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Re-evaluation of *Amphidiniopsis* (Dinophyceae) morphogroups based on phylogenetic relationships, and description of three new sand-dwelling species from the NW Mediterranean¹

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ABSTRACT

Amphidiniopsis is a diverse genus of thecate heterotrophic dinoflagellates within the benthic, sand-dwelling species, with more than 20 currently described. Although molecular information about members of this genus is still scarce, morphological heterogeneity suggests the genus is paraphyletic. We investigated the diversity of *Amphidiniopsis* species in the NW Mediterranean Sea by morphological and molecular approaches, which led to the description of three new species, *A. bulla* sp. nov., *A. erinacea* sp. nov., and *A. selene* sp. nov. Phylogenetic reconstructions based on SSU and LSU rDNA sequences obtained from individual cells and the observed morphological characters confirm, as previously suggested, the paraphyly of the genus and the existence of at least four phylogenetic subgroups, instead of the three main subgroups defined to date. We also morphologically characterize *Herdmania litoralis*, suggesting the existence of more than one species belonging to this monotypic genus. *Herdmania* is a sister taxon to *Amphidiniopsis*, both morphologically and phylogenetically, and given the paraphyly of the latter, it should be considered a member of the newly termed *Amphidiniopsis* genus-complex. The finding of the three new species highlights that the Mediterranean harbors distinctive, sand-dwelling dinoflagellates and needs further investigations of its unexplored diversity.

Keywords: *Amphidiniopsis*; Benthic; dinoflagellate; *Herdmania*; Phylogeny; Protists

Abbreviations: BPP, Bayesian posterior probability; BS, bootstrap; HMDS, hexamethyldisilazane; APC, apical pore complex; Po, apical pore plate; X, canal plate; 1', first apical plate; 2', second apical plate; 3', third apical plate; 4', fourth apical plate; 1a, first intercalary plate; 2a, second intercalary plate; 3a, third intercalary plate; 1'', first precingular plate; 2'', second precingular plate; 3'', third precingular plate; 4'', fourth precingular plate; 5'', fifth precingular plate; 6'', sixth precingular plate; 7'', seventh precingular plate; c1, first cingular plate; c2, second cingular plate; c3, third cingular plate; c4, fourth cingular plate; Sa, anterior sulcal plate; Sad, right anterior sulcal plate; Sas, left anterior sulcal plate; Sd, right sulcal plate; Ss, left sulcal plate; Ssa, anterior left sulcal plate; Ssp, posterior left sulcal plate; Sp, posterior sulcal plate; Sm, median sulcal plate; 1''', first postcingular plate; 2''', second postcingular plate; 3''', third postcingular plate; 4''', fourth postcingular plate; 5''', fifth postcingular plate; 1''', first antapical plate; 2''', second antapical plate.

INTRODUCTION

Amphidiniopsis is one of the most diverse benthic, sand-dwelling, dinoflagellate genera with more than 20 species currently described (Hoppenrath et al. 2014, Selina and Morozova 2017). It encompasses thecate, heterotrophic, species and shows a remarkable morphological heterogeneity that can broadly be divided into three main groups: I) laterally flattened species; II) dorsoventrally flattened species with complete cingulum, sulcus not displaced, no apical hook and one or two anterior intercalary plates; and III) dorsoventrally flattened species with complete or incomplete cingulum, sulcus displaced to the left, with apical hook and three intercalary plates (Hoppenrath et al. 2014; Fig. 1). However, some species belonging to group III show no apical hook and a relatively smooth theca, leading to a further subdivision of subgroups within this group (Selina and Morozova 2017; group IV in Fig. 1). The ventral area of *Amphidiniopsis* members of group III and *Herdmania litoralis* (group V in Fig. 1) is characterized by non-depressed plates and depressed plates conforming the displaced longitudinal furrow. Those non-depressed plates were interpreted as the “X” and a post-cingular plate respectively (Hoppenrath 2000a,b, Murray and Patterson 2002; i.e., the cingulum was considered incomplete). Recent thecal interpretations of the ventral area consider both non-depressed and depressed plates forming the displaced longitudinal furrow as sulcal plates (Yamaguchi et al. 2011, Selina and Hoppenrath 2013, Selina and Morozova 2017), non-depressed plates being usually interpreted as Sa and Sd respectively. Under this view, all members of group III are thought to have a complete cingulum, the left border being in contact with the longitudinal furrow and the right border being in contact with the non-depressed sulcal plates.

Phylogenetically, the genus is related to the Protoperidiniaceae, and within this family, most *Amphidiniopsis* species with molecular information available cluster with *Herdmania litoralis*, *Archaeperidinium* species and species of the *Protoperidinium* “*monovelum*” and “*americanum*” sections (Yamaguchi et al. 2011, 2016). The exception is *A. cf. arenaria*, which clusters outside this clade (Yamaguchi et al. 2016). The *Protoperidinium* “*sensu stricto*” clade containing the type species of the genus (*P. pellucidum*) is not directly related with the latter sections / clades of the genus. Although molecular information available for *Amphidiniopsis* representatives is still scarce, such information, together with morphological heterogeneity suggests that *Amphidiniopsis* is a paraphyletic genus (Hoppenrath et al. 2009, Yamaguchi et al. 2016), herein named *Amphidiniopsis* genus-complex.

Studies on benthic dinoflagellates from the Mediterranean Sea have mainly focused on epiphytic toxic species (Vila et al. 2001, Aligizaki and Nikolaidis 2006, Aligizaki et al. 2009, Penna et al. 2012), whereas sand-dwelling dinoflagellates have been poorly studied and information is scarce or nonexistent. Our study examined the diversity of *Amphidiniopsis* species in unexplored sediments from the NW Mediterranean Sea, obtained missing morphological information for *Herdmania litoralis*, and increased the information available to infer phylogenetic relationships within the *Amphidiniopsis* genus-complex and with *Herdmania* and other Protoperidiniaceae members.

MATERIAL AND METHODS

Sampling, cell extractions and microscopy.

Sediment samples from the Catalan Coast were obtained at L'Estartit beach (42°3'6.3" N, 3°12'6.7" E), Barceloneta beach (41°23'4.4" N, 2°11'53" E), and Castelldefels beach (41°15'37" N, 1°55'48.8" E), mostly during spring and summer months, from 2015 to 2017, with monthly samplings from April to September in 2017. Sediment samples from Sardinia beaches were randomly obtained at Platamona beach (40°49'27.13" N, 8°31'36.36" E) during summer months, from 2014 to 2017. Plastic bottles were dragged along the seafloor while snorkeling at a depth of 1.5-2 m to obtain samples containing the upper five centimeters of sediment. Sediments were kept at room temperature in the dark and immediately taken to the laboratory. Cells were extracted from around 100 g of sediment using the melting seawater-ice method (Uhlig 1964). Resulting sub-samples (~30-50 mL) were fixed with Lugol's iodine and preserved in the dark at 4°C.

Living and fixed samples from the Catalan coast were observed under a phase-contrast Leica DM-IRB inverted microscope (Leica Microsystems, Wetzlar, Germany), equipped with an epifluorescence filter set, and connected to a ProgRes C10 (Jenoptik Laser, Optik Systeme GmbH, Jena, Germany) digital camera. Epifluorescence microscopic examination of the thecal plate tabulation was performed on fixed cells stained with two-three drops of Calcofluor white at a final concentration of 10-20 $\mu\text{m} \cdot \text{mL}^{-1}$ (Fritz and Triemer 1985). Cellular measurements were conducted using the ProgRes CapturePro software (JENOPTIK Laser, Optik, Systeme GmbH, Jena, Germany).

Live samples from the Sardinian coast were observed under a Leica DMRB (Leica Microsystems GmbH, Wetzlar, Germany), a Zeiss 100, and a Zeiss 10 (Carl Zeiss, Oberkochen, Germany) inverted microscope equipped with differential interference contrast and

epifluorescence optics. Digital photos were taken using a Leica DFC420C camera (Leica Microsystems GmbH), a Zeiss AxioCam (Carl Zeiss, Oberkochen, Germany; Axiovert 100), and a Spot Flex digital camera (Spot imaging, Sterling Heights, Michigan, USA; Axiovert 10). Cell measurements were obtained using a calibrated eyepiece micrometer and from images using the ImageJ software (1.47v; W. Rasband, USA).

For SEM, fixed sub-samples were filtered onto a 3.0-5.0 μm polycarbonate filter, and washed in seawater and distilled water for 15 min. Dehydration was carried out in a 25, 50, 75, 90, 96, and 100% ethanol series for ca. 10 min. The final step of 100% ethanol was repeated twice. Filters were critical-point dried (Catalan samples) or rinsed twice in HMDS and dried for 5 min at 60°C (Sardinian samples). Dried filters were mounted on stubs and sputter-coated with gold-palladium. Catalan samples were examined under a HITACHI S-3500N SEM (Hitachi High Technologies Corp., Japan) at the Servei de Microscopia Electrònica (ICM-CSIC), whereas Sardinian samples were examined under a Zeiss-EVO SEM (Carl Zeiss, Oberkochen, Germany). SEM stubs were deposited in the Centre of Excellence for Dinophyte Taxonomy (CEDiT, Wilhelmshaven, Germany), which is part of the Herbarium Senckenbergianum Frankfurt/M. (FR), with the accession numbers CEDiT2019RM100, CEDiT2019RM101 and CEDiT2019RM102.

Polymerase chain reactions and phylogenetic analyses.

For the phylogenetic analyses, *Amphidiniopsis* cells were isolated using a capillary micropipette and, after being photographed, placed in 0.2 mL PCR-tubes and immediately frozen at -20°C. The tubes with cells from Sardinia contained 5 μL of lysis buffer containing 400 $\text{ng} \cdot \mu\text{L}^{-1}$ Proteinase K and 0.005% SDS. When the samples were ready for processing, PCR tubes were firstly pre-treated to facilitate cell lysis by freezing and thawing 3-4 times, followed by around 5 s of heating in a microwave oven at minimum power (samples from Catalonia) or freezing at -20°C for 10 min, heating at 60°C for 30 min and then at 95°C for 10 min (samples from Sardinia).

