Taxonomic revision, phylogeny, and cyst wall composition of the dinoflagellate cyst genus *Votadinium* Reid (Dinophyceae, Peridiniales, Protoperidiniaceae)

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Abstract :

The taxonomy of the dinoflagellate cyst-based genus *Votadinium* has been in need of revision. This was accomplished here by integrating morphology, LSU rDNA sequences, and cyst wall composition analyses through micro-Fourier transform infrared spectroscopy. Three new species, *Votadinium pontifossatum*, *V. rhomboideum* and *V. reidii*, were described as cyst stages of *Protoperidinium paraoblongum*, *P. quadrioblongum*, and *P. steidingeriae*, respectively. A previously undescribed type of ornamentation was reported for *V. pontifossatum*. A fourth new species was assigned to *V. concavum*. Furthermore, *Lejeunecysta psilodora* Benedek (= *Lejeunecysta psuchra* Matsuoka), was re-described as *V. psilodora* and the holotype re-illustrated, as was done for the holotype of *V. spinosum* Reid. New illustrations were provided for *V. elongatum* and *V. nanhaiense*. LSU rDNA sequences of *V. concavum* revealed its placement in the *Oceanica* section of the genus *Protoperidinium*, supporting earlier evidence that the *Oceanica* section and the cyst based genus *Votadinium* form a monophyletic clade. Thus, *Votadinium* could be considered the cyst equivalent of the motile-defined *Oceanica* section. The cyst walls of *Votadinium spinosum*, *V. calvum* and *V. pontifossatum* are built from carbohydrate-based macromolecules with N-containing functional groups in agreement with the heterotrophic nature of their motile stage. Comparison with related genera *Trinovantedinium* and *Quinquecuspidis* reveals that the species and genera can be separated based on cyst wall composition, supporting the phylogeny based on molecular data and/or ecological differences. Furthermore, *Trinovantedinium applanatum* spectra unexpectedly show an additional absorption at 1510 cm⁻¹ indicating an aromatic group that is commonly reported for sporopollenin and not seen in dinoflagellate cysts so far. This study stabilizes the taxonomy

of the cyst genus *Votadinium*, clarifies its relationship to the *Oceanica* section of *Protoperidinium*, and further supports the application of whole cyst wall chemistry in helping address problems in dinoflagellate cyst taxonomy and ecology.

Keywords : taxonomy, dual nomenclature, dinocyst, ornamentation, micro-FTIR, cyst wall composition, sporopollenin

1. Introduction

The life cycle of ~200 living dinoflagellate species involves a cyst stage or hypnozygote (e.g., Head 1996). The existence of these two morphologically-distinct stages, motile and cyst, has led to the usage of a dual nomenclature (i.e., paleontological/biological; e.g., Ellegaard et al. 2018). The relationship between the stages is increasingly better understood through incubation experiments and molecular analyses (e.g., Sarai et al. 2013; Gu et al. 2015; Mertens et al. 2017a). However, this duality persists and is sanctioned by the international nomenclatural code (e.g., Head et al. 2016) because of the differing approaches of biologists, working mainly on the motile stages, and paleontologists that work primarily with the cyst stages. In particular, a cyst-based taxonomy is indispensable for paleontologists working with extinct taxa where no biological approach or molecular identification is possible.

The dinoflagellate cyst genus *Lejeunecysta*, originally *Lejeunia*, see Artzner and Dörhöfer (1978), was erected by Gerlach (1961) with the Oligocene age *Lejeunecysta hyalina* (Gerlach) Artzner et Dörhöfer as the type species. The description given by Gerlach encompasses cysts with a bilateral symmetry, a pentagonal ambitus with one apical and two antapical horns, no reflection of tabulation but with an indication of a spiral cingulum and a sulcus. The genus was emended by Kjellström (1972, p. 467) to include features of the archeopyle, and by Lentin and Williams (1976, p. 68–69) who detailed the wall structure. Stover and Evitt (1978, p.111) emended the genus to include only forms with autophragma (i.e. excluding cavate species). The emendation by Bujak et al. (1980) to stress the symmetrical position of the archeopyle relative to the middorsal line was rejected by Head (1993). The genus currently comprises 39 species (Williams et al. 2017).

Reid (1977) erected the genus *Votadinium* to include dinoflagellate cysts similar to *Lejeunecysta* but without an expression of the cingulum as an indentation. Two species were described from modern surface sediments around the British Isles: *Votadinium calvum* (type species) and *Votadinium spinosum*. Later, He and Sun (1991) included two more species: *Votadinium elongatum* and *Votadinium nanhaiense* recovered from Quaternary sediments around the Spratly Islands (South China Sea). Although the genus was considered illegitimate and superfluous by Lentin and Williams (1993), it was retained by later authors (Head et al. 2016; Williams et al. 2017).

Votadinium calvum has been frequently recorded in marine Quaternary sediments (e.g., Zonneveld et al. 2013), and Reid (1977) related the cyst form to the cysts of motile-based species *Protoperidinium oblongum* (Aurivillius) Parke et Dodge reported by Wall and Dale (1968). Wall and Dale (1968) illustrated two other distinct morphotypes that they also related to *P. oblongum*. Since then, *P. oblongum* has been shown to be a species complex, and has been split into several closely related species that previously were considered varieties of *P. oblongum*: *Protoperidinium quadrioblongum* (= *P. oblongum* var. *symmetricum* Dangeard), *Protoperidinium latidorsale* and *Protoperidinium paraoblongum* (= *Protoperidinium oblongum* var. *inaequale* Dangeard) and their respective cysts. However, new cyst-based species were not erected (Sarai et al. 2013).

In the subdivision of the large genus *Protoperidinium*, the *P. oblongum* complex belongs to the *Oceanica* section. This section was defined by Jörgensen (1912) based on thecate morphology with a typical *Protoperidinium* tabulation (Po, X, 4', 1–3a, 7'', 4c(3+t), 6s, 5''', 2'''''), an ortho 1' and a usually four-sided (quadra) second intercalary plate. Balech (1974) adopted the original definition of the *Oceanica* section, but the shape and number of anterior intercalary plates were not a stable taxonomic characteristic as they significantly

differ in number and shape (Abé 1981). Instead, details from the sulcal area and the nature of the antapical horns were considered more stable characteristics (Abé 1981).

Harland (1982), stressing the importance of cyst morphology, specifically the archeopyle, in dinoflagellate classification, called this ortho-quadra configuration “section *Votadinium*”. Although the hierarchies in classifications based on cysts and motile stages are different, Harland (1982) found that both systems were in agreement and that different cyst morphologies reflected the sections within the genus *Protoperidinium*.

Molecular phylogenies of *Protoperidinium* increasingly support the morphological classifications based on motile stages and their reconciliation with cyst-based taxonomy (Matsuoka and Kawami 2013; Sarai et al. 2013; Mertens et al. 2017c). In these phylogenies, species of the *Oceanica* section form a clade, sister to a “*Protoperidinium sensu stricto*” clade which includes the cyst species *Trinovantedinium applanatum* (cyst of *Protoperidinium shanghaiense*) and *Quinquecuspis concreta* (cyst of *Protoperidinium leonis*) (Mertens et al. 2017c) that possess morphological similarities to *Votadinium* cysts (Table 1). For the genus *Lejeunecysta*, which also possesses morphological similarities, the phylogenetic position is unknown so far, since molecular data are lacking, although *Protoperidinium latissimum*, the cyst of which could be assigned to *Lejeunecysta*, belongs to the *Conica* clade 1 (Gu et al. 2015).

Cyst walls of dinoflagellates are composed of dinosporin, which has been suggested, predominantly through micro-Fourier transformed infrared (FTIR) spectroscopic data, to be a suite of complex carbohydrate-based macromolecules (e.g., Versteegh et al. 2012; Bogus et al. 2014; Mertens et al. 2015). The overall geochemical composition of the cyst wall seems mainly determined by the phylogeny (e.g., Gurdebeke et al. 2018a) and by the nutritional strategy of the dinoflagellate species (e.g., Mertens et al. 2017b, c; Luo et al. 2018). However, the number of dinoflagellate cyst species from surface sediments for which spectra are

available remains very small (a total of 20 in published or accepted publications), and an expanded sampling of taxa is desirable to further explore its utility for chemotaxonomy and an increased understanding of dinoflagellate ecology.

Overall, the taxonomy of genus *Votadinium* is in need of revision and stabilization. This is done here by emending the genus and re-evaluating type material (holotypes and type region sediments) of attributable species. Furthermore, new LSU rDNA sequences of *Votadinium* species allow further evaluation of the molecular phylogeny and the relation between motile and cyst-based taxonomies. Micro-FTIR spectroscopy was used to characterize the cyst wall chemistry of selected *Votadinium* species and to explore the concept of chemotaxonomy for dinoflagellate cysts, by comparison with the cyst composition of related (*Protoperidinium* sensu stricto clade) genera *Trinovantodinium* and *Quinquecupis*.

2. Material and methods

2.1 Sampling and imaging

Cyst specimens were studied from surface, sediment trap and core samples that were mostly used in previous studies (Table 2). These include samples from the coastal waters of southern Vancouver Island (Krepakevich and Pospelova 2010), Saanich Inlet (Price and Pospelova 2011, Mertens et al. 2012, Price et al. 2016), western Vancouver Island (Gurdebeke et al. 2017, 2018b, in prep.), the Vilaine estuary (France; Mertens et al. 2009), the South China Sea (Li et al., in prep.) and Bohai Sea. The reader is referred to the respective publications for details on sampling and sediment processing. Specimens of the cysts of *Protoperidinium steidingeriae* from the culture study of Gribble et al. (2009) on Vineyard Sound (Massachusetts, USA) samples were re-examined (Table 2).

New transmitted light microscopy (LM) photomicrographs of a topotype of *Lejeunecysta psuchra* from Akkeshi Bay were made from slides studied by Matsuoka (1987);

the holotype could not be located. The holotype of *Lejeunecysta psilodora* Benedek 1972 is housed in the repository of the Institute of Geology and Mineralogy (Köln University, Germany), and was restudied. Reid's (1977) holotype of *Votadinium spinosum*, stored in the repository at the Micropalaeontology Laboratory (University of Sheffield, UK), was also observed.

