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MORPHOLOGY AND MOLECULAR PHYLOGENY OF *ANKISTRODINIUM* GEN. NOV. (DINOPHYCEAE), A NEW GENUS OF MARINE SAND-DWELLING DINOFLAGELLATES FORMERLY CLASSIFIED WITHIN *AMPHIDINIUM*[1]

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The classical athecate dinoflagellate genera (*Amphidinium*, *Gymnodinium*, *Gyrodinium*) have long been recognized to be polyphyletic. *Amphidinium* sensu lato is the most diverse of all marine benthic dinoflagellate genera; however, following the redefinition of this genus ~100 species remain now of uncertain or unknown generic affiliation. In an effort to improve our taxonomic and phylogenetic understanding of one of these species, namely *Amphidinium semilunatum*, we re-investigated organisms from several distant sites around the world using light and scanning electron microscopy and molecular phylogenetic methods. Our results enabled us to describe this species within a new heterotrophic genus, *Ankistrodinium*. Cells of *A. semilunatum* were strongly laterally flattened, rounded-quadrangular to oval in lateral view, and possessed a small asymmetrical epicone. The sulcus was wide and characteristically deeply incised on the hypocone running around the antapex and reaching the dorsal side. The straight acrobase with hook-shaped end started at the sulcal extension and continued onto the epicone. The molecular phylogenetic results clearly showed that *A. semilunatum* is a distinct taxon and is only distantly related to species within the genus *Amphidinium* sensu stricto. The nearest sister group to *Ankistrodinium* could not be reliably determined.

Key index words: **acrobase; *Amphidinium* s.l.; *A. semilunatum*; benthic; dinoflagellates; LSU rDNA; SSU rDNA; taxonomy**

The genus *Amphidinium* Claparède et Lachmann sensu lato is among the largest and most diverse of all marine benthic dinoflagellate genera, containing about 120 species; however, the genus is polyphyletic (Dodge 1982, Larsen 1985, Larsen and Patterson 1990, Hoppenrath 2000a, Murray and Patterson 2002). To distinguish *Amphidinium* from other athecate genera, overly generalized criteria, such as episome dimensions (shorter than 1/3 of the cell length) and the displacement of the cingulum (Steidinger and Tangen 1997) were used in the past. Modern methods have been used to re-investigate the type species of the athecate genera *Gymnodinium* Stein, *Gyrodinium* Kofoid et Swezy (Daugbjerg et al. 2000, Hansen et al. 2000, Hansen and Daugbjerg 2004, Takano and Horiguchi 2004), and also *Amphidinium* (Fløe Jørgensen et al. 2004a, Murray et al. 2004). More precise re-definitions of these genera have caused many of the species formerly assigned to them to be considered ''sensu lato (s.l.) taxa''. Several new genera have been described accordingly, such as *Akashiwo* Hansen et Moestrup, *Karenia* Hansen et Moestrup, *Karlodinium* Larsen, and *Takayama* de Salas, Bolch, Botes et Hallegraeff (Daugbjerg et al. 2000, De Salas et al. 2003) for *Gymnodinium* s.l. taxa and *Togula* Fløe Jørgensen, Murray et Daugbjerg, *Prosoaulax* Calado et Moestrup, and *Apicoporus* Sparmann, Leander et Hoppenrath (Fløe Jørgensen et al. 2004b, Calado and Moestrup 2005, Sparmann et al. 2008) for *Amphidinium* s.l. taxa.

After re-investigations of *A. operculatum* Claparède et Lachmann, the type species, and putative relatives (Fløe Jørgensen et al. 2004a, Murray et al. 2004), the genus *Amphidinium* was redefined as dorso-ventrally flattened, athecate dinoflagellates with a minute

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epicone that overlays the anterior ventral part of the hypocone and deflects to the left (Flø Jørgensen et al. 2004a). The epicone can be irregular, triangular-shaped or crescent-shaped. Cells may or may not be photosynthetic. Of the taxa previously classified within *Amphidinium* only ~20 species fall into the 'sensu stricto' (s.s.) definition, leaving ~100 species of uncertain or unknown generic relationship (Murray 2003). Three new genera have been described already, as mentioned above, and *Amphidinium pellucidum* Herdman was transferred into the genus *Gymnodinium*, as *G. venator* Flø Jørgensen et Murray (Flø Jørgensen et al. 2004a,c).

Another *Amphidinium* species that does not fit the above description is *A. semilunatum* Herdman. This heterotrophic species with a characteristic morphology was originally described by Herdman (1924) from beach sand at Port Erin. Lebour (1925) copied the original description from Herdman without adding any new information, and she modified the drawing from Herdman by giving the right lateral view (Lebour 1925, p. 28, Fig. 9B) as interpretation of the original drawing showing the left lateral side (Herdman 1924, a, p. 61, Fig. 7 – reproduced here as Fig. 1A); also Schiller reproduced the information provided by Herdman (Schiller 1933). Bursa (1968) recorded *A. semilunatum* from the Canadian Arctic and Alaska. New observations by Baillie (1971) provided additional morphological information. His drawing of the ventral view shows the extension of the sulcus onto the episome (reproduced here as Fig. 1Ba). In our opinion, Baillie (1971) drew the species side-reversed (reproduced here as Fig. 1B). Larsen (1985) depicted *A. semilunatum* with light micrographs showing all characteristic features, and his observations were in agreement with the original description, only amending it by describing the sulcal extension onto the epicone and the slight girdle displacement. As the species was originally not described from the ventral side, because this is very difficult to observe in the light microscope, these ''differences'' were judged to not be critical. Later, the species was found in tropical sediments, showing exactly the same morphology as specimens described from temperate sites (Larsen and Patterson 1990, reproduced here as Fig. 1C). The last additions to the morphological description of *A. semilunatum* were performed by Murray, who observed a narrow ventral ridge and an apical groove running as straight line along the left side of the apex (Murray and Patterson 2002, Murray 2003). Moreover, she observed a morphotype containing large extrusomes (Murray and Patterson 2002, Murray 2003). Hoppenrath (2000a) noticed specimens showing morphological variability – e.g., cells strongly pointed in the posterior dorsal end – and relatively small and short cells. Generally, the morphology of *A. semilunatum* is distinct and the morpho-species is well established.