Single-cell PCRs were conducted in order to obtain the SSU and LSU rDNA sequences, following a series of nested or semi-nested PCRs. PCR amplifications followed Reñé and Hoppenrath (2019), and details on isolates and primers used for amplification are provided in Table 1. SSU rDNA sequences encompassed approximately positions from 450 to 1500 that included V3 to V8 regions. LSU rDNA sequences obtained from Catalan Coast organisms encompassed approximately positions from 1 to 800 that included D1 to D3 regions, while those from Sardinian encompassed approximately positions from 1 to 600 that included D1 to D2

regions. Purification of PCR products and sequencing were carried out by external services (Genewiz, United Kingdom; Macrogen, Korea; Genoscreen, France). Sequencing was done using forward and reverse primers and an ABI 3730XL DNA. Partial sequences were merged and aligned with a selection of sequences gathered from GenBank covering the Protoperidiniaceae family, as well as some representatives of other dinoflagellate groups, using the MAFFT v.7 online server (Kato et al. 2002) under “auto” option. The alignment was manually checked with BioEdit v. 7.0.9 (Hall 1999), containing 1,795 base pairs for SSU rDNA sequences and 1,571 bp for LSU rDNA sequences. Alignments were trimmed using Gblocks with the less stringent options (Castresana, 2000) to remove poorly aligned regions, resulting in a final alignment of 1,711 and 1,116 bp respectively. Phylogenetic relationships were determined as described in Reñé et al. (2017). Briefly, they were computed using maximum-likelihood (ML) using GTRGAMMA model and 1000 runs with distinct starting trees. Bootstrap (BS) ML analysis was done with 1000 pseudo-replicates. Bayesian inference was also run with a GTR model, and four Markov chains with one million cycles for each chain. Bayesian posterior probabilities (BPP) were obtained from the post-burn-in consensus tree. All sequences obtained were deposited in GenBank under the Accession Numbers MK940547-MK940550 for SSU rDNA and MK940551-MK940556 for LSU rDNA sequences (Table 1).

RESULTS

Nomenclatural considerations

The International Code of Nomenclature for algae, fungi and plants (Shenzhen Code) indicates the holotype of a name of a species must be a specimen (Chapter V, Section 2, Art. 40.4). But “the type of a name of a new species of microscopic algae or microfungi may be an effectively published illustration if there are technical difficulties of specimen preservation or if it is impossible to preserve a specimen that would show the features attributed to the taxon by the author of the name” (Chapter V, Section 2, Art. 40.5; Turland et al. 2018)

Illustrations have been designated as holotype in the following new species descriptions because the study deals with rare heterotrophic specimens isolated from the environment, there is no possibility to work with cultures, and a limited number of cells are available for study, causing technical problems to preserve one specimen on an SEM stub that show all features attributed to the taxa. Specimens from the illustrations can be found in SEM stubs of mixed field samples, which have been designated as reference material and deposited in a type material collection.

***Amphidiniopsis erinacea* Reñé, Satta & Hoppenrath sp. nov.**

Description: Cells ovoid, laterally compressed, 23-28 μm long and 20-24 μm wide. Cells are not pigmented. The epitheca is much smaller than the hypotheca and the cingulum is slightly ascending. Thecal plates are covered with small protuberances. No apical hook. Its plate formula is APC 4' 3a 7" 3c 5s 5''' 2''''.

Holotype: Fig. 2D,

Reference material: SEM-stub of a mixed/field sample from the type locality deposited at the dinoflagellate type collection in the Centre of Excellence for Dinophyte Taxonomy (CEDiT, Wilhelmshaven, Germany), which is part of the Herbarium Senckenbergianum Frankfurt/M. (FR), with the accession number CEDiT2019RM100

Type locality: La Barceloneta beach, Catalonia, NW Mediterranean Sea (41°23'4.4" N, 2°11'53" E)

Habitat: Marine, benthic, sandy sediment

Etymology: From latin *erinaceus*, meaning hedgehog, referring to the short processes covering the cell.

Cells are 23-28 μm long and 20-24 μm wide (mean 25 μm and 21 μm respectively, n=6). Cells are laterally compressed and not pigmented. The epitheca is much smaller than the hypotheca (Figs. 2A; 3D) and the cingulum is slightly ascending, less than one time its width. The nucleus is round, and occupies a central position in the cell, displaced to the dorsal side (Fig. 2B). Large pusules are commonly present (Fig. 2, A and B). All plates possess small projections and protuberant sutures (Figs. 2, C and D; 3G), except the sulcal and cingular ones. Pores are present in all plates. Its plate formula is APC 4' 3a 7" 3c 5s 5''' 2'''' . It possesses four apical plates (Fig. 3, A-C). The first apical plate (1') is elongated and extends from the sulcus to the apical pore complex (APC). The second (2') and third apical plates (3') are small, encircling the APC. The fourth apical plate (4') is much larger and joins with the 1' plate. The apical pore complex (APC) is located centrally, comprising a circular apical pore plate (Po) and a lenticular X-plate seen as a cavity (Fig. 3C). It has seven precingular plates and three intercalary plates. The first (1a) and third (3a) intercalary plates are large, while the second intercalary plate (2a) is smaller, rhomboidal and placed laterally, between the other intercalary plates, and the third (3'') and fourth (4'') precingular plates (Fig. 3, A-D). It has

three cingular plates. The first cingular plate (c1) is short, and follows the suture between 1''' and 2''' (Fig. 3, D and E). The second cingular plate (c2) comprises almost the entire cingulum (Fig. 3, G and H), continued by a short third cingular plate (c3), coinciding with the suture separating 4''' and 5''' plates (Fig. 3, D and E). The right (Sd) and left (Ss) sulcal plates are elongated and smooth (Fig. 3E). The Sd plate contacts with 1' plate and possess a well-developed wing running along the sulcal area, and extending antapically (Fig. 3, D - F). There is a small wing in the anterior sulcal area, in touch with plate 1'', but a suture is present between them, meaning that it belongs to a sulcal plate (Fig. 3E). Two posterior spines are present (Fig. 3D). The first one is formed in the posterior 1' plate edge and probably extending into the posterior sulcal (Sp) plate (Fig. 3, D and F). The second one is located below the Sd wing and probably belonging to Sp plate (Fig. 3D). This second spine is visible in lateral view (Figs. 2B; 3G). An additional sulcal plate (Sm) is partly visible below the Sd wing (Fig. 3, E and F). The posterior sulcal plate (Sp) is quite sunk and smooth (Fig. 3F). Growth sutures are often visible on the hypotheca. First and fifth postcingular plates (1''' and 5''') are narrow and elongated. Second and fourth postcingular plates (2''' and 4''') are quadrangular and placed laterally. The pentagonal third postcingular plate (3''') is narrow, mostly symmetrical, slightly pointed posteriorly and occupies a dorsal position (Figs. 2C; 3, G and H). The two antapical plates are large, occupying half the length of the hypotheca.

Amphidiniopsis erinacea was detected in all three sampled locations from the Catalan Coast at the end of summer months. However, the number of cells observed was always low, and it could be considered as rare. In Sardinia, it was detected at Platamona beach in June 2015.

A cell resembling *A. arenaria* (laterally compressed, no protuberances or processes observed, length 28 µm, depth 27 µm) was detected during an opportunity sampling in La Fosca beach (41°51'27.5" N, 3°08'38.7" E) from the Catalan Coast in March 2017 (Fig. 2E; Table 1). Its morphology could not be established in detail but it was individually isolated and its SSU and LSU rDNA sequences were successfully obtained.

***Amphidiniopsis bulla* Reñé, Satta & Hoppenrath sp. nov.**

Description: Cells dorso-ventrally compressed, 17-26 µm long and 15-21 µm wide. Cells are not pigmented and quite pentagonal in shape. The epitheca is much smaller than the hypotheca, with an apical hook. It possesses a characteristic smooth loop-shaped second precingular plate. The cingulum is ascending, and the deepened part of the sulcus is curved, narrow and slightly

displaced to the left side of the cell. Thecal plates with small spines or protuberances, more developed in apical plates. Its plate formula is APC 3' 3a 7'' 3c 5s 5''' 2''''.

Holotype: Figure 4A

Reference material: SEM-stub of a mixed/field sample from the type locality deposited at the dinoflagellate type collection in the Centre of Excellence for Dinophyte Taxonomy (CEDiT, Wilhelmshaven, Germany), which is part of the Herbarium Senckenbergianum Frankfurt/M. (FR), with the accession number CEDiT2019RM101

Type locality: Castelldefels beach, Catalonia, NW Mediterranean Sea (41°15'37'' N, 1°55'48.8'' E)

Habitat: Marine, benthic, sandy sediment

Etymology: From latin *bull*a, meaning bubble, referring to the characteristic shape of its second pre-cingular plate.

Cells are 17-26 μm long and 15-21 μm wide (mean 20 μm and 18 μm respectively, n=10). Cells are highly dorsoventrally compressed, with a quite pentagonal shape. The epitheca is much smaller than the hypotheca and possesses an apical hook (Figs. 2, F and G; 4A). Cells are not pigmented and the nucleus is large and placed centrally (Fig. 2F). Two large pusules are sometimes present (Fig. 2G). The cingulum ascends about two times its width, and the deepened part of the sulcus is curved, narrow and slightly displaced to the left side of the cell (Fig. 2, F and H; 4A). All plates except 2'' have pores, and are ornamented with small spines or protuberances, more developed in apical plates (Fig. 4, A-B). The plate formula is APC 3' 3a 7'' 3c 5s 5''' 2''''.

The APC is formed by a round Po and an X-plate in front of it (Fig. 4B). The plate 1' is pentagonal and runs from the Sad plate to the APC (Fig. 4C). The 2' plate is small and rectangular (Fig. 4D). The 3' plate runs dorsally and possesses a characteristic crest bent to the left (apical hook) that partially covers the APC (Fig. 4C, D). *Amphidiniopsis bulla* possesses seven precingular plates. 1'' and 3'' are narrow, elongated, and encircling the characteristic 2'' plate, which is roundish (loop-shaped), smooth, and with no pores (Figs. 2H; 4C). Plates 4'' and 5'' are small, in a lateral position, while plate 6'' is large and runs dorsally (Fig. 4, C and E). Finally, plate 7'' joins with Sad and 1' plates (Fig. 4, A and C). It possesses five postcingular plates. Plates 1''' and 5''' are narrow and placed ventrally (Fig. 4, A and F). Dorsally, the plates show a high symmetry (Fig. 4G). Plate 3''' occupies a central position dorsally, with a typical inverted pentagonal shape, surrounded by plates 2''' and 4''' laterally, and 1'''' and 2'''' antapically. The cingulum consists of three cingular plates: a

small c1 in ventral position in touch with the sulcus, a long c2 which encompasses most dorsal area, and a small c3 present in the ventral distal area of the cingulum (Fig. 4, A, C and G). The central ventral area of the cell is occupied by a large Sd plate. The Sad plate is narrow and elongated. Both plates are ornamented, with pores (Fig. 4A). Inside the sulcal cavity, there is a small left anterior sulcal plate (Sas), followed by an elongated Ss plate (Fig. 4, A and C). Antapically, there is the Sp plate, which is smooth, but some pores are present, and it possesses several well developed antapical spines, both ventrally and dorsally (Fig. 4F).

This organism was commonly detected in all samplings performed in Castelldefels beach (Catalan Coast), but rarely reported in the other Catalan locations. Water temperature ranged from 17°C to 23°C during its presence. *Amphidiniopsis bulla* was frequently observed in late summer samples from Platamona beach (Sardinian coast).

***Amphidiniopsis selene* Reñé, Satta & Hoppenrath sp. nov.**

Description: Cells dorso-ventrally compressed, round in shape, 18-23 µm long and 16-22 µm wide and not pigmented. Epitheca shorter than hypotheca, with no apical hook. The central ventral area is occupied by a large Sa and Sd plates and the deepened part of the sulcus is shifted to the left side of the cell. Thecal plates are smooth, with pores and some small protuberances mostly in the hypotheca. No antapical projections. Its plate formula is APC 3' 3a 7" 4c 4-5s 5''' 2''''.