The photomicrographs were produced with a Zeiss Axio Imager A1 equipped with a MRc 5 camera in the Department of Geology (Ghent University, Belgium) and Nikon Eclipse 80i microscopes and coupled Nikon DS Camera Heads (DS-Fi1)/DS with Camera Control Units DS-L2 in the Paleoenvironmental Laboratory (the School of Earth and Ocean Sciences) at the University of Victoria (Canada). The photomicrographs of *L. psuchra* were made with an Olympus BX51 light microscope with a digital camera (Qimaging, Burnaby, Canada) and the *V. spinosum* holotype was photographed at the University of Sheffield with an Infinity 1 camera mounted on a Meiji MT 5300H microscope.

For scanning electron microscope (SEM) images, single cysts were picked from the residue, mounted on polycarbonate membrane filters (Millipore, Billerica, MA, USA, GTTP Isopore, 0.22 µm pore size) and sputter coated with Au. The images were made with a Zeiss SIGMA300 Gemini field emission SEM at the Station de Biologie Marine (Ifremer, Concarneau, France).

2.2 Synonymy

The John Williams Index of Palaeopalynology (Riding et al. 2012), based at the British Museum (Natural History), was consulted as a base for establishing species synonymies. This data base includes entries for *Lejeunecysta psilodora*, *L. psuchra*, *Votadinium calvum* and *V. spinosum*. The illustrated specimens were re-interpreted along the lines of the taxonomy

proposed here. The references in the synonymy lists can be consulted in Williams et al. (2017) and are not reproduced here for the sake of brevity.

2.3 Cyst wall composition analysis

For micro-FTIR analysis, cysts of *Votadinium spinosum*, *V. calvum*, *V. pontifossatum*, *Quinquecuspis concreta*, *Trinovantedinium applanatum* and *T. pallidifulum* were extracted from surface sediment samples from Kyuquot Sound (see Gurdebeke et al. 2017 for details), Saanich Inlet (see Mertens et al. 2012 for details), Izmir Bay (see Mertens et al. 2015 for details), Diana Lagoon (Corsica, France) (see Luo et al. 2017 for details), German Wadden Sea (see Bogus et al. 2014 for details) and the Vilaine Estuary in Brittany (see Gurdebeke et al. 2018a for details) (Table 2).

The palynological residues were processed as described in Gurdebeke et al. (2018b). The residues were ultrasonicated for 30 s and rinsed three times with organic solvents (methanol and dichloromethane, 1:1 in volume) and MilliQ water over a 20 µm mesh-size filter to remove polar and apolar compounds that might have adhered to the cyst walls. Visually clean, empty individual whole cysts were manually isolated, placed on an Au-coated mirror and dried. Specimens were analyzed with a Bruker Hyperion 2000 microscope coupled to a Bruker Vertex 80v FTIR spectrometer in the Department of Solid State Sciences (Ghent University, Belgium). The objective magnification of the microscope was set at 15× and the aperture at 100×100 µm. The combination of detector (liquid N₂ cooled MCT), source (Globar) and beamsplitter (KBr) settings restricted the effective infrared spectral range examined to ~4000–650 cm⁻¹. All presented spectra were recorded in reflection mode, at a resolution of 2 cm⁻¹, and averaged over 100 scans.

Data were analyzed with the OPUS[®] software (Bruker 2014). The presented absorbance spectra were obtained after background (direct reflection on the Au mirror)

subtraction, atmospheric correction and baseline correction with a rubberband method using polynomes. Functional groups were identified based on comparison with Colthup et al. (1990) and published data (e.g., Bogus et al. 2014; Cárdenas et al. 2004). Data reduction was performed as a principal component analysis (PCA) using PAST 3.2 (Hammer et al. 2001).

2.4 LSU sequencing and molecular phylogeny

Votadinium cysts were isolated from recent sediments of the South China Sea, East China Sea and Bohai Sea to 96 well plates for germination with f/2-Si medium (Guillard and Ryther 1962) at 20°C, $90 \mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ under a 12:12 h light:dark cycle. Germinated cells or living cysts were rinsed several times in sterilized distilled water, broken by squeezing the coverslip above and then transferred into a polymerase chain reaction (PCR) tube. The single cell/cyst was used as the template to amplify ~1,430 bp of the LSU rDNA (D1-D6 domains) using the primers D1R (forward, 5'-ACCCGCTGAATTTAAGCATA-30) (Scholin et al. 1994), 28-1483R (reverse, 5'-GCTACTACCAAGATCTGC-30) (Daugbjerg et al. 2000). A 50 μl PCR cocktail containing 0.2 μM primers, PCR buffer, 50 μM dNTP mixture and 1 U of Ex Taq DNA polymerase (Takara, Dalian, China) was subjected to 35 cycles using a Mastercycler PCR (Eppendorf, Hamburg, Germany). The PCR protocol was initial denaturation for 3.5 min at 94°C, followed by 35 cycles of 50 s denaturation at 94°C, 50 s annealing at 45°C and 80 s extension at 72°C, plus a final extension of 10 min at 72°C.

The amplified products were run on a 1% agarose gel. Positive bands were excised and purified using a DNA extraction kit (Sangon, Shanghai, China). The purified PCR products were sequenced directly using the ABI Big-Dye dye-terminator technique (Applied Biosystems, Foster City, California, USA), according to the manufacturer's recommendations.

Newly obtained LSU rDNA sequences were incorporated into a systematically representative set of dinoflagellates available in GenBank. Sequences were aligned using the

MAFFT v7.110 (Katoh and Standley 2013) online program (<http://mafft.cbrc.jp/alignment/server/>) with default settings. Alignments were manually checked with BioEdit v. 7.0.5 (Hall 1999). For Bayesian inference (BI), the program jModelTest (Posada 2008) was used to select the most appropriate model of molecular evolution with Akaike Information Criterion (AIC). Bayesian reconstruction of the data matrix was performed using MrBayes 3.2 (Ronquist and Huelsenbeck 2003) with the best-fitting substitution model (GTR+G). Four Markov chain Monte Carlo (MCMC) chains ran for 2,000,000 generations, sampling every 100 generations. The first 10% of burn-in trees were discarded. A majority rule consensus tree was created to examine the posterior probabilities of each clade. Maximum likelihood (ML) analyses were conducted with RaxML v7.2.6 (Stamatakis 2006) on the T-REX web server (Boc et al. 2012) using the model GTR+G. Node support was assessed with 1000 bootstrap replicates.

3. Results

3.1 Systematic paleontology

Division Dinoflagellata (Bütschli) Fensome et al.

Class Dinophyceae Pascher

Order Peridiniales Haeckel

Family Protoperidiniaceae Bujak and Davies

Subfamily Protoperidinioideae (Autonym)

Genus *Votadinium* Reid, emend.

Type species. *Votadinium calvum* Reid 1977, p. 444, pl. 2, fig. 21.

Original diagnosis (Reid 1977). “Peridinoid-shaped cysts with a rounded apex or elongate apical horn and with two antapical, rounded horns or lobes separated by an antapical

depression which may be deep or shallow. Test compressed dorsoventrally, no evidence for a girdle. Archeopyle intercalary.”

Emended diagnosis. Dorsoventrally compressed peridinioid cysts consisting of a single wall layer with a rounded apex or an elongated apical horn and two rounded or elongated, blunt antapical horns separated by a deep or shallow antapical concavity. The equatorial area is usually the broadest part of the cyst. The thin wall (~1 μm) is pale brown to dark brown and can be smooth or microgranular and can have a discrete surface ornamentation or spines. No cingular indentation present, such as thickened or elevated cingular lists, although the cingulum is sometimes delineated by the absence of ornamentation. Sulcal depression present to absent, and may bear flagellar scars. Archeopyle intercalary, reflecting a 1a or 2a plate, basically hexagonal or pentagonal sometimes with rounded corners, positioned dorsally or involving the entire apical section of the cyst.

Stratigraphic range. Eocene to recent.

Votadinium calvum Reid, 1977

Pl. 1, Fig. 1–7.

Synonymy.

1882 *Peridinium divergens*: Bergh, Pl. 5, Fig. 40.

1965 *Peridinium* cyst-form 3: Wall, Fig. 13–15.

1968 *Peridinium oblongum* (Aurivillius) Paulsen: Wall and Dale, Pl. 1, Fig. 25–28.

1975 Cyst of *Peridinium oblongum*: Davey and Rogers, Pl. 1, Fig. 11.

1977 *Votadinium calvum* gen. nov., sp. nov.: Reid, p. 444, Pl. 2, Fig. 21–23.

1982 *Protoperidinium* (*Protoperidinium* sect. *Votadinium*) *oblongum*: Harland, p. 380, Pl. 40, Fig. 10–12.

