compiled. Meta-barcoding analyses uncovering global occurrences have been carried out (Le Bescot et al. 2016), which is a significant step towards more extensive knowledge on protist distributions. However, these methods are insufficient for detailed species-based distribution assessments, as their taxonomic resolution is not high enough. Furthermore, contemporary DNA-based occurrence surveys show limitations in this respect, as they use only SSU or LSU (Cuvelier et al. 2010; Kohli et al. 2014; Massana et al. 2015; Elferink et al. 2017) but not ITS sequence information,

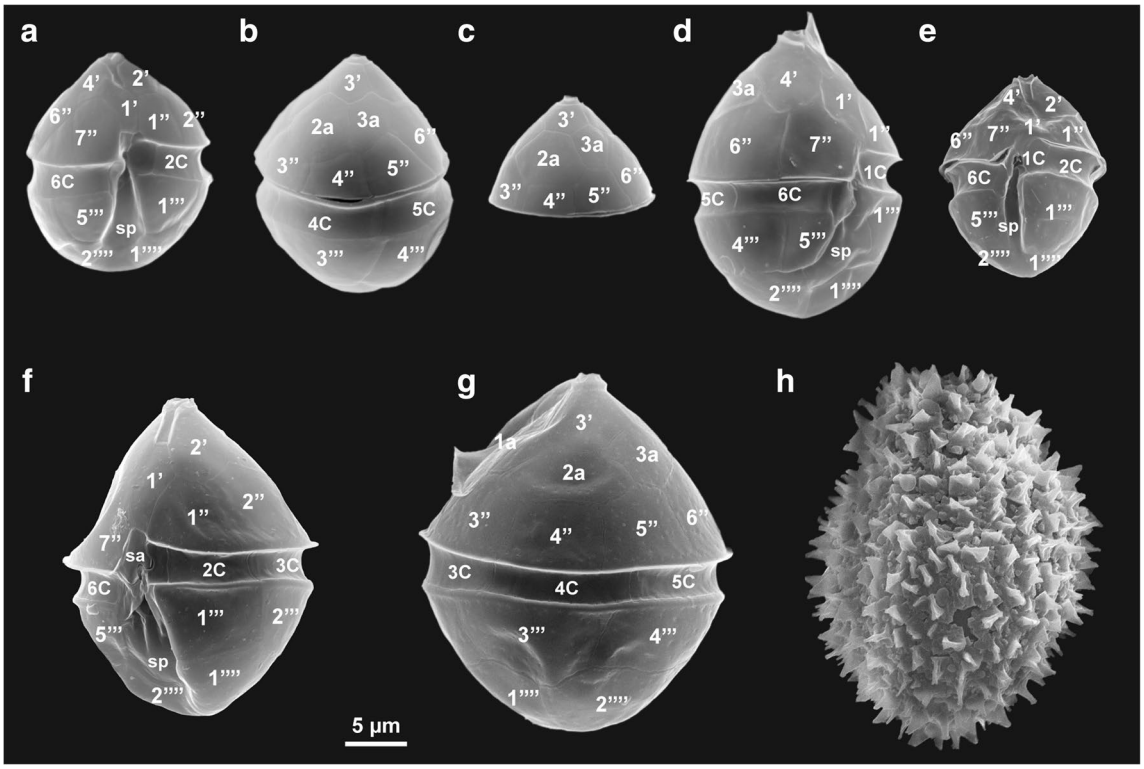

Fig. 2 Motile and coccoid stages of *Scrippsiella lachrymosa* and *S.* aff. *acuminata* (SEM; all images at the same scale; same scale as in Fig. 3). **a**–**c** Motile cells of *S. lachrymosa* (GeoM*575). **a** Ventral view. **b** Dorsal view. **c** Epitheca on dorsal view. **d**–**h** Motile and coccoid cells of the *S.* aff. *acuminata.* **d**, **e** Motile cells of GeoM*549 showing a variably in size. **d** Lateral-ventral view. **e** Ventral view. **f**, **g** Motile cells of GeoM*553. **f** Ventral-lateral view. **g** Dorsal view. **h** Coccoid cell of GeoM*553 showing a spiny surface. *n*' apical plate. *n*" precingular plate. *n‴* postcingular plate. *n⁗* antapical plate. *na* anterior intercalary plate. *nC* cingular plate. *sa* anterior sulcal plate. *sp* posterior sulcal plate

which is essential for species delimitation due to its high variability, not only in *Scrippsiella* but also in other dinophyte lineages (Litaker et al. 2007; Gottschling 2008; Gottschling and Kirsch 2009). In general, the data basis with a high taxonomic resolution is anything but extensive. Due to the scarce knowledge of distribution on lower taxonomic levels, *Scrippsiella* is a good study group in this respect, as we extensively compiled occurrence data of the constituent species during the past two decades. In total, we established ten new strains (eight of which were monoclonal) from different localities in the Black Sea off Romania (Table S1).