In an effort to improve our taxonomic and phylogenetic understanding of this marine athecate dinoflagellate, we re-investigated it from several distant sites around the world. The uncultured morphotype was isolated from marine sand collected in Germany, Canada, and Australia. We evaluated whether the species belonged to the *Amphidinium* s.s. or a different genus altogether, using light and scanning electron microscopy and molecular phylogenetic analyses of small and large subunit ribosomal DNA (SSU and LSU rDNA) sequences.

MATERIALS AND METHODS

Sampling and cell isolation. A spoon was used to collect the top 5 cm of sand exposed during low tides. Samples were then brought back to the laboratory and the melting seawater-ice method (Uhlig 1964) was used with a 45 μm mesh size filter to extract organisms from the sand. Dinoflagellates were gathered in a Petri dish and then investigated at 40–250× magnification. Micropipetting was used for further processing of the cells as described below.

Collections of marine sand in Canada took place in summer 2006 at sites in Vancouver (Boundary Bay). First observations and cell isolations in Germany were performed from 1997 to 1999 in the German Wadden Sea around Sylt (Hoppenrath 2000a, Saldarriaga et al. 2001). Cell isolations for DNA extraction were performed in 2009 from samples from Wilhelmshaven

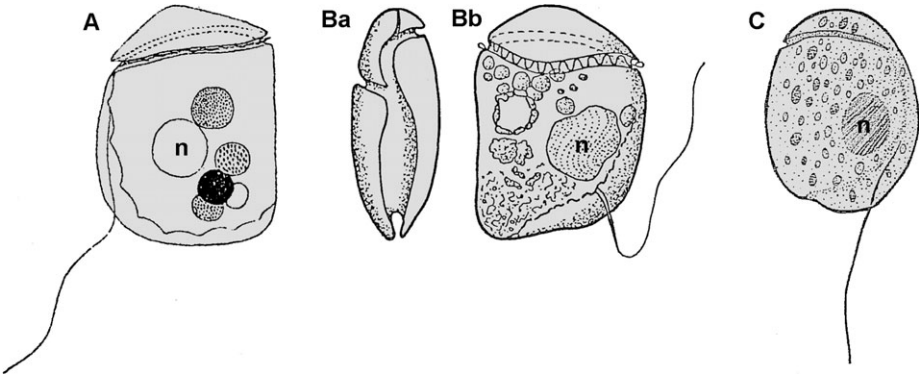

FIG. 1. Drawings from the literature showing *Amphidinium semilunatum*. (A) Modified after Herdman 1924, left lateral view; (B) modified after Baillie 1971, (a) side-reversed ventral view, (b) side-reversed left lateral view; (C) modified after Larsen and Patterson 1990, right lateral view. n = nucleus.

and Helgoland, Germany. Specimens were observed and documented at sites in Sydney, Australia, from 2000 to 2002 (Murray and Patterson 2002).

For documentation with differential interference contrast (DIC) light microscopy, the cells of interest were micropipetted onto glass specimen slides and covered with cover slips. A Zeiss Axioplan 2 imaging microscope connected to a Leica DC500 color digital camera was used to capture images in Canada. In Germany cells were examined with a Leica DMRB microscope using DIC, and a Leitz Orthoplan microscope, using a seawater-immersion objective (SW 50). In Australia, images were taken using a Leica DMR compound light microscope with DIC optics.

Scanning electron microscopy. Environmental samples extracted from the sand were first fixed with evaporating OsO_4 for about 25 min and then by directly adding eight drops of OsO_4 (4% solution) to the sample for about 20 min. Following this, the cells were transferred onto a 5-μm polycarbonate membrane filter (Corning Separations Div., Acton, MA, USA), first washed with distilled water and then gradually dehydrated with increasing amounts of ethanol. After the final step with 100% ethanol the filter was critical point dried using CO_2, mounted on stubs, sputter-coated with gold and examined using a Hitachi S4700 Scanning Electron Microscope. The SEM images were isolated onto a black background using image processing with Adobe Photoshop (Adobe Systems, San Jose, CA, USA).

DNA extraction, PCR amplification, and sequencing. The Epicentre MasterPure complete DNA & RNA Purification Kit (EPICENTRE, Madison, WI, USA) was used for the DNA extraction. Between five and 20 cells were isolated using micropipetting, washed three times in filtered (eukaryote free) autoclaved seawater and then added together. After slight centrifugation and removal of the seawater, 2× lysis (cell tissue) solution mixed with proteinase K was added. The manufacturer's protocol for cell samples was followed.