1983 Cyst of *Protoperidinium oblongum*: Dale, Fig. 9.

- 1987 Cyst of *Protoperidinium oblongum* (Aurivillius) Parke et Dodge: Akselman, Pl. 2, Fig. 1.
- 1987 *Votadinium carvum* [sic]: Matsuoka, Pl. 7, Fig. 1–8.
- 1988 *Votadinium calvum* Reid: Bint, p. 335, Fig. 3K.
- 1992 *Votadinium calvum* Reid: de Vernal et al., p. 328, Pl. 7, Fig. 3.
- 1993 Cyst of *Protoperidinium oblongum*: Nehring, Fig. 5N.
- 1994 Cyst of *Protoperidinium oblongum*: Ellegaard et al., Fig. 58.
- 1994 Cyst of *Protoperidinium oblongum*: Nehring, Fig. 1Q–R.
- 1994 *Votadinium carvum* [sic.]: Matsuoka and Lee, Pl. 2, Figs. 6–8.
- 1996 Cyst of *Protoperidinium oblongum*: Nehring, Fig. 12A–D.
- 1997 Cyst of *Protoperidinium oblongum*: Sonneman and Hill, p. 165, Fig. 26.
- 1999 *Votadinium calvum* Reid: Rochon et al., p. 52, Pl. 14, Fig. 1–2.
- 2000 *Protoperidinium oblongum* type a: Rubino et al., Fig. 2p–q.
- 2003 Cyst of *Protoperidinium oblongum*: Morquecho and Lechuga-Devéze, Fig. 5.
- 2004 *Protoperidinium oblongum*: Harland et al., Pl. 1, Fig. 1–2.
- 2004 *Votadinium calvum* Reid: Pospelova et al., Pl. 2, Fig. 11a.
- 2004 *Votadinium calvum* Reid: Radi and de Vernal, Pl. 1, Fig. 10.
- 2005 *Votadinium calvum*: Pospelova et al., Fig. 7–6.
- 2006 *Votadinium calvum*: Brenner, Fig. 4I.
- 2006 Cyst of *Protoperidinium oblongum*: Fuji and Matsuoka, Fig. 4d.
- 2007 *Votadinium calvum*: Prasad et al., Fig. 3J.
- 2010 *Votadinium calvum*: Limoges et al., Pl. 3, Fig. 12.
- 2010 *Votadinium calvum*: Pospelova and Kim, Pl. 2, Fig. G.
- 2010 Cyst of *Protoperidinium oblongum*: Satta et al., Pl. 3, Fig. e.
- 2011 *Votadinium calvum*: Price and Pospelova, Pl. 4, Fig. 4.

2012 Cyst of *Protoperidinium oblongum*: Furio et al., Fig. 2–25.

2012 *Protoperidinium oblongum*: Srivilai et al., Fig. 2–P.

2016 *Votadinium calvum*: Bringué et al., Pl. 3, Fig. 5.

Motile stage equivalent. *Protoperidinium latidorsale* Balech, according to Sarai et al. (2013).

Remarks. The specimen in Pl. 1, Figs. 1–3 shows the general characteristics of the species: broad (wider than long) heart-shaped cyst with a thin wall and broadly rounded antapical horns separated by a shallow antapical concavity. The surface may be smooth (Pl. 1, Figs. 4–7) or microgranular (Pl. 1, Figs. 1–3). The SEM images (Pl. 1, Figs. 4–7) show that the cyst presented here is deformed, which somewhat alters the appearance of its outline in ventral or dorsal view (e.g. depth of the antapical concavity or the shape of the horns). It is not known how general this phenomenon is, however, and whether the deformation took place before settling, in the sediment or during processing. The defining characteristics of the species remain valid. The SEM images (Pl. 1, Fig. 4–7) also show sutural delineations suggesting that the entire apex is involved in archeopyle formation or that the apex is not clearly distinguishable. The archeopyle reflects a hexagonal dorsal intercalary plate (cf. the large 1a plate, Sarai et al. 2013). Although the apex is involved in the formation of the hexagonal archeopyle, it probably does not involve apical plates, as more than sutures would be expected in that case. A flagellar scar is present on the right side of the sulcal depression (Pl. 1, Fig. 5, 7).

Comparison with other species. *Votadinium calvum* differs from *V. spinosum* by the absence of spines, from *V. rhomboideum* by its well-rounded shape, and from all other species in the genus by having the apex involved in the archeopyle. Specimens of *Quinquecuspis concreta* have been incorrectly identified as *V. calvum* in cases of poor cyst orientation (e.g., Matsuoka 1987, Pl. 8, Figs. 1–3; McMinn 1991, Pl. 1, Figs. 6 and 8).

Distribution and stratigraphic range. The modern distribution is global. Reports published with illustrations are mainly limited to latest Pleistocene to recent sediments. Older occurrences have been reported up to Burdigalian age (Londeix and Jan du Chêne 1998) but these are not illustrated so the identification cannot be confirmed.

Votadinium concavum sp. nov.

Gurdebeke, Mertens, Matsuoka, Gu, Louwye

Pl. 1, Figs. 8–10.

Synonymy.

2011 Cyst of *Protoperidinium* sp.: Attaran-Fariman et al., Fig. 12–15.

2012 *Votadinium calvum*: Liu et al., Fig. 3v.

Motile stage equivalent. Unidentified.

Holotype. SEM stub of WN142 from the Bohai Sea (39°55.864'N, 119°44.128'E).

Repository. Third Institute of Oceanography, State Oceanic Administration, Xiamen, 361005, China.

Diagnosis. Broad cysts with concave outline on both epi- and hypocyst and shallow antapical concavity. Cyst wall smooth and pale brown. Archeopyle seems to partly involve the apex.

Description. The concavity of the ambitus may be less obvious in some specimens (Pl. 1, Fig. 8). No obvious flagellar scar was observed (Pl. 1, Fig. 8), but the sulcal depression is often roughened, which might obliterate such structure.

Remarks. The small number of cysts observed does not allow an extensive formal description. This, along with the identification of the motile stage using incubation experiments, will be the focus of future work.

Dimensions. Length 65–85 µm; width 65–85 µm (N=2), with an average L/W of 0.96.

Comparison. *Votadinium concavum* mostly resembles *V. calvum*, but is broader and typically has a less rounded and more concave outline. *V. concavum* has a more typical concave outline and a more shallow antapical concavity than *V. rhomboideum*. Absence of ornamentation distinguishes *V. concavum* from *V. spinosum* and *V. pontifossatum*. The lower L/W distinguishes *V. concavum* from *V. elongatum*, *V. nanhaiense*, *V. pontifossatum* and *V. reidii*.

Distribution and stratigraphic range. In modern sediments from the East China Sea and Bohai Sea. Based on the synonymy also in off coast Iran and in the Yellow Sea.

Votadinium elongatum He and Sun, 1991

Pl. 2, Figs. 1–6.

Synonymy.

1965 *Peridinium* sp. cyst-form 1: Wall, Fig. 8-9.

2004 “Pre-excysted protoperidinioid”: Kawamura, Fig. 16.

2007 “Protoperidinioid dinocysts (*Trinovantedinium* sp.)”: Prasad et al., Fig. 3-K.

Motile stage equivalent. Unknown.

Diagnosis (He and Sun 1991, p. 297). “Cyst proximate, elongate peridinioid, compressed dorsoventrally with cingular area rounded. Epicyst bearing a conspicuous apical horn, cylindrical to conical. Hypocyst extending straight-backwards two blunt antapical horns of approximately equal size, forming U-shaped antapical concavity. Wall thin, 0.5–1 μm thick, single-layered or with two layers closely appressed, light brown to pale yellow in color, surface smooth or with several secondary folds. Paracingulum sometimes faintly expressed or marked, on the type specimen, by a transverse equatorial fold. Parasulcus deep, restricted to hypocyst and broader posteriorly. Archeopyle, when present, intercalary, subcircular in outline, formed by loss of large paraplate 2a. Operculum free or lying within cyst.”

Dimensions. He and Sun (1991, p. 297): Length 70–77 μm , width 40–49 μm , apical horn length 6–10 μm , antapical horns 11–14 μm and 16 μm long, respectively. Own measurements: Length 69.9–79.2 μm , width 40.4–49.8 μm , apical horn 13.4–14.7 μm , antapical horns: 14.1–17.0 μm , L/W 1.58–1.73 (N=2).

Remarks. The identification of *V. elongatum* with the cyst of *Protoperidinium oblongum* in Wall and Dale (1968, Pl. 1, Figs. 23-24) by He and Sun (1991) is not followed here, since the difference in morphology is clear (e.g., rounded instead of straight, more conspicuous apical horn). As such, the motile stage equivalent of *V. elongatum* remains unknown. The original illustrations are reproduced in Pl. 2, Figs. 1, 2 as this publication is not widely available.

Comparison. Distinguished from other *Votadinium* species by the large size, elongated (high L/W) and rounded body shape, and rounded archeopyle. *Votadinium elongatum* is comparable to *V. reidii* in size, but *V. reidii* typically has a straight outline and a hexagonal archeopyle.

Distribution and stratigraphic range. This species has been rarely reported. It was recorded in recent sediments from around the Spratly Islands (type area) and the Sunda Shelf (Kawamura 2004), the southwestern waters off Taiwan and the shelf off Hainan Island (South China Sea), and from the west coast of India (Prasad et al. 2007). Wall (1965) recorded *V. elongatum* from Woods Hole (Massachusetts, USA).

Votadinium nanhaiense He and Sun, 1991.

Pl. 2, Figs. 7–15.

Synonymy.

2003 *Votadinium nanhaiense*: Jurkschat, p. 14, Pl. 13, Fig. 4-7.

2013 *Protoperidinium* sp. 2: Narale et al., Fig. 2n.

Motile stage equivalent. Unknown.

Diagnosis (He and Sun 1991, p. 297). “Cyst proximate, elongate peridinioid, always with shoulders, compressed dorsoventrally, equatorial area prominently bulging outwards. Epicyst and hypocyst of approximately equal size. Epicyst bell-shaped with a triangular apical horn, blunt at tip. Hypocyst trapezoidal, with a gently arc-shaped antapical margin and two strongly divergent antapical horns of nearly the same size and blunt at free ends. Autophragm pale yellow in color, surface smooth or folded. Both paracingulum and parasulcus faint or absent. Archeopyle very large, intercalary, rounded six-sided or subcircular in outline, and situated in the mid-dorsal surface just below apex. Operculum free or falling into cyst cavity.”

Dimensions. He and Sun (1991, p. 298): Length 65–75.8 μm ; width 49–53.5 μm ; apical horn length 9–11 μm ; antapical horn length 6.7–11 μm . Own measurements: Length 59.7 (72.1) 79.4 μm , width 44.4 (54.4) 67.3 μm , apical horn 10.4 (11.5) 12.1 μm , antapical horns 11.5 (14.7) 16.8, L/W 1.17 (1.33) 1.47 (N=4).

Remarks. The original photograph of the holotype is reproduced in Pl. 2, Figs. 7–9 as this publication is not widely available.

Comparison. The divergent antapical horns and epicystal shoulders clearly distinguish *V. nanhaiense* from other species within the genus.

