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PLATE PATTERN CLARIFICATION OF THE MARINE DINOPHYTE *HETEROCAPSA TRIQUETRA* SENSU STEIN (DINOPHYCEAE) COLLECTED AT THE KIEL FJORD (GERMANY)[1]

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One of the most common marine dinophytes is a species known as *Heterocapsa triquetra*. When Stein introduced the taxon *Heterocapsa*, he formally based the type species *H. triquetra* on the basionym *Glenodinium triquetrum*. The latter was described by Ehrenberg and is most likely a species of *Kryptoperidinium*. In addition to that currently unresolved nomenclatural situation, the thecal plate composition of *H. triquetra* sensu Stein (1883) was controversial in the past. To clarify the debate, we collected material and established the strain UTKG7 from the Baltic Sea off Kiel (Germany, the same locality as Stein had studied), which was investigated using light and electron microscopy, and whose systematic position was inferred using molecular phylogenetics. The small motile cells (18–26 µm in length) had a biconical through fusiform shape and typically were characterized by a short asymmetrically shaped, horn-like protuberance at the antapex. A large spherical nucleus was located in the episome, whereas a single pyrenoid laid in the lower cingular plane. The predominant plate pattern was identified as apical pore complex (Po, cp?, X), 4′, 2a, 6″, 6c, 5s, 5‴, 2⁗. The triradiate body scales were 254–306 nm in diameter, had 6 ridges radiating from a central spine, 9 peripheral and 3 radiating spines, and 12 peripheral bars as well as a central depression in the basal plate. Our work provides a clarification of morphological characters and a new, validly published name for this important but yet formally undescribed species of *Heterocapsa*: *H. steinii* sp. nov.

Key index words: **morphology; plate pattern; taxonomy; variability**

***Abbreviations*: APC, apical pore complex; BPP, Bayesian posterior probabilities; C, cingular plate; cp, cover plate; GTR, generalized time reversible; HMDS, hexamethyldisilazane; ICN, International code of nomenclature for algae, fungi, and plants; LBS, Maximum Likelihood bootstrap support; LF, longitudinal flagellum; ML, Maximum Likelihood; OTU, operational taxonomic unit; Po, pore plate; Sa, anterior sulcal plate; Sd, right sulcal plate; Sp, posterior sulcal plate; Ssa, anterior left sulcal plate; Ssp, posterior left sulcal plate; TF, transverse flagellum; X, canal-plate**

About 20 species have been assigned to dinophycean *Heterocapsa* (Iwataki 2008, Guiry 2017) including cosmopolitan bloom formers such as *H. rotundata* (Hansen 1989) and the toxic *H. circularisquama* (Nagai et al. 1996). The latter has caused severe bivalve mortalities in Japan in 1992 (Matsuyama et al. 1997) and since then, it is a serious

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threat to the mussel industry in western Japan and Hong Kong (Iwataki et al. 2002). Another species, currently known as *Heterocapsa triquetra*, is one of the most abundant bloom-forming dinophyte species in coastal and estuarine waters, with a wide distribution around the world (Carstensen et al. 2015). It is regularly recorded from the North Sea, the Baltic Sea, the North and South Atlantic, along the west and east coast of Greenland, the Mediterranean and in the eastern Pacific (Lohmann 1908, Lebour 1925, Braarud 1935, Grontved and Seidenfaden 1938, Braarud and Pappas 1951, Balech 1988). Dense blooming populations frequently occur in estuaries and harbor areas, but are also recorded from a brackish lake below thick surface ice (Baek et al. 2011). Typical bloom densities range from 1–20 $\times$ 10^6 cells $\cdot$ L^{-1} (Lindholm and Nummelin 1999, Litaker et al. 2002a, Tas 2016). Further studies of *H. triquetra* sensu Stein (1883) comprise a wide array of investigations in ecophysiology (Braarud and Pappas 1951, Litaker et al. 2002b), fatty acid composition (Matsuyama and Suzuki 1998), phagotrophy (Legrand et al. 1998), life-history (Olli 2004), vertical migration (Jephson et al. 2011), phylogenetics (Salas et al. 2014, Price and Bhattacharya 2017), and entire genome assessment (McEwan et al. 2008).

Heterocapsa triquetra sensu Stein (1883) is thus one of the most frequently encountered and best studied marine representatives of unicellular dinophytes. It is relatively small (ca. 16–30 μm long, 9–18 μm wide) but characteristic because of its unique fusiform shape. Furthermore, the horn-like hypothecal protuberance is a highly diagnostic trait making the recognition and identification of the species exceptionally easy despite its small size. In the initial descriptions and minute illustrations, Stein (1883) referred to thecal plates (and sutures between them) of the epitheca only. In fact, he regarded this hemispheric tabulation as the main difference between his new *Heterocapsa* and other thecate taxa recognized during his time, such as *Peridinium* (plates visible in light microscopy: LM) and *Glenodinium* (plates not visible in LM).

Heterocapsa triquetra sensu Stein (1883) became established fast (Bütschli 1885, Schütt 1895, Delage and Hérouard 1896, Lohmann 1908, Paulsen 1908, Meunier 1910, 1919). Schütt (1895) was the first who observed and depicted plates of the hypotheca, and was followed by Meunier (1919) showing hypothecal plates in ventral and dorsal views. Lindemann (1924) re-examined Stein's species based on plankton material from the Mediterranean Golden Horn, as well as from the Baltic Sea off Kiel and Rostock, and presented the complete tabulation pattern of both epi- and hypotheca for the first time in detail. He did not resolve cingular and sulcal plates, but his descriptions and figures corresponded to a Kofoidean formula of 4′, 2a, 6″ for the epitheca and 5‴, 2⁗ for the hypotheca. Shortly after, Lebour (1925) largely confirmed the plate pattern described by Lindemann (1924), considering that she regarded the midventral plate as belonging to the precingular series. Based on Lindemann (1924) and Lebour (1925), Schiller (1937) thus registered somewhat indecisively the plate pattern of *H. triquetra* sensu Stein (1883) as [sic] 4′, 2a, 7″, (6″), 5‴, 2⁗ in his seminal pre-war book.

The next important addition came in 1977 when, for the first time, Pennick and Clarke (1977) described the presence of three-dimensional body scales for *H. triquetra* sensu Stein (1883). Subsequently, Morrill and Loeblich (1981) detected similar scales in two species assigned to *Cachonina*. These scales are unique in their three-dimensional architecture, which led Morrill and Loeblich (1981) to aim at the comparison of the plate patterns in *H. triquetra* sensu Stein (1883) and in species assigned to *Cachonina*. Based on cultivated material they reported a large variability regarding the number of plates and described the "most common" plate formula of *H. triquetra* sensu Stein (1883) as having 2 pr, 5′, 3a, 7″, 6c, 7s, 5‴, 1p, 2⁗ (whereas "pr" refers to preapical plates; i.e., a pore plate Po and a canal plate X). This plate pattern notably consists of one additional plate in each of the three epithecal plate series in comparison to the results of Lindemann (1924). However, the discrepancy was not even mentioned by Morrill and Loeblich (1981). Their plate pattern determined for *H. triquetra* sensu Stein (1883) in fact is congruent to that of *Cachonina* (Loeblich 1968), and this congruence, together with the presence of the characteristic body scales, was the reasons to bring *Cachonina* into synonymy with *Heterocapsa* (Morrill and Loeblich 1981).

Seven years later, Balech (1988) published thecal plates of *H. triquetra* sensu Stein (1883) in detail based on material of South Atlantic origin. It remains unclear whether Balech (1988) was not aware of, or whether his practical work preceded the study of, Morrill and Loeblich (1981). In any case, his work using field samples basically confirmed the results of Lindemann (1924) for the conformation of epi- and hypothecal plates. Additional work on cingular and sulcal plates led Balech (1988) to conclude the complete plate formula as Po, 4′, 2a, 7″, 6c, 4s, 5‴, 2⁗. As Lebour (1925), Balech (1988) considered the plate in the midventral area as plate number 7 of the precingular series, whereas Lindemann (1924) regarded this area as part of the cingular and sulcal groove system. The latest revision of plate patterns goes back to the revisionary work of Iwataki (2002), who concluded the same plate pattern for all species of *Heterocapsa*, including *H. triquetra* sensu Stein (1883), namely Po, X, 5′, 3a, 7″, 6c, 5s, 5‴, 2⁗. Thus, the emendation of *Heterocapsa* (Iwataki et al. 2003) is in conflict with the delicate work of Lindemann (1924), Lebour (1925) and Balech (1988).

Parallel to this work, we became aware that *H. triquetra* is not only challenging because of divergent interpretations of the plate formula, but also because of a nomenclatural pitfall that has not been recognized for more than a century (Gottschling

et al. 2017). Briefly, the type of *H. triquetra* formally is that of its basionym, *Glenodinium triquetrum* (Ehrenberg 1840), being most likely a species of *Kryptoperidinium* (see the original drawing of Ehrenberg, *Glenodinium triquetrum* BHUMP drawing 674, available at http://download.naturkundemuseum-berlin.de/Ehrenberg/Ec%20Drawings/Ec%20draw%20001-999/Ec%20draw%20600-699/ECdraw674.jpg). Despite this actual nomenclatural ambiguity, we present a morphological clarification of divergent plate pattern concepts and other morphological details of the taxon Stein (1883) reported and depicted. An appropriate solution for the inference of the organism that Stein (1883) in fact studied more than a century ago is to collect at the same locality in the Baltic Sea off Kiel (Germany) to established monoclonal strains for thorough morphological and molecular investigations.

MATERIAL AND METHODS

Sampling, cell isolation, cultivation. A surface water sample (temperature: 20°C, salinity: 14.5) was taken at the Kiel Fjord (Germany) from a pier at 54.32° N and 10.15° E on August 7th, 2013. Single dinophycean cells were isolated by micro-capillary into 96-well plates filled with 0.2 mL filtered water from the sample site. Plates were incubated at 15°C under a photon flux density of 80 $\mu mol \cdot m^{-2} \cdot s^{-1}$ on a 16:8 h light:dark photocycle in a controlled environment growth chamber (Sanyo Biomedica MIR 252, Wood Dale, IL, USA). A total of five clonal strains of *Heterocapsa triquetra* sensu Stein (1883) (UTKG1, UTKG3, UTKG4, UTKG5, UTKG7) were established and subsequently grown at the culture conditions described above in a natural seawater medium consisting of sterile filtered (0.2 µm VacuCap filters; Pall Life Sciences, Dreieich, Germany) and diluted North Sea water with a salinity of ~16 containing nutrients corresponding to 50% of K-medium (Keller et al. 1987) slightly modified by omitting addition of ammonium ions. Strains were grown for subsequent DNA harvest by centrifugation (Eppendorf 5810R, Hamburg, Germany) in 50 mL centrifugation tubes at 3,220 *g* for 10 min. Cell pellets were transferred to 1 mL microtubes, then again centrifuged (Eppendorf 5415, 16,000 *g*, 5 min) and stored frozen (−80°C) for subsequent DNA extraction.

In addition to the strains from Kiel Fjord, plate pattern and plate variability in a field population of *Heterocapsa triquetra* sensu Stein (1883) were analyzed. Corresponding samples from a natural bloom were kindly provided by Rafael Salas (Marine Institute, Galway, Ireland). They were collected during the Irish Water Framework directive monitoring program in the Bandon lower river estuary in County Cork, Ireland (51.69° N 8.53° W), on July 23rd, 2012.