In Canada and Germany, the isolated genomic DNA was then used for the following PCR amplification protocol with the universal eukaryotic primers: PF1–R4 for SSU (PF1: GCGCTACCTGGTTGATCCTGCC; R4: GATCCTTCTGCAGG TTCACCTAC) and D1R–R2 (initial PCR) followed by D1R–25R1 and D3A–R2 (seminested PCR; Scholin et al. 1994, Nunn et al. 1996) for LSU (D1R: ACCCGCTGAATTTAAGCATA; R2: ATTCGGCAGGTGAGTTGTTAC; 25R1: CTTGGTCCGTGTTT CAAGAC; D3A: GACCCGTCTTGAAACACGGA). Primer sequences for cytochrome b were used (Lin et al. 2009) to amplify the cytochrome b dinoflagellate 'barcode' region: Dinocob4F- 5′-AGCATTTATGGGTTATGTNTTACCTTT; Dinocob3R- 5′-AGCTTCTANDGMATTATCTGGATG. The PCR consisted of an initial denaturing period (95°C for 2 min); 35 cycles of denaturing (92°C for 45 s), annealing (50°C for 45 s), and extension (72°C for 1.5 min); and a final extension period (72°C for 5 min). The sequences were PCR amplified using puReTaq Ready-to-go PCR beads (GE Healthcare, Quebec, Canada). PCR products of the right size were gel isolated and cloned into pCR2.1 vector with the use of a TOPO TA cloning kit (Invitrogen Corporation, CA, USA). New sequences were completely sequenced with ABI big-dye reaction mix (Applied Biosystem, Foster City, CA, USA) using both vector primers and two internal primers oriented in both directions. In Australia typical cycling conditions for PCRs consisted of an initial denaturing step of 94°C for 2 min, followed by 35 cycles consisting of a denaturation step at 94°C for 20 s, an annealing step at 56°C for 30 s, and an extension step at 72°C for 1 min, followed by a final extension step of 7 min, and then a hold at 20°C. PCR products were separated using agarose gel electrophoresis, and then stained with ethidium bromide and visualized using UV transillumination. Fragments to be sequenced were excised from the gel. DNA was purified using ULTRA CLEAN reaction (in Canada), QIAquick gel extraction kit (in Germany), or a Bioline gel purification kit (in Australia), eluted in 12 μL dH_2O (in Canada), 30 μL DEPC treated water (in Germany), or 2 × 10 μL of elution buffer (in Australia). The concentration checked by nanodrop and ~40 ng of PCR product was then used for direct sequencing with the same primers used for the initial amplification of the product (in Australia). Sequences were checked against the NCBI nucleotide database before use in phylogenetic analysis. GenBank accession codes for *Ankistroduinium*: JQ179861 SSU Wilhelmshaven, Germany; JQ179860 SSU Canada clone 4; JQ179859 SSU Canada clone 7; JQ179865 LSU Canada clone 1; JQ179864 LSU Canada clone 2; JQ179863 LSU Canada clone 4; JQ179862 LSU Helgoland, Germany; JQ179866 for cob for Helgoland, Germany; sequence and new sequence for *Apicoporus glaber* JQ179867 LSU Sylt, Germany.

Alignment and phylogenetic analyses. The new sequences were aligned with other dinoflagellate sequences (SSU: 53 taxa, LSU: 62 taxa). Alignments were performed using ClustalW and checked by eye (SSU: 1638 bp, LSU: 928 bp included in the analysis). FindModel was used to analyze alignments and determine which phylogenetic model to use prior to tree generation (SSU: GTR + gamma model, LSU: GTR + I + gamma model). Maximum likelihood trees were constructed with PhyML (Guindon and Gascuel 2003) using the general time-reversible model with a gamma distribution (SSU: Ln likelihood -9869.103, LSU: Ln likelihood -15303.55916), bootstrapped 1000 times. The trees were rooted using a colpodellid (*Colpodella pontica*), another alveolate (*Voromonas pontica*) in the SSU analyses, and the apicomplexan *Besnoitia besnoiti* in the LSU analyses.

Bayesian analyses were conducted using MrBayes 3.2 (Ronquist et al. 2012), using the same model as previously determined to be optimum (SSU: GTR + gamma model, LSU: GTR + I + gamma model). Analyses were run for 2,000,000 generations, sampling every 1000 generations, with a burnin of 25%. The posterior probabilities based on the majority rule consensus phylogeny of the sampled Bayesian trees are reported.

RESULTS

Taxonomy. Ankistrodinium Hoppenrath, Murray, Sparmann et Leander gen. nov.

Etymology: Greek ''ankistri'' (αγκίστρι), meaning fish-hook; referring to the shape of the acrobase that is characteristic for the genus.

Diagnosis: Athecate laterally flattened cells with a small asymmetric epicone. Epicone higher on left lateral than on right lateral side. Fish-hook shaped acrobase. Sulcus wide and deeply incised running around the antapex, reaching the dorsal side. Sulcal extension onto epicone. Ventral ridge.

Type: *Ankistrodinium semilunatum* (Herdman) Hoppenrath, Murray, Sparmann et Leander comb. nov.

Basionym: *Amphidinium semilunatum* Herdman 1924, Notes on dinoflagellates and other organisms causing discolouration of the sand at Port Erin. III. *Proc. Trans. Liverpool Biol. Soc.* 38: p. 59, Fig. 7.