Distribution and stratigraphic range. He and Sun (1991) described *Votadinium nanhaiense* from recent sediments from the South China Sea (type area). The species was also recorded in recent sediments from the Arabian Sea by Jurkschat (2003), off the coast of India by Narale et al. (2013) and, as this study shows, Holocene sediments from the South China Sea.

Votadinium pontifossatum sp. nov.

Gurdebeke, Mertens, Pospelova, Matsuoka, Li and Louwye

Pl. 3, Figs. 1–13.

Synonymy.

1972 “*Votadinium* cyst form 1”: Reid, Pl. 11, Fig. 13–15.

2011 Cyst of *Protoperidinium oblongum*: Price & Pospelova, Pl. 4, Fig. 3.

2013 Cyst of *Protoperidinium paraoblongum*: Sarai et al., Pl. 2, Fig. 1–3, Pl. 5.

Motile stage equivalent. *Protoperidinium paraoblongum* Sarai et al., 2013, according to Sarai et al. (2013).

Holotype. Patricia Bay, Saanich Inlet, SIPB-EOS313-09 core 4-5 cm sample, UVic 2009-179-1 (48°39.997’N, 123°29.014’W), EF V37/0, Pl. 3, Figs. 1–4.

Repository. Royal Belgian Institute of Natural Sciences (RBINS, Brussels), accession n° IRSNB b7155.

Etymology. From Latin *pons*, meaning bridge, and *fossa*, meaning small depression, in reference to the ornamentation described below.

Diagnosis. The brownish cysts have a dorso-ventrally compressed pentagonal ambitus. The length is greater than the width ($L/W=1.18$, $N=9$). The epicyst has straight or slightly convex sides with a pronounced apical horn. The antapical concavity is deep and U-shaped and the antapical horns elongated. The ornamentation consists of small rod-like elements in circular depressions, covering the entire cyst including the operculum. Archeopyle saphopylic and pentagonal, though occasionally the entire apical part (the horn) can be missing. The cingular area is sometimes devoid of ornamentation.

Description. The thin-walled (less than 1 μm) cysts are fragile and often strongly folded and torn (Pl. 3, Figs. 5, 9). The right antapical horn is often longer and broader than the left antapical horn. The ornamentation consists of small (length 0.83 (1.01) 1.14 μm , width 0.18 (0.20) 0.24 μm , $N=8$) rod-like elements bridging a circular crater-like depression in the cyst wall (Pl. 3, Fig 10). The depressions do not form holes. The rods are mostly fused with the cyst wall but sometimes appear as unattached elements lying in the depression (Pl. 3, Fig. 10). The rods are randomly oriented and distributed over the cyst’s wall (11/100 μm^2 , $N=5$). This

kind of wall ornamentation is unique and is here named a pontifossate wall texture, in reference to the ornamentation resembling a bridge spanning a depression.

Remarks. The characteristic surface ornamentation of *Votadinium pontifossatum* sp. nov. is visible in the illustrations of the cyst of *Protoperidinium paraoblongum* provided by Sarai et al. (2013), and was described as “granules” in that publication. The ornamentation was also observed on a number of cysts from a sediment trap (UVic 2009-179-1 in Price and Pospelova, 2011) and surface sediments in Saanich Inlet (Pl. 3, Figs. 5–7, 9), as well as in the Holocene sediment samples from Kyuquot Sound (Pl. 3, Fig 8). Other examples of this species were documented from the Kiel Bight (German Wadden Sea) and the South China Sea. When describing the new species *Votadinium calvum*, Reid (1977) stated “careful observation under phase contrast showed ‘crater-like’ pits on many specimens,” which relates to the ornamentation described here, though no rods were reported. The ornamentation is not illustrated by Reid (1977) but Figs. 13–15 on Plate 11 in Reid (1972) depicts the ornamented type. Some specimens observed here indeed have a number of craters that lack rods, but quantitative information on the range of this presence-absence is not available at present (e.g. Pl. 3, Fig. 11–13).

Dimensions. Sarai et al. (2013): length 65 (80) 90 μm , width 63 (72) 80 μm , L/W 1.19–1.39 (N=4). Our measurements on the cysts from the NE Pacific are: Holotype: length 85 μm , width 73 μm , range: length 71.6 (79.2) 86.6 μm , width 61.1 (67.7) 75.7 μm , L/W 1.09 (1.18) 1.33 (N=9).

Comparison. This species differs from all other *Votadinium* species by its characteristic surface ornamentation. In addition, the new species is distinguished from *V. calvum* and *V. spinosum* by a differing L/W (generally >1), and from *V. psilodora* in that the latter has a more rounded shape and lower L/W. *Votadinium elongatum* generally has a higher L/W. *Votadinium nanhaiense* differs in having typical shoulders in the epicyst outline, diverging

antapical horns and a triangular apical horn. *V. reidii* has more pronounced apical and antapical horns.

Distribution and stratigraphic range. Holocene, surface and trap sediments from Vancouver Island waters in Canada, as well as from recent sediments from Omura Bay in Japan (Sarai et al. 2013), Fangchenggang Port (South China Sea), and coastal waters from Europe (United Kingdom and northern Germany).

Votadinium psilodora (Benedek, 1972) comb. nov.

Pl. 4, Figs. 1–11.

Synonymy.

- 1972 *Lejeunecysta psilodora*: Benedek, Pl. 6, Fig. 5.
- 1981 *Selenopemphix nephroides*, pars: Benedek and Sarjeant, Fig. 8, 3–4.
- 1987 *Lejeunecysta psuchra*: Matsuoka, Pl. 9, Fig. 7–8.
- 1988 *Votadinium calvum* Reid: Bint, Fig. 3-J.
- 1988 *Lejeunecysta psilodora* (Benedek) Artzner and Dorhofer: Gruas-Cavagnetto and Barbin, Pl. V, Fig. 12-13.
- 1989 Cyst of *Protoperidinium oblongum*: Kojima, Fig. 6-2.
- 2000 *Protoperidinium* sp. 1: Cho, p. 24, Pl. 2, Fig. 8.
- 2004 *Lejeunecysta psuchra*: Louwye et al., Fig. 10r–s.
- 2010 Cyst of *Protoperidinium* spp.: Pospelova et al., Pl. 5, Fig. 5.

Motile stage equivalent. Unknown.

Holotype. Mid-Oligocene of Tönisbergen (Germany). The holotype is stored in the repository of the Institute of Geology and Mineralogy, University of Köln, Germany. Re-illustrated here in Pl. 4, Figs. 1–8.

Remarks. No diagnostic characteristics separate '*Lejeunecysta psuchra*' Matsuoka from '*L. psilodora*' Benedek. *Lejeunecysta psuchra* is slightly smaller (length 80–84 µm; width 68–72 µm, Matsuoka 1987) than *Lejeunecysta psilodora* (length 90–96 µm, width 75–78 µm, Benedek 1972). Therefore, synonymy of *Lejeunecysta psilodora* and *Lejeunecysta psuchra* is proposed, with *Lejeunecysta psuchra* as the junior synonym. Furthermore, no expression of the cingulum as an equatorial indentation of the cyst body, was observed. As the presence of a cingulum was the main characteristic that distinguishes *Votadinium* from *Lejeunecysta*, *Lejeunecysta psilodora* is transferred to the genus *Votadinium*. Also, the archeopyle index of '*L. psilodora*' is too large and the antapical horns are wide and rounded, as previously noted by Stover and Evitt (1978, p. 112); these are characteristics more suited for *Votadinium*. The holotype of *V. psilodora* is re-illustrated in Pl. 4, Figs. 1–8. The holotype of '*L. psuchra*' was not found, but observations were made on cysts from the type material (Pl. 4, figs 9–11).

Comparison. *Votadinium psilodora* is different from *Votadinium reidii* in having a more rounded outline and (ant)apical horns, and from *V. pontifossatum* by the absence of a pontifossate ornamentation. *V. psilodora* is also distinguished from *V. calvum*, *V. spinosum*, *V. rhomboideum* and cyst of *P. venustum* by the dorsal position of the archeopyle.

Distribution and stratigraphic range. Mid-Oligocene deposits from Tönisbergen (NW Germany; Benedek 1972), Pliocene deposits from Belgium (Louwye et al. 2004) and late Quaternary sediments from Japan (Matsuoka 1987, 1992). Williams and Bujak (1977, p. 30) reported *L. psilodora* as having its highest occurrence in their "*Epicephalopyxis indentata* assemblage," which ranges into the early Miocene. They did not provide illustrations, so the identification cannot be confirmed.

Votadinium reidii sp. nov.

Gurdebeke, Mertens, Pospelova, Matsuoka, Gribble and Louwye

Pl. 5, Figs. 1–13.

Synonymy:

1965 *Peridinium* sp. cyst-form 1: Wall

1966 *P. oblongum* (Aur.) Cleve: Wall and Dale, fig 6

1968 Cyst of *Protoperidinium oblongum* (Aurivillius) Paulsen: Wall and Dale, Pl. 1, Figs. 23–24.

2005 *Protoperidinium oblongum*: Pospelova et al., Fig. 6.8.

2009 Cyst of *Protoperidinium steidingeriae*: Gribble et al., Fig. 4, 5.

Motile stage equivalent: *Protoperidinium steidingeriae*, according to Gribble and Anderson (2006) and Gribble et al. (2009).

Holotype. Cyst from culture experiment based on material sampled at Martha's Vineyard Coastal Observatory, Vineyard Sound, Massachusetts (41°19.5'N, 70°34.0' W; MV0923-PO-1 in Gribble et al. 2009) mounted on SEM stub GR-2, illustrated in Pl. 5, Figs; 1–3.

Repository: Royal Belgian Institute of Natural Sciences (RBINS, Brussels), accession n° IRSNB b7156.

Etymology. Named in honor of P.C. Reid, who erected the genus *Votadinium*.

Diagnosis: Brownish elongated cyst with smooth wall. Outline with straight edges, especially on the left side of the cyst; right cyst variably more rounded. Apical horn variable, ranging from long and narrow to small and rounded. Antapical horns ranging from elongated to well-rounded; right antapical horn mostly longer than the left one. Sulcal area with flagellar scar. Archeopyle hexagonal in dorsal position, not involving apex.