The strains showed two distinct morphologies of species belonging to *Scrippsiella*. One morphotype corresponded to *S. acuminata* exhibiting characteristic spiny coccoid cells (Figs 1d, e, g, i, k, m, 2h, 3d, h, i). The other morphotype was determined as *Scrippsiella lachrymosa* Lewis ex Head, which can be distinguished from *S. acuminata* based on smaller size of the motile thecate cells (Figs 1a, 2a–c) and on teardrop-shaped coccoid cells with a smooth surface (Lewis 1991; though not observed in strains GeoM*575 and GeoM*576). Under cultivation conditions, motile thecate cells of both species were predominant, whereas immotile coccoid cells were rare, if formed at all. The epitheca of motile cells were conical and had a slightly acuminate, hyaline apex, while the hypotheca's outline ranged from polygonal through hemispheric (Figs 1a–c, f, h, j, l, 2a–g, 3a–c, e–g, j–m). However, the identical basic thecal plate arrangement of *S. acuminata* and *S. lachrymosa* (i.e., Po, cp, X, 4′, 3a, 7″, 6c, 5s, 5‴, 2⁗; Figs 2a–g, 3a–c, e–g, j–m), and a high variability in size of the motile cells of *S. acuminata*, made the motile cells of both morphotypes indistinguishable.

DNA-barcoding (36 new sequences were deposited into GenBank under the entry numbers KY996760–KY996801) confirmed the systematic placement of all ten strains in the *Scrippsiella* lineage, and three distinct ITS ribotypes could be determined. One ribotype was at least 94% similar (but not identical) to other sequences determined as *S. lachrymosa* as inferred from a NCBI Blast Search, while the other two corresponded to species exhibiting a morphology consistent with *S. acuminata*. However, both ribotypes differed from each other (84% similarity between each other) as well as from the ITS sequence of the epitypified 'true' *S. acuminata* (88% and 90% similarity, respectively). Unambiguous scientific names cannot currently be assigned to the two ribotypes and therefore, we refer to them as *S.* cf. *erinaceus* and *S.* aff. *acuminata*, respectively, in the following.