Microscopy. Observation of living or fixed cells (formaldehyde: 1% final concentration, or neutral Lugol-fixed: 1% final concentration) was carried out using an inverted microscope (Axiovert 200M; Zeiss, Munich, Germany) and a compound microscope (Axiovert 2; Zeiss), both equipped with epifluorescence and differential interference contrast optics. Light microscopic examination of thecal plates was performed on fixed cells (neutral Lugol) stained with calcofluor white (Fritz and Triemer 1985). Images were taken with a digital camera (Axiocam MRc5; Zeiss). Cell length and width were measured at 1,000× microscopic magnification using freshly fixed cells (neutral Lugol) from dense but healthy and growing strains (based on stereomicroscopic inspection of the living material) at late exponential phase and the Axiovision software (Zeiss).

For scanning electron microscopy (SEM), cells were collected by centrifugation (Eppendorf 5810R; 3,220 *g* for 10 min) from 2 to 15 mL of the culture, depending on cell density. The supernatant was removed and the cell pellet resuspended in 60% ethanol prepared in seawater (final salinity ca. 13) in a 2 mL microtube at 4°C for 1 h to strip off the outer cell membrane. Subsequently, cells were pelleted by centrifugation (Eppendorf 5415R; 16,000 *g* for 5 min) and re-suspended in a 60:40 mixture of deionized water and seawater (final salinity ca. 13) at 4°C for 30 min. After centrifugation and removal of the diluted seawater supernatant, cells were fixed with formaldehyde (2% final concentration in a 60:40 mixture of deionized water and seawater) and stored at 4°C for 3 h.

The following methods were applied to cultivated cells having pre-treated as described above as well as to formalin-fixed field samples: Cells were collected on polycarbonate filters (Millipore Merck, Darmstadt, Germany; 25 mm Ø, 3 µm pore-size) in a filter funnel, in which all subsequent washing and dehydration steps were carried out. A total of eight washing steps (2 mL MilliQ-deionized water each) were followed by a dehydration series in ethanol (30%, 50%, 70%, 80%, 95%, 100%; 10 min each). Filters were dehydrated with hexamethyldisilazane (HMDS), first in 1:1 HMDS:EtOH, followed by twice 100% HMDS, and then stored in a desiccator under gentle vacuum. Finally, filters were mounted on stubs, sputter coated (Emscope SC500, Ashford, UK) with gold-palladium and viewed under a SEM (FEI Quanta FEG 200, Eindhoven, the Netherlands). Micrographs were presented on a black background using Photoshop 6.0 (Adobe Systems, San Jose, CA, USA).

For transmission electron microscopy (TEM), cells from strain UTKG7 were concentrated in a microfuge tube by slow centrifugation (8 *g* for 1.5 min). The pellet was prefixed with 2.5% glutaraldehyde in filtered seawater (salinity 16) at 4°C for 30 min. Cells were washed twice in filtered seawater before postfixation with 1% OsO_4 in filtered seawater at room temperature for 40 min. Fixed cells were dehydrated through a graded series of ethanol (30%, 50%, 70%, 85%, 90%, 95%, 2× 100%; 10 min each), followed by 2× 100% propylene oxide, infiltrated with propylene oxide-resin mixtures (2:1, 1:1, 1:2), and embedded in EMBed-812 resin (Science Services, Munich, Germany). The block was polymerized at 60°C for 22 h and sectioned with a diamond knife on a Reichert Ultracut microtome (Reichert-Jung, Vienna, Austria). Thin sections were directly viewed under an EM 902A TEM (Zeiss) operated at 80 kV. Digitized images were taken with a 1 k Proscan High Speed SSCCD camera (Proscan, Lagerlechfeld, Germany) operated by the iTEM Five software (Olympus, Münster, Germany).

For negative staining TEM, 50 µL of an old culture were used. The detached body scales were allowed to adsorb onto Formvar-coated grids for 5 min. The grids were stained with 1% (w/v) uranyl acetate for 1 min, washed in two drops of distilled water and air-dried. The sample was investigated in a JEM2100F TEM (Jeol, Tokyo, Japan) operated at 120 kV. The cameras Orius SC200D and Orius SC600 CCD were operated using Digital Micrograph software (Gatan, Pleasanton, CA, USA). The body scale structure of *H. triquetra* sensu Stein (1883) was analyzed using the morphological descriptors and definitions of Iwataki et al. (2004).

DNA sequencing and molecular phylogenetics. Genomic DNA was extracted from cell pellets with a NucleoSpin® Plant II Kit (Macherey Nagel, Düren, Germany) according to the manufacturer's instructions. Various ribosomal RNA loci (rRNA; 18S or small subunit: SSU; Internal Transcribed Spacer region including ITS1, 5.8S rRNA, ITS2: ITS; D1/D2 region of 28S or large subunit: LSU) were amplified from total DNA by polymerase chain reaction (PCR). Primers and PCR setting corresponded to the descriptions in Tillmann et al. (2017). To assess intragenomic variability, ITS and LSU PCR products were purified,

cloned into a TOPO® TA sequencing vector (Invitrogen, Life Technologies, Darmstadt, Germany) and transformed into One Shot® TOP10 chemically competent *Escherichia coli* (Invitrogen). Purified plasmids of several positive bacterial colonies were sequenced using M13 primers on an ABI 3130XL Genetic Analyzer (Applied Biosystems, Darmstadt, Germany). Forward and reverse sequences were assembled into a contig and edited using the program Sequencher 5.1 (Gene Codes Corporation, Ann Arbor, MI, USA). In total, 57 new GenBank sequences were deposited in the course of the study (Table S1 in the Supporting Information).

For alignment constitution, we defined the four regions of the rRNA: SSU, ITS, LSU D1→D2, LSU D3→D10, and included virtually all rRNA sequences available for Heterocapsaceae including many GenBank entries (Table S1). The data matrix included 13 of 16 known species of *Heterocapsa* (81%), and nine of them (56%) were represented by type material or equivalents. As outgroup, we compiled all Peridiniales and Amphidomataceae, from which SSU+ITS+LSU sequences were complete. Where available, we added sequences from nuclear (β-tubulin), mitochondrial (MT-CYB, MT-CO1) and plastid (*psb*A) loci, which have been identified suitable for phylogenetic analyses (Saldarriaga et al. 2003, Zhang et al. 2007, Fukuda and Endoh 2008, Orr et al. 2012, Fawcett and Parrow 2014). Not-homologous mitochondrial gene copies (Orr et al. 2012) were treated separately. Single-locus matrices were aligned using "MAFFT" v6.502a (Katoh and Standley 2013) and were concatenated afterward. The absence of significantly contradicting phylogenetic signals between loci was confirmed by single-partition analyses. The aligned matrix is 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) and using the resources available from the CIPRES Science Gateway (Miller et al. 2010). The Bayesian analysis was performed using "MrBayes" v3.2.2 (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 ML calculation, the MPI version of "RAxML" v8.0.24 (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

Morphological description. Using light and epifluorescence microscopy, all five clonal strains were identical in terms of morphology and plate pattern (observed on calcofluor stained cells). The selected strain UTKG7 will be described and depicted in detail including size measurements and SEM. Motile cells were biconical through fusiform, slightly elongated and somewhat irregular in outline (Fig. 1). Cells ranged from 17.8 to 25.9 μm in length (mean length: 21.3 ± 1.6 μm, $n = 157$) and 13.0–17.6 μm in width (mean width 15.0 ± 1.0 μm, $n = 157$), with a mean length/width ratio of 1.4 ($n = 157$). The dome-shaped epitheca was slightly larger than the hypotheca. The latter was variable in shape, ranging from conical (Fig. 1, A and O) through more pyramidal (Fig. 1, B, C, N) and irregularly acuminate (Fig. 1, D–F, P–Q), and typically had a small, asymmetrically shaped, horn-like posterior protuberance (Fig. 1, D–F, Q–S). The cingulum was incised and wide, slightly descending and confined by small lists (Fig. 1, N–S). The broad sulcus did not exhibit any list (Fig. 1, N and S).

A large and spherical nucleus occupied most of the epitheca (Figs. 1, A–E; 2A) and contained thick, dinokaryotic chromosomes (Fig. 2A). Sometimes, a nucleolus was visible (data not shown). A single spherical pyrenoid surrounded by a starch sheath was located in the hypotheca (Figs. 1, A, C, D, F; 2A). The pyrenoid matrix was penetrated by many cytoplasmic tubules being invaginations of the pyrenoid envelope (Fig. 2, B and C), but not by thylakoids. Associated starch (the surrounding starch sheath visible in LM) was not detected by TEM. A presumably single, but multiply lobed and retiform, brownish chloroplast was parietally located in both epi- and hypotheca (Figs. 1, G–I; 2A). The chloroplast contained parallel arranged lamellae (Fig. 2, A–B, D) consisting of thylakoids in stacks of three (Fig. 2E). Trichocysts were scarce (Fig. 2D), and mitochondria had tubular cristae (Fig. 2F).

Cells were covered by a sturdy theca (Fig. 1D), whose plates were visible in LM (Fig. 1, L and M). Dividing cells kept their motility, and division was by desmoschisis (i.e., the parent theca was shared by the two daughter cells; Fig. 1, J and K). Thecal plates were separated along an oblique fission line separating an anterosinistral from a posterodextral part (Fig. 1K). The basic thecal plate formula was initially determined by fluorescence LM using calcofluor white (Fig. 1, L and M) and in more detail by SEM (Figs. 1, N and O; 3; 4) being APC (Po, cp?, X), 4′, 2a, 6″, 6c, 5s, 5‴, 2⁗. The basic plate pattern is schematically illustrated in Figure 3. Thecal plates' surface was smooth and free of any ornamentation. A number of small pores were present that were mainly arranged adjacent to the sutures (Fig. 1, N and O). The small X-plate was consistently free of pores (Fig. 4, G and H).

Within the epitheca (Fig. 4), four apical plates surrounded the apical pore plate. The ventral (1′) and dorsal (3′) apical plates were hexagonal and triangular at their distal ends. The lateral apical plates 2′ and 4′ were hexa- and octagonal, respectively, with plate 4′ being distinctly larger in size. Two large anterior intercalary plates of almost the same size were located dorsally. Plate 1a was hexagonal and was in contact to three precingular plates, whereas plate 2a was pentagonal and in contact with two precingular plates. Among the series of six precingular plates, plate 3″ was the narrowest. The most ventral plate in the precingular series was considered as the anterior sulcal plate.