Description. There is considerable variability in the cyst shape, ranging from elongated with straight edges (Pl. 5, Figs. 3, 4) to nearly equidimensional and more rounded (Pl. 5, Figs. 10–13). The archeopyle shape is consistent, however, and the outline of the left side of the cyst is always straight. The archeopyle reflects a 2a intercalary plate.

Dimensions (Gribble et al. 2009). Length 68 (83) 95 μm , width 48 (59) 68 μm , L/W: 1.2 (1.4) 1.8 (N=30).

Remarks. Most of the information available stems from incubation experiments (Gribble et al. 2009). Palynological records are rare and limited to the same region (Pospelova et al. 2005). The relationship of *P. steidingerae* with the morphologically closely related motile-based species *P. concinna* and *P. novella* described by Faust (2006) in the subgenus *Testeria* is unclear (Gribble et al. 2009, p. 102) and needs further investigation.

Comparison. *Votadinium elongatum* has a more rounded outline and archeopyle. It differs from *V. calvum* and *V. spinosum* by having a straight outline and a length that is generally greater than the width, and a dorsal archeopyle. *Votadinium spinosum* and *V. pontifossatum* are different because they have ornamentation. The apex is not involved in the operculum, which differentiates it from *V. rhomboideum*.

Distribution and stratigraphic range: Rare species, presently known from cultures based on material recovered from Vineyard Sound (Massachusetts, USA) and from surface sediments from New England (USA).

Votadinium rhomboideum sp. nov.

Gurdebeke, Mertens, Pospelova, Matsuoka, Li and Louwye

Pl. 6, Figs. 1–14.

Synonymy.

1983 Cyst of *Protoperidinium oblongum*: Dale, Fig. 11-12.

1987 *Protoperidinium oblongum*: Baldwin, Fig. 14.

2000 Cyst of *Protoperidinium oblongum* (Aurivillius) Parke & Dodge: Della Tommasa et al., Pl. 1, Fig. 7.

2000 *Protoperidinium oblongum* type b: Rubino et al., Fig. 2r.

- 2001 Cyst of *Protoperidinium oblongum*: Dale, Fig. 1–7, 8.
- 2004 Cyst of *Protoperidinium oblongum*: Joyce, p. 178, Fig. 18.
- 2004 “Pre-excysted protoperidinioid cyst”: Kawamura, Fig. 15.
- 2004 Cyst of *Protoperidinium oblongum*: Morquecho and Lechuga-Devéze, Fig. 12.
- 2004 Cyst of *Protoperidinium oblongum*, Orlova et al., Fig. 58.
- 2006 Cyst of *Protoperidinium oblongum*: Fujii and Matsuoka, Fig. 4-e.
- 2008 Cyst of *Protoperidinium oblongum*: Ribeiro and Amorim, Pl. 2, Fig. b.
- 2008 *Votadinium calvum*: Vilanova et al., Pl. 1, Fig. J.
- 2010 *Votadinium calvum*: Pospelova et al., Pl. 6, Fig. 3.
- 2010 *Votadinium calvum*: Uzar et al., Fig. 25.
- 2011 *Votadinium carvum* [sic.]: Aydin et al., Fig. 27.
- 2011 *Votadinium calvum*: Pasuram et al., Pl. 1, Fig. 8.
- 2011 *Votadinium calvum*: Price and Pospelova, Pl. 4, Fig. 4.
- 2013 Cyst of *Protoperidinium oblongum*, Orlova and Morozova, Fig. 4f, g.
- 2013 Cyst of *Protoperidinium quadrioblongum*: Sarai et al., Pl. 3, Fig. 1-4.

Motile stage equivalent. *Protoperidinium quadrioblongum* Sarai et al., 2013, according to Sarai et al. (2013).

Holotype. Patricia Bay, Saanich Inlet (48°39.005'N, 123°29.027'W, station PB 3 in Mertens et al., 2012), surface sediments, UVic 2012-197-1 (EF R20/4, label to the right), Pl. 6, Figs. 1–8.

Repository. Royal Belgian Institute of Natural Sciences (RBISN, Brussels), accession n° IRSNB b7157.

Diagnosis. Brownish cysts, rhomboidal in outline in ventral or dorsal view, length and width comparable. Epi- and hypocyst sides are straight or slightly concave, yielding the cyst an angular outline. Cyst wall smooth. Two rounded antapical horns rounded and generally

symmetrical, sometimes right horn slightly broader. Sulcal area incised and carrying flagellar scar. Antapical concavity deeply incised and U-shaped. Archeopyle (type IA, 2a) large, trapezoidal, saphopylic and involving the apex.

Description. Rhomboidal/pentagonal cyst with straight outlines. Cyst width and length comparable, with $L/W = 0.98$ ($0.87-1.05$, $N=4$), although the length is obscured when the apex is lost with archeopyle. Cyst is smooth and has a single wall.

Dimensions. Sarai et al. (2013): Length 61 (70) 84 μm , width 59 (67) 80 μm , L/W 0.88-1.05 ($N=4$). Own measurements: Holotype: length 65.4 μm , width 62.2 μm ; Range: length 65.4–73.1 μm , width 62.2–69.5 μm , L/W 0.95 ($N=2$).

Remarks. *Votadinium rhomboideum* is not identical to the cyst of *Protoperidinium oblongum* (Aurivillius) Paulsen reported by Wall and Dale (1968, Pl. 1, Fig. 29), as was suggested by Sarai et al. (2013).

Comparison. *V. rhomboideum* sp. nov. differs from *V. calvum* and *V. spinosum* in that it has a higher L/W ratio and straight outlines for the epi and hypocyst. It lacks ornamentation, which distinguishes it from *V. spinosum* and *V. pontifossatum*, and it differs from *V. pontifossatum* and *V. reidii* in having the apex involved in the operculum. It can be distinguished from *V. nanhaiense* and *V. elongatum* by the position of the archeopyle and general outline. Finally, *V. concavum* has concave outlines of the epi- and hypocyst, rather than straight edges.

Distribution and stratigraphic range. The species was observed here in the South China Sea and Saanich Inlet. Based on the synonymy list, *Votadinium rhomboideum* occurs globally, but with most reports from the North Atlantic and North Pacific domains.

Votadinium spinosum Reid, 1977

Pl. 7, Fig. 1–15.

Synonymy:

- 1965 *Peridinium* sp. (cysts-form 5): Wall, p. 307, Fig. 16.
- 1967 Cyst of *Peridinium claudicans* Paulsen: Wall and Dale, p. 350, Pl. 1, Fig. R.
- 1968 *Peridinium claudicans* Paulsen: Wall and Dale, p. 273, Pl. 2, Fig. 1–2.
- 1975 Cyst of *Peridinium claudicans* Paulsen: Davey and Rogers, p. 217, Pl. 1, Fig. 10.
- 1977 *Votadinium spinosum* Reid: Reid, p. 445–446, Pl. 2, Fig. 24–26.
- 1985 *Votadinium spinosum* Reid: Matsuoka, p. 58–59, Pl. 14, Fig. 1–6.
- 1987 *Votadinium spinosum*: Matsuoka, p. 65–66, Pl. 14, Fig. 1–5.
- 1987 Cyst of *Protoperidinium claudicans*: Akselman, Pl. 1, Fig. 7–9.
- 1989 *Votadinium spinosum*; Kojima, Fig. 6.5.
- 1990 Cyst of *Protoperidinium claudicans* (Paulsen) Balech: Bolch and Hallegraeff, p. 183, Fig. 25.
- 1992 *Votadinium spinosum* Reid: Matsuoka, p. 51, Pl. 2, Fig. 9.
- 1993 Cyst of *Protoperidinium claudicans*; Nehring, Fig. 5–J.
- 1994 *Votadinium spinosum* Reid: Matsuoka, Pl. 2, Fig. 3a–b.
- 1996 Cyst of *Protoperidinium claudicans* Qi et al., p. 96, Pl. 3, Fig. Q.
- 1997 Cyst of *Protoperidinium claudicans*: Nehring, p. 313, Fig. 17.
- 1998 Cyst of *Protoperidinium claudicans*: Montresor et al., Fig. 3a.
- 2000 *Votadinium spinosum*: Cho, p. 27, Pl. 3, Fig. 1.
- 2000 Cyst of *Protoperidinium claudicans*: Ishikawa and Taniguchi, Fig. 4–J.
- 2000 *Protoperidinium claudicans*: Rubino et al., p. 276, Fig. 2g.
- 2002 *Votadinium spinosum* Reid: Prauss, Pl. 3, Fig. 7.
- 2003 *Protoperidinium claudicans*: Morquecho and Lechuga-Devéze, Fig. 3.
- 2004 Cyst of *Protoperidinium claudicans*: Harland et al., Pl. 1, Fig. 3.
- 2004 Cyst of *Protoperidinium claudicans*: Joyce, p. 176, Fig. 14a,b.
- 2004 *Protoperidinium claudicans* (Paulsen) Balech: Orlova et al., Figs. 44–46.