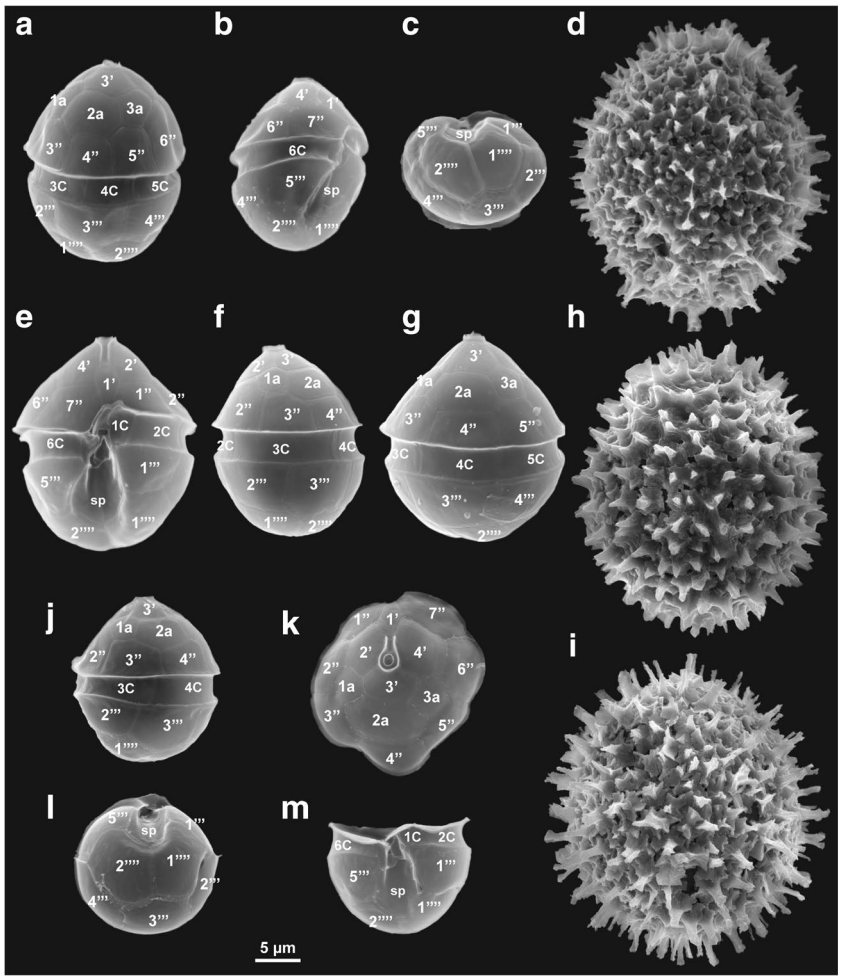

Fig. 3 Motile and coccoid stages of *Scrippsiella* cf. *erinaceus* (SEM; all images at the same scale; same scale as in Fig. 2). **a**–**d** Motile and coccoid cells of GeoM*551. **a** Motile cell in dorsal view. **b** Motile cell in lateral view. **c** Motile cell in antapical view. **d** Spiny coccoid cell. **e**–**i** Motile and coccoid cells of GeoM*552. **e** Motile cell in dorsal view. **f** Motile cell in dorsal-lateral view. **g** Motile cell in dorsal view. **h**, **i** Coccoid cells showing a spiny surface. **j**–**m** Motile cells and thecae of GeoM*554. **j** Motile cell in lateral-dorsal view. **k** Apical view of epitheca. **l** Motile cell in antapical view. **m** Ventral view of hypotheca with cingulum and sulcal region. *n′* apical plate. *n″* precingular plate. *n‴* postcingular plate. *n⁗* antapical plate. *na* anterior intercalary plate. *nC* cingular plate. *sp* posterior sulcal plate

Global distribution of *Scrippsiella* cryptic species

We placed occurrence data of three ribotypes corresponding to *S.* aff. *acuminata* and *S.* cf. *erinaceus* as well as to 'true' (i.e., epitypified: Kretschmann et al. 2015a) *S. acuminata* on a global map (Fig. 4). They all showed a wide, partly overlapping distribution and shared the presence primarily at coastal localities (the only exception was a single record of *S.* aff. *acuminata* from the middle of the Northern Atlantic). With respect to our study area, we cannot confirm the presence of 'true' *S. acuminata* in the Black Sea as stated by Gómez and Boicenco (2004). Nevertheless, we found two distinct species with a highly similar morphology collected at the same site (i.e., Costineşti) on the same day (i.e., July 4, 2014). Furthermore, we found *S. lachrymosa*, which has not been previously recorded at the Black Sea (Gómez and Boicenco 2004).

During the past two decades, we have been collecting samples with no regard to particular habitats, therefore the observed variation in abundance of species cannot be explained as an artefact. *Scrippsiella* aff. *acuminata* occurred at 11 localities, while *S.* cf. *erinaceus* was found at 6 localities around the world. The ITS ribotype associated with *S. lachrymosa* is currently only known from the Black Sea off Romania, which is in agreement with other only sporadically documented dinophytes such as *Spiniferodinium limneticum* (Wołosz.) Kretschmann & Gottschling from Poland (Kretschmann et al. 2015b). The most frequently encountered of all *Scrippsiella* species is 'true' *S. acuminata*, which is known from 22 localities all over the world—and therefore almost 'everywhere' (Fenchel 2005; Finlay 2002)—but not from the Black Sea. *Scrippsiella acuminata* is also the species with occurrences at the highest latitudes (i.e., North Sea off Norway; Table S1; the same or at least a very similar species is even documented from Baffin Bay; Elferink et al. 2017), while all other investigated species were restricted to either temperate and/or subtropical regions.

An extensive amount of distribution data are available based on morphology also from online repositories. However, without a more reliable DNA-based support, this data cannot be indisputably used for further research, at least not in the microbial distribution assessments. Furthermore, such large online databases include data of