Holotype: Figure 5A

Reference material: SEM-stub of a mixed/field sample from the type locality deposited at the dinoflagellate type collection in the Centre of Excellence for Dinophyte Taxonomy (CEDiT, Wilhelmshaven, Germany), which is part of the Herbarium Senckenbergianum Frankfurt/M. (FR), with the accession number CEDiT2019RM102

Type locality: L'Estartit beach, Catalonia, NW Mediterranean Sea (42°3'6.3" N, 3°12'6.7" E)

Habitat: Marine, benthic, sandy sediment

Etymology: *Selene* is the goddess of the moon in Greek mythology, referring to the spherical shape of the cell, covered with protuberances, cavities and multiple pores.

Cells are 18-23 µm long and 16-22 µm wide (mean 20 µm and 18 µm respectively, n=10). Cells are roundish and dorsoventrally compressed. The epitheca is slightly shorter than the hypotheca, with no apical hook (Figs. 2, I and L; 5A). The cingulum is complete but appears incomplete in the light microscope because the sulcus is wide and the curved deepened part displaced to the left

(Fig. 2, I and M). The nucleus is large and round. It is centrally located and slightly displaced to the epitheca. Cells have no chloroplasts (Fig. 2I). The plate formula is APC 3' 3a 7'' 4c 4-5s 5''' 2''''.

The plates are generally smooth with pores, but some scattered protuberances are present, mainly on hypothecal plates, some of them forming lists running in parallel to the sutures of the hypothecal plates (Fig. 5, A, F and G). The plate 1' is large and six-sided, running from the Sa plate to the APC (Fig. 5, A and D). The plates 2' and 3' are also large and encircle the APC (Fig. 5, D and E). The APC is not covered, drop-like and located centrally. The apical pore is placed in the center of a protuberant circular area of the pore plate Po, inside the APC. An additional round area is located ventrally in the APC, representing the canal plate (X; Fig. 5, C and D). The 1'' plate is large and pentagonal. Plates 2'' to 5'' are wider than high, encompassed below the three intercalary plates (Fig. 5, D and E). The plate 6'' is higher, and in contact with plates 4'', 7'', 3a and 3' (Fig. 5E). The plate 7'' is the largest precingular one (Fig. 5, A and D). The first intercalary plate is hexagonal, while the 2a plate is smaller and pentagonal and the 3a plate is longer and hexagonal (Fig. 5, D and E). It possesses four cingular plates. The plates c1 and c2 are short, placed in the left ventral area (Fig. 5A). The plate c3 is long and encircles the cingulum until the suture between 3''' and 4''' (Fig. 5, E and H). Finally, the plate c4 runs through the right ventral side and connecting with the Sd plate, which occupies the central ventral area (Fig. 5A). The wide sulcal area is composed by a Sa and Sd plate occupying a central ventral position in the cell. Sa has a depression running obliquely conforming the continuation of the cingulum. The suture between Sa and Sd plate is located in the posterior part of the depression (Fig. 5B). The Sd plate is larger and forms a wing that partially covers the sulcal furrow, which is narrow and displaced to the left side of the cell (Fig. 5, A, F and G). The Ss plate is narrow, elongated and partially covered by the wing of the Sd plate. There is a small lenticular plate between the c1, Ss and Sa plate (Fig. 5, A and B) that could represent the Sas plate (and thus Sa representing Sad). However, the suture between this plate and Sa was not observed and it could be part of it. The Sp is placed antapically, below the Sd and Ss plates, and surrounded by plates 1''', 5''', 1'''' and 2'''' (Fig. 5, F and G) The hypotheca shows symmetry in the plates, with the pentagonal plate 3''' occupying a central position, and surrounded by 2''' and 1'''' in the left side, and 4''' and 2'''' in the right side (Fig. 5H).

This organism was detected in all sampling locations from the Catalan Coast, but not regularly and at low numbers. It was also detected during winter in Barceloneta beach.

Herdmania litoralis. Even though specimens of this taxon have been observed in many locations worldwide, some aspects about their plate pattern are still uncertain and are clarified below. Cells are 15-17 μm long and 12-15 μm wide (mean 16 μm and 13 μm respectively, $n=4$), colorless and dorsoventrally compressed. Cells are round and possess a characteristic apical notch (Figs. 2N; 6, A and B), and the theca is smooth with scattered round pores. The cingulum is median and the sulcus is wide, being only partially depressed like a furrow and running straight through the left side of the hypotheca. The material obtained allowed us to do a detailed observation of cingular and sulcal plates, resulting in the plate formula: APC 4' 3a 7" 4c, 6s 5''' 2'''. The ventral center of the theca is occupied by a large plate, interpreted as the Sa. Below of this plate, we can find the plate interpreted as the Sd, which has a small list in its left border. In the left side of the sulcus, there is the Ssp plate, which also has a list that joins with a small spine formed by the Sp plate. In the deeper part of the sulcus, a small lenticular plate, the Ssa plate, can be observed between the c1 and Ssp plates. Between the Sd and Ssp plates, another suture is visible, that could separate the Sm plate. A small spine is visible below of Ssp plate and belonging to Sp plate (Fig. 6A). It has four cingular plates. The cingulum is complete, with a c3 plate occupying the central dorsal position of the cell (Fig. 6A). Plates c1 and c2 are narrow and located ventrally. The plate c3 runs from the left ventral side and encircles the cell almost completely in dorsal side (Fig. 6B). The plate c4 runs from the right dorsal side to the right ventral side, where the cingulum ends. This organism was rarely detected in Castelldefels and Barceloneta beaches during summer months.

Phylogenetic relationships. The SSU rDNA phylogeny (Fig. 7) showed that all sequences belonging to *Amphidiniopsis* representatives clustered within the so-called "Clade X" (Yamaguchi et al. 2016), showing high support (96%/1; bootstrap [BS]/ Bayesian Posterior Probability [BPP]). The only exception was the sequence belonging to *Amphidiniopsis* cf. *arenaria*, which clustered outside of this clade, and together with *Protoperidinium sensu stricto* clade, formed a sister group to "Clade X", but with low support. All morphogroups included within the "Clade X" were organized in two main clades. The first one, with low support, was formed by the *Amphidiniopsis* morphogroup 2 (86%/1), including *A. swedmarkii*, *A. hirsuta* and *A. hexagona*, and by *Herdmania litoralis* sequences (99%/1). The second main clade, also with low support, included by the one side the *Archaeoperidinium* "minutum" section (98%/1). By the other side, a first cluster (99%/1) was obtained containing morphogroup 2 *A. dragescoi*, *A. rotundata* sequences (100%/1), and

Protoperidinium “monovellum” section (100%/1). The second cluster obtained (100%/1) contained the *Protoperidinium* “americanum” section” (100%/1), and the *Amphidiniopsis* morphogroup 1 (100%/1) and morphogroup 3, showing low support. The *Amphidiniopsis* morphogroup 1 included the sequences of *A. erinacea*, *Amphidiniopsis* sp. AR19 and *Amphidiniopsis* cf. *kofoidii*. The *Amphidiniopsis* morphogroup 3 included the sequences of *A. bulla* and *A. uroensis*, clustering together (83%/0.99), *A. korewalensis* and *A. elongata*. The LSU rDNA phylogeny (Fig. 8) showed that all sequences of *Amphidiniopsis* representatives were included within the so-called “Clade Y” (Yamaguchi et al. 2016), which received high support (99%/1). Two main clades conformed “Clade Y”. The first clade showed low statistical support (-/0.97) and contained a cluster including representatives of the *Archaeperidinium* genus (100%/1), and a second cluster (84%/1) including the *Protoperidinium* “americanum” section (100%/1), and *Amphidiniopsis* subgroups 1 and 3 (87%/0.99). The clade of *Amphidiniopsis* morphogroup 1 (100%/1) included the sequences of *A. erinacea* sp. nov. and *Amphidiniopsis* sp. AR19. The sequences of *A. erinacea* specimens from the Catalan Coast were identical, and showed a 99.8% similarity with the sequence from Sardinia specimen. The clade of *Amphidiniopsis* morphogroup 3 (96%/1) included *A. korewalensis*, and a cluster containing *A. bulla* sp. nov. and *A. uroensis* (100%/1). The sequences of *A. bulla* obtained from the Catalan Coast and Sardinia showed a 99.7% similarity. The second sub-clade of “Clade Y” (96%/1) included a sequence of *Amphidiniopsis rotundata*, belonging to the morphogroup 4, *Herdmania litoralis*, and the *Protoperidinium* “monovellum” section (100%/1). Finally, and as observed for the SSU rDNA phylogeny, the sequences of *Amphidiniopsis* cf. *arenaria* clustered unrelated to “Clade Y”.

DISCUSSION

Amphidiniopsis erinacea.

This species shows a plate pattern almost identical for all members of the morphological subgroup 1 (Fig. 9), encompassing laterally compressed species with complete cingulum, and including *Amphidiniopsis arenaria*, *A. dentata* and *A. galericulata* (Hoppenrath et al. 2012, 2014). It also includes the type species of the genus *A. kofoidii*. However, it presents some significant morphological differences with the other species, mostly at the level of epithelial plates. A comparison of morphological characters with closely related species is shown in Table 2.

Besides their plate pattern, these species differ in some morphological characters. Those can be related to the thecal ornamentation, being small projections in *Amphidiniopsis erinacea* and *A. dentata*, smooth or dotted in *A. kofoidii*, dotted in *A. galericulata*, rugose in *A. arenaria* and smooth in *A. sibbaldii* (Selina and Hoppenrath 2008). *Amphidiniopsis galericulata* is the only species with an apical hook, pointing dorsally (Hoppenrath 2000b). *Amphidiniopsis erinacea* has no antapical projections, but like *A. arenaria*, *A. dentata* or *A. galericulata*, the Sd plate forms posterior spines visible in lateral view. Additionally, *A. dentata* and *A. galericulata* have an antapical row of tooth-like and spine-like projections respectively. *A. galericulata* has a squared 2", while it is asymmetrical in the other species.

The SSU phylogenetic tree showed that the sequences available of species belonging to morphogroup 1 clustered together. The exception is the sequence belonging to *Amphidiniopsis* cf. *arenaria*, which clustered not only independently of these morphologically close species, but outside of the "Clade X" (Yamaguchi et al. 2016). Thus, a detailed morphological characterization of this species is needed, but in any case, its independent phylogenetic position is enigmatic. The sequence of *Amphidiniopsis* sp. AR19 also clustered within this clade, but unfortunately, we could not determine with certainty its morphology.

Amphidiniopsis bulla.