The apical pore complex (APC; Fig. 4, F–I) was composed of a round through slightly rectangular pore plate (Po). On its ventral side, a small plate

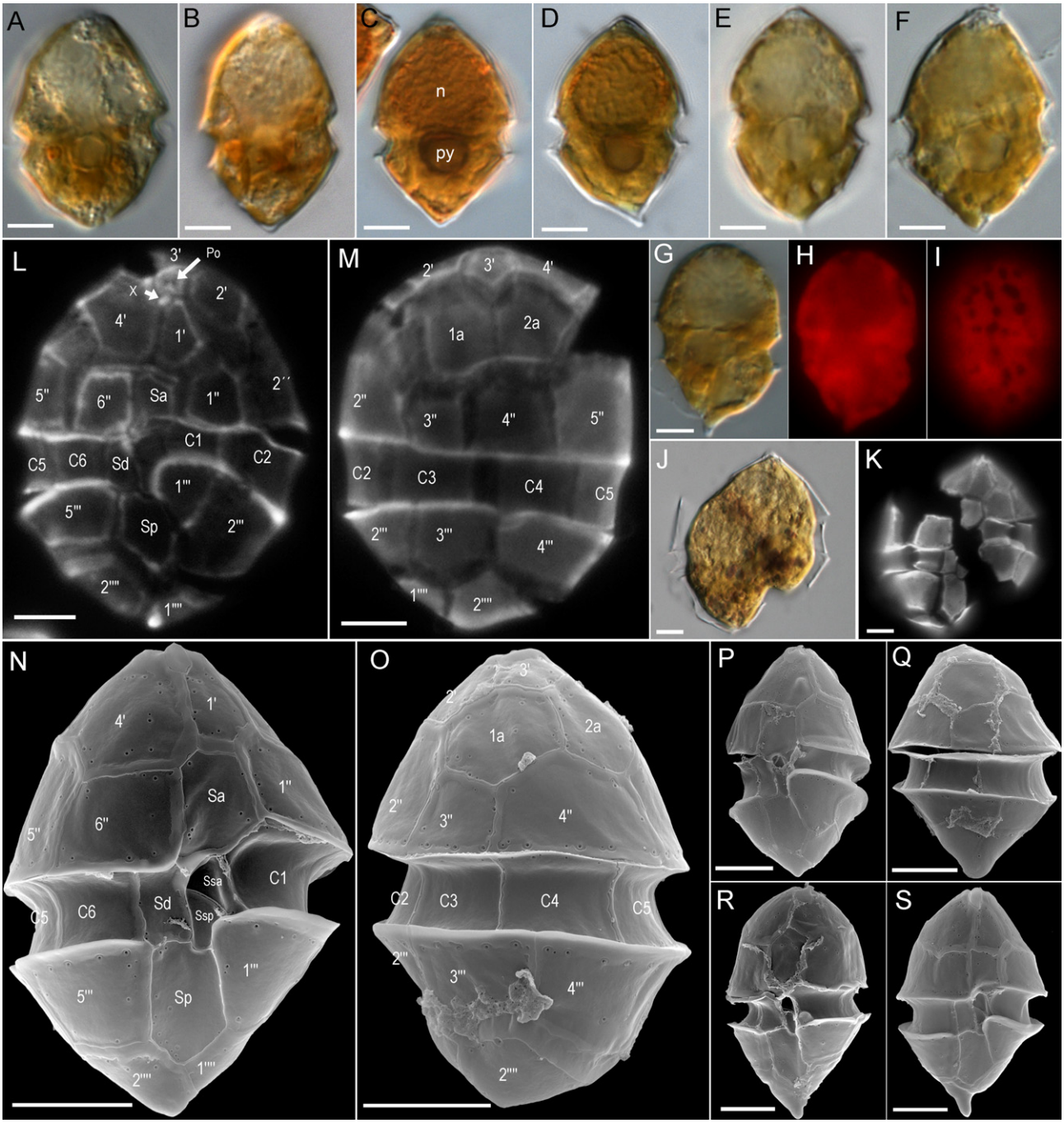

FIG. 1. Light microscopy (A–M) and electron microscopy (N–S) images of *Heterocapsa triquetra* sensu Stein (1883) (strain UTKG7). (A and B) Living cells. (C and D) Lugol-fixed cells showing the anterior position of the large nucleus (n) and the posterior position of the pyrenoid (py). (E–I) Formalin-fixed cells. (J–M) Lugol-fixed cells stained with calco-fluor in brightfield (J) and with UV excitation (K–M), with J and K showing the same cells. (G–I) The same cell in brightfield (G) or in two focal planes with epifluorescence (H and I), where chlorophyll autofluorescence indicated chloroplast structure. (L–S) Total view of different cells in ventral (L, N, P, R and S) or dorsal (M, O, Q) view. Plate labels according to the Kofoidean system. Scale bars = 5 μm. [Color figure can be viewed at wileyonlinelibrary.com]

was located, which we regarded as the X- or canal plate. This plate was generally small, but variable in size and shape when seen from outside but in internal view, it was always narrow and rectangular (Fig. 4I). The X-plate was slightly displaced to the cell's right hand side (abutting the apical plates 1′ and 4′), but still allowed contact of plate 1′ to the pore plate. The apical pore was located in the center of the Po plate. A roundish rim in the middle of the pore plate was present. When observed from inside, however, the actual pore seemed to be rather small. It was somehow covered by a plate-like

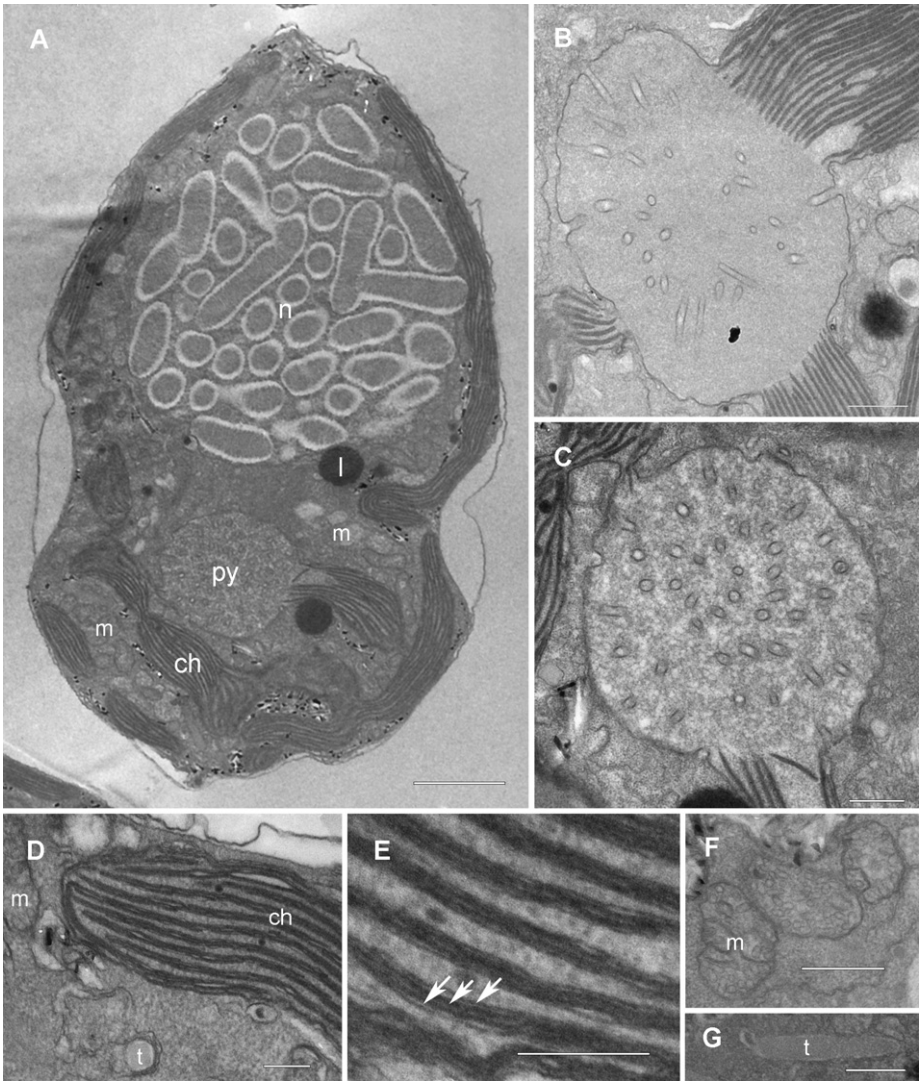

FIG. 2. Transmission electron microscopy of *Heterocapsa triquetra* sensu Stein (1883) (strain UTKG7). (A) Longitudinal section through a cell showing the large nucleus (n), the pyrenoid (py) without starch sheath, the chloroplast (ch), mitochondria (m), and lipid droplets (l). (B, C) Details of the pyrenoid with many tubular invaginations. (D, E) Details of the chloroplast (ch) with parallel lamellae consisting of thylakoids in stacks of three (arrows). (F) Mitochondria (m) with tubular cristae. (G) Trichocyst (t) in longitudinal section. Scale bars = 2 μm (A), 500 nm (B and C, F and G), 200 nm (D, E).

structure extending from the X-plate to the apical pore. In lateral view, this structure always seemed to shield rather than to tightly cover the pore (Fig. 4F).

The hypotheca (Fig. 5, A–C) consisted of five postcingular plates, with plate 4‴ being the widest. The two antapical plates were different in size, of which plate 2⁗ was the larger one bearing the horn-like antapical projection. The cingulum (Fig. 5, B and C) was composed of six cingular plates having all comparable size. In the sulcus (Fig. 5, C–E), five plates were identified. The large anterior sulcal plate (Sa) extended into the epitheca (Figs. 1, L and N; 4D). Usually, this plate contacted both apical plates 1′ and 4′ (Fig. 1N) but rarely, the suture between Sa and 4′ was short and almost indiscernible (Fig. 1S). The posterior sulcal plate (Sp) was large and pointed in its distal part and extended into the hypotheca for more than 2/3 of its height (Fig. 5C). A right sulcal plate (Sd) completed the cingulum from the right lateral side. The left lateral side of the central sulcal area was formed by two plates, an anterior and a posterior left sulcal plate (Ssa and Ssp). The anteriorly located Ssa plate formed an inwardly directed concave pocket (Figs. 1N; 5, D and F), the cavity from which both flagella emerged (Fig. 5G). On the anterior end of this pocket, there was an additional structure most clearly visible from internal view (Fig. 5, D and E). Its granular and wrinkled appearance was distinctly different from all other plates being smooth and plane. It is thus not considered to represent another sulcal plate herein (see Discussion).

Plate overlap (Fig. 3, C and D) was identified individually for each suture by inspecting cells with slightly dissociated plates, those with clearly visible growth bands, and by internal theca views as well (examples are given in Fig. S1 in the Supporting Information). Keystone plates (i.e., those overlapping all their neighbors) were the dorsal plates 4″ and 3‴ for the precingular- and postcingular series, respectively. With respect to the keystone plate of the cingular series, two different morphae were observed, namely cells having either plate C3 or C4