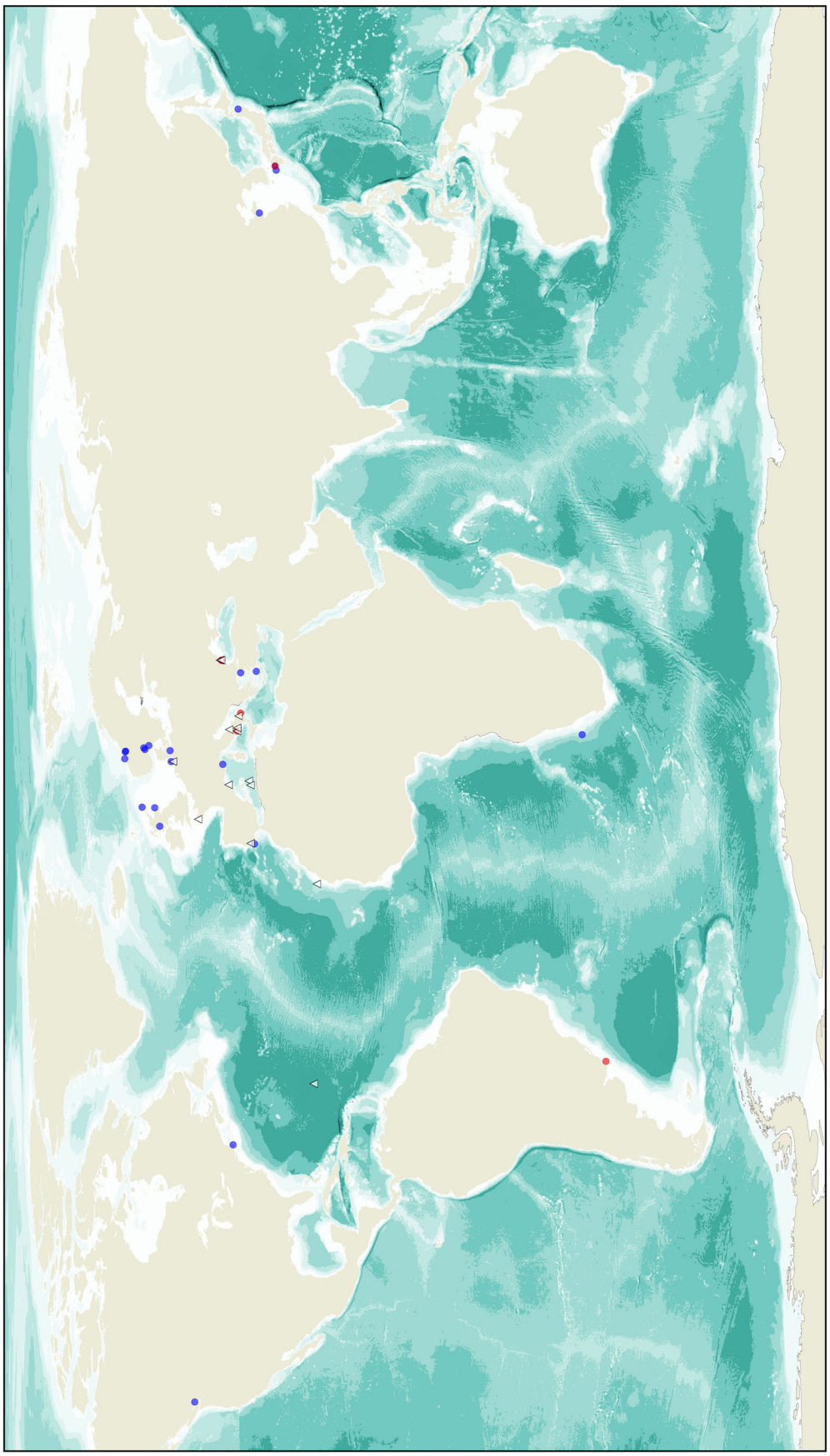

Fig. 4 Cryptic species of *Scrippsiella* with a wide, partly overlapping distribution, found primarily at coastal localities (distribution map). *Blue circles S. acuminata* (incl. epitype locality off Kiel), *red circles S.* cf. *erinaceus*, *white triangles S.* aff. *acuminata*. Note that *S. lachrymosa*, also found in the Black Sea at Costineşti, is not mapped

low taxonomic resolution and/or of uncertain expertise (Dolan 2011). GenBank (http://www.ncbi.nlm.nih.gov/genbank/) provides free access to extensive DNA sequence information about various taxonomic groups, but sequence submission policy is lax for crucial input fields. Not only is species (and occasionally strain) nomenclature often inconsistent, but it is also not mandatory to supply the DNA sequence with voucher information, which reduces the reliability of the data. Particularly in the microbial world, DNA sequence information is necessary for the precise application of scientific names, and it is not available for the majority of the historical names. This greatly challenges the taxonomy (Boenigk et al. 2012), but at least for cultivatable species, epitypification appears to be a good approach to resolve this problem (Kosmala et al. 2009; Kretschmann et al. 2015a, b). In the future, we should aim towards compiling a database with curated entries found for a certain strain or species—the Global catalogue of microorganisms (Wu et al. 2013) appears to be a good starting point in this respect. Otherwise, we will be left with parallel sets of incomplete data, of limited biological value.