- 2004 *Votadinium spinosum*: Pospelova et al., Pl. 2, Fig. 12.
- 2004 *Votadinium spinosum*: Radi and de Vernal, Pl. 1, Fig. 11.
- 2005 *Protoperidinium claudicans*: Joyce et al., Fig. 11.
- 2005 *Votadinium spinosum*: Pospelova et al., Fig. 7.7.
- 2007 *Votadinium spinosum*: Cremer et al., Fig. 191–192.
- 2008 *Votadinium spinosum*: Vilanova et al., Pl. 1, Fig. K.
- 2010 *Votadinium spinosum*: Krepakevich and Pospelova, Pl. 3f.
- 2010 *Votadinium spinosum*: Limoges et al., Pl. 3, Fig. 11.
- 2010 *Votadinium spinosum*: Matsuoka and Shin, Fig. 2E.
- 2010 *Votadinium spinosum*: Pospelova et al., Pl. 6, Fig. 4.
- 2010 *Votadinium spinosum*: Satta et al., Pl. 2, Fig. n.
- 2010 *Votadinium spinosum*: Shin et al., Fig. 4-H.
- 2010 *Votadinium spinosum*: Uzar et al., Fig. 26.
- 2011 *Votadinium spinosum*: Aydin et al., Fig. 12a,b.
- 2011 Cyst of *Protoperidinium claudicans*: D'Silva et al., Fig. 2.15.
- 2011 *Votadinium spinosum*: Price and Pospelova, Pl. 4, Fig. 5.
- 2012 *Votadinium spinosum*: Candel et al., Fig. 2.8.
- 2012 Cyst of *Protoperidinium claudicans*: Furio et al., Fig. 2.17.
- 2012 *Protoperidinium claudicans*: Srivilai et al., Fig. 2Q.
- 2013 *Votadinium spinosum*: Bringué et al., p. 41, Pl. 3, Fig. 6.
- 2013 *Votadinium spinosum*: Candel et al., Pl. 2, Fig. 6.
- 2013 *Votadinium spinosum*: Limoges et al., Pl. 2, Fig. 4.
- 2013 Cyst of *Protoperidinium claudicans*: Narale et al., Fig. 3g.
- 2013 *Protoperidinium claudicans* (Paulsen) Balech: Orlova and Morozova Fig. 3n-o.
- 2016 *Votadinium spinosum* Bringué et al., p. 805, Pl. 3, Fig. 6..

2018 *Votadinium spinosum* Reid: Matsuoka et al., p. 128, Fig. 5.g.

Motile stage equivalent. *Protoperidinium claudicans* (Paulsen 1907) Balech 1974, according to Reid (1977, p. 445).

Remarks. The holotype of Reid (1977) was re-photographed and is illustrated here for the purpose of completeness (Pl. 7, Fig. 1–4). It shows a broad (wider than long) heart-shaped cyst with broadly rounded antapical horns separated by a shallow antapical concavity and a rounded apex. The thin smooth wall is ornamented with short (3–7 μm ; Reid 1977) solid spines. There is a large 2a archeopyle, which may involve apical plates. These general characteristics are also visible in the SEM image (Pl. 7, Fig. 13), where the archeopyle is open and the operculum has been shed. The cyst wall and spines appear to be microgranular, and the solid spines have minute fenestrae at their bases (Pl. 3, Figs. 5–7). It is possible that *V. spinosum* represents a species complex, as suggested by Matsuoka and Head (2013). This hypothesis needs to be further evaluated through cyst-theca and morphological studies.

Comparison. This species differs from all other *Votadinium* species in having a granular wall and bearing spines, and from *V. psilodora*, *V. elongatum*, *V. nanhaiense*, *V. pontifossatum* and *V. reidii* in having a $L/W < 1$ and the archeopyle involving the apical part of the cyst.

3.2 Sequencing and molecular phylogeny

Three sequences were retrieved from cysts from surface sediments in the South China Sea: two for *Votadinium concavum* ([genbank numbers]), and one for *Votadinium calvum* (cyst of *Protoperidinium latidorsale*, [genbank number]). The molecular phylogeny based on LSU rDNA is shown in Fig. 1. Within the phylogeny, the three new sequences are placed in the *Oceanica* clade of *Protoperidinium*, which is formed by *P. latidorsale* (motile stage of *Votadinium calvum*), *P. paraoblongum* (*V. pontifossatum*), *P. steidingeriae* (*V. reidii*), *P. claudicans* (*V. spinosum*), *P. quadrioblongum* (*V. rhomboideum*) and *P. depressum* (no cyst

known). The sequence of *P. latidorsale* clusters with the known molecular data of this species, while the sequences of *Votadinium concavum* form a sister cluster to *P. latidorsale*. The *Oceanica* clade forms a clade with maximal support (Fig. 1).

3.3 Cyst wall chemistry

A total of 19 spectra were measured and representative spectra are shown in Fig. 2: three specimens of *Votadinium spinosum* (M9S9 and M12S13 from Kyuquot Sound, M1S8 from Izmir Bay), two of *V. calvum* (MXS20 and MXXS5 from Saanich Inlet), one of *V. pontifossatum* (MXS8 from Saanich Inlet), eight specimens of *Quinquecuspis concreta* (MXS3, MXS7, MXS16 and MXS17 from Saanich Inlet, M9S5 and M9S7 from Kyuquot Sound and M13S15 from Corsica), one specimen of *T. pallidifulvum* (M6S4 from the German Wadden Sea), and four specimens of *Trinovantedinium applanatum* (M4S3 and M4S33 from the Vilaine estuary, M1S7 from Izmir Bay and M6S13 from the German Wadden Sea). The main absorptions are summarized in Table 3. The broad peak between 3600 and 3000 cm^{-1} is due to OH-stretching with the maximum mostly centered around 3270 cm^{-1} . The centering of the absorption at 3270 cm^{-1} , along with a faint shoulder at 3100 cm^{-1} , is indicative of N-H stretching, although further detail in this region is obscured by the broad peak. Two consistent absorptions are present at 2925 and 2860 cm^{-1} that indicate aliphatic methyl and methylene (CH_3 and CH_2) stretching.

In the fingerprint region (1700–800 cm^{-1}), the dominant absorptions for most specimens are between 1700 and 1500 cm^{-1} . The amide II band, located between 1550 and 1590 cm^{-1} , is one of the dominant absorptions in all spectra. Absorptions around 1660 cm^{-1} (amide I band) is the strongest absorption in *Votadinium spinosum* spectra, while it is a secondary peak or shoulder for *Q. concreta* and *T. applanatum*. Unusually for dinoflagellate cysts, a consistent absorption is recorded for the cysts of *T. applanatum* at 1510 cm^{-1} .

The spectral region from 1500 to 1200 cm^{-1} is, in most cases, less pronounced. A consistent small absorption or shoulder at 1255 cm^{-1} indicates N-H bending, as does the 1312 cm^{-1} absorption (amide III). The latter also contains absorptions characteristic for C-N stretching. Absorptions at 1420 and 1370 cm^{-1} indicate C-H bending.

The absorptions between 1200 and 1030 cm^{-1} , which are minor in the *V. spinosum* spectra but more prominent in the others, arise from C-O-type stretching. Finally, absorption at the amide V band ($\sim 700 \text{ cm}^{-1}$) consistently occurs in all spectra.

A PCA analysis on the fingerprint region of the spectra (1700–800 cm^{-1} , see Bogus et al. 2014) yields PC1 and PC2 that represent 72.9% of the variability and reveals that each of the species' spectra cluster, regardless of sample location (Fig. 3).

4. Discussion

4.1 Taxonomy, phylogeny and evolution of the genus *Votadinium*

The previously poorly understood taxonomy of the cyst genus *Votadinium* was emended in this study to include brown peridinioid cysts without expression of a cingulum and with a dorso-apical archeopyle. Based on morphology, nine species were attributed to *Votadinium*, which are schematically represented in Fig. 4. A cyst that should possibly also be assigned to *Votadinium* has been reported for *Protoperidinium brochii* by Blanco (1989), but new observations are required to evaluate such a placement. Perhaps further species may be transferred to *Votadinium*, e.g. *Lejeunecysta sabrina* of Reid (1977), but new observations are needed for this.

Besides the species reported in this study, the *Oceanica* section includes other species such as *P. oceanicum*, *P. larsenii*, *P. murrayi* (Balech 1974; Taylor 1976; Abé 1981; Phan-Tan et al. 2017), for which molecular data or information on possible cyst stages are lacking (Gribble and Anderson 2006). Cysts belonging to *Votadinium psilodora*, *V. elongatum* and

Votadinium nanhaiense most probably have motile stages assignable to *Protoperidinium* (also suggested by Matsuoka 1987, p. 61 for '*Lejeunecysta psuchra*'). Incubation experiments and/or molecular analysis can resolve this relationship.

The new molecular data confirm earlier phylogenetic studies (e.g., Gribble and Anderson 2006; Sarai et al. 2013; Gu et al. 2015; Mertens et al. 2017c) that, based on LSU rDNA sequences, the *Oceanica* section of the genus *Protoperidinium* (i.e. *P. latidorsale*, *P. claudicans*, *P. steidingeriae*, *P. paraoblongum*, *P. quadrioblongum* and *P. depressum*) forms a monophyletic clade (Fig. 1). In addition, the topology of these earlier phylogenetic trees is confirmed (e.g. Mertens et al. 2017c).

The placement of the cysts of the *Oceanica* section *Protoperidinium* species into a single genus (*Votadinium*) indicates that the morphological classification of the cysts reflects the phylogenetic relationships of the motile stage species (Harland 1982). It must be noted however, that for other cyst-based genera related to *Protoperidinium*, the same conclusions cannot always be drawn. For example, Mertens et al. (2017c) found that two species in the cyst-based genus *Selenopemphix* have motile stages belonging to different clades within the genus *Protoperidinium*. It was argued that, since the morphological difference between both cyst species is considerable, they belong to different cyst genera. Also, the cyst-based genus *Brigantedinium* is known to be polyphyletic with respect to motile stages in the genus *Protoperidinium* (Matsuoka et al. 2006). Compared with other protoperidinioid cyst genera, *Brigantedinium* species have relatively few characteristics (e.g., archeopyle) upon which to base classification (Matsuoka 2002). That explains the previously unrecognized diversity within this group. Other clades were found within the *Protoperidinium* genus that have morphologically similar cysts; for example, the cyst genera *Trinovantedinium* and *Quinquecuspsis* in the *Conica*-section (Mertens et al., 2017c). Overall, this indicates coherence

between the differing cyst- and motile-based taxonomic concepts. Future discoveries of cyst new stages for species of the *Oceanica* section will provide a further test for this.