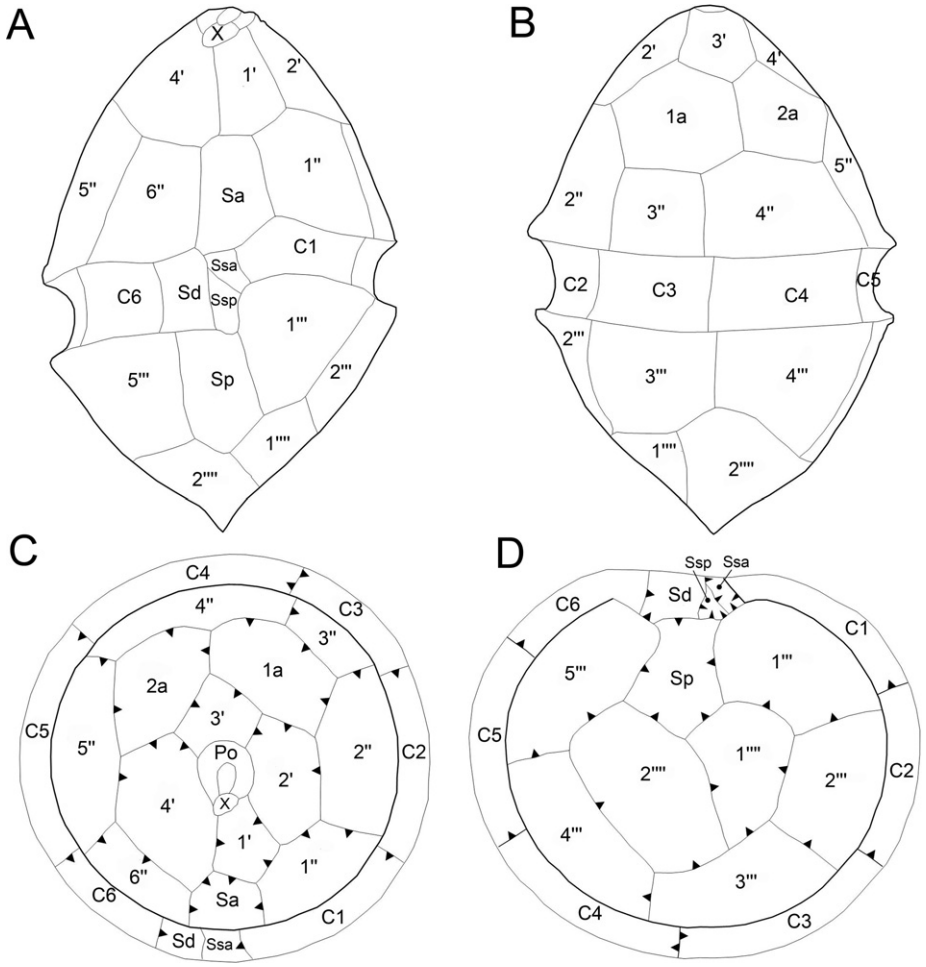

FIG. 3. Thecal plate pattern (schematic) of *Heterocapsa triquetra* sensu Stein (1883) (strain UTKG7). (A) Ventral view. (B) Dorsal view. (C) Apical view. (D) Antapical view. Plate labels according to the Kofoidean system. Arrowheads in C and D indicate direction of plate overlap. Two arrowheads on the suture of plates C3 and C4 indicate that both, cells having C3 or C4 as keystone plate of the cingulum, were observed.

overlapping all adjacent cingular plates (Fig. S1, G–L). On the epitheca, the left intercalary plate 1a overlapped plate 2a. The mid-ventral plate Sa was overlapped by all adjacent plates of the epitheca. On the hypotheca, the second antapical plate 2′′′′ overlapped plate 1′′′′. Among sulcal plates, the large posterior sulcal plate was overlapped by all hypothecal plates, and the small plate Ssa was overlapped by the adjacent plates Sd, Ssp, 1′′′ and C1 (Fig. 5E). Overlap of the sulcal plates Sd and Ssa to the plates of the epitheca (6′′, Sa) could not be uncovered unequivocally.

In strain UTKG7, deviations from the abundant plate pattern described above were observed, as it is exemplarily shown in Figures S2–S4 in the Supporting Information. To quantitatively estimate the number of plates in each series, a SEM stub was systematically scanned, and the number of plates in each series was scored for all cells, in which all plates of a series were visible. The results are summarized in Table 1. Plate number variability was highest for the apical series, in which 22.5% of cells with five instead of four apical plates were encountered. For the intercalary and precingular series, the amount of deviating plate numbers was 18.6% and 14.4%, respectively. Variability in plate number was lower for hypothecal plates (11.7% and 5.9% for the postcingular and antapical series) and for the cingulum (5.9%). Variations in the plate pattern primarily resulted from additional sutures between plates (Figs. S2 and S4), but a reduced number of plates due to plate fusions (Figs. S3 and S4) was also observed. Table 1 also summarizes the results for thecate cells in a natural population of *H. triquetra* sensu Stein (1883) (see also Fig. S5 in the Supporting Information), in which the number of plates in each series was rather consistent. The presence of three instead of two intercalary plates, of seven instead of six precingular plates, and of four instead of five postcingular plates was observed once out of >50 cases. Within the apical, antapical, and cingular series, no deviating pattern was observed.

Body scales were 254–306 nm in diameter (Fig. 6) and had an obtusely triangular basal plate with finely reticulate perforations (Fig. 6, A and B). Six ridges on the basal plate radiated from a large central upright (spine) to peripheral shorter uprights (spines; Fig. 6, B and C). At the triangle's corners, peripheral short spines were additionally present (Fig. 6, D and E), as though the scale had nine

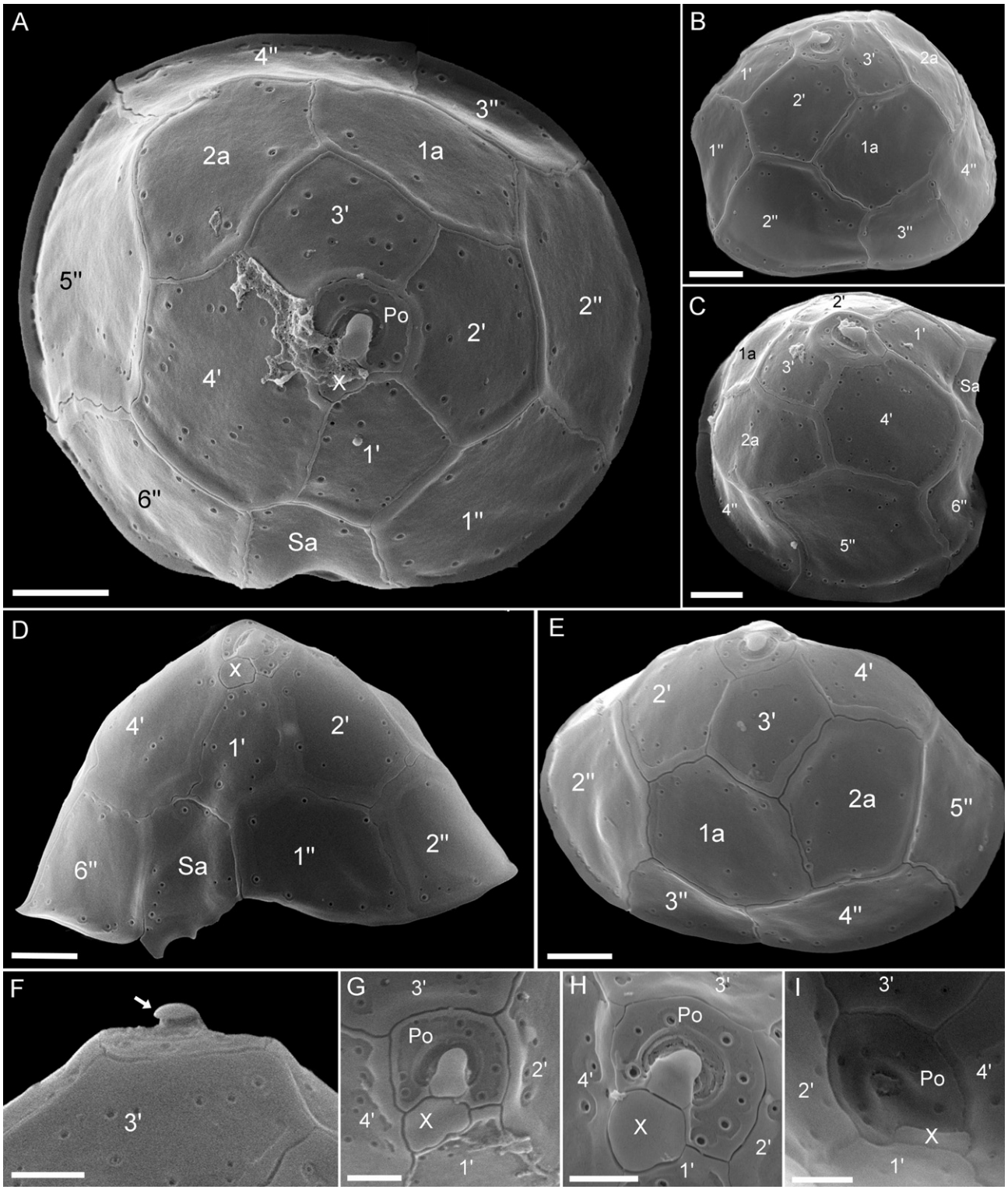

FIG. 4. Electron microscopy of *Heterocapsa triquetra* sensu Stein (1883) (strain UTKG7). (A–E) Epithecal plates in (A) apical view, (B) left lateral view, (C) right lateral view, (D) ventral view, and (E) in dorsal view. (F–I) The apical pore complex in (F) dorsal view, (G–H) apical view, and (I) in internal view. White arrow in F indicates the plate-like structure of the APC which like an umbrella seem to shield the apical pore. Scale bars = 2 μm (A–E), 1 μm (F–I).

peripheral spines. Three peripheral spines in each triangle corner were directly connected with peripheral bars (Fig. 6, D–F). At a particular level, three bars radiated from the large central spine terminating in a radiating spine (Fig. 6, A–E). These three radiating spines were each connected by peripheral