Nomenclatural (homotypic) synonym: *Thecadinium semilunatum* (Herdman) Dodge 1982

Emended description: Cells are strongly laterally flattened, rounded-quadrangular to oval in lateral view, with a small asymmetric epicone (Figs. 2; 3A; 4A). The epicone is higher on the left lateral side than on the right lateral side (Figs. 2A, C; 4) because the cingulum is rising from its origin over

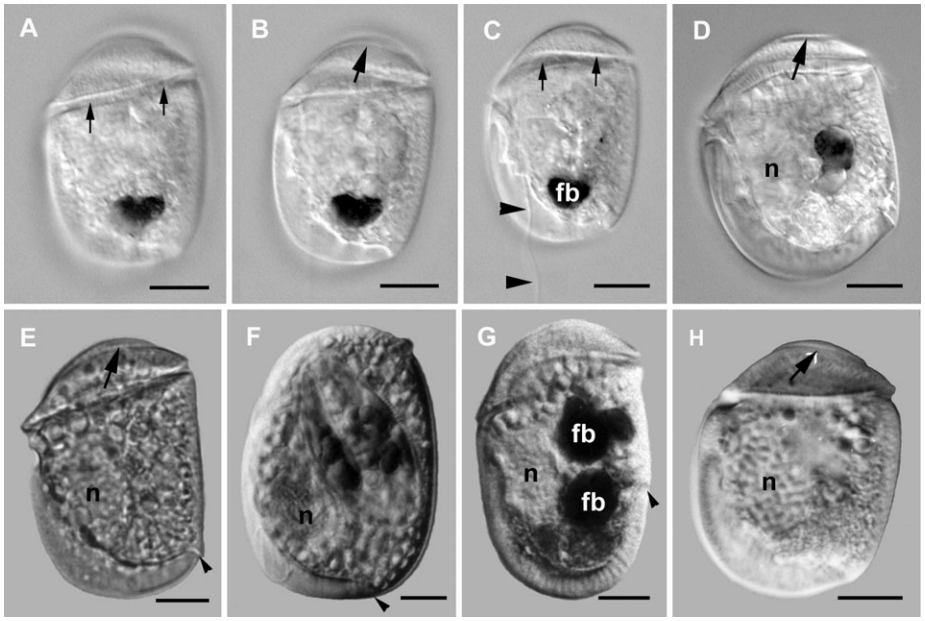

FIG. 2. Light micrographs of *Ankistrodinium semilunatum* from different sites. (A–D) Cells from Canada; (A) left lateral view, note the cingulum path (small arrows); (B) same cell in mid cell focus, note the acrobase (arrow); (C) same cell in right lateral focus, note the path of the cingulum (small arrows), the food body (fb), and the longitudinal flagellum (arrowheads); (D) focus on the right lateral side, note the acrobase (arrow) and nucleus (n); (E–G) cells from Germany; (E) left lateral view, showing acrobase (arrow) and nucleus (n), note the end of the sulcus (arrowhead); (F) mid cell focus, note the large ingested diatom cell, the nucleus (n), and the end of the sulcus (arrowhead); (G) mid cell focus, showing food bodies (fb) and the nucleus (n), note the end of the sulcus (arrowhead); (H) cell from Australia, left lateral view, note the acrobase (arrow) and the nucleus (n). Scale bars, 10 μm.

the left lateral side to the dorsal side (Figs. 2A, E; 3A), and around the dorsal side (Fig. 3B), first keeping the height on the right lateral side (for about three quarters of the epicone depth) and finally descending to the sulcus again (Fig. 2C, D). The cingulum is deeply incised and slightly ascending, about one cingular width (Fig. 3D, F). The large hypocone is ventrally convex, dorsally nearly straight to slightly convex, posteriorly rounded, and dorsal higher than ventral (Figs. 2 and 3A). The sulcus is wide and very deeply incised on the hypocone running around the antapex and reaching the dorsal side (Figs. 2 and 3B–E). This striking feature is visible in the light microscope because of the transparent (hyaline) hypoconal flanges (lists of cytoplasm) running along the sulcus giving the typical ''semilunate'' appearance (Fig. 2). The length of the sulcus is variable, from ending at the antapex (Fig. 2B, F and H) – the most common morphology – over reaching the dorsal side (Figs. 2E; 3B and E) to running up the dorsal side (Fig. 2G). The sulcus extends onto the epicone as narrow and deep groove (Figs. 3C, D, F; 4B, C). At the end of the sulcal extension, the acrobase (apical groove) starts (Fig. 3F, G). The acrobase runs as straight line over the left apex to the dorsal epicone side, curving back into the ventral direction in a steep way and forming a short hook-like end (Figs. 2B, D, E, H, 3F–H; 4B, C). The complete shape of the acrobase was shown in this study for the first time. A short ventral ridge starts at the beginning of the cingulum and runs down the sulcus (Fig. 3C, D). It seems to be connected with the upper left edge of the hypocone (Fig. 3D). The nucleus is located in the mid-ventral area of the hypocone (Fig. 2D–H). Dark colored food bodies can be present in the cell (Fig. 2C, G) and sometimes whole ingested diatoms can be identified (Fig. 2F). A cell full of ingested cyanobacteria was observed in German samples (not shown). The cells neither contain large extrusomes nor chloroplasts. In this study, cells were 29–60 μm long, 20–40 μm deep, and about 6 μm wide (Table 1). The size range reported in the literature is 29–64 μm long, 20–48 μm deep, and about 6–20 μm wide (Table 1).

Molecular phylogenetic inferences. The results of the phylogenetic analyses based on LSU rDNA and SSU rDNA show that *A. semilunatum* is a distinct taxon and only distantly related to species of the genus *Amphidinium* sensu stricto.