A next logical step would be niche modelling, which is already a common practice in terrestrial organisms such as plants (Heibl and Renner 2012; Smith and Donoghue 2010) and even in harmful bacteria (Mullins et al. 2013). However, there are only a handful of studies on niche modelling of unicellular organisms, and even those are based on refutable morphological data and not on a global scale (Aguilar et al. 2014; Aguilar and Lado 2012; Langer et al. 2013). Despite the fact that abiotic data on marine environments are available online (http://www.noaa.gov/), though with a rather low resolution, no niche global-scale modelling has been carried out so far for genetically-determined protist species. Predicting the distribution based on 'ecological licence' of the species and 'ecological potence' of the environment (Osche 1966) is a key for a reliable monitoring of species, with a high invasive potential. In 2005, there were already five dinophyte species considered invasive in the Black Sea, probably brought there either via ship ballast waters or river run-offs (Terenko 2005). Additionally, the increased size of cargo vessels, together with increased eutrophication of many coastal waters, have extended the possibilities of successful species transport across long distances (Hallegraeff and Bolch 1992). Monitoring of invasive species is thus nowadays getting a crucial role in maintaining the world's biodiversity. Due to global warming, massive changes in biogeographical ranges of protists have already been observed (Gobler et al. 2017; Pettay et al. 2015), and studying these provides crucial data on dealing with events representing a threat to biodiversity in general and global human health.

Conclusion

Despite the fact that we still lack extensive DNA-based (and therefore reliable) data with additional occurrence records, our study shows that such an approach is possible, and that it helps to understand the biogeography of unicellular dinophytes in more detail. Some species such as *S. acuminata* appear to be abundant, widely distributed and almost 'everywhere' at any time; others such as *S. erinaceus* are rarer and more restricted, while a considerable fraction is known from a single locality as DNA sequence records. Whether such observations correspond to true endemism, or rather reflect our incomplete knowledge, remains a yet unanswered question. Furthermore, the biological background (e.g., seasonality and dormancy stages) and the specific ecological requirements (e.g., temperature, salinity, nutrient availability, water-depth preference) shaping protist distribution remain to be explored. These will then represent the basis for further research on dynamics of individual colonisation/invasion processes and species establishment. This collective approach is of great importance particularly because of the fact that ongoing climate change and maritime transport levels (e.g., ballast water) heavily influence the distribution of all living beings on the planet. Much work still lies ahead of us, but it is necessary to obtain an overview on distribution of such organisms, which may be microscopic, but can cause macroscopic problems on a global scale.

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Compliance with ethical standards

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

Ethical approval This article does not contain any studies with animals performed by any of the authors.

Sampling and field studies The study was performed in compliance with the Convention on Biological Diversity (CBD).

References

Aguilar M, Lado C (2012) Ecological niche models reveal the importance of climate variability for the biogeography of protosteloid amoebae. ISME J 6:1506–1514

Aguilar M, Fiore-Donno AM, Lado C, Cavalier-Smith T (2014) Using environmental niche models to test the 'everything is everywhere' hypothesis for *Badhamia*. ISME J 8:737–745

Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 25: 3389–3402

Bakan G, Büyükgüngör H (2000) The Black Sea. Mar Pollut Bull 41:24–43

Boenigk J, Ereshefsky M, Hoef-Emden K, Mallet J, Bass D (2012) Concepts in protistology: species definitions and boundaries. Eur J Protistol 48:96–102

Caron DA (2009) Past president's address: Protistan biogeography: why all the fuss? J Eukaryot Microbiol 56:105–112

Cuvelier ML, Allen AE, Monier A, McCrow JP, Messie M, Tringe SG, Woyke T, Welsh RM, Ishoey T, Lee JH, Binder BJ, DuPont CL, Latasa M, Guigand C, Buck KR, Hilton J, Thiagarajan M, Caler E, Read B, Lasken RS, Chavez FP, Worden AZ (2010) Targeted metagenomics and ecology of globally important uncultured eukaryotic phytoplankton. Proc Natl Acad Sci U S A 107:14679–14684

Dolan JR (2011) The legacy of the last cruise of the Carnegie: a lesson in the value of dusty old taxonomic monographs. J Plankton Res 33:1317–1324

Elbrächter M, Gottschling M, Hildebrand-Habel T, Keupp H, Kohring R et al. (2008) Establishing an agenda for calcareous dinoflagellates research (Thoracosphaeraceae, Dinophyceae) including a nomenclatural synopsis of generic names. Taxon 57:1289–1303

Elferink S, Gottschling M, John U, Töbe K, Voß D, Neuhaus S, Zielinski O, Lundholm N, Koch B, Krock B, Cembella A, Wohlrab S (2017) Molecular diversity patterns among various phytoplankton size-fractions in West Greenland in late summer. Deep-Sea Res I 121:54–69

Evans KM, Chepurnov VA, Sluiman HJ, Thomas SJ, Spears BM, Mann DG (2009) Highly differentiated populations of the freshwater diatom *Sellaphora capitata* suggest limited dispersal and opportunities for allopatric speciation. Protist 160:386–396