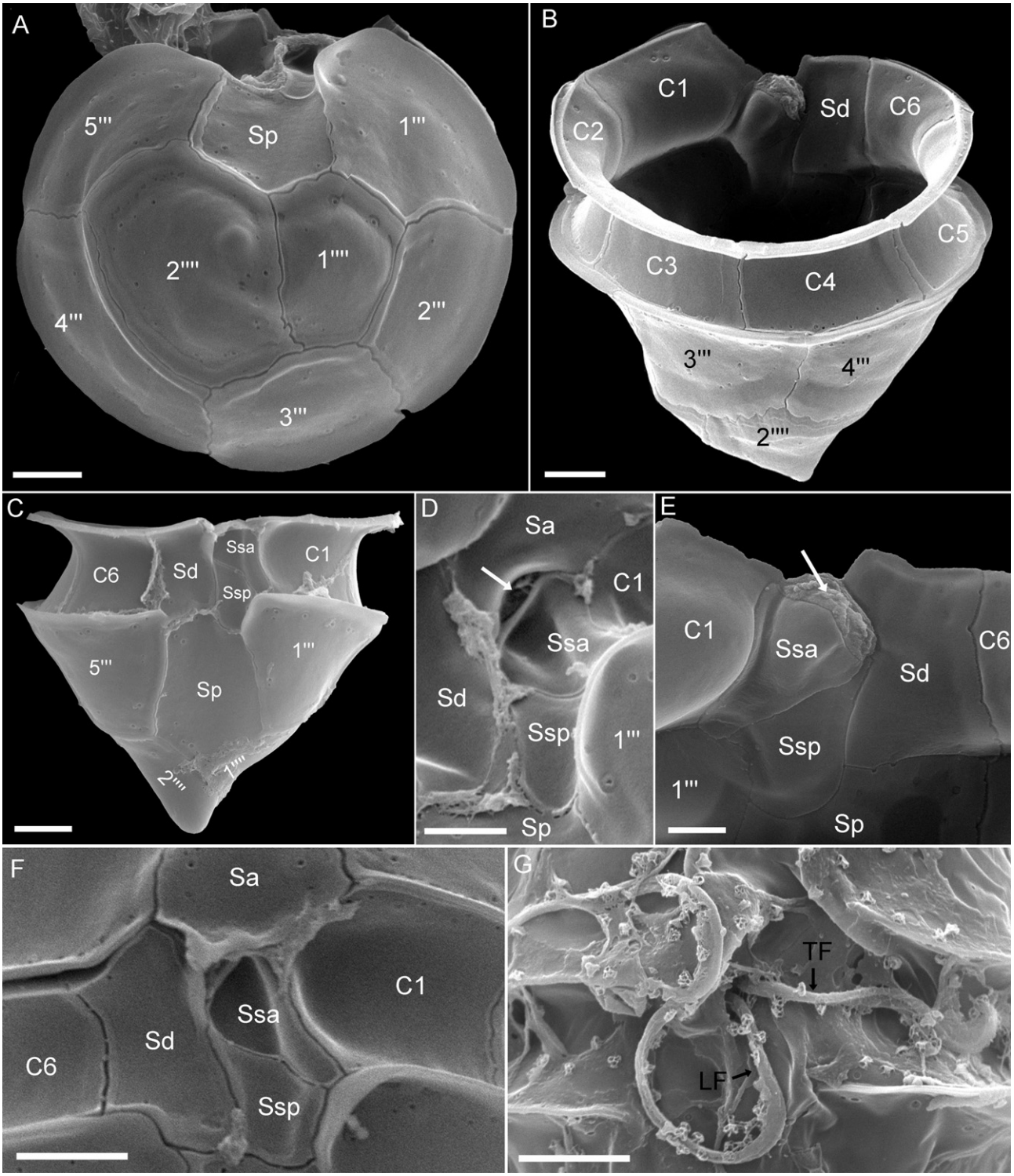

FIG. 5. Electron microscopy of *Heterocapsa triquetra* sensu Stein (1883) (strain UTKG7). (A) Hypothecal plates in antapical view. (B) Cingular plates in dorsal view. (C) Hypothecal plates in ventral view. (D–E) Sulcal plates in ventral (D) or in internal (E) view. White arrows in D and E point to an additional structure of the sulcus not considered to represent a sulcal plate. (F, G) Detailed view of the sulcal area to illustrate the position where both flagella (LF, longitudinal flagellum; TF, tranverse flagellum) emerge. Scale bars = 2 μm (A–C, F and G), 1 μm (D and E).

bars with peripheral spines (Fig. 6, C–F). In sum, each scale had twelve peripheral bars. In TEM (Fig. 6, A–C), the central cavity of the basal plate was not observed as in SEM. In SEM, scales in upside-down orientation revealed the presence of a small central depression in the basal plate (Fig. 6, D

and E). This depression was located directly below the large central upright. Whether this depression was the basal opening of a hollow central spine or a depressed area below the spine with sunken basal plate, or in fact a cavity in the basal plate, was not discernible.

Molecular phylogeny. The alignment was 11,715 bp long and comprised 2,260 parsimony informative sites (19%, 10.23 per terminal taxon) and 4,029 RAxML distinct alignment patterns. Figure 7 (as cut-off of Fig. S6 in the Supporting Information) shows the best-scoring ML tree (−ln=85,261.36), with *Heterocapsa* retrieved as monophyletic (100LBS, 1.00BPP). The internal topology was not always well supported, but a number of lineages could be distinguished corresponding to established species of *Heterocapsa*. They included either OTUs corresponding to a single voucher (*H. huensis*, *H. lanceolata*, *H. ovata*, *H. psammophila*) or forming clades of several accessions (*H. arctica*: 100LBS, 1.00BPP, *H. horiguchii*: 100LBS, .99BPP, *H. illdefina*, *H. minima*: 100LBS, 1.00BPP, *H. niei*: 76LBS, *H. pseudotriquetra*: 71LBS, 1.00BPP, *H. pygmaea*). Furthermore, sequences from 16 old and newly collected strains constituted a monophyletic group together with all 5 Kiel strains obtained here, including strain UTKG7 (100LBS, 1.00BPP). Branch length variation between different strains as well as different LSU and ITS clones of UTKG7 was overall low within this clade. The sister species of *H. triquetra* sensu Stein (1883) was *H. pseudotriquetra* (94LBS, 1.00BPP) represented by six different vouchers.

Sequences from *Heterocapsa circularisquama* and *H. rotundata* only did not constitute monophyletic groups because of sequences that did not overlap in the alignment. A few species determinations of GenBank entries were to be corrected (e.g., *H. pseudotriquetra* instead of *H. triquetra* for strain KJ34-3-05, *H. pygmaea* instead of *C. hallii* for strain NCMA2770). Sequences of strain NCMA448 as inferred from corresponding GenBank entries (Table S1) were polyphyletic and were assigned to either *H. triquetra* sensu Stein (1883; GU594638, AF527816, EU165307) or *H. pygmaea* (AF352363, AF352364). The DNA tree also presented a considerable species diversity within *Heterocapsa* that has been formally not recorded at present (i.e., many GenBank determinations as "*Heterocapsa* sp." or "Heterocapsaceae sp.").

DISCUSSION

Re-collection of an old species. The cells of strain UTKG7 are consistent to a high degree with the description and the drawings of *Heterocapsa triquetra* sensu Stein (1883; p. 13, pl. III, figs. 30–40). This consistency refers to the general shape with the horn-like, hypothecal protuberance and to the position of the nucleus in the epitheca, although the size of the nucleus as drawn by Stein (1883) is rather small (Fig. 8A; "n" in his pl. III, fig. 30). Consistency, moreover, refers to the arrangement and size of epithecal plates between Stein's drawings and our observations of strain UTKG7 (Fig. 8B). *Heterocapsa triquetra* sensu Stein (1883) has been continuously documented at the Baltic Kiel Fjord over the past century (Lohmann 1908, Lenz 1977, Wasmund et al. 2008). The high abundances as reported by Stein (1883) and Lohmann (1908) seem to be reversed nowadays (Wasmund et al. 2008), but environmental conditions may have changed over the past 100 years as though this observation is not surprising.

Morphological descriptions of *Heterocapsa triquetra* sensu Stein (1883) in the literature support the view that they all refer to the same species, but some minor inconsistencies in the various descriptions and illustrations must be stated. The size range of

TABLE 1. Numbers of thecal plates in each series (values in percentages, N = total number of cells examined) of cells harvested from *Heterocapsa triquetra* sensu Stein (1883) strain UTKG7 (upper section) and of cells harvested from a natural population of *Heterocapsa triquetra* sensu Stein (1883) collected at the Bandon lower river estuary, Ireland (lower section). Gray shades mark the dominant plate number in each series.

	Number of plates							
Plate series	1	2	3	4	5	6	7	*N*
Strain UTK G7								
Apical plates	–	–	4.6	69.5	22.9	3.1	–	131
Anterior intercalaries	2.3	81.4	16.3	–	–	–	–	129
Precingular plates	–	–	–	–	1.7	85.6	12.7	118
Postcingular plates	–	–	–	4.9	88.3	6.8	–	103
Antapical plates	1.0	94.2	4.9	–	–	–	–	103
Cingular plates	–	–	–	–	1.5	94.1	4.4	135
Field sample, Ireland								
Apical plates	–	–	0	100	0	–	–	53
Anterior intercalaries	0	98.1	1.9	–	–	–	–	54
Precingular plates	–	–	–	–	0	98.1	1.9	52
Postcingular plates	–	–	–	1.9	98.1	0	–	54
Antapical plates	0	100	0	–	–	–	–	54
Cingular plates	–	–	–	–	0	100	0	51

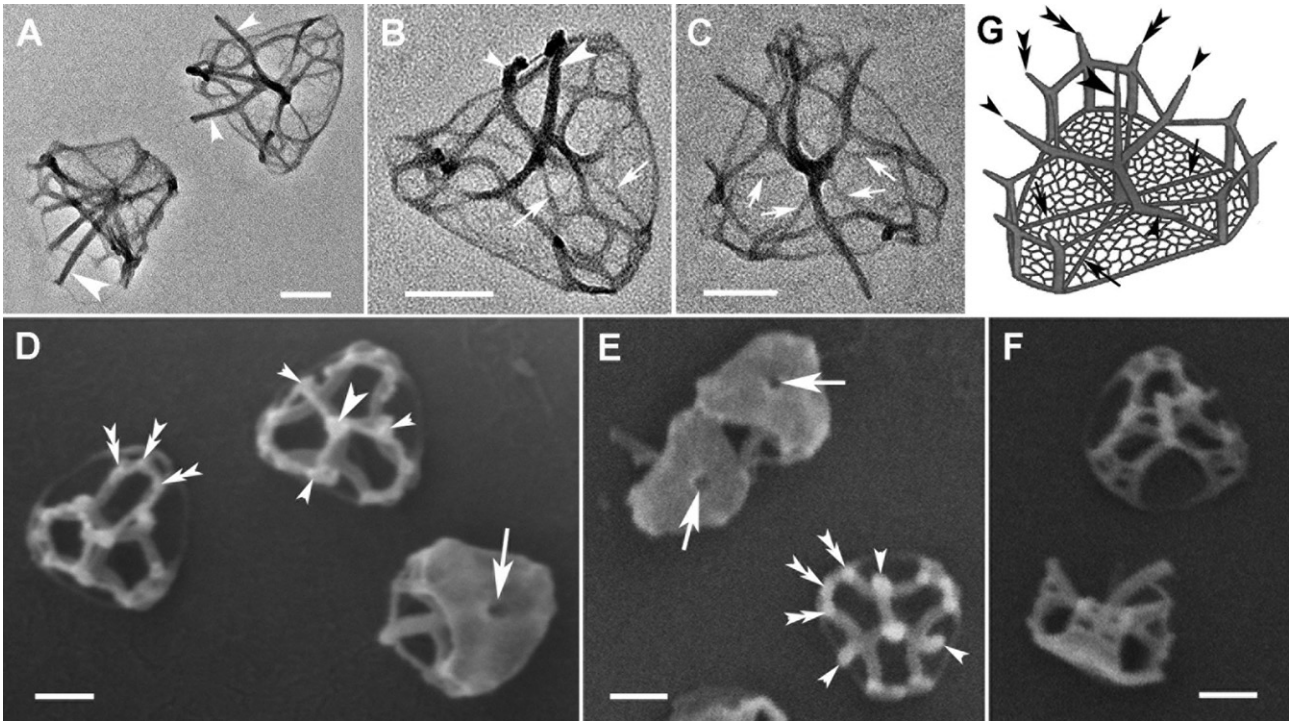

FIG. 6. Body scale morphology of *Heterocapsa triquetra* sensu Stein (1883) (strain UTKG7) as observed by transmission (A–C) and scanning electron microscopy (D–F). (D–E) Note the central depression in the basal plate (large arrow). Scale bars = 100 nm. (G) Scale reconstruction (Iwataki et al. 2004). Large arrowhead = central upright (spine), large arrow = central depression in the basal plate, small arrowhead = radiating spine, small double-arrowhead = peripheral spine, small arrow = ridge.

the strain from Kiel Fjord (18–26 μm in length) reflects very well size ranges given in the literature though rarely, a non-overlapping larger size range has been reported (Paulmier 1992; 38–43 μm cell length). *Heterocapsa triquetra* sensu Stein (1883) has a characteristic shape with a horn-like hypothecal protuberance. *Scrippsiella ramonii* has a similar horn in the hypotheca but, apart from the different plate pattern, is slightly larger with a lower length/width ratio, is dorso-ventrally compressed, and has the nucleus located in the cingular level (Montresor 1995). In the Kiel Fjord material of *H. triquetra* sensu Stein (1883), this horn and its development is variable from nearly unrecognizable through elongated, finger-like and –if present– without exception part of the second antapical plate (i.e., right lateral part of the antapex). The position of the posterior horn has not always been documented carefully. Both, Schütt (1895) and Meunier (1919) have shown the two conformations in different figures, on the left lateral side (Schütt: fig. 62.1; Meunier: figs. 47, 49) or on the right lateral side (Schütt: fig. 62.4; Meunier: fig. 48). Dodge (1982) has drawn it correctly in ventral view, but on the wrong plate in dorsal view. As this putative variability in the position of the posterior horn has been documented by drawings only, it is interpreted here as inaccuracy but not as real morphological variability.