The four SSU rDNA sequences of *A. semilunatum* clustered together with boot strap (BS) support of 73% and Bayesian posterior probability of 0.96 (Fig. 5). The sequence from Wilhelmshaven branched as the sister lineage to a clade containing the sequences from Canada and Sylt (Fig. 5). The sister group relationship between *A. semilunatum* and other dinoflagellate clades was not well supported.

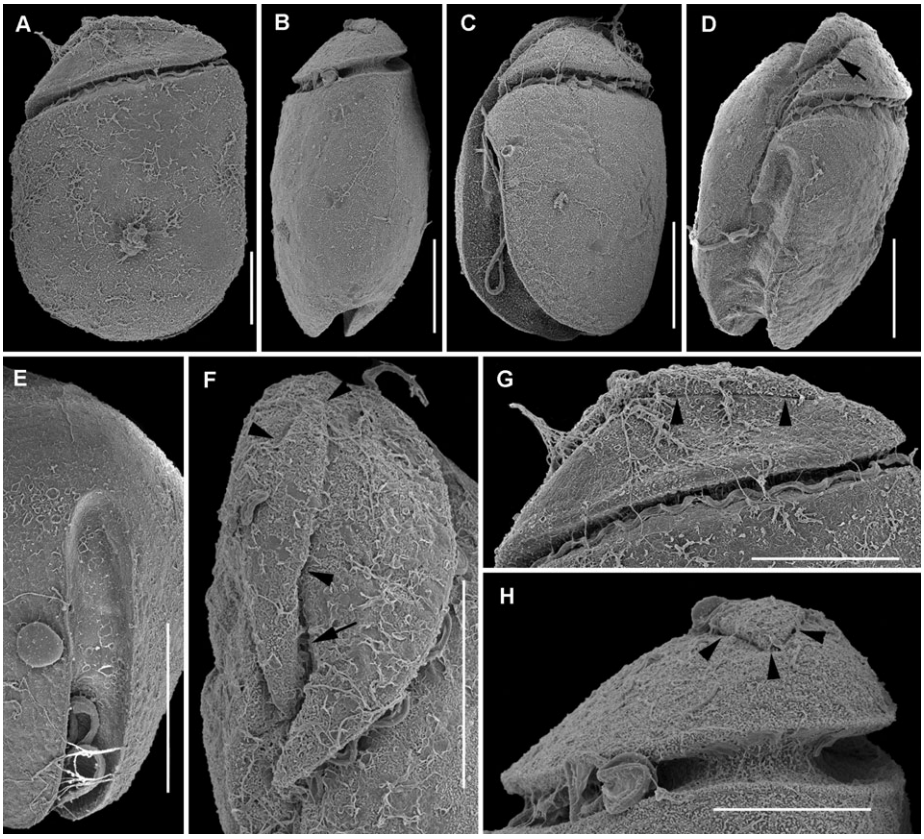

FIG. 3. Scanning electron micrographs of *Ankistrodinium semilunatum* from Canada. (A) Left lateral side; (B) dorsal side; (C) oblique ventral to left lateral view; (D) ventral view showing the wide sulcus, the sulcal extension onto the epicone (arrow), and the ventral ridge; (E) antapical view showing the sulcus running to the dorsal cell side; (F) apical view showing the sulcal extension (arrow) and the hook-shaped acrobase (arrowheads); (G) left epicone side, note the straight path of the acrobase (arrowheads); (H) dorsal epicone side, note the acrobase (arrowheads); scale bars, 10 μm.

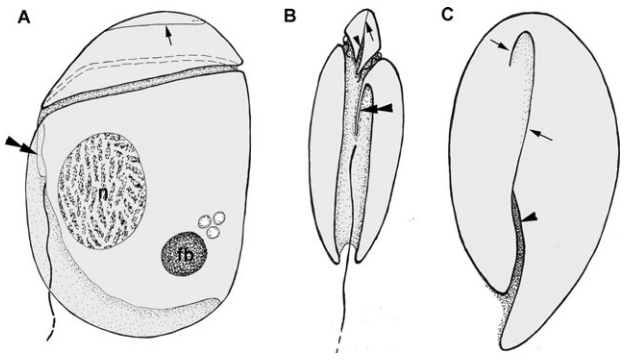

FIG. 4. Line drawings of *Ankistrodinium semilunatum*. (A) Lateral view (mainly from left) of a cell; (B) ventral view of a cell, (C) apical view of a epicone. Note the acrobase (arrow), the sulcal extension (arrowhead), and the ventral ridge (double arrowhead). n = nucleus, fb = food body.

In the analyses based on LSU rDNA, the five sequences formed a well supported clade (99% and 1.00 pp, Fig. 6). This clade formed part of a larger clade that included species of the Kareniaceae and *Apicoporus*; however, this larger clade was not well supported. The sequences from Canada fell into a separate clade to those from the North Sea region.

TABLE 1. Cell sizes of *Ankistrodinium semilunatum* cells from different geographical regions/studies.

	Length [μm]	Width [μm]	Depth [μm]	*n* = sample size
Germany	34–60	NA	25–35	16
Canada	30–50	NA	20–40	23
Australia[a]	29–49	6	20–30	5
Port Erin, England[b]	~50	NA	NA	NA
Folkstone, England[c]	50–64	NA	30–48	NA
Denmark[d]	31–37	12–15	25–29	NA
Canada[e]	38–55	12–20	28–40	NA

[a]Murray and Patterson (2002), [b]Herdman (1924), [c]Dodge (1982), [d]Larsen (1985), [e]Baillie (1971), NA = no data.