This species shows high resemblance with *Amphidiniopsis cristata* (Fig. 10). However, they possess significant differences in the shape and disposition of some plates (Table 2). The 1" plate is narrow and elongated in *A. bulla*, while it is pentagonal in *A. cristata* (Hoppenrath, 2000b, Hoppenrath et al. 2014). This difference is mainly due to the shape of the 2" plate. It is smaller and slightly displaced to the left side of the cell in *A. cristata*. In *A. bulla*, it is larger, almost the same length than 1", and occupying a more central position in ventral view. It also affects the shape of the 3" plate that has the same length than the 2" in both species, thus being short in *A. cristata* and elongated in *A. bulla*. *Amphidiniopsis cristata* is described as having four apical plates, while *A. bulla* has three, as in the case of the close species *A. uroensis*. *Amphidiniopsis bulla* clearly possesses three cingular plates, but *A. cristata* possess four. Other significant differences are also observed. But in this case, and taking into account the available published material, a re-investigation is needed for *A. cristata* epithelial plates of the left side of the cell. Old unpublished images did not provide further information and an attempt to find cells in samples from Germany for evaluation failed. *Amphidiniopsis bulla* presents three intercalary and seven pre-cingular

plates, but only one intercalary and six pre-cingular were described in *A. cristata*. The 2a and 4" plates are small, in touch, and located at the left border between the ventral and dorsal side of the cell in *A. bulla*. Furthermore, the sutures dividing the 1a and 2a plates from the 3a plate are also located in the same region. The material collected by Hoppenrath (2000b) likely did not allow the observation of the presence of such plates and sutures. In both SSU and LSU rDNA phylogenies, the sequences of *A. bulla* clustered with species belonging to the morphological subgroup 3 like *A. uroensis*, *A. korewalensis*, and a sequence of specimens not identified at the species level at the time of publication, but being morphologically conspecific to *A. elongata* (Yamaguchi et al. 2016, Selina and Morozova 2017). However, it clustered independently of other subgroup 3 representatives showing no apical notch like *A. rotundata*. Both *A. bulla* and *A. cristata* show high similarity with *A. uroensis*, which shows a spiny ornamentation in all plates except 2" that is completely smooth and depressed. This appearance is identical to 2" plate of both *A. bulla* and *A. cristata*. *A. uroensis* and *A. bulla* show the same number and disposition of apical and intercalary plates. However, *A. uroensis* lack the loop-shaped smooth 2" plate and only has six pre-cingular plates (Toriumi et al., 2002). The close similarity with *A. uroensis* is also reflected in the phylogeny, with both species clustering together, and *A. korewalensis* forming a sister clade. In contrast to previous species, cells of *A. korewalensis* are rounded, the thecal plates are smooth, and plates Sa (as "x") and Sd (as 6") are larger than in *A. aff. cristata* (Murray and Patterson 2002). Finally, *A. bulla* is also close to *A. pectinaria*, but the latter is characterized by a row of short antapical spines and pectinations in the antapical plates (Toriumi et al. 2002) .

Amphidiniopsis selene.

This species shows intermediate features with *Amphidiniopsis rotundata* (Fig. 11) and *Herdmania litoralis* (Fig. 12). A close phylogenetic relationship is not observed among both species in SSU phylogeny, but they cluster closely in LSU phylogeny, together with representatives of *Protoperidinium* "monovelum" section. Unfortunately, we could not obtain phylogenetic information for *A. selene*. Together with *A. ovalis*, *A. rotundata*, and *A. dragescoi*, it forms a morphogroup of dorsoventrally compressed species, with no apical hook, a similar shape of the three intercalary plates, and a relatively smooth theca (Selina & Morozova, 2017), representing the morphogroup 4. However, these four species show quite distinctive features. Plate 3''' is symmetrical in *A. selene* and *A. ovalis*, while it is asymmetrical in *A. rotundata* and *A. dragescoi*. The cingulum is descending in *A. dragescoi* while ascending in the rest of species and the sulcus is

only slightly displaced to the left in *A. ovalis*. Furthermore, *A. selene* shows a large central sulcal plate, similar to the Sa plate of *H. litoralis*, that penetrates into the epitheca, and it has four cingular plates, while the other species of the morphogroup have three or five. Additionally, and in contrast to the other species, it only has three apical plates instead of four and the first intercalary plate is in touch with 1', while not in the others.

Additionally, it shows similarities with some members of the third morphogroup with apical hook. The presence of two large central sulcal plates is also found in *Amphidiniopsis korewalensis* (Murray and Patterson 2002) and *A. elongata* (Selina and Morozova 2017), both have smooth theca, the sulcal furrow displaced to the left and the latter also possesses a small lenticular sulcal plate.

Herdmania litoralis.

The monospecific genus *Herdmania* was described by Dodge (1981) without providing details of the thecal plate pattern, even though the species was previously documented by several authors like Herdman (1922) as *Gymnodinium agile*, or Baillie (1971) as *Gymnodinium* sp. 1. Hoppenrath (2000a) reinvestigated the species *H. litoralis* and established the plate formula APC 3' 2a 6" x 7c 3s 6" 1p 1"" (Fig. 12). The tabulation would be interpreted as APC 3' 2a 6" 7c, 5(6?)s, 5" 2"" today. The size range of the original species description was 25-37 µm long and 23-24 µm wide (Dodge 1981) conforming to measurements recorded later (Hoppenrath (2000a): 25-36 µm long and 20-35 µm wide). Canadian specimens of *H. litoralis* were in average smaller (14-30 µm in length and width) and had an additional plate in all epithecal plate series (Yamaguchi et al. 2011). Small cells were also observed in the Mediterranean (15-17 µm long, 12-15 µm wide). The tabulation documented in the present study conforms to Yamaguchi's description (APC 4' 3a 7" 5?c, 5?s 5" 2""), only adding a further sulcal plate and recording one less cingular plate APC 4' 3a 7" 4c, 6s 5" 2"". The possibility that sutures on the left lateral epitheca side were unnoticed in German specimens has been discussed (Yamaguchi et al. 2011). Additionally, intraspecific variability in SSU and LSU rDNA sequences was reported by Yamaguchi et al. (2011). The uncertainty about the completeness of the thecal plate observations done by Hoppenrath (2000a), prevented the delimitation of a second smaller species by morphology. The consistency of cell sizes and shape of Mediterranean specimens and their agreement with those from Canada suggest they could be conspecific and represent a second *Herdmania* species. However, it must be confirmed in future studies.

New species groupings supported by morphological and phylogenetic analyses.

The *Amphidiniopsis* genus-complex has been repeatedly claimed as polyphyletic / paraphyletic, based in both morphological characters and phylogenetics (Hoppenrath et al. 2012, Yamaguchi et al. 2016). The genus-complex is characterized by thecate cells with small epitheca and large hypotheca with characteristic plate pattern (Hoppenrath et al. 2014) and two of the new *Amphidiniopsis* species are not directly related to the type species (sensu stricto genus), but as discussed to members of the respective morpho-group. It stresses the need to collect as much information as possible of *Amphidiniopsis* representatives to erect new genera. However, the number of available sequences of *Amphidiniopsis* members is still scarce and some morphogroups are only represented by sequences of one species. The inclusion of the new sequences obtained in this study complements the information available for some of the groups. Both SSU and LSU rDNA phylogenies obtained support the parphyly of the genus, with species clustering in four independent clades, plus a sequence clustering completely unrelated to others (*A. cf. arenaria*). It implies that there are, at least, four groups of species instead of the three originally described (Hoppenrath et al. 2012), that are representing different genera (Fig. 1). This *Amphidiniopsis* genus-complex comprises the laterally flattened species *A. kofoidii* (the type species of the genus), *A. erinacea*, and likely *A. arenaria*, *A. dentata*, *A. galericulata*, and *A. sibbaldii*, that represents the *Amphidiniopsis* sensu stricto group. The features of *A. cf. arenaria*, which clusters unrelated to other *Amphidiniopsis* representatives, remain to be solved, and some species here included in this morphogroup could be related to it. The second one (“hirsuta-group”) comprises dorsoventrally flattened species, with sulcal furrow positioned in the middle of the cell and no apical hook, and includes *A. aculeata*, *A. hexagona*, *A. hirsuta*, *A. konovalovae*, *A. striata*, *A. swedmarkii*, and *A. yoshimatsui*. The third cluster (“uroensis-group”) comprises dorsoventrally flattened species with sulcal depression positioned in the middle of the cell or slightly displaced to the left, with an apical hook and three anterior intercalary plates, and includes *A. korewalensis*, *A. uroensis*, *A. pectinaria*, *A. hoppenrathae* and *A. elongata* and at present *A. bulla* and *A. cristata* (the number of intercalary plates needs to be reinvestigated). It is possible that a “cristata-group” will get separated in future when further molecular data will be available. The morphologically unique character of the group will be the loop-shaped second precingular plate. Finally, the fourth “rotundata-group” comprises dorsoventrally flattened species, without apical hook, sulcal depression shifted to the left side, and relatively smooth theca, and includes *A. rotundata*, *A. dragescoi* and likely *A. selene*, and *A.*

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676 **Table 1:** Details of specimens isolated, samplings and rDNA sequences obtained. Shaded areas represent the
 677 performance of nested PCR.

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679	Species	Isolate	Location	Isolation Date	1st PCR	2n PCR	3rd PCR	rDNA	Length (bp)	GenBank Number
680	<i>Amphidiniopsis erinacea</i>	AR41	Castelldefels (Catalan Coast)	July 24, 2017	EK82F - 28S_1611R	EK82F - 1520R	DIN464F - 1520R	SSU	1,181	MK940548
28S1F-803R						ITS4F-803R	LSU	834	MK040551	
681	<i>Amphidiniopsis erinacea</i>	AR48	Castelldefels (Catalan Coast)	Aug 14, 2017	EK82F - 28S_1611R	EK82F - 1520R	DIN464F - 1520R	SSU	1,195	MK940549
28S1F-803R						ITS4F-803R	LSU	1,107	MK940554	
682	<i>Amphidiniopsis erinacea</i>	Sard30	Platamona (Sardinia)	Jun 2, 2015	D1R - D2C			LSU	599	MK940552
683	<i>Amphidiniopsis bulla</i>	AR44	Castelldefels (Catalan Coast)	July 24, 2017	EK82F - 28S_1611R	EK82F - 1520R	DIN464F - 1520R	SSU	807	MK940550
28S1F-803R						ITS4F-803R	LSU	826	MK940553	
684	<i>Amphidiniopsis bulla</i>	Sard11	Platamona (Sardinia)	Aug 22, 2017	DinFi-DinRi			LSU	603	MK940555
685	<i>Amphidiniopsis</i> sp.	AR19	Fosca (Catalan Coast)	Apr 20, 2017	EK82F - 28S_1611R	EK82F - 1520R	DIN464F - 1520R	SSU	1,196	MK940547
28S1F-803R						ITS4F-803R	LSU	831	MK940556	

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							LSU	834	MK040551
<i>Amphidiniopsis erinacea</i>	AR48	Castelldefels (Catalan Coast)	Aug 14, 2017	EK82F - 28S_1611R	EK82F - 1520R 28S1F-803R	DIN464F - 1520R ITS4F-803R	SSU	1,195	MK940549
							LSU	1,107	MK940554
<i>Amphidiniopsis erinacea</i>	Sard30	Platamona (Sardinia)	Jun 2, 2015	D1R - D2C			LSU	599	MK940552
<i>Amphidiniopsis bulla</i>	AR44	Castelldefels (Catalan Coast)	July 24, 2017	EK82F - 28S_1611R	EK82F - 1520R 28S1F-803R	DIN464F - 1520R ITS4F-803R	SSU	807	MK940550
							LSU	826	MK940553
<i>Amphidiniopsis bulla</i>	Sard11	Platamona (Sardinia)	Aug 22, 2017	DinFi- DinRi			LSU	603	MK940555
<i>Amphidiniopsis</i> sp.	AR19	Fosca (Catalan Coast)	Apr 20, 2017	EK82F - 28S_1611R	EK82F - 1520R 28S1F-803R	DIN464F - 1520R ITS4F-803R	SSU	1,196	MK940547
							LSU	831	MK940556

Table 2: Morphological features of *Amphidiniopsis* species (except those belonging to morphogroup 2).