Fenchel T (2005) Cosmopolitan microbes and their 'cryptic' species. Aquat Microb Ecol 41:49–54

Fensome RA, Taylor FJR, Norris G, Sarjeant WAS, Wharton DI, Williams GL (1993) A classification of living and fossil dinoflagellates. Micropaleontol Spec Publ:1–245

Finlay BJ (2002) Global dispersal of free-living microbial eukaryote species. Environ Microbiol 296:1061–1063

Foissner W (2007) Dispersal and biogeography of protists: recent advances. Jpn J Protozool 40:1–16

Fritz SA, Schnitzler J, Eronen JT, Hof C, Böhning-Gaese K, Graham CH (2013) Diversity in time and space: wanted dead and alive. Trends Ecol Evol 28:509–516

Gillespie RG, Baldwin BG, Waters JM, Fraser CI, Nikula R, Roderick GK (2012) Long-distance dispersal: a framework for hypothesis testing. Trends Ecol Evol 27:47–56

Gobler CJ, Doherty OM, Hattenrath-Lehmann TK, Griffith AW, Kang Y, Litaker RW (2017) Ocean warming since 1982 has expanded the niche of toxic algal blooms in the North Atlantic and North Pacific oceans. Proc Natl Acad Sci U S A 144:4975–4980

Gómez F, Boicenco L (2004) An annotated checklist of dinoflagellates in the Black Sea. Hydrobiologia 517:43–59

Gottschling M (2008) Aktuelle Herausforderungen für Diversitätserfassung und Systematik: Blütenpflanzen, Kalkige Dinoflagellaten und Papillomviren im Vergleich. FU Berlin, Berlin (Habilitation thesis; www.sysbot.biologie.uni-muenchen.de/en/people/gottschling/gottschling_habil.pdf), 33p

Gottschling M, Kirsch M (2009) Annotated list of Scandinavian calcareous dinoflagellates collected in fall 2003. Berl Paläobiol Abh 10:193–198

Gottschling M, Knop R, Plötner J, Kirsch M, Willems H, Keupp H (2005) A molecular phylogeny of *Scrippsiella sensu lato* (Calciodinellaceae, Dinophyta) with interpretations on morphology and distribution. Eur J Phycol 40:207–220

Gottschling M, Söhner S, Zinßmeister C, John U, Plötner J, Schweikert M, Aligizaki K, Elbrächter M (2012) Delimitation of the Thoracosphaeraceae (Dinophyceae), including the calcareous dinoflagellates, based on large amounts of ribosomal RNA sequence data. Protist 163:15–24

Gu H, Kirsch M, Zinßmeister C, Söhner S, Meier KJS, Liu T, Gottschling M (2013) Waking the dead: morphological and molecular characterization of extant †*Posoniella tricarinelloides* (Thoracosphaeraceae, Dinophyceae). Protist 164:583–597

Hall TA (2011) BioEdit: an important software for molecular biology. GERF Bull Biosci 2:60–61

Hallegraeff GM, Bolch CJ (1992) Transport of diatom and dinoflagellate resting spores in ships' ballast water: implications for plankton biogeography and aquaculture. J Plankton Res 14:1067–1084

Heibl C, Renner SS (2012) Distribution models and a dated phylogeny for Chilean *Oxalis* species reveal occupation of new habitats by different lineages, not rapid adaptive radiation. Syst Biol 61:823–834

Janofske D (2000) *Scrippsiella trochoidea* and *Scrippsiella regalis*, nov. comb. (Peridiniales, Dinophyceae): a comparison. J Phycol 36:178–189

John U, Litaker RW, Montresor M, Murray S, Brosnahan ML, Anderson DM (2014) Formal revision of the *Alexandrium tamarense* species complex (Dinophyceae) taxonomy: the introduction of five species with emphasis on molecular-based (rDNA) classification. Protist 165:779–804

Kohli GS, Neilan BA, Brown MV, Hoppenrath M, Murray SA (2014) Cob gene pyrosequencing enables characterization of benthic dinoflagellate diversity and biogeography. Environ Microbiol 16:467–485

Kosmala S, Karnkowska-Ishikawa A, Milanowski R, Kwiatowski J, Zakrys B (2009) Phylogeny and systematics of *Euglena* (Euglenaceae) species with axial, stellate chloroplasts based on morphological and molecular data-new taxa, emended diagnoses, and epitypifications. J Phycol 45:464–481

Kremp A, Tahvanainen P, Litaker W, Krock B, Suikkanen S, Leaw CP, Tomas C, De Clerck O (2014) Phylogenetic relationships, morphological variation, and toxin patterns in the *Alexandrium ostenfeldii* (Dinophyceae) complex: implications for species boundaries and identities. J Phycol 50:81–100