We constructed an alignment of 25 sequences of various dinoflagellates of 338 bp of the short 'barcoding' region of the mitochondrial gene cytochrome b, including *Ankistrodinium semilunatum* from Helgoland, Germany. In a pairwise comparison, this sequence was found to be only 0.67–0.75 similar to aligned sequences of cytochrome b from the species of *Amphidinium* ss, *A. carterae* and *A. operculatum*. It was most similar (0.90–0.94) to aligned sequences from the species *Karenia brevis* and *Karlodinium micrum*.

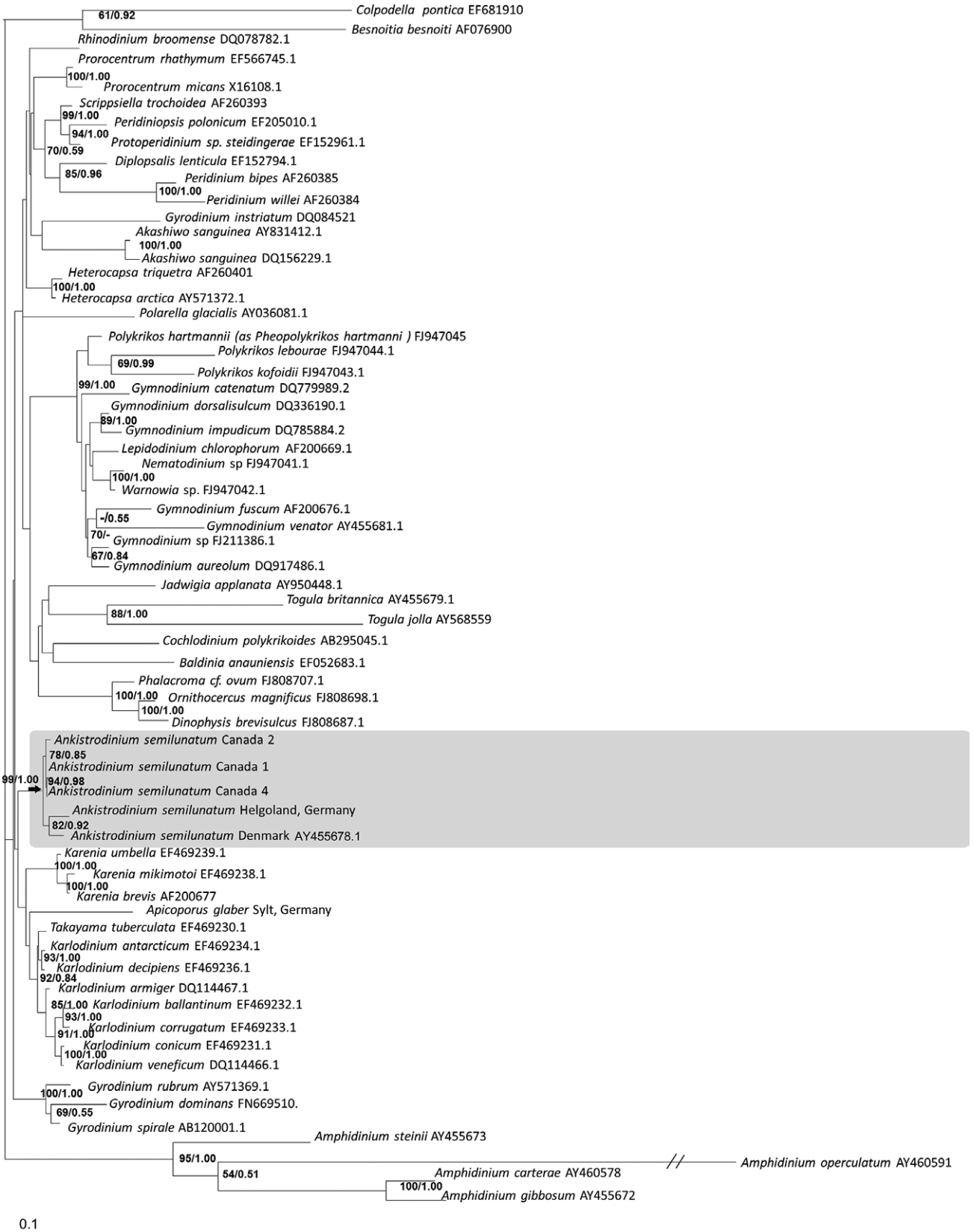

FIG. 5. The most likely phylogenetic tree based on an Maximum Likelihood (ML) analysis of partial sequences of SSU rDNA from species of dinoflagellates, with an emphasis on unarmoured species. The values at nodes represent bootstrap (BS)/Bayesian posterior probability (PP) values. Only values above 50% are shown. Sequences from *Ankistrodinium* are highlighted. Likelihood = 9,869.103.