Group	Species	Cell compression	Apical hook	Apical plates	Intercalary plates	Precingular plates	Third postcingular plate	Distinctive feature	Shift of the sulcus cavity	Thecal ornamentation	Reference
1	<i>A. erinacea</i>	lateral	absent	4	3	7	symmetric	Cell roundish	no	short processes and spines	This study
1	<i>A. arenaria</i>	lateral	absent	4	3	7	symmetric	Cell elongated	no	rugose	Hoppenrath et al. 2014
1	<i>A. dentata</i>	lateral	absent	4	3	8	symmetric	Antapical row of projections	no	small projections	Hoppenrath et al. 2014
1	<i>A. galericulata</i>	lateral	present	4	3	7	symmetric	Presence of an apical hook	no	papillated	Hoppenrath et al. 2014
1	<i>A. kofoidii</i>	lateral	absent	2	2	6	?	Epithelial plate pattern	no	smooth	Hoppenrath et al. 2014
3	<i>A. bulla</i>	dorsoventral	present	3	3	7	symmetric	2'' smooth and rounded	slight	short spines	This study
3	<i>A. cristata</i>	dorsoventral	present	4	1	6	symmetric	2'' smooth and rounded	slight	short spines	Hoppenrath et al. 2014
3	<i>A. uroensis</i>	dorsoventral	present	3	3	6	symmetric	2a smooth	slight	short spines	Hoppenrath et al. 2014
3	<i>A. elongata</i>	dorsoventral	present	4	3	6	asymmetric	Elongated shape	yes	smooth to reticulate	Selina and Morozova

3	<i>A. hoppenrathae</i>	dorsoventral	present	4	3	6	asymmetric	Ventral intercalary plates	slight	short spines	Selina and Morozova 2017
3	<i>A. korewalensis</i>	dorsoventral	present	4	3	6	asymmetric	cells rounded	yes	smooth	Hoppenrath et al. 2014
3	<i>A. pectinaria</i>	dorsoventral	present	4	3	6	symmetric	Pectination of antapical plates	slight	short processes and spines	Hoppenrath et al. 2014
4	<i>A. selene</i>	dorsoventral	absent	3	3	7	symmetric	Sa long	yes	small dots (pusticulate)	This study
4	<i>A. rotundata</i>	dorsoventral	absent	4	3	7	asymmetric	Sad short	yes	small dots (pusticulate)	Hoppenrath et al. 2014
4	<i>A. ovalis</i>	dorsoventral	absent	4	3	7	symmetric	Sa depressed	slight	smooth to reticulate	Selina and Morozova 2017
4	<i>A. dragescoi</i>	dorsoventral	absent	4	3	7	asymmetric	3a separated from 1a and 2a	slight	small pimples	Hoppenrath et al. 2014
5	<i>Herdmania litoralis</i>	dorsoventral	present	4	3	7	symmetric	Large central Sa	slight	smooth	This study

Figure 1: Drawings of plate patterns showing morphological characteristics of different morpho-groups of *Amphidiniopsis* genus-complex (modified from Hoppenrath et al. 2014).

Figure 2: Light microscopy images of studied species. *Amphidiniopsis erinacea* in A) ventral view, B) left lateral view (arrow indicates the ventral spine), C) dorsal view, and D) ventral to left lateral view under epifluorescence. E) *Amphidiniopsis* sp. AR19 in right lateral view. *Amphidiniopsis bulla* in F, G) ventral view in different focal planes, H) ventral view under epifluorescence. *Amphidiniopsis selene* in I) ventral view, L) dorsal view, M) ventral view under epifluorescence. *Herdmania litoralis* in N) ventral view. Scale bars = 10 μm .

Figure 3: Scanning electron microscopy images of *Amphidiniopsis erinacea* showing its plate pattern. A) Left apical view. B) Right apical view. C) Detail of the apical area. D) Ventral view. E) Detail of the sulcal area. F) Antapical view. G) Left lateral view. H) Right lateral view. Arrows indicate the ventral posterior spines. Po = apical pore plate, Sd = right sulcal plate, Sm = median sulcal plate, Sp = posterior sulcal plate, Ss = left sulcal plate. All scale bars = 10 μm , except C) and F) = 5 μm .

Figure 4: Scanning electron microscopy images of *Amphidiniopsis bulla* showing its plate pattern. A) Ventral view. B) Detail of the APC. C) Left ventro-lateral view of the epitheca. D) Dorsal view of the epitheca. E) Left dorso-lateral view of the cell. F) Antapical view. G) Dorsal view. Arrowheads indicate the apical hook, and arrows indicate the antapical spines. Po = apical pore plate, Sad = right anterior sulcal plate, Sas = left anterior sulcal plate, Sd = right sulcal plate, Sp = posterior sulcal plate, Ss = left sulcal plate. Scale bar = 5 μm , except B) Scale bar = 1 μm .

Figure 5: Scanning electron microscopy images of *Amphidiniopsis selene* sp. nov. showing its plate pattern. A) Ventral view. Arrowheads indicate thecal protuberances. B) Detail of the sulcal area. Arrowheads mark the suture between Sa and Sd plates. Arrow indicates the putative Sas plate. C) Detail of the apical pore complex (APC). D) Apical ventral view of the epitheca. E) Dorsal view of the epitheca. F) Oblique ventral view. G) Antapical view. Arrowheads indicate hypothecal thecal lists. H) Dorsal view of the hypotheca. Po = apical pore plate. Sa = anterior sulcal plate, Sd

= right sulcal plate, Sp = posterior sulcal plate, Ss = left sulcal plate. Scale bar = 5 μm , except C) Scale bar = 1 μm .

Figure 6: Scanning electron microscopy images of *Herdmania litoralis* showing its plate pattern. A) Ventral view. B) Dorsal view. Sa = anterior sulcal plate, Sd = right sulcal plate, Sm = median sulcal plate, Sp = posterior sulcal plate Ssa = anterior left sulcal plate, Ssp = posterior left sulcal plate. Scale bars= 5 μm .

Figure 7: Maximum likelihood phylogenetic tree inferred from the SSU rDNA sequences of the family Protoperidiniaceae, as well as other dinoflagellate representatives. The sequence of the Syndiniales member *Amoebophrya* sp. served as outgroup. Sequences obtained in this study are indicated in bold. The bootstrap values (% BS) and Bayesian posterior probabilities (BPP) are provided at each node (BS/BPP). Only BS and BPP values >80% and >0.95, respectively, are shown and maximum support (100%/1) is indicated by black circles.

Figure 8: Maximum likelihood phylogenetic tree inferred from the LSU rDNA sequences of the family Protoperidiniaceae, as well as other dinoflagellate representatives. The sequence of the Syndiniales member *Amoebophrya* sp. served as outgroup. Sequences obtained in this study are indicated in bold. The bootstrap values (BS) and Bayesian posterior probabilities (BPP) are provided at each node (% BS/BPP). Only BS and BPP values >80% and >0.95, respectively, are shown and maximum support (100%/1) is indicated by black circles.

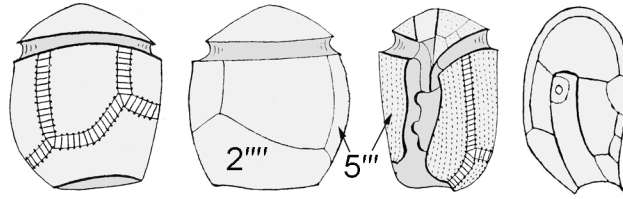
Figure 9: Plate pattern of *Amphidiniopsis erinacea* sp. nov. in A) left lateral view, B) right lateral view, C) and apical view. Apical view, modified from Hoppenrath *et al.* (2014), of D) *A. arenaria*, E) *A. dentata*, F) *A. galericulata*, and G) *A. kofoidii*.

Figure 10: Comparison of plate pattern of *Amphidiniopsis bulla* in A) ventral view, B) dorsal view, and C) apical view, and *Amphidiniopsis cristata*, modified from Hoppenrath *et al.* (2014), in D) ventral view, E) dorsal view, and F) apical view.

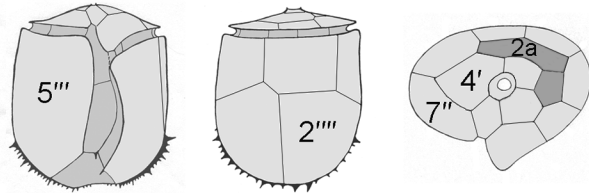
Figure 11: Comparison of plate pattern in ventral and apical view respectively of *Amphidiniopsis selene* sp. nov. (A, D), *Amphidiniopsis rotundata* (B, E) from Hoppenrath et al. (2012), and *Amphidiniopsis ovalis* (C, F) from Selina and Morozova (2017).

Figure 12: Comparison of recorded plate patterns of *Herdmania litoralis* in ventral and dorsal view respectively, (A, D) present study, (B, E) modified from Yamaguchi et al. (2011), (C, F) modified from Hoppenrath (2000a).