Kretschmann J, Zinßmeister C, Gottschling M (2014) Taxonomic clarification of the dinophyte *Rhabdosphaera erinaceus* Kamptner, ≡ *Scrippsiella erinaceus* comb. nov. (Thoracosphaeraceae, Peridiniales). Syst Biodivers 12:393–404

Kretschmann J, Elbrächter M, Zinßmeister C, Söhner S, Kirsch M, Kusber W-H, Gottschling M (2015a) Taxonomic clarification of the dinophyte *Peridinium acuminatum* Ehrenb., *Scrippsiella acuminata*, comb. nov. (Thoracosphaeraceae, Peridiniales). Phytotaxa 220:239–256

Kretschmann J, Filipowicz NH, Owsianny PM, Zinßmeister C, Gottschling M (2015b) Taxonomic clarification of the unusual dinophyte *Gymnodinium limneticum* Wolosz. (Gymnodiniaceae) from the Tatra Mountains. Protist 166:621–637

Krupnick GA, Kress WJ (2005) Plant conservation: a natural history approach. University of Chicago Press, Chicago

LaJeunesse TC, Thornhill DJ (2011) Improved resolution of reef-coral endosymbiont (*Symbiodinium*) species diversity, ecology, and evolution through psbA non-coding region genotyping. PLoS ONE 6:e29013

Langer MR, Weinmann AE, Lotters S, Bernhard JM, Rodder D (2013) Climate-driven range extension of *Amphistegina* (Protista, Foraminiferida): models of current and predicted future ranges. PLoS ONE 8: e54443

Le Bescot N, Mahé F, Audic S, Dimier C, Garet MJ, Poulain J, Wincker P, de Vargas C, Siano R (2016) Global patterns of pelagic dinoflagellate diversity across protist size classes unveiled by metabarcoding. Environ Microbiol 18:609–626

Lewis J (1991) Cyst-theca relationships in *Scrippsiella* (Dinophyceae) and related orthoperidinoid genera. Bot Mar 34:91–106

Litaker RW, Vandersea MW, Kibler SR, Reece KS, Stokes NA, Lutzoni FM, Yonish BA, West MA, Black MND, Tester PA (2007) Recognizing dinoflagellate species using ITS rDNA sequences. J Phycol 43:344–355

Martiny JB, Bohannan BJ, Brown JH, Colwell RK, Fuhrman JA et al. (2006) Microbial biogeography: putting microorganisms on the map. Nat Rev Microbiol 4:102–112

Massana R, Gobet A, Audic S, Bass D, Bittner L et al. (2015) Marine protist diversity in European coastal waters and sediments as revealed by high-throughput sequencing. Environ Microbiol 17: 4035–4049

Matsuoka K, Cho HJ (2000) Morphological variation in cysts of the gymnodinialean dinoflagellate *Polykrikos*. Micropaleontology 46: 360–364

Mayer GC, Coyne JA, Losos JB, Foufopoulos J, Shubin N, Futuyma DJ, Campbell BC, Edwards SV (2013) Museums' role: increasing knowledge. Science 339:1148–1149

McCauley LAR, Erdner DL, Nagai S, Richlen ML, Anderson DM (2009) Biogeographic analysis of the globally distributed algal bloom species *Alexandrium minutum* (Dinophyceae) based on rRNA gene sequences and microsatellite markers. J Phycol 45:454–463

Milchakova NA, Phillips RC (2003) Black Sea seagrasses. Mar Pollut Bull 46:695–699

Montresor M, Sgrosso S, Procaccini G, Kooistra WHCF (2003) Intraspecific diversity in *Scrippsiella trochoidea* (Dinopbyceae): evidence for cryptic species. Phycologia 42:56–70

Mullins J, Garofolo G, Van Ert M, Fasanella A, Lukhnova L, Hugh-Jones M, Blackburn J (2013) Ecological niche modeling of *Bacillus anthracis* on three continents: evidence for genetic-ecological divergence? PloS ONE 8: e72451

Murray JW, Stewart K, Kassakian S, Krynytzky M, DiJulio D (2006) Oxic, suboxic and anoxic conditions in the Black Sea. In: Gilbert A, Yanko-Hombach V, Panin N (eds) Climate change and coastline migration as factors in human adaptation to the circum-pontic region: from past to forecast. Kluwer, New York, pp 437–452

Osche G (1966) Die Welt der Parasiten. Zur Naturgeschichte des Schmarotzertums. Springer, Berlin

Ozsoy E, Di Iorio D, Gregg MC, Backhaus JO (2001) Mixing in the Bosphorus Strait and the Black Sea continental shelf: observations and a model of the dense water outflow. J Mar Syst 31:99–135