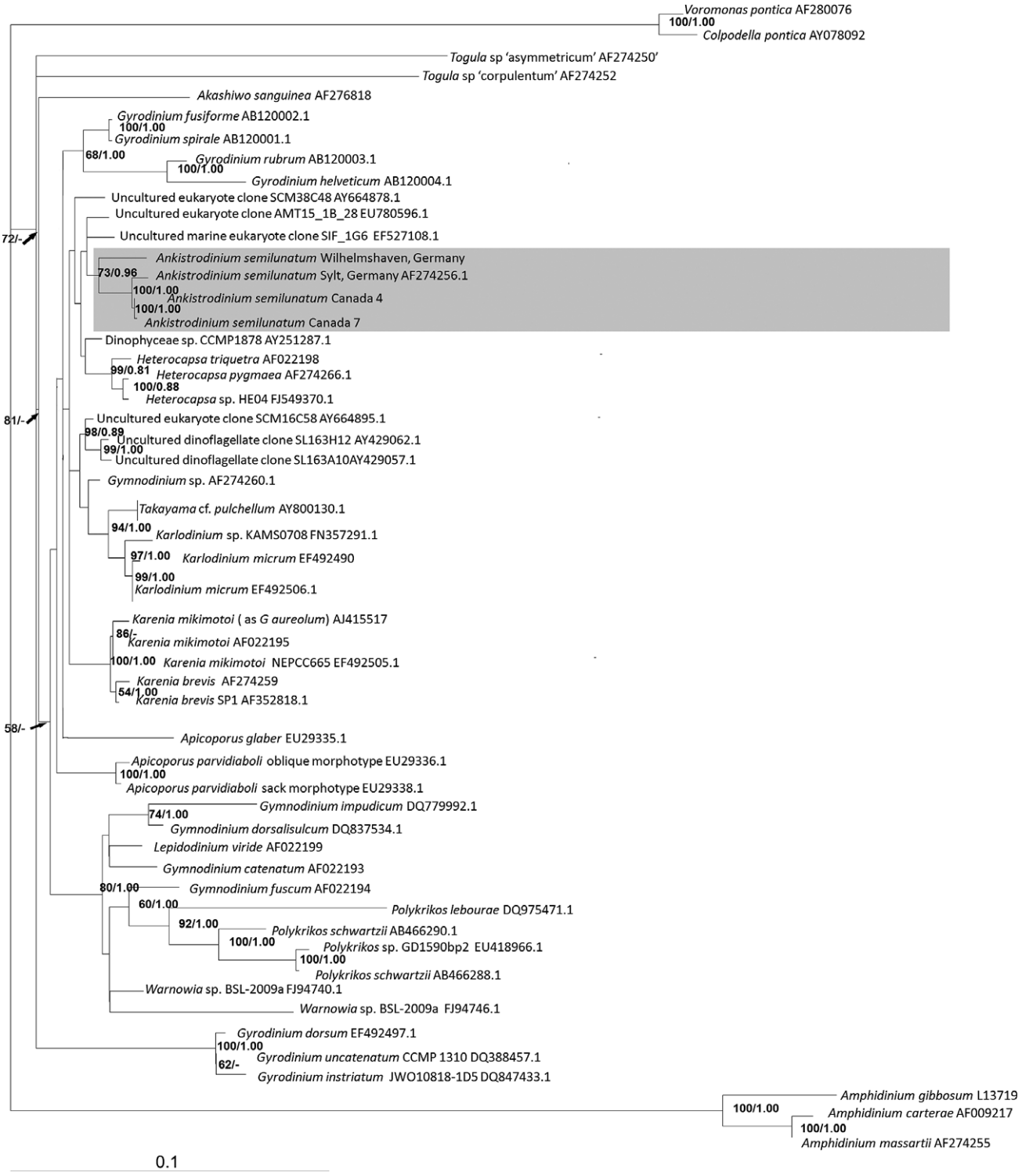

FIG. 6. The most likely phylogenetic tree based on an Maximum Likelihood (ML) analysis of partial sequences of LSU rDNA from species of dinoflagellates, with an emphasis on unarmoured species. The values at nodes represent bootstrap (BS)/Bayesian posterior probability (PP) values. Only values above 50% are shown. Sequences from *Ankistrodinium* are highlighted. Likelihood = 15,303.559.

DISCUSSION

Taxonomy and morphology. The morpho-species criteria for *Ankistrodinium semilunatum* are distinctive and well established. However, Dodge (1982) argued that the species possesses a delicate theca (without investigating plates) and transferred it into the genus *Thecadinium*, as *T. semilunatum*; he also

regarded *Thecadinium inclinatum* as conspecific. The new combination was premature, as discussed by Larsen (1985), Hoppenrath (2000a), and also Dodge (Saunders and Dodge 1984). Balech (1956) originally described *Thecadinium inclinatum*, and Hoppenrath et al. (2004) demonstrated the thecal tabulation of this species. In this study, we demonstrated for the first time using SEM that *A. semilunatum* is truly naked.

Specimens with a slightly different morphology were described and documented from Australia (Murray and Patterson 2002). These specimens contained a row of large extrusomes in the posterior hyposome (Murray and Patterson 2002, Fig. 61). This conspicuous feature was never observed in German or Canadian specimens. Interestingly, Balech (1956) briefly mentioned that he found *A. semilunatum* with visible trichocysts (Balech 1956, p. 29: ''… *A. semilunatum* (très abundant, avec de beaux trichocysts qui n'ont pas été signalés) …''). A more detailed description or drawings were not provided. Despite looking for this morphotype in Australia, we were unable to find cells for reinvestigation. If the presence of extrusomes is a stable character, then it is possible that these morphologically slightly different specimens represent a second species of *Ankistrodinium*.