From an evolutionary point of view, Bujak and Davies (1983) hypothesized that *Votadinium psilodora* from Oligocene age sediments (as '*Lejeunecysta psilodora*') is an early representative of the *Votadinium* evolutionary lineage, represented in their scheme by *Votadinium spinosum* and *Votadinium calvum*. This scheme is updated in Fig. 5. Cyst morphology in the *Oceanica/Votadinium* clade is characterized by the absence of cingular excavation, which was present in ancestral forms like *Senegalinium* or *Phelodinium* (cf. Bujak and Davies 1983) and was retained in the extant *Lejeunecysta*, *Trinovantedinium* and *Quinquecuspis*. The earliest representative, *V. psilodora*, had a dorsally positioned archeopyle, which also is an ancestral trait. In some of the younger members of the clade (i.e. *V. calvum*, *V. concavum*, *V. spinosum*, *V. rhomboideum*), the archeopyle has shifted to include at least a part of the apical region. Judging from the molecular phylogeny (Fig. 1), this would have taken place multiple times. The records of all *Votadinium* species except for *V. psilodora* and possibly *V. calvum* are limited to the Holocene and Upper Pleistocene. *Votadinium calvum* has been reported from Miocene sediments (Londeix and Jan Du Chêne 1998), but that observation could not be confirmed since it was not illustrated. *Votadinium psilodora* consistently occurs from the late Eocene onwards. This mainly Neogene and younger occurrence of *Votadinium* cysts is in line with other protoperidiniacean groups, appearing after the decline of the Deflandreaceae in the Miocene (Bujak and Davies 1983). The late appearance of other *Votadinium* species may be due to a late radiation in the Plio/Pleistocene or poor preservation in the geological record. Though a poor preservation of peridinioid cysts was suggested by Zonneveld et al. (1997), the presence of e.g. *Votadinium psilodora* in Oligocene deposits shows that there are settings that allow exquisite preservation (Pl.4, Fig. 1–8). The Plio/Pleistocene is known as a period of intense global environmental change (e.g.,

Lisiecki and Raymo 2007; De Schepper et al. 2014), offering a drive for evolutionary radiations. This radiation may also be related to enhanced prey availability, since dinoflagellates of the '*P. oblongum*' species complex are known to feed on diatoms (Jacobson and Anderson 1986; Sherr and Sherr 2007), which had their highest diversities during this interval (e.g., Lewituss et al. 2018). More research is needed on the *Votadinium* fossil record and on the feeding habits and molecular phylogeny of *Oceanica* clade species to support this tentative evolutionary scenario. The morphological evolution of the motile stages of the *Oceanica* section started with the divergence from an ancestral *Protoperidinium* with an ortho 1' plate and three anterior intercalary plates, from which species with ortho-quadra arrangement diverged to form the *Oceanica* clade (Yamaguchi et al. 2006). A reduction in the number of anterior intercalary plates to just one has taken place in *P. latidorsale* and *P. steidingeriae*, in line with the general trend of plate reduction in dinoflagellate evolution (e.g., Bujak and Williams 1981).

4.2 Cyst wall chemistry of *Votadinium* and related genera

The cyst wall composition of *Votadinium spinosum*, *V. calvum*, *V. pontifossatum*, *Trinovantedinium pallidifulvum* and *Quinquecuspis concreta* are broadly consistent with the dinosporin compositions measured by Bogus et al. (2014) for cysts of heterotrophic dinoflagellates; that is, a carbohydrate-based macromolecule with nitrogen-containing functional groups. This result is also consistent with the known heterotrophic nature of the corresponding motile stages belonging to the genus *Protoperidinium*. The spectrum of *T. pallidifulvum* reported here differs from that of Mertens et al. (2017c), measured from cysts from the same sample location. Their spectrum shows a strong absorption peak around 850–900 cm⁻¹ while the spectrum reported here has its maximum absorption in the amide II (1570 cm⁻¹) band (Fig. 2, Table 3). It is at present not known whether this might be due to different

FTIR methods or to other factors (e.g., ecologic or taphonomic). More measurements are needed to resolve this, highlighting the need for a more systematic cataloguing of cyst wall chemistry.

The absorption at 1510 cm^{-1} in *Trinovantedinium applanatum* is the first record of this aromatic absorption in a dinoflagellate cyst. All reported spectra were measured from specimens prepared identically, and the 1510 cm^{-1} absorption was measured for specimens from different locations, so this is a species-specific feature. This is the most prominent absorption in the spectra of specimens from Germany and Izmir, and is clearly different from the amide II band in other specimens (sharper) and represents the C=C bond of single aromatic rings. This absorption has been indicative of sporopollenin (Watson et al. 2007; Jardine et al. 2015). Interestingly, *Trinovantedinium applanatum* is the only marine species with a transparent cyst wall that is known to be produced by a heterotrophic dinoflagellate (Reid 1977; Gu et al. 2015), and the absorptions characteristic for nitrogen-containing functional groups are present. Thus, this result both illustrates the utility of micro-FTIR in revealing the nutritional strategy of a species, as well as showing, for the first time, that there may be a sporopollenin-like component in some dinoflagellate cyst walls.

The PCA ordination of compositional data (Fig. 3) indicates that at least part of the cyst wall composition is species-specific. The remaining variability is arguably assigned to environmental or taphonomic differences, since specimens from the same location often plot closer to each other than specimens of the same species from other locations (e.g., the *T. applanatum* specimens from Brittany and the *Q. concreta* specimens from BC), or perhaps to cryptic speciation (Fig. 3). This compositional separation is further support for the taxonomy implied by morphology and molecular phylogeny and increases the potential of micro-FTIR in taxonomic studies (i.e. chemotaxonomy). For dinoflagellate cysts, this chemotaxonomy has

up to now been underexplored despite a similar approach being used successfully for other organic walled microfossils such as grass pollen (Julier et al. 2016).

5. Conclusions

The taxonomy of the dinoflagellate cyst genus *Votadinium* was stabilized in this study. The genus *Votadinium* previously had included four species: *Votadinium calvum* (cyst of *Protoperidinium latidorsale*), *Votadinium spinosum* (cyst of *P. claudicans*), *Votadinium elongatum* and *Votadinium nanhaiense*. *V. elongatum* and *V. nanhaiense* were re-illustrated from South China Sea (type region) material and the holotype of *V. spinosum* was re-illustrated. Additionally, the cysts '*Lejeunecysta psuchra*' and '*Lejeunecysta psilodora*' were re-studied, found to be synonymous, and transferred to the genus *Votadinium* as *V. psilodora*; the holotype was also re-illustrated.

Furthermore, two new *Votadinium* species, *V. pontifossatum* and *V. rhomboideum*, were erected here to encompass the cysts of *Protoperidinium paraoblongum* and *Protoperidinium quadrioblongum*, respectively, and a new type of ornamentation was described for *V. pontifossatum*. Another new species, *V. reidii*, was erected for the cyst of *Protoperidinium steidingerae* (Gribble et al. 2009), and a new cyst was described as *V. concavum*.

LSU rDNA sequences of the motile stage of *V. concavum* and a new sequence of *P. latidorsale* confirmed placement of these taxa in the *Oceanica* clade. As a consequence, the genus *Votadinium* includes all of the cysts produced by *Protoperidinium* species belonging to the *Oceanica* section reported by Sarai et al. (2013). Cyst-based taxonomy thus reflected the taxonomy of the motile-stage based on morphology and molecular phylogeny.

The cyst wall chemistry of *V. spinosum*, *V. calvum* and *V. pontifossatum* and related species *Quinquecuspis concreta*, *Trinovantedinium applanatum* and *T. pallidifulvum* was

evaluated using micro-FTIR spectroscopy. It is characterized in all but *T. applanatum* by a carbohydrate-based macromolecule with nitrogen-containing functional groups, consistent with the heterotrophic nature of the dinoflagellates involved. The spectra of *Trinovantedinium applanatum* indicate the presence of single aromatic rings, previously found in sporopollenin, and demonstrated for the first time in a dinoflagellate cyst species. This suggests that it is possible there is a sporopollenin-like component to the dinosporin comprising *T. applanatum*. This is an exciting finding, but will require additional investigation in order to understand its origin. Overall, the chemical composition of the cyst walls may also allow for species discrimination (chemotaxonomy), which is supported by morphology-based taxonomy and molecular phylogeny.

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Table 1. Generic characteristics of <i>peridinioid</i> dinoflagellate cysts that are bilaterally symmetrical, pentagonal and have one apical and two antapical horns.				
Genus	<i>Votadinium</i>	<i>Lejeunecysta</i>	<i>Quinquecuspis</i>	<i>Trinovantedinium</i>
Appearance
General shape	Dorsoventrally compressed, pentagonal to rounded.	Peridinioid with dorsoventral compression and a pentagonal ambitus. Epi- and hypocyst of approximately equal length	Pentagonal, peridinioid, acavate.	Proximochorate, protoperidiniacean, pentagonal to subcircular, dorsoventrally compressed.
Tabulation	Not expressed	Only indicated near the archeopyle and paracingulum.	Absent	Dorsally reflected by archeopyle, ventrally variable.
Wall structure	Smooth or microgranular single wall	Leaevigata to chagrinata.	Autophragm, acavate, thickens at apex and antapex	Biphragmal, with or without pericoels, usually closely adpressed; pericoel development not pronounced or spectacular. The surface of the phragma may be psilate or carry a simple ornament, but it does not possess any reticulum.
Wall thickness	Thin wall	Thin wall	Thick wall	-
Ornamentation	None, small processes or pontifossate* ornamentation.	None	None	Surface variably covered by short, penitabular and intratabular, but never sutural, solid or hollow spines or processes. Hollow processes may be distally open or closed
Paracingulum	No indentation, at most absence of ornamentation	A planar or very slightly helicoidal paracingulum is delimited by two folds and a shallow indentation. The folds are distally entire and may be continuous or partite. When partite, the paracingulum is separated into seven anterior and five posterior divisions.	Conspicuous and planar, usually delimited by low ridges	Well to poorly defined
Parasulcus	Absent to expressed as shallow depression	Marked by a shallow depression.	Very deeply indented, with reflection of flagellar pores	Well to poorly defined
Epicystal characters	Rounded apex or elongated apical horn.	Apex rounded or prolonged into a small distinct boss.	Conical or with shoulder	Lateral sides convex or straight, apex rounded or angular with or without apical horn or boss.
Hypocystal characters	Rounded broad or elongated antapical horns separated by a shallow or deep concavity.	Two approximately equal, symmetrically located horns which are small, pointed and solid.	Symmetric horns or flat antapically	Antapical horns symmetrical or asymmetrical
Archeopyle	Intercalary 1a or 2a, dorsal or involving apical section. Hexagonal or rounded.	Intercalary 2a and is symmetrically located on the middorsal line. It extends almost to the paracingulum. Operculum free or attached along the posterior margin.	Basically intercalary, ?2a, but may also involve apical plate ?3'	Simple hexa 2a intercalary archeopyle, operculum free.
Reference	Reid (1977); this paper	Bujak et al. (1980)	Harland (1977)	de Verteuil & Norris (1992)

* Defined in this paper.