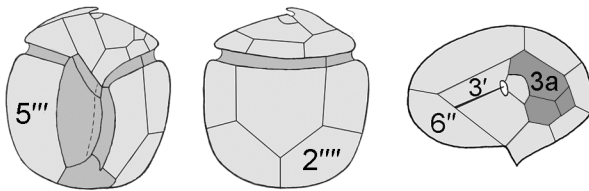
group I ('*kofoidii* group') - laterally flattened



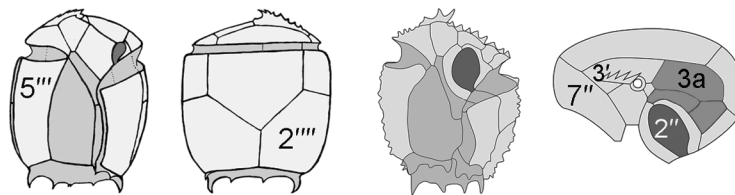
group II ('*hirsuta* group') - dorsoventrally flattened,
sulcus not displaced, no apical hook



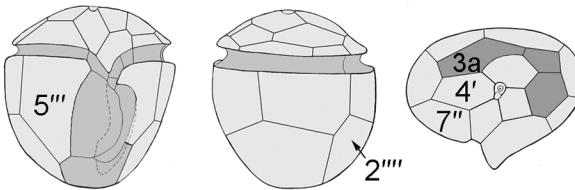
group III ('*uroensis* group') - dorsoventrally flattened,
sulcus not or slightly displaced, apical hook



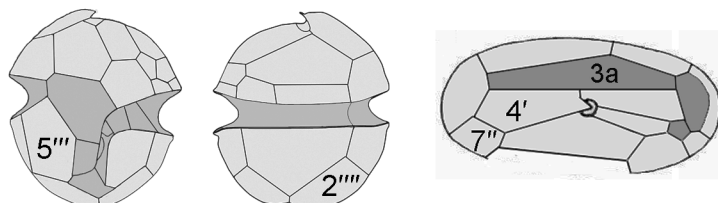
group IIIa ('*cristata* group') - dorsoventrally flattened,
sulcus displaced, apical hook loop-shaped 2''

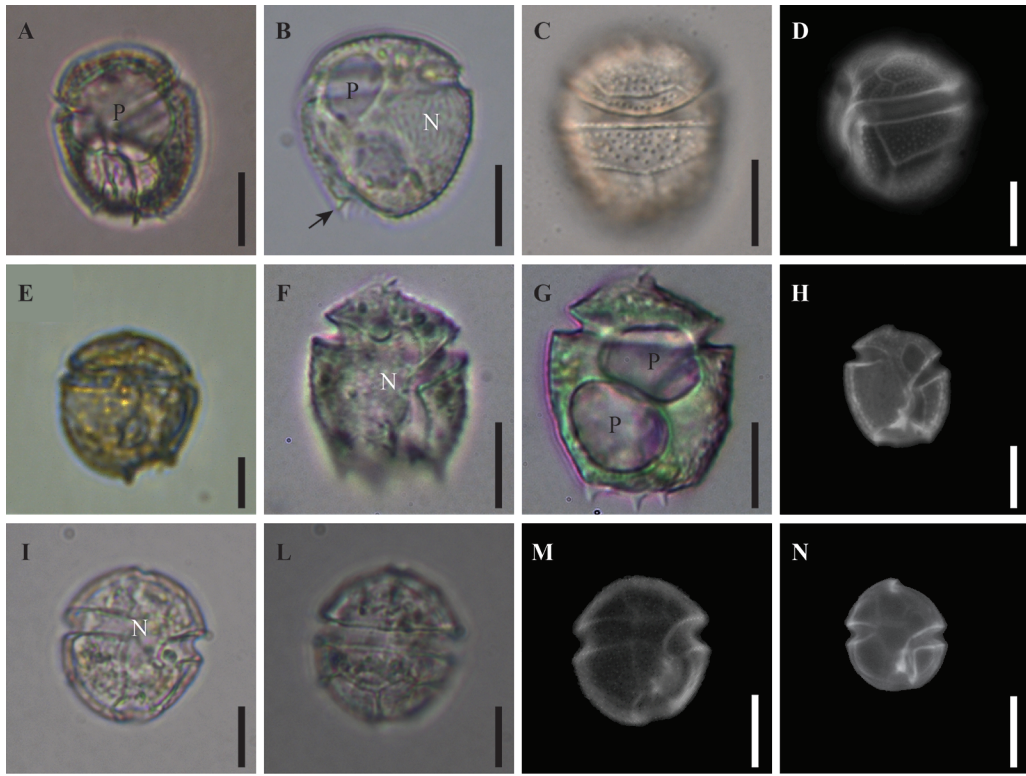


group IV ('*rotundata* group') - dorsoventrally flattened,
sulcus displaced, no apical hook

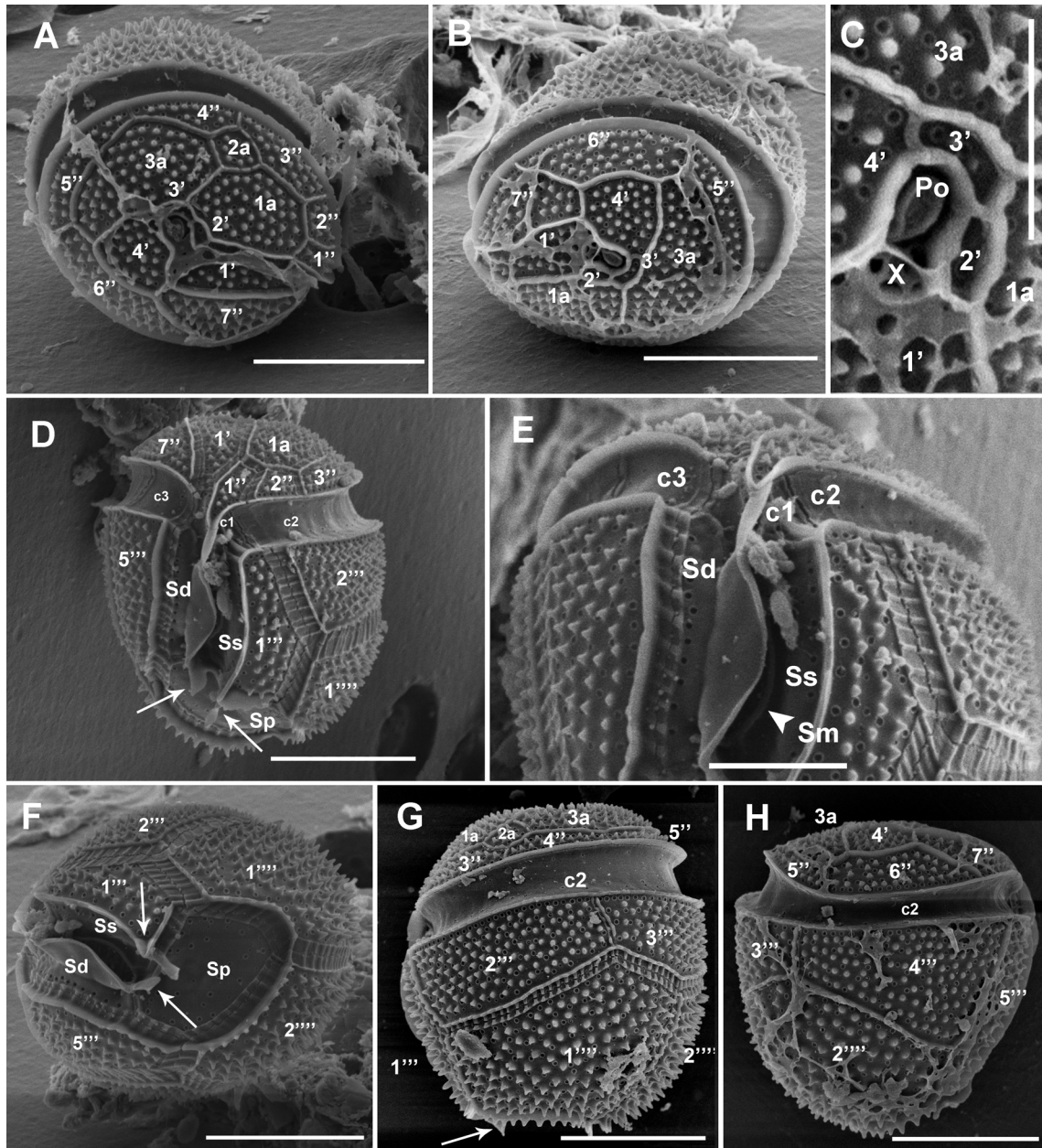


group V ('*Herdmania* group') - dorsoventrally flattened,
sulcus displaced, apical hook