There is one '*Amphidinium*' species of similar size that resembles *A. semilunatum* in right lateral view, namely *A. sulcatum* Kofoid (Kofoid 1907). The species is laterally flattened, the epicone is very small and low, and the sulcus ''deeply channeled'', giving a similar appearance than in *Ankistrodinium*. However, unlike *Ankistrodinium*, the epicone is not asymmetrical, the cingulum is wide and deep, and the right sulcal flange is higher than the left (Kofoid 1907). A deep sulcal extension starts, similar to that in *Ankistrodinium*, but runs further over the apex. An acrobase has not been described (Kofoid 1907). *A. sulcatum* was reported to contain small yellowish chromatophores, interpreted as chloroplasts, which are not present in *A. semilunatum*. Dodge (1989) recorded *'Amphidinium' sulcatum* as separate from *Ankistrodinium semilunatum*, indicating that they are different and morphologically distinguishable species. These two species should not be confused with the taxon Herdman (1921) identified as *A. sulcatum* and later (Herdman 1922) transferred to *Amphidinium kofoidii* var. *petasatum* that is today known as *Thecadinium kofoidii* (Hoppenrath 2000b).

The morphological features of *Ankistrodinium*, especially the characteristic straight acrobase with a short hook-like end, suggest that this genus may be related to *Karenia* Hansen et Moestrup and *Karlodinium* Larsen, genera possessing a straight acrobase (Daugbjerg et al. 2000). The general morphology of *Ankistrodinium* does not suggest any close relationship to the redefined genus *Amphidinium* whatsoever (Flø Jørgensen et al. 2004a, Murray et al. 2004).

Molecular phylogenetic relationships. The phylogenetic results clearly show that *A. semilunatum* is a distinct taxon and is only distantly related to species of the genus *Amphidinium*. These molecular phylogenetic results are consistent with our morphological reinvestigation. The different lineages of *A. semilunatum* from different geographical locations formed a monophyletic group in all analyses. However, molecular differences in the geographic isolates suggest that cryptic diversity may be present within this taxon. Comprehensive investigation of the species diversity within the new genus was not within the scope of this study. Future work may uncover ultrastructural or other molecular differences that distinguish the Canadian strains of *A. semilunatum* from the majority of the strains in German and Danish waters. At least one of the genotypes appears to have a cosmopolitan distribution because the same SSU rDNA sequence was found in both Canadian and German waters.

The specific morphological features of this genus, in particular, the lack of thecal material in the amphiesmal vesicles and the possession of an acrobase, indicate that this taxon is a member of the order Gymnodiniales. In phylogenetic analyses based on ribosomal genes and mitochondrial genes (e.g., cox1 and cytochrome b), this order has been polyphyletic, even when excluding the genus *Amphidinium* (Flø Jørgensen et al. 2004b, Saldarriaga et al. 2004, Murray et al. 2005, 2009, Zhang et al. 2005, 2007, Sparmann et al. 2008).

The morphological features of *Ankistrodinium*, in particular, the sulcal extension onto the epicone(Fig. 3C, D, F) and the possession of a straight acrobase with a short hook-like end, suggest that this taxon may be related to genera with a straight acrobase, like in the Kareniaceae (De Salas et al. 2004a,b, Bergholtz et al. 2005), or species that possess an anti-clockwise circular acrobase encircling the epicone, like in the *Gymnodinium s.s.* clade (e.g., *Gymnodinium*, *Lepidodinium*, and *Polykrikos*; Daugbjerg et al. 2000, Hoppenrath and Leander 2007a,b). This interpretation is consistent with some of our molecular phylogenetic data. For instance, LSU rDNA sequences show *Ankistrodinium* forming a clade with members of the Kareniaceae, which are characterized by the possession of novel (haptophyte-derived) plastids (*Karenia*, *Takayama* and *Karlodinium)*, and the heterotrophic gymnodinioid genus *Apicoporus*. However, this clade was not well supported. Further information using additional nuclear and mitochondrial genes is necessary to determine whether a relationship among these taxa is apparent.

Biogeography. *Ankistrodinium semilunatum* is most likely occurring worldwide in marine sandy sediments from temperate to tropical regions; so far, the species has been recorded from England (Port Erin, Herdman 1924, a; Folkstone, Dodge 1982), Scotland (North Sutherland, Dodge 1989), the Danish and German Wadden Sea (Rejsby, Denmark, Larsen 1985, Sylt, Wangerooge, Wilhelmshaven,

Germany, Hoppenrath 2000a and this study), the German Bight (Helgoland, Germany, Hoppenrath 2000a), Brittany and Normand, France (Roscoff, Balech 1956, Concarneau, Hoppenrath and Chomérat unpubl. data; Cotentin, Paulmier 1992), Gdansk Bay, Baltic Sea, Poland (Pankow 1990), Elba, Italy (Hoppenrath unpubl. data), Crete, Greece (Hoppenrath unpubl. data), British Columbia, Canada (Boundary Bay, Pachena Beach, Brady's Beach, Wilson Creek, Willows Bay, Baillie 1971 and this study), New South Wales, Queensland and Western Australia, Australia (Botany Bay, Chowder Bay, Durras Lake, Narrabeen Lagoon, Sydney, Murray and Patterson 2002 and Hoppenrath unpubl. data; Bowling Green Bay, Larsen and Patterson 1990, Shark Bay, Broome, Al-Qassab et al. 2002, Murray and Hoppenrath unpubl. data), Kuwait (Al-Yamani and Saburova 2010), Alaska, USA (Bursa 1968).

Data about the seasonality at a site are only known from Germany. *A. semilunatum* has been registered year round at Sylt in all eulittoral areas and also the sublittoral zone. Highest abundance was observed in late summer and autumn (Hoppenrath 2000a).

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