Heterocapsa triquetra sensu Stein (1883) divides by desmoschisis (Braarud and Pappas 1951, Morrill and Loeblich 1981), with the same oblique fission line similar to other species of *Heterocapsa* (Loeblich et al. 1981, Morrill and Loeblich 1984) and other dinophyte species (Tillmann and Elbrächter 2013). However, the life-history of *H. triquetra* sensu Stein (1883) is poorly studied so beyond some vague and ambiguous reports of "spore formation" (Paulsen 1908, Lebour 1925), more detailed documentation of "temporary cyst formation" (Olli 2004), and the formation of thick shelled and spiny coccoid cells in culture (Braarud and Pappas 1951). All these observations are in need of confirmation by detailed studies of the *H. triquetra* sensu Stein (1883) life-history.

The principal plate pattern. The re-investigation enables us to clarify a long-lasting debate about the correct tabulation pattern present in *H. triquetra* sensu Stein (1883). The dominant plate pattern is APC (Po, cp?, X), 4′, 2a, 6″, 6c, 5s, 5‴, 2⁗ and confirms the interpretations of Lebour (1925), Campbell (1973), Balech (1988) and Lewis and Dodge (1990) (epithecal plates only are here reported). The plate pattern of *H. triquetra* sensu Stein (1883) also agrees largely with the work of Lindemann (1924), but he considered some variation in the species, leading to the formal descriptions of new varieties and forms. He put particular attention to (i)

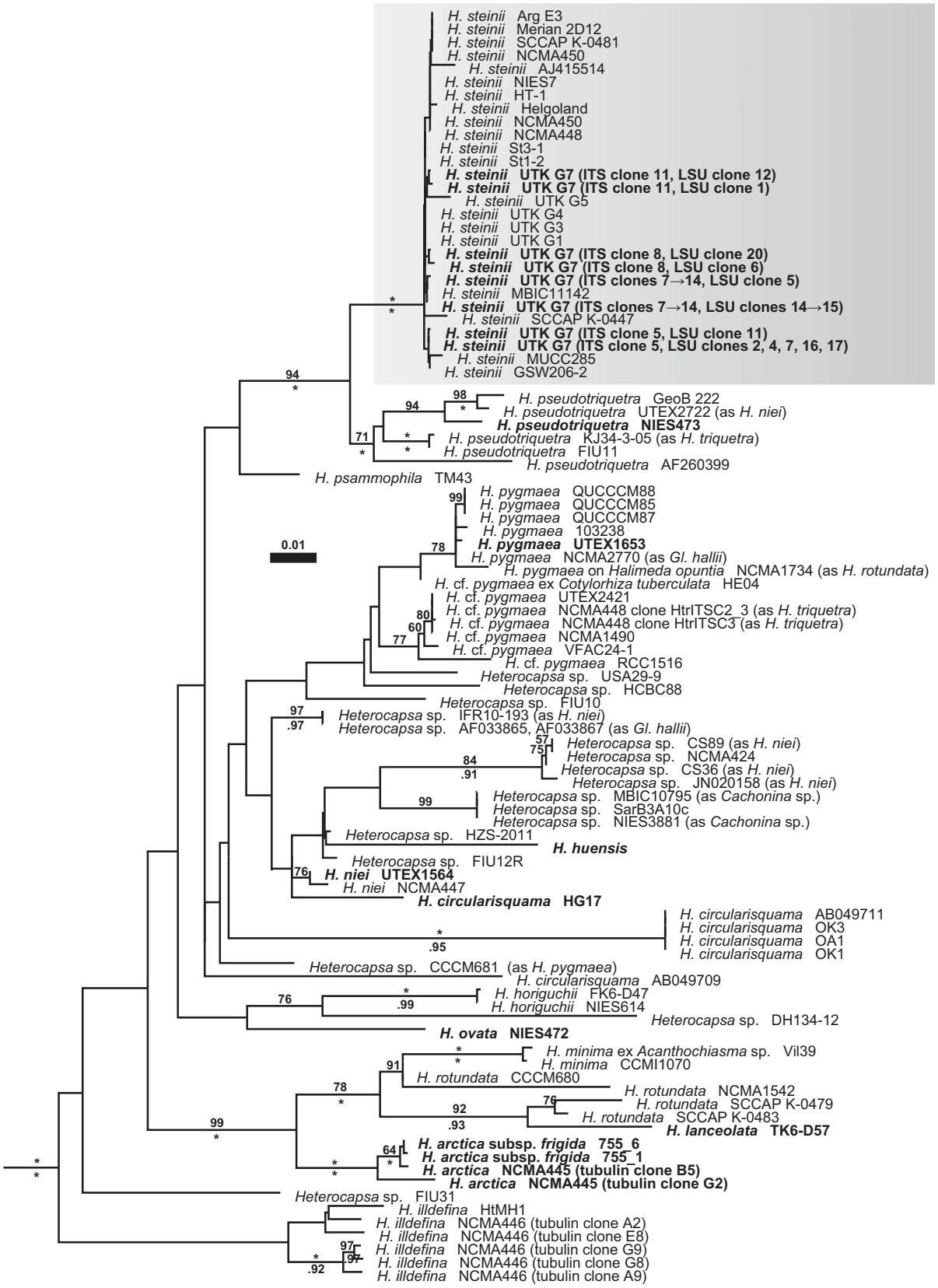
H. steinii Arg E3
H. steinii Merian 2D12
H. steinii SCCAP K-0481
H. steinii NCMA450
H. steinii AJ415514
H. steinii NIES7
H. steinii HT-1
H. steinii Helgoland
H. steinii NCMA450
H. steinii NCMA448
H. steinii St3-1
H. steinii St1-2
H. steinii UTK G7 (ITS clone 11, LSU clone 12)
H. steinii UTK G7 (ITS clone 11, LSU clone 1)
H. steinii UTK G5
H. steinii UTK G4
H. steinii UTK G3
H. steinii UTK G1
H. steinii UTK G7 (ITS clone 8, LSU clone 20)
H. steinii UTK G7 (ITS clone 8, LSU clone 6)
H. steinii UTK G7 (ITS clones 7→14, LSU clone 5)
H. steinii MBIC11142
H. steinii UTK G7 (ITS clones 7→14, LSU clones 14→15)
H. steinii SCCAP K-0447
H. steinii UTK G7 (ITS clone 5, LSU clone 11)
H. steinii UTK G7 (ITS clone 5, LSU clones 2, 4, 7, 16, 17)
H. steinii MUCC285
H. steinii GSW206-2
H. pseudotriquetra GeoB 222
H. pseudotriquetra UTEX2722 (as H. niei)
H. pseudotriquetra NIES473
H. pseudotriquetra KJ34-3-05 (as H. triquetra)
H. pseudotriquetra FIU11
H. pseudotriquetra AF260399
H. psammophila TM43
H. pygmaea QUCCCM88
H. pygmaea QUCCCM85
H. pygmaea QUCCCM87
H. pygmaea 103238
H. pygmaea UTEX1653
H. pygmaea NCMA2770 (as Gl. hallii)
H. pygmaea on Halimeda opuntia NCMA1734 (as H. rotundata)
H. cf. pygmaea ex Cotylorhiza tuberculata HE04
H. cf. pygmaea UTEX2421
H. cf. pygmaea NCMA448 clone HtrITSC2_3 (as H. triquetra)
H. cf. pygmaea NCMA448 clone HtrITSC3 (as H. triquetra)
H. cf. pygmaea NCMA1490
H. cf. pygmaea VFAC24-1
H. cf. pygmaea RCC1516
Heterocapsa sp. USA29-9
Heterocapsa sp. HCBC88
Heterocapsa sp. FIU10
Heterocapsa sp. IFR10-193 (as H. niei)
Heterocapsa sp. AF033865, AF033867 (as Gl. hallii)
Heterocapsa sp. CS89 (as H. niei)
Heterocapsa sp. NCMA424
Heterocapsa sp. CS36 (as H. niei)
Heterocapsa sp. JN020158 (as H. niei)
Heterocapsa sp. MBIC10795 (as Cachonina sp.)
Heterocapsa sp. SarB3A10c
Heterocapsa sp. NIES3881 (as Cachonina sp.)
Heterocapsa sp. HZS-2011
H. huensis
Heterocapsa sp. FIU12R
H. niei UTEX1564
H. niei NCMA447
H. circularisquama HG17
H. circularisquama AB049711
H. circularisquama OK3
H. circularisquama OA1
H. circularisquama OK1
Heterocapsa sp. CCCM681 (as H. pygmaea)
H. circularisquama AB049709
H. horiguchii FK6-D47
H. horiguchii NIES614
Heterocapsa sp. DH134-12
H. ovata NIES472
H. minima ex Acanthochiasma sp. Vil39
H. minima CCMI1070
H. rotundata CCCM680
H. rotundata NCMA1542
H. rotundata SCCAP K-0479
H. rotundata SCCAP K-0483
H. lanceolata TK6-D57
H. arctica subsp. frigida 755_6
H. arctica subsp. frigida 755_1
H. arctica NCMA445 (tubulin clone B5)
H. arctica NCMA445 (tubulin clone G2)
Heterocapsa sp. FIU31
H. illdefina HtMH1
H. illdefina NCMA446 (tubulin clone A2)
H. illdefina NCMA446 (tubulin clone E8)
H. illdefina NCMA446 (tubulin clone G9)
H. illdefina NCMA446 (tubulin clone G8)
H. illdefina NCMA446 (tubulin clone A9)
0.01

FIG. 7. Maximum likelihood (ML) tree of 94 Heterocapsaceae s.str. OTUs, derived from the comparison of concatenated rRNA, nuclear (β-tubulin), mitochondrial (MT-CYB, MT-CO1) and chloroplast gene (*psb*A) sequences. Major clades are indicated, bold lettering indicate OTUs corresponding to type material, and OTUs assigned to *Heterocapsa steinii*, sp. nov., are shaded in gray. 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 <0.90 are not shown). Asterisks indicate maximal support.

whether the first apical and precingular plates share an extensive suture (*H. triquetra* var. *litoralis*), or not ("true" *H. triquetra* sensu Stein 1883), and (ii) whether the APC is distinctly visible in LM (his formae <*apiculata*>), or not (using the pure names for taxa with "pseudoapex").

The concept of such a "pseudoapex" has never acted on a suggestion later, and a dinophyte species exhibiting variability regarding this feature (i.e., thecate cells with and without APC in the same population) is not documented unequivocally. The observations of Lindemann (1924) can thus be explained that he has most likely overlooked the flat and inconspicuous APC (particularly in his early reports; Lindemann 1918). In contrast to his clear depictions of the general plate pattern, Lindemann's erection of these varieties and forms remain obscure, especially since Stein's original illustrations clearly show plate 1′ in contact with plate 1″ (i.e., corresponding to var. *litoralis*). In Lindemann's categories, our material from Kiel Fjord (and from the Irish field samples as well) refers without exception to *H. triquetra* var. *litoralis* forma *apiculata* described from the Golden Horn. Anyhow, there is no single observation, in which plate 1′ has not been in contact with plate 1″ (in a very few cases, the right suture of plate 1′ contacting the last apical plate 4′ is shortened leading to contact of plate 1′ with plate 6″; Fig. S2A).