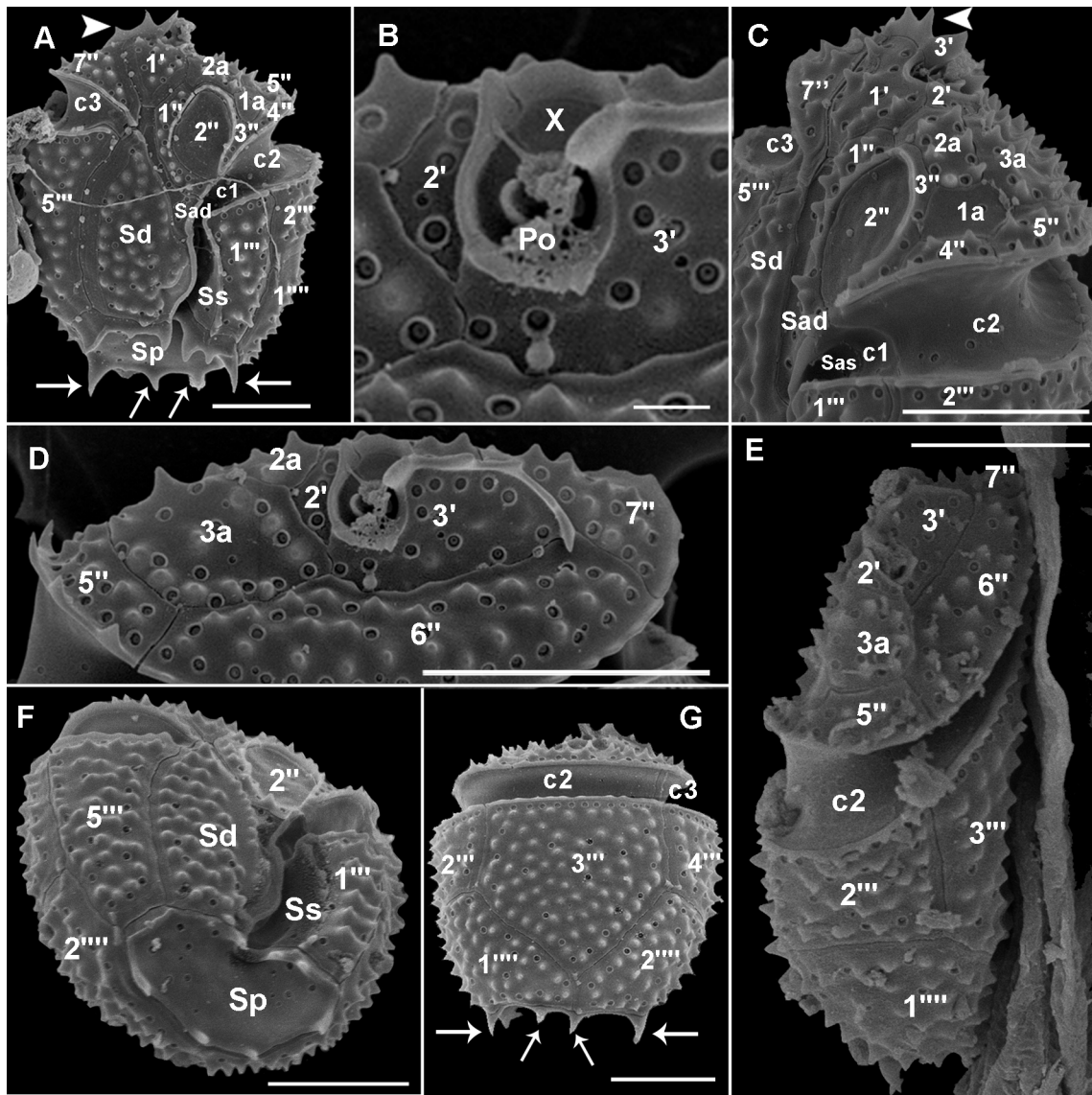




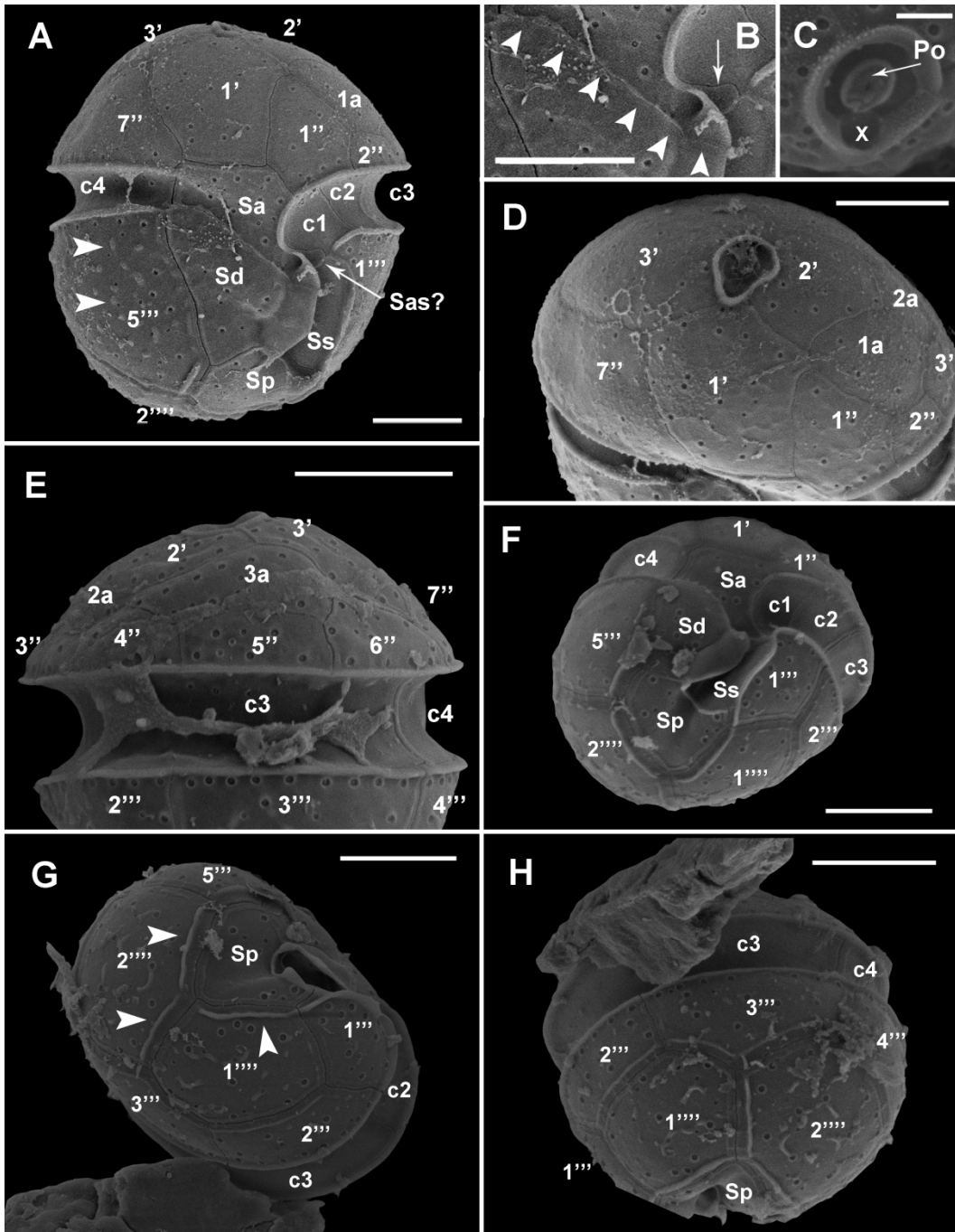
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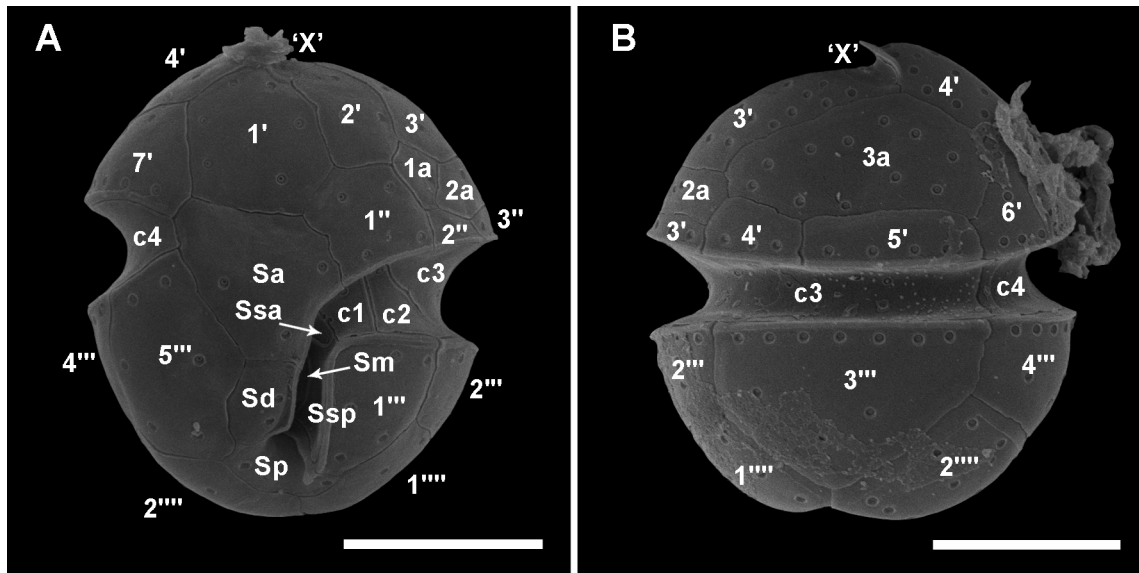
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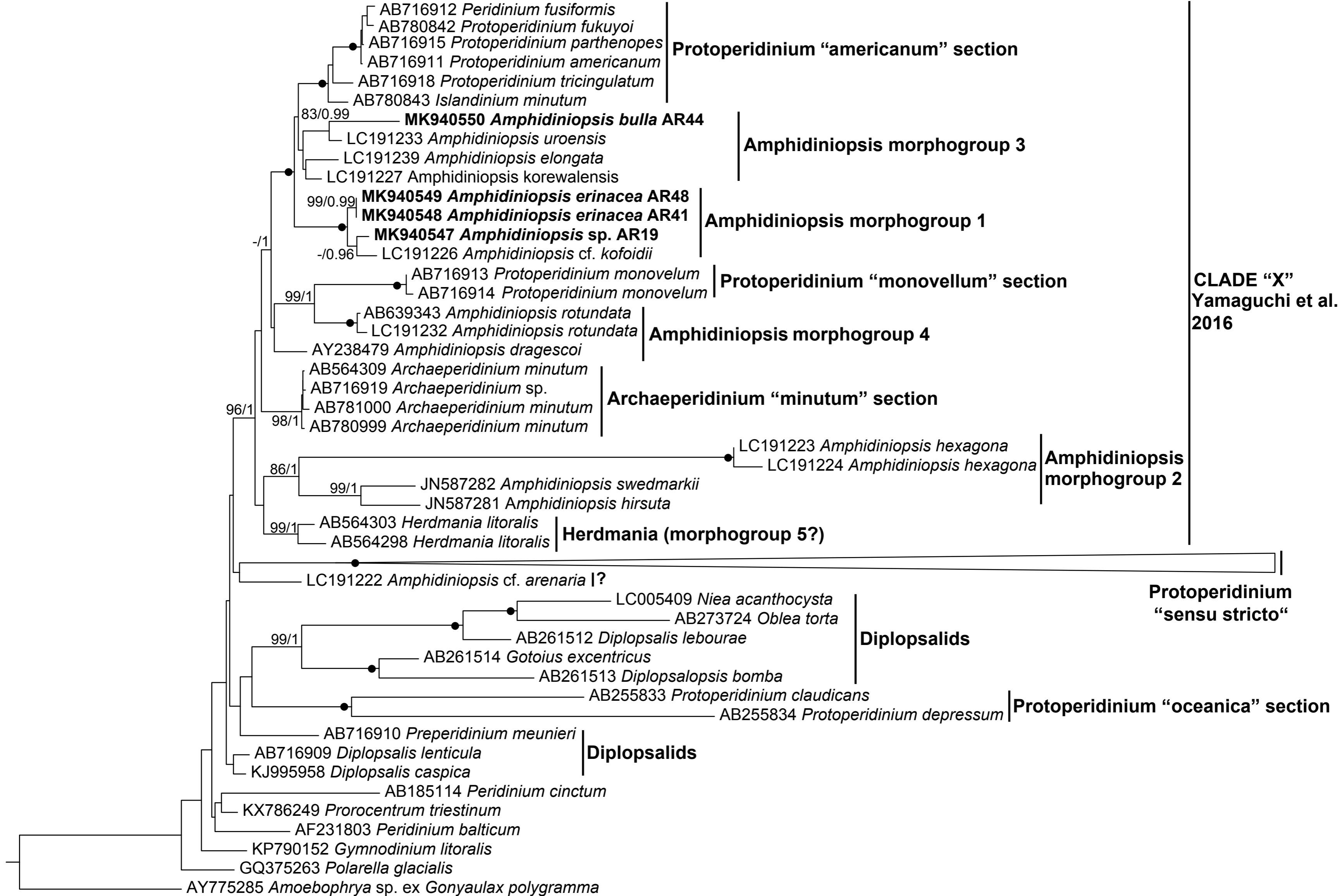
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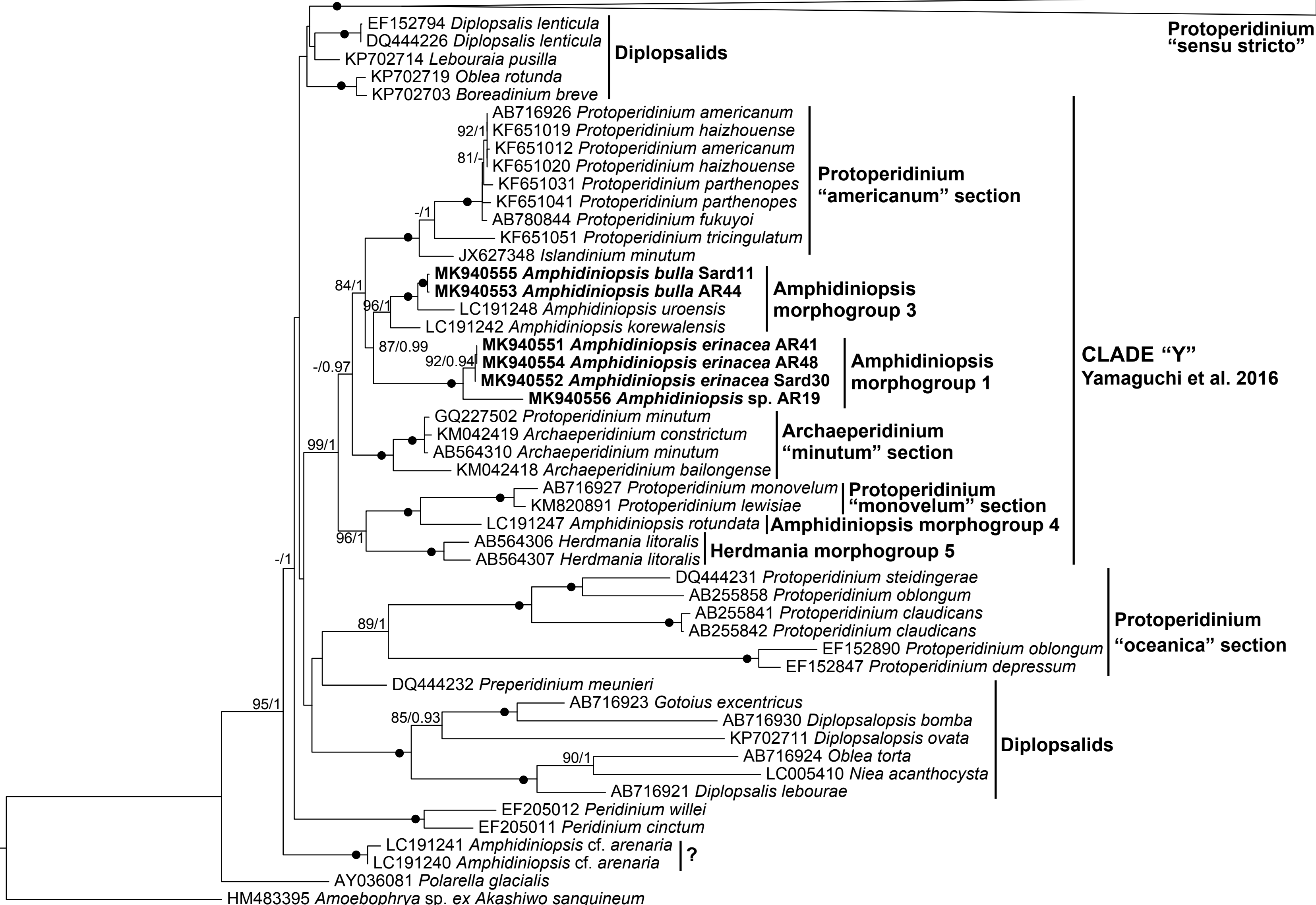