Pettay DT, Wham DC, Smith RT, Iglesias-Prieto R, LaJeunesse TC (2015) Microbial invasion of the Caribbean by an indo-Pacific coral zooxanthella. Proc Natl Acad Sci U S A 112:7513–7518

Renner SS (2004) Plant dispersal across the tropical Atlantic by wind and sea currents. Int J Plant Sci Suppl 165:23–33

Rintala J-M, Hällfors H, Hällfors S, Hällfors G, Majaneva M, Blomster J (2010) *Heterocapsa arctica* subsp. *frigida* subsp. nov. (Peridiniales, Dinophyceae) – description of a new dinoflagellate and its occurrence in the Baltic Sea. J Phycol 46:751–762

Rocha LA, Aleixo A, Allen G, Almeda F, Baldwin CC et al. (2014) Specimen collection: an essential tool. Science 344:814–815

Said MA, Gerges MA, Maiyza IA, Hussein MA, Radwan AA (2011) Changes in Atlantic water characteristics in the south-eastern Mediterranean Sea as a result of natural and anthropogenic activities. Oceanologia 53:81–95

Smith SA, Donoghue MJ (2010) Combining historical biogeography with niche modeling in the *Caprifolium* clade of *Lonicera* (Caprifoliaceae, Dipsacales). Syst Biol 59:322–341

Söhner S, Zinßmeister C, Kirsch M, Gottschling M (2012) Who am I – and if so, how many? Species diversity of calcareous dinophytes (Thoracosphaeraceae, Peridiniales) in the Mediterranean Sea. Org Divers Evol 12:339–348

Soininen J (2012) Macroecology of unicellular organisms – patterns and processes. Environ Microbiol Rep 4:10–22

Sunagawa S, Coelho LP, Chaffron S, Kultima JR, Labadie K et al. (2015) Structure and function of the global ocean microbiome. Science 348: 1261359

Tang YZ, Gobler CJ (2012) Lethal effects of Northwest Atlantic Ocean isolates of the dinoflagellate, *Scrippsiella trochoidea*, on eastern oyster (*Crassostrea virginica*) and northern quahog (*Mercenaria mercenaria*) larvae. Mar Biol 159:199–210

Tang YZ, Egerton TA, Kong L, Marshall HG (2008) Morphological variation and phylogenetic analysis of the dinoflagellate *Gymnodinium aureolum* from a tributary of Chesapeake Bay. J Eukaryot Microbiol 55:91–99

Taylor FJR (1980) On dinoflagellate evolution. BioSystems 13:65–108

Terenko L (2005) New dinoflagellate (Dinoflagellata) species from the Odessa Bay of the Black Sea. Oceanol Hydrobiol Stud 34 (Suppl 3): 205–216

Tsarenko PM, Vasser SP, Nevo E (2006) Algae of Ukraine: diversity, nomenclature, taxonomy, ecology and geography. Vol. 1. Cyanoprocaryota, Euglenophyta, Chrysophyta, Xanthophyta, Raphidophyta, Phaeophyta, Dinophyta, Cryptophyta, Glaucocystophyta, and Rhodophyta. Gantner, Ruggel

Vargas C, Audic S, Henry N, Decelle J, Mahé F et al. (2015) Eukaryotic plankton diversity in the sunlit ocean. Science 348:1261605

Vellend M (2010) Conceptual synthesis in community ecology. Q Rev Biol 85:183–206

Vink A (2004) Calcareous dinoflagellate cysts in south and equatorial Atlantic surface sediments: diversity, distribution, ecology and potential for palaeoenvironmental reconstruction. Mar Micropaleontol 50:43–88

Weiner A, Aurahs R, Kurasawa A, Kitazato H, Kucera M (2012) Vertical niche partitioning between cryptic sibling species of a cosmopolitan marine planktonic protists. Mol Ecol 21:4063–4073

Wu L, Sun Q, Sugawara H, Yang S, Zhou Y, McCluskey K, Vasilenko A, Suzuki K, Ohkuma M, Lee Y, Robert V, Ingsriswang S, Guissart F, Philippe D, Ma J (2013) Global catalogue of microorganisms (GCM): a comprehensive database and information retrieval, analysis, and visualization system for microbial resources. BMC Genomics 14:933

Zinßmeister C, Söhner S, Facher E, Kirsch M, Meier KJS, Gottschling M (2011) Catch me if you can: the taxonomic identity of *Scrippsiella trochoidea* (F.Stein) A.R.Loebl. (Thoracosphaeraceae, Dinophyceae). Syst Biodivers 9:145–157