While the APC may be overlooked in LM, our SEM study has revealed a number of structural details. The APC of *Heterocapsa triquetra* sensu Stein (1883) has a canal plate (X) resembling peridinioid taxa (Fensome et al. 1993), but the position differs when the first apical plate still has contact to Po (Fig. 4). Anteriorly to the X-plate, there is an additional tongue-like structure with a plate-like appearance (see also pl. 6B in Steidinger and Tangen 1996), which seems to cover the apical pore in lateral view (Fig. 4F) like an umbrella. In internal view of the APC, however, there are no indications that this structure is a separate plate but rather is an outer extension of the pore plate. Structures anteriorly of the X-plate are also known from Amphidomataceae (Tillmann et al. 2009, 2012) as well as from *Adenoides* and *Pseudadenoides* (Hoppenrath et al. 2003, 2017, Gómez et al. 2015). In published micrographs of *H. minima* (Salas et al. 2014) and *H. niei* (pl. 6C in Steidinger and Tangen 1996), a similar though smaller structure is visible, which function as a hinge or connection of the X-plate to a more exposed and thinner coverplate. Here, the coverplate seems to cover and close the apical pore completely. In *H. triquetra* sensu Stein (1883), a similar thin cover plate in the pore is likely present as well but is obscured by the overlaying tongue-like structure.

Compared with epithecal, hypothecal, and cingular plates, the architecture of the sulcal region is difficult to determine. The five sulcal plates reported here are not a matter of dispute and are confirmed by numerous studies (Morrill and Loeblich 1981, Balech 1988, Iwataki 2002). However, the presence of additional minute accessory plates has been claimed. Morrill and Loeblich (1981) reported for *H. triquetra* two extra small arc-like plates in the sulcal series as part of the conjunction area, where plates Sa, Sd, and Ssa form the concave pocket with

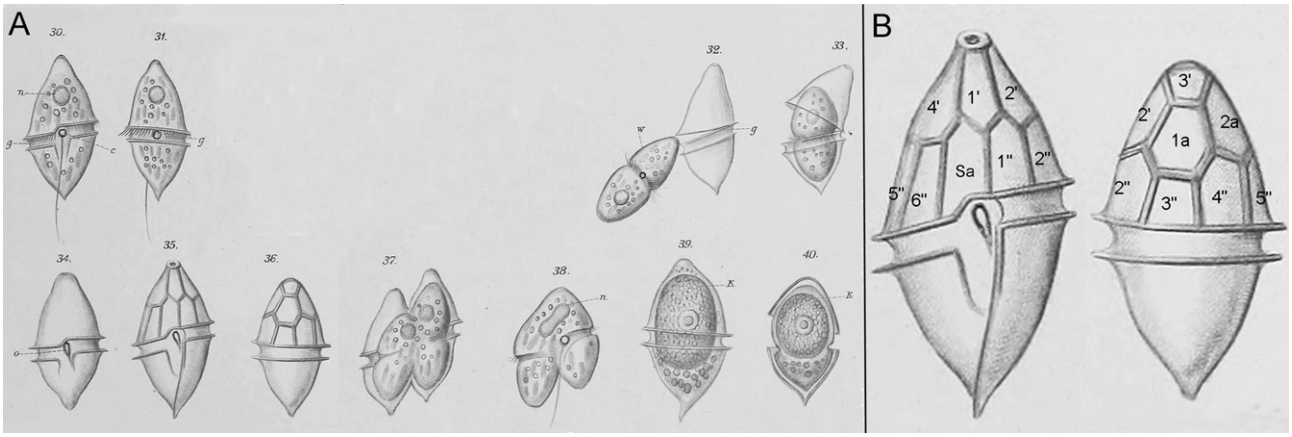

FIG. 8. (A) Stein's original material of *Heterocapsa triquetra* sensu Stein (1883). Reproduction of Stein (1883), pl. III 30–40. (B) Stein's figure 35 and 36 complemented with Kofoidean plate labels in our interpretation.

the two emerging flagella. These observations are based on LM and disintegrated thecal plates, and corresponding micrographs (Morrill and Loeblich 1981) show tiny dark structures. It is difficult to decide whether these are additional plates or simply artifacts but using LM, we cannot confirm additional plates in the sulcal area. Nevertheless, SEM clearly reveals the presence of an additional structure in the contact area of plates Sa and Ssa (Fig. 5, D and E), which might correspond to the structure termed an accessory sulcal plate (Morrill and Loeblich 1981). However, the appearance is granular and wrinkled and different from other thecal plates, and we interpret this structure as a conglomerate of fibers connected to the flagellar pore rather than an extra plate.

Plate overlap pattern. In addition to the number and arrangement of thecal plates, we determined the plate overlap or imbrication pattern. Our observations are congruent to the epitheca overlap pattern of *Heterocapsa triquetra* sensu Stein (1883) reported by Lewis and Dodge (1990) and correspond to a general gradient from dorsal to ventral and from cingulum to poles. Generally, the plate overlap pattern may be a useful aid in determining plate homologies and phylogenetic relationships (Netzel and Dürr 1984). For example, the central ventral plate is overlapped by all adjacent epithecal plates including the first apical plate 1′. Usually, plates of the precingular series overlap the first apical plate (Dickensheets and Cox 1971, Below 1987a, b, Fensome et al. 1993, Elbrächter and Meyer 2001, Tillmann and Elbrächter 2010) and thus, the imbrication pattern of the ventral epitheca provides evidence for our interpretations that the central plate is homologous with the anterior sulcal plate (Sa) but not with a precingular plate. For *H. triquetra* sensu Stein (1883), the fourth precingular plate is the keystone plate. This is comparable to species of Peridiniales with seven precingular plates (Elbrächter and Meyer 2001), but different to species of Gonyaulacales, where the third precingular plate is the keystone plate (Dodge 1988, Fensome et al. 1993). It is also different from species of the Amphidomataceae (whose taxonomic affiliation remains unclear, for a discussion see Tillmann et al. 2014b), in which the third precingular plate is the keystone plate (Tillmann et al. 2012, 2014a).

Plate overlap pattern have been described being highly constant (Netzel and Dürr 1984, Elbrächter and Meyer 2001, Tillmann and Elbrächter 2010), but inverted plate overlap between the cingular plates C3 and C4 has been detected in this study commonly. Such a flip-flop pattern in overlap is also present in field samples of *H. triquetra* sensu Stein (1883; Fig. S5, G–L) and is thus not a culture artifact. It rather seems to represent a rare case of intra-specific variability regarding the plate overlap pattern.

Plate pattern deviations. The pattern of the main epithecal and hypothecal plates of *Heterocapsa triquetra* sensu Stein (1883) reported here conforms to many other reports (as discussed above), but Morrill and Loeblich (1981) and Iwataki (2002) list three additional plates on the epitheca (one in each the apical, the intercalary, and the precingular plate series) as the dominant pattern, and Morrill and Loeblich (1981) even listed an additional hypothecal posterior intercalary plate (1p). It is unlikely that the authors have investigated an alternate species because of the very characteristic cell shape and nucleus/pyrenoid positions of *H. triquetra* sensu Stein (1883), and with the micrographs, drawings, and additional information on body scale morphology found in Morrill and Loeblich (1981) and Iwataki (2002). Explanatory evidence for their observations is that deviating plate patterns can be abundant in dinophyte cultivated material and have also been commonly observed in strain UTKG7 (Figs. S2–S4). In most cases, these can be interpreted as fragmentation of specific plates.

More rarely among hundreds of inspected cells, highly aberrant plate patterns also occurred indicating an in principle high level of flexibility in the process of plate formation and organization. Generally, it is likely that growth in culture often leads to enhanced levels of deviating plate number and arrangement, as has been discussed for *H. niei* (Balech 1977a), *Peridinium* (Elbrächter and Meyer 2001), *Azadinium* (Tillmann et al. 2010), and *Scrippsiella* (Gottschling et al. 2005). Supporting evidence for this assumption is provided by our analysis of the Irish field bloom sample: plate pattern is highly conserved, although even here, a few cases of additional plates in most plate series are present (Table 1). As a conclusion, we consider previous reports of deviating plate patterns in *H. triquetra* sensu Stein (1883) as misinterpretation of an unnaturally increased number in abnormal patterns of cells in cultures.

Ultrastructure and scales. Our LM observations indicate the presence of a single but reticulate plastid in parietal arrangement. Other descriptions of organelles for *H. triquetra* sensu Stein (1883) in the literature differ and range from the presence of many small plate-like chloroplasts (Paulsen 1908, Lebour 1925, Hoppenrath et al. 2009) through a single lobed or star-shaped chloroplast (Campbell 1973, Horiguchi 1990, Iwataki 2002). Admittedly, it is difficult to show unequivocally whether there is a single or more plastid(s) present, even using fluorescence microscopy, and confocal laser scanning microscopy and/or extensive TEM would be needed for a final evaluation. Thus, deviating report about the number of chloroplasts in *H. triquetra* sensu Stein (1883) may refer to observational differences rather than reflecting true and significant differences in cell morphology sufficient to assume different species.

All *Heterocapsa* species possess one or several pyrenoids (Iwataki 2008, Iwataki et al. 2009, Salas et al.

2014), and the number, the position in the cell, and the ultrastructure are species-specific and useful for species identification (Tamura et al. 2005, Iwataki 2008, Iwataki et al. 2009). In terms of ultrastructure, five pyrenoid types are distinguished in dinophytes (Dodge and Crawford 1971). The presence or absence of tubular invaginations in the pyrenoid matrix has been further used to characterize and to distinguish species (Horiguchi 1985, Tamura et al. 2005). The posterior position and the ultrastructure ("stalked pyrenoid with invaginations") as described here conform to previous descriptions of *H. triquetra* sensu Stein (1883) (Dodge and Crawford 1971).

The fine structure of the organic body scales is an important taxonomic trait for identification of the very similar small *Heterocapsa* species (Morrill and Loeblich 1981, Hansen 1995, Iwataki et al. 2004, Iwataki 2008, Rintala et al. 2010). The body scales of strain UTKG7 confirm previous descriptions for *H. triquetra* sensu Stein (1883; Pennick and Clarke 1977, Morrill and Loeblich 1981, Iwataki et al. 2004, Table S2 in the Supporting Information, including a detailed discussion about ambiguity in the original studies). Intraspecific variability in scale morphology has been documented (Iwataki et al. 2004) and interpreted as different ontogenetic stages. Anyhow, scales show some degree of different appearance in our study, but there is no conclusive evidence for intraspecific morphological variation in *H. triquetra* sensu Stein (1883). Rather, scale morphology is diagnostic for species delimitation in *Heterocapsa* (Iwataki et al. 2004). An exception are *H. triquetra* sensu Stein (1883) and *H. pseudotriquetra* having indistinguishable scales (Iwataki et al. 2004), and the sister species relationship shown in the molecular tree identifies the trait as syn-apomorphy of both species.