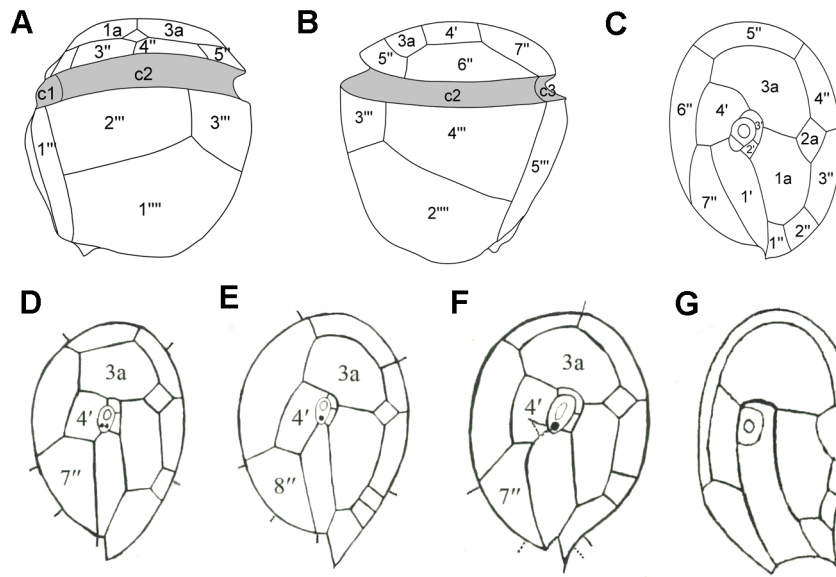
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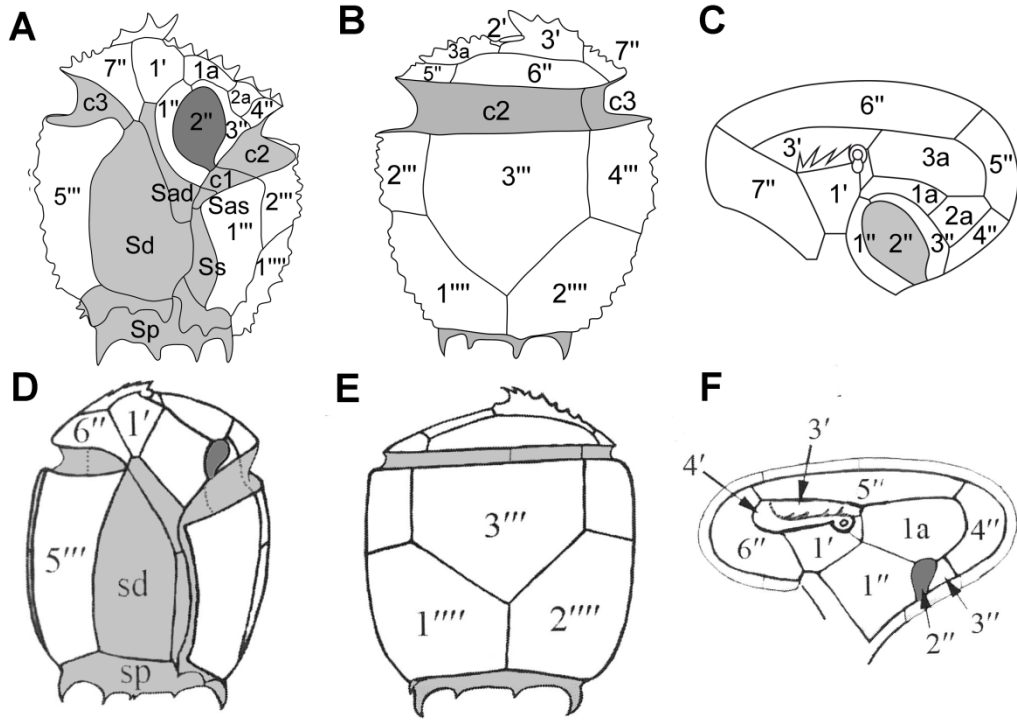
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Yamaguchi et al.
2016

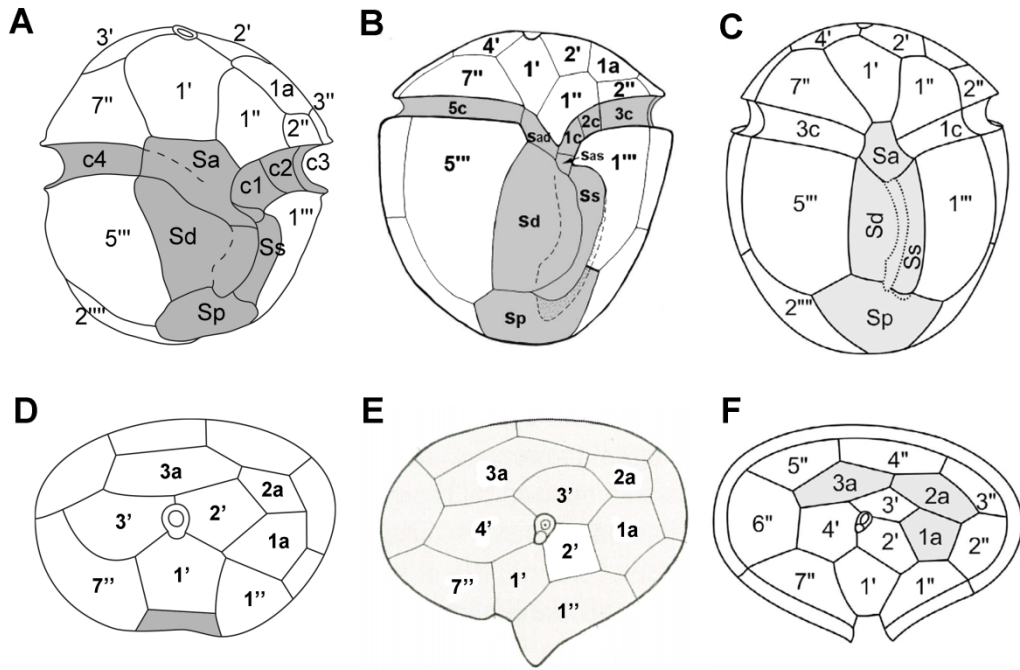




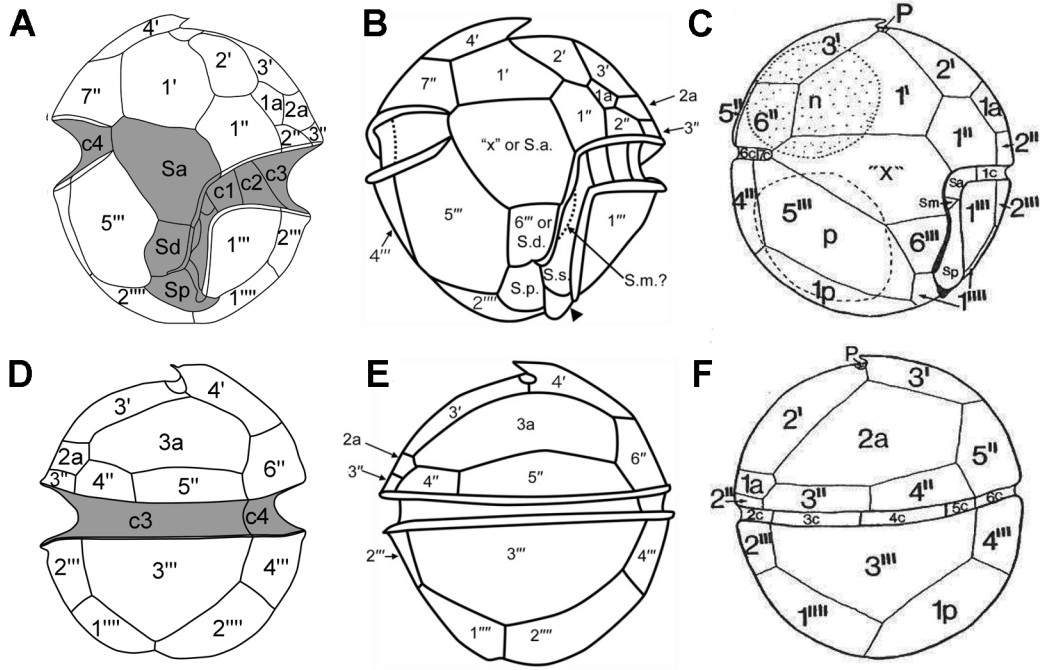
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