Table 2. Location, coordinates, sample nature and references to literature for samples used in this study.

Sample ID	Locality	Latitude	Longitude	Sample nature	Reference(s)
Samples for taxonomy - LM					
UVic 2008-5-1	off Albert Head, near Metchosin, Strait of Juan de Fuca	48°23.226'N	123°28.214'W	Palynological slide, surface sediment	Krepakevich and Pospelova (2010)
UVic 2009-179-1	Patricia Bay, Saanich Inlet	48°39.997'N	123°29.014'W	Palynological slide, core sediment	Price and Pospelova (2011)
UVic 2009-408-1	Patricia Bay, Saanich Inlet	48°39.997'N	123°29.014'W	Palynological slide, sediment trap	Price and Pospelova (2011)
UVic 2012-197-1	Patricia Bay, Saanich Inlet	48°39.005'N	123°29.027'W	Palynological slide, surface sediment	Mertens et al. (2012)
UVic 2013-326-1	Patricia Bay, Saanich Inlet	48°35.554'N	123°30.034'W	Palynological slide, surface sediment	Price et al. (2016)
UVic 2015-622-2	Kyuquot Sound	50°05.463'N	127°09.046'W	Palynological slide, core sediment	Gurdebeke et al. (in prep.)
UVic 2015-684-2	Kyuquot Sound	50°05.463'N	127°09.046'W	Palynological slide, core top	Gurdebeke et al. (in prep.)
UVic 2014-112	South China Sea	20°01.965'N	115°27.882'E	Palynological slide, core sediment	Li et al. (2017)
UVic 2014-149	South China Sea	20°01.965'N	115°27.882'E	Palynological slide, core sediment	Li et al. (2017)
UVic 2015-287	South China Sea	22°39.817'N	119°55.342'E	Palynological slide, sediment trap	Li et al. (2018)
UVic 2017-11	South China Sea	18°38.210'N	111°14.480'E	Palynological slide, surface sediment	Li et al. (in prep.)
WN142	Bohai Sea	39°55.864'N	119°44.128'E	Culture experiment	Present study
Samples for taxonomy - SEM					
BV5 (SEM stub 16C6)	Vilaine estuary, Brittany, France	47°30.152'N	2°23.481'W	Residue for SEM, surface sediment	Mertens et al. (2009)
MV0923-PO-1	Vineyard Sound, Massachusetts	41°19.500'N	70°34.000'W	SEM mount from culture experiment	Gribble et al. (2009)
WN142	Bohai Sea	39°55.864'N	119°44.128'E	SEM mount from culture experiment	Present study
UVic 2009-659	Saanich Inlet	48°33.148'N	123°31.969'W	Residue for SEM, surface sediment	Price et al. (2016)
SCS-FCG	Fangchanggang Port, South China Sea	21°29.967'N	108°13.883'E	Residue for SEM, surface sediment	Li et al. (in prep.)
CJ17-B703. 9	South China Sea	21°19.503'N	111°10.452'E	Residue for SEM, surface sediment	Li et al. (in prep.)
Samples for micro-FTIR					
UVic 2012-31	Patricia Bay, Saanich Inlet	48°39.150'N	123°26.976'W	Residue for FTIR, surface sediment	This study
IB St. 29	Izmir Bay	38°27.08'N	27°08.28'E	Residue for FTIR, surface sediment	Mertens et al. (2015)
Schillig	German Wadden Sea	53°42.933'N	7°58.199'E	Residue for FTIR, surface sediment	Bogus et al. (2014)
UVic 16-320	Diana Lagoon, Corsica	42°7.660'N	9°31.720'E	Residue for FTIR, surface sediment	Luo et al. (2017)
BV5, 16C6	Vilaine estuary, Brittany, France	47°30.152'N	2°23.481'W	Residue for FTIR, surface sediment	Mertens et al. (2009)
UVic 2015-684	Kyuquot Sound	50°05.463'N	127°09.046'W	Residue for FTIR, core top sediment	Gurdebeke et al. (in prep)

Table 3. Absorption peaks identified in the micro-FTIR spectra.

Wave number (cm ⁻¹)	Origin	Interpretation
3600-3000	vOH	Broad band
3270-3100	vNH	
2925-2860	vCH	
1660	vC=O	Amide I band
1550-1570-1590	vCN+δNH	Amide II band
1510	C=C	Aromatic ring
1420	δCH ₂	δCH + δC-CH ₃
1370		
1312	vCN + δNH	Amide III band
1255	δNH	Ring
~1160	vC-O-C	
~1113	vC-O	
~1060	vC-O	Glucose ring
~1030	vC-O	
~900	γCH	β-glycosidic bond
698	γNH	Amide V band

Fig. 1. LSU rDNA phylogenetic tree of the family Protoperidiniaceae, with the three new sequences for *Votadinium concavum* and *Votadinium calvum* shown in bold and indicated by arrows. Node numbers indicate bootstrap support values (1000 replicates), * means these nodes are found in all replicates. [Genbank to be inserted in proof]

Fig. 2. Representative FTIR spectra of *Trinovantedinium applanatum*, *T. pallidifulum*, *Quinquecuspis concreta*, *Votadinium spinosum*, *V. pontifossatum*, *V. calvum*. Grey shading indicates the fingerprint region ($1700\text{--}800\text{ cm}^{-1}$) comprising the data used for the PCA analysis (see Fig. 3). See Table 3 for explanation of the absorption peaks.

Fig. 3. Ordination plot of principal components PC1 and PC2 of FTIR spectral data in the fingerprint range (1700–800 cm^{-1}). Genera and species are delineated.

Fig. 4. Line drawings of *Votadinium* species as discussed in this paper.

Plate 1. 1–7: *Votadinium calvum*. 1–3: LM, Kyuquot Sound (UVic 2015-684-1), dorsal view, incrementally lowering focus. 4–7: SEM, the Vilaine Estuary, Bretagne (16C6), 4: oblique apical view; 5 ventral view; 6: lateral view; 7: detail of sulcal area, arrows in 4–6 indicating archeopyle outline, arrow in 7 indicating flagellar scar. 8–10: *Votadinium concavum*. 8: Holotype, SEM, Bohai Sea, WN142. 9, 10: LM, Bohai Sea, WN142.

Plate 2. 1–6: *Votadinium elongatum* from the South China Sea, LM. 1, 2: specimens illustrated by He and Sun (1991, Pl. 2, Figs. 11, 12); 1: Holotype. 3, 4: UVic 2015-287. 5, 6: UVic 2017-11 . 7–9: *Votadinium nanhaiense* from the South China Sea. 7–9: Specimens illustrated by He and Sun (1991, Pl. 2, Figs. 15, 16 and 17); 8: Holotype. 10–12: UVic 2014-149, incrementally lowering focus. 13–15: UVic 2014-112, incrementally lowering focus. 1, 2 and 5–7 are reproduced with permission of Prof. He Chengquan.

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Plate 3. 1–13: *Votadinium pontifossatum* sp. nov. 1–4: LM, Holotype, 4–5 cm core sample, UVic 2009-179-1, EF. P38/2, incrementally lowering focus. 5–7: LM, Saanich Inlet, surface sediments, UVic 13-326-1. 8: LM, Kyuquot Sound, core sample UVic 2015-622, Holocene, folded ambitus. 9, 10: SEM, Saanich Inlet, surface sediments, UVic 2009-659, 9: folded ambitus, 10: detail of pontifossate ornamentation. 11–13: SEM, Fangchanggang Port, the South China Sea 17G04, surface sediment SCS-FCG, 3D preserved specimen. Scale bars represent 10 μm unless indicated otherwise.

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Plate 4. 1–11: *Votadinium psilodora* comb. nov. 1–8 Holotype, Middle Oligocene, Tönisberg (Germany), single grain slide 747/130, dorsal view, incrementally lowering focus; 9–11: Slide AK2-2, Akkeshi Bay, Hokaido Province, Japan, incrementally lowering focus. Arrow points to the sulcal depression, as in Matsuoka (1987).

Plate 5. 1–13: *Votadinium reidii* sp. nov., SEM, sample MV0923-PO-1 from Vineyard Sound, Massachusetts. 1–3: Holotype. 4–13: Illustration of variability. Arrow in Fig. 5 indicates flagellar scar in the sulcal area. Granulae appearing on the cyst wall, especially near (ant)apex is a methodological artifact from SEM coating.

Plate 6. 1–14: *Votadinium rhomboideum*. 1–8: Holotype, LM, Saanich Inlet UVic 2012-197-1 (EF R20/4). 9–14: SEM, the South China Sea CJ17-B703. 9, 10: ventral view, arrows in 9 indicate archeopyle trace; 11: detail of sulcal area showing flagellar scar; 12, 13: apical view; 14: oblique ventral-apical view. Scale bar 10 μ m.

Plate 7. 1–15: *Votadinium spinosum*. 1–4: Holotype, LM, re-illustrated from Reid (1977), slide 179K4. 5–8: LM, Saanich Inlet, UVic 2009-408-1, incrementally lowering focus; specimen with operculum in place and apex visible. 9–12: LM, off Albert Head near Metchosin in the Strait of Juan de Fuca, UVic 2008-5-1, with archeopyle visible, truncating the apex. 13–15: SEM, Saanich Inlet, UVic 2009-659; 13: ambitus, dorsal view; 14, 15: detail of wall and processes; arrow shows the minute fenestrae at the process bases. Scale bar 10 μ m unless indicated otherwise.