Phylogenetic considerations. Our clarification of the plate pattern of *H. triquetra* sensu Stein (1883) inevitably brings up the point that the plate pattern of the type is different to the plate pattern of all other species currently placed in *Heterocapsa*. They have without exception the plate pattern of APC, 5′, 3a, 7″, 6c, 5s, 5‴, 2⁗ (Iwataki 2008), which initially has been considered characteristic for *Cachonina* (Loeblich 1968). From a phylogenetic perspective, this does not argue for a taxonomic separation of *Cachonina* and *Heterocapsa* (Morrill and Loeblich 1981), but identifies the epithecal plate pattern of 4′, 2a, 6″ as aut-apomorphy of *H. triquetra* sensu Stein (1883). Variation in epithecal plate number within a taxon at the generic level is not restricted to *Heterocapsa* but refers also to the concept of *Amphidiniopsis*, *Pyrophacus*, *Protoperidinium* (Steidinger and Tangen 1996), and *Peridiniella* (Balech 1977b). Another case comparable to *Heterocapsa* is *Azadinium* from the Amphidomataceae. Here the predominant epithecal plate conformation is 4′, 3a, 6″, whereas the species *A. dalianense* has only 3 apical and 2 intercalary plates (Luo et al. 2013), and *A. zhuanum* has 4 apical and 2 intercalary plates (Luo et al. 2017).

Irrespectively of the epithecal plate number, all species of *Heterocapsa* share a peculiar location of the first apical plate 1′, which is in contact with a single plate only that is interpreted as belonging to the precingular series (i.e., plate 1″). Moreover, the presence of body scales of a very similar architecture represents another striking apomorphy of entire *Heterocapsa*. This view is strongly supported by the molecular phylogeny, which unambiguously shows that all species designated as *Heterocapsa* analyzed so far constitute a monophyletic group of closely related species (Fig. 8; see also Yoshida et al. 2003, Stern et al. 2012, Salas et al. 2014).

A case of dispute in the past is *Peridinium chattonii* from the Mediterranean Sea, which was described with the same plate pattern as *H. triquetra* sensu Stein (1883), namely 4′, 2a, 6″, 5‴, 2⁗ (Biecheler 1952). A corresponding combination under *Heterocapsa* by Campbell (1973) was not validly published (ICN Art. 41.5.), but Morrill and Loeblich (1981) and Iwataki (Iwataki 2002, 2008) also rejected the transfer because of *Heterocapsa* having a greater number of apical, intercalary, and precingular plates. Anyhow, *P. chattonii* has the same plate pattern as *H. triquetra* sensu Stein (1883), and the species is in need of reinvestigation as possible member of *Heterocapsa*. Another interesting taxon is a species designated as "*Peridinium* 1" by Barker (1935) with a plate pattern illustrated as 3′, 3a, 6″, 5‴, 2⁗. It was described as rather similar to *H. triquetra* sensu Stein (1883), though certainly being a distinct species. Also this taxon is in need of re-investigation but if confirmed to be a member of *Heterocapsa*, it would represent another type of epithecal plate numbers.

Taxonomic conclusion. There can be little doubt that we, based on material from the same locality, successfully have established strains of the same species illustrated more than a century ago as specified in Stein (1883). The past confusion about the identity of *H. triquetra* sensu Stein (1883) and precise morphological interpretations illustrate that the species is an ambiguous taxon and therefore in need of taxonomic clarification. If there is no contradiction to the protologue, the International Code of Nomenclature for algae, fungi, and plants (ICN; McNeill et al. 2012) provides the effective tool to designate an interpretative epitype. This procedure has been successfully applied for several taxa in the past (Zinßmeister et al. 2011, Kretschmann et al. 2015a,b, 2017) but in case of *H. triquetra* sensu Stein (1883), there is nothing to epitypify. The formal type of this name refers to a species of *Kryptoperidinium* and has been collected in the Baltic Sea off Wismar (Gottschling et al. 2017) being more than 100 km distant from Kiel. Even when Stein published images of a new species with legend, he failed in formally describing this species. Astonishingly, generations of

dinophyte specialists referred to the taxonomic concept of an undescribed species. To overcome these shortcomings, we formally describe here the species behind the well-known concept using Stein's figure as part of the protologue. In an all evidence approach, we provide an authentic strain, LM, SEM, TEM, and molecular data for comparative purposes.

Heterocapsa steinii Tillmann, Gottschling, Hoppenrath, Kusber & Elbrächter, ***sp. nov.***—

Description: Small, phototrophic thecate dinophyte; cells 17.8–25.9 μm long and 13.0–17.6 μm wide; biconical through fusiform with a characteristically short, horn-like protuberance at the antapex; nucleus large, located in the episome; pyrenoid 1, large, located in the lower cingular plane; tabulation formula: APC (Po, cp?, X), 4′, 2a, 6″, 6c, 5s, 5‴, 2⁗. *Heterocapsa steinii*, sp. nov., shares body scale morphology with its sister species *H. pseudotriquetra*, but differs by its epithecal plate pattern, its shape, and molecular sequence characters.

Holotype, designated here: [illustration] pl. III: fig. 35! in Stein (1883), showing a non-fossil individual, see also our Fig. 8.

Holotype locality: Baltic Sea, off Germany: either Kiel or Wismar (Stein 1883), probably late summer 1879 according to Wetzel (1885).

Epitype, designated here: [non-fossil] SEM-stub prepared from clonal strain UTKG7 (designated CEDiT2017E65, see Fig. 1N), deposited at the Senckenberg Research Institute and Natural History Museum, Centre of Excellence for Dinophyte Taxonomy, Senckenberg am Meer Wilhelmshaven, Germany); duplicates: [non-fossil] formalin-fixed sample prepared from clonal strain UTKG7 (designation CEDiT2017I66) deposited at the Senckenberg Research Institute and Natural History Museum, Centre of Excellence for Dinophyte Taxonomy, Senckenberg am Meer Wilhelmshaven, Germany).

Epitype locality: Baltic Sea, off Germany: Schleswig-Holstein, Kiel (54.32° N, 10.15° E)

Habitat: marine and brackish water, plankton.

Strain establishment: sampled by A. Tillmann on August 7, 2013, isolated by U. Tillmann on August 8, 2013.

Etymology: The present species was illustrated (Stein 1883) but hitherto not formally described as new species. The epithet thus refers to the distinguished person who first observed this species of *Heterocapsa*.

Registration: http://phycobank.org/100010

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web site:

Figure S1. Plate overlap pattern of *Heterocapsa triquetra* sensu Stein (1883) (strain UTKG7) as inferred from thecae with growth bands, slightly disarranged plates or interior views of the epitheca (A–C), hypotheca (D–E), and cingular plates (G–L). Black or white arrows indicate direction of plate overlap. Note that in (A–B) and (D–E), broad bulging growth bands are present on overlapping plate margins only, whereas underlapping plate margins are characterized by a narrow bulge. Note that within the cingular series both plate C3 (G–I) and plate C4 (J–L) overlap both neighboring thus being keystone plates. Scale bars = 2 μm.

Figure S2. Variations in plate pattern of *Heterocapsa triquetra* sensu Stein (1883) observed in cultivated material (strain UTKG7). (A–C) Basic plate pattern, but unusual shape/size of particular plates. (A) Plate 4′ much smaller than usual. (B) Plate 3′ with aberrant shape. (C) Apical plates 2′ and 3′ with aberrant shape. (D–L) Variations in plate number. (D–F) Five apical plates due to an additional suture dividing plate 4′. Note that one of the dived plates may have lost contact to the pore plate (black arrow in F) formally becoming an epithecal ventral intercalary plate. (G) Subdivision of plate 3′. (H) Subdivision of both plates 2′ und 4′. (I) Presence of 7 precingular plates due to subdivision of plate 5″. (J) Presence of three dorsal intercalary plates due to subdivision of plate 1a. (K) Subdivision of both plates 3′ and 1a. (L) Multiple subdivisions of epithecal plates 3′, 1a, and 4″ leading to 5 apicals, three intercalary plates, and 7 precingular plates. Scale bars = 2 μm.

Figure S3. Variations in plate pattern of *Heterocapsa triquetra* sensu Stein (1883) observed in cultivated material (UTKG7). (A–C) Reduction in plate number due to fusion of plates. (A–B) Fusion of plates 2′ and 3′. Note that plate arrangement can be disturbed as well, an intercalary plate can exceed, for example, exceptionally into contact to the pore plate (white arrow in A), or plate 1″ can exceptionally contact plate 1a (black arrow in A). (C) Fusion of plates 2a and 4′. (D) Fusion of plates 1′ and Sa. Note that there is an additional subdivision of plate 4′. (E) Fusion of plates 1′ and 2′. (F) Fusion of plates 2′ and 1″. (G) Fusion of plates 3″ and 4″. Note that there is an additional subdivision of plate 4′. (H) Deviating plate pattern, here interpreted that plate 2′ had lost contact to the pore plate. (I) Deviating plate pattern interpreted that plate 1′ had lost contact to the pore plate with a subdivision of plate 4′. (J) Deviating plate patter interpreted that plate 2a exceptionally got into contact to the pore plate (white arrow in J). Note the multiple subdivisions of precingular plates (4″ and 5″). (K) Deviating plate pattern interpreted here as extremely aberrant size of apical plates, subdivision of intercalary plate 1a, and fusion of plates 3″ and 4″. (L) Deviating plate pattern interpreted here as aberrant size of apical plates, fusion of plates 2′ and 3′, and fusion of plates 1a and 2a. Scale bars = 2 μm.

Figure S4. Variations in plate pattern of *Heterocapsa triquetra* sensu Stein (1883) observed in cultivation (strain UTKG7). (A–B) Presence of six precingular plates due to subdivision of plate 1‴ (A) or plate 5‴ (B). (C–D) Presence of four precingular plates due to fusion of plates 2‴ and 3‴ (C), or of plates 4‴ and 5‴ (D). (E) Presence of one antapical plate (possible fusion of plates 1⁗ and 2⁗). (F) Presence of three antapical plates due to a subdivision of plate 1⁗. (G) Presence of seven cingular plates due to subdivision of plate C1. (H) Presence of three small median sulcal plates due to subdivision of plate Ssp. (I) Epitheca in ventral view showing fusion of plates 1′ and Sa. Scale bars = 2 μm.

Figure S5. Electron microscopy of *Heteropcapsa triquetra* sensu Stein (1883) harvested from a natural population collected at the Bandon lower river estuary, Ireland. (A, B) Whole cell in (A) ventral and (B) dorsal view. (C–E) Epithecal plates in (C, D) external or (E) internal view. (F) Hypothecal plates. (G–L) Cingular plates indicating plate overlap (black arrows) of the dorsal cingular plates C3 and C4. (G–I) Plate C3 overlaps plate

C4. (J–L) Plate C4 overlaps plate C3. Scale bars = 2 μm.

Figure S6. Maximum Likelihood (ML) tree of 94 Heterocapsaceae (plus 127 outgroup) OTUs, derived from the comparison of concatenated rRNA, nuclear (β-tubulin), mitochondrial (MT-CYB, MT-CO1) and chloroplast gene (*psb*A) sequences. Major clades are indicated, bold lettering indicate OTUs corresponding to type material, and OTUs assigned to *Heterocapsa steinii*, sp. nov., are shaded in gray. 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: dt, Kryptoperidiniaceae; E/Pe, clade including *Ensiculifera* and *Pentapharsodinium*; PER, Peridiniaceae *s.str.*; POP, Peridiniopsidaceae; T/Pf, clade including *Pfiesteria* and *Thoracosphaera*).

Table S1. Voucher list. All names are given under the rules of the ICN, the author standard forms follow Brummitt and Powell (1992).

Table S2. Published sizes and characteristics for scales of *Heterocapsa triquetra* sensu Stein (1883).