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Molecular phylogeny of the marine dinoflagellate genus *Heterodinium* (Dinophyceae)

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The dinoflagellate genus *Heterodinium* has unusual morphological characters such as a mid-ventral intercalary plate with a pore, a small plate in the left side of the dorsal epitheca, three antapical plates, and a well-developed anterior cingular list. We obtained the first SSU rDNA sequences from single cells of six species of *Heterodinium* from Mediterranean coastal and open waters. They included the type species *H. scrippsii* and *H. rigdeniae* and representatives of the other subgenera, *Sphaerodinium* (*H. doma*, *H. milneri*, *H. globosum*) and *Platydinium* (*H. pavillardii*). SSU rDNA phylogeny showed that *Heterodinium* spp. formed a well-supported monophyletic group (100% bootstrap support) composed of two subclades: one comprising *H. doma*, *H. pavillardii*, *H. globosum* and *H. rigdeniae*, and another comprising *H. milneri* and *H. scrippsii*. This whole heterodiniacean clade branched among the poorly resolved short-branching sequences of the lineage comprising groups of Gymnodiniales, Peridiniales, Dinophysales and Prorocentrales. The current classification into subgenera, and even into morphological groups, is not supported by the molecular data. In contrast to previous classifications, our SSU rDNA phylogeny suggests that the genus *Heterodinium* is divergent from the clade of Gonyaulacales. Accordingly, the supposed homology of pores and plate patterns of *Heterodinium* and gonyaulacoids may require revision. In *Heterodinium*, the first antapical and postcingular plates may be interpreted as sulcal plates, suggesting a more typical hypothecal tabulation (5‴, 2⁗). Our new data and analysis indicate that the systematic position of *Heterodinium* is uncertain at present.

Key words: Dinoflagellata, Gonyaulacales, gonyaulacoid dinoflagellate, Gymnodiniales–Peridiniales–Prorocentrales lineage, *Heterodinium*, single-cell PCR, SSU rDNA phylogeny

Introduction

The genus *Heterodinium* contains a large number of species most often reported from deep waters of warm temperate and tropical seas (e.g. Kofoid & Adamson, 1933). The first *Heterodinium* species were described as *Peridinium* and *Goniodoma* from the tropical Atlantic Ocean (Murray & Whitting, 1899). Kofoid (1906) erected a separate genus, *Heterodinium*, for these species and later added up to 30 new species from the tropical Pacific Ocean (Kofoid & Adamson, 1933). Other species were described from the Mediterranean Sea (Pavillard, 1916; Schiller, 1937; Rampi, 1941).

The distinctive features of the genus *Heterodinium* are the anterior cingular list, which is usually well developed and often supplemented by an angular projection of the body wall itself, the two separated anterior intercalary plates with a pore in the mid-ventral plate, and the three antapical plates (Balech, 1988). Following Kofoid's original scheme (Kofoid, 1906), Kofoid & Adamson (1933) divided the genus *Heterodinium* into three subgenera. One subgenus, *Sphaerodinium* Kofoid, was erected for species with a spherical or rotund body and either no antapical horns [*H. doma* (G. Murray & Whitting) Kofoid], or spines or weakly developed horns [*H. milneri* (G. Murray & Whitting) Kofoid, *H. globosum* Kofoid]. The more distinctive members of the genus *Heterodinium* are characterized by an elongated body, flattened dorsoventrally and with strong antapical horns. These species were placed into two subgenera: the subgenus *Heterodinium* for species possessing an epitheca that narrows towards a more or less conical truncate apex, and an apical horn (the type species *H. scrippsii* Kofoid and *H. rigdeniae* Kofoid.

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The latter is often misspelled as 'rigdenae' but should be corrected according to ICBN Article 60.11, Recommendation 60C.1, and Article 32.7: see McNeill *et al*., 2006), and *Platydinium* Kofoid for species with a strongly flattened epitheca that expands scoop-like with a rounded apex (*H. pavillardii* Kofoid & A.M. Adamson). While these groups appeared 'natural', uniting forms with shared morphological characteristics, it was admitted that the characters employed represented gradients among species in the two subgenera and thus the subdivision was perhaps mainly one of convenience (Kofoid & Adamson, 1933, p. 26).

Kofoid gave the plate formula 3′, 1a, 6″, 6–7?c, 7‴, 3⁗ for the genus *Heterodinium* (Kofoid & Adamson, 1933). However, Balech (1962, p. 150) found only six cingular plates, and he interpreted the seventh postcingular one as a sulcal plate. He also identified two intercalary plates, a mid-ventral plate with a pore, and a small plate in the left side of the dorsal epitheca. Later, Balech (1988, p. 153) proposed the plate formula 3′, 2a, 6″, 6c, 6‴, 3⁗. The plates of the apex (apical pore complex, APC) of *Heterodinium* have a pore and a canal plate, as is usual in gonyaulacoids, and lack the plate 'X' that is typical of peridinioids (see Table 1 in Steidinger & Tangen, 1997).

Historically, the genus *Heterodinium* has been allied to various groups (Lindemann, 1928; Kofoid & Adamson, 1933; Schiller, 1937). In modern classifications, the family Heterodiniaceae is placed alongside the gonyaulacoid Ceratocoryaceae, Goniodomataceae and Ceratiaceae (Taylor, 1976; Sournia, 1986; Balech, 1988). The Heterodiniaceae are placed within Gonyaulacales when this order is separated from Peridiniales (Taylor, 1987; Steidinger & Tangen, 1997). Fensome *et al*. (1993, p. 114) listed the morphological characters that justified the placement of *Heterodinium* within the order Gonyaulacales, and they interpreted the distinctive morphological features of *Heterodinium* to be homologues of those in the typical Gonyaulacales.

The assumed close relatives of *Heterodinium* – members of Ceratocoryaceae, Goniodomataceae and Ceratiaceae – are well nested in the monophyletic gonyaulacoid lineage in phylogenies based on small subunit (SSU) and large subunit (LSU) ribosomal DNA (rDNA) (see for example, Saldarriaga *et al*., 2004; Logares *et al*., 2007; Moestrup & Daugbjerg, 2007). Therefore, one would also expect *Heterodinium* to be well-nested within the gonyaulacoids. In this study we examine the systematic position of *Heterodinium* based on a molecular phylogeny and an analysis of morphological features. In particular, we provide 12 new SSU rDNA sequences of six species of the genus *Heterodinium*; among these are representatives of all three subgenera and the type species, *H. scrippsii*.

Materials and methods

Sampling and isolation of material

Specimens were isolated from water samples collected at three coastal sites in the north-western Mediterranean Sea (Marseille, Banyuls-sur-Mer, Villefranche-sur-Mer) and two open-water sites in the eastern Mediterranean Sea. At the Marseille site, surface water was sampled from the pier (water depth 3 m) of the Station Marine d'Endoume, Marseille (43°16′48″N, 5°20′57″E) from October 2007 to September 2008. Ten to 100 litres of seawater, according to the concentration of particles, were slowly filtered using a plankton concentrator fitted with Nitex screening (20, 40 or 60 μm mesh-size). In addition, we also studied samples collected during several monitoring research cruises to the SOMLIT (Service d'Observation en Milieu LITtoral) station in the Bay of Marseille (43°14′30″N, 05°17′30″E, bottom depth 60 m). Seawater samples were collected with a 12-litre Niskin bottle at 40 and 55 m depth and filtered as described above. The concentrated sample was examined in Utermöhl chambers at 100× magnification with a Nikon inverted microscope (Nikon Eclipse TE200) and was photographed at 200× or 400× magnification with a digital camera (Nikon Coolpix E995). Sampling continued from October 2008 to August 2009 in the surface waters of the port (depth of 2 m) of Banyuls-sur-Mer, France (42°28′50″N, 3°08′09″E), and from September 2009 to February 2010 in the Bay of Villefranche-sur-Mer, Ligurian Sea. For the latter location, sampling was performed at the long-term monitoring site Point B (43°41′10″N, 7°19′00″E, water column depth ~80 m). This site, located on the slope of a submarine canyon, is well known to be rich in deep-water plankton organisms associated with the upwelling of deep water (Bougis, 1968). Water column samples (0–80 m) were obtained using a phytoplankton net (53 μm mesh size, 54 cm diameter, 280 cm length). Samples were prepared according to the same procedure as described above and specimens were observed with an Olympus inverted microscope (Olympus IX51) and photographed with an Olympus DP71 digital camera.

Open-water samples were collected during the BOUM (Biogeochemistry from the Oligotrophic to the Ultra-oligotrophic Mediterranean) cruise in the Mediterranean Sea between the Gulf of Lions and Cyprus in June–July 2008. Ten litres of seawater were collected from the surface with a bucket and filtered by using a strainer of 20 μm netting aperture. The material retained was fixed with absolute ethanol to a final concentration of 50% concentrated sample and 50% ethanol. In the laboratory, the ethanol sample was examined following the procedure described above.

After being photographed, each *Heterodinium* specimen was micropipetted individually with a fine capillary into a clean chamber and washed several times in a series of drops of 0.2 μm-filtered and sterilized seawater (live specimens from coastal waters) or ethanol (ethanol prefixed specimens from open waters). Finally, the

specimen was placed in a 0.2 ml Eppendorf tube filled with several drops of absolute ethanol. The sample was kept at room temperature and in darkness until the molecular analysis could be performed.

PCR amplification of small subunit rRNA genes (SSU rDNAs) and sequencing

The specimens fixed in ethanol were centrifuged for 5 min at 504 × *g*. Ethanol was then evaporated in a vacuum desiccator, and single cells were resuspended directly in 25 µl of Ex TaKaRa buffer (TaKaRa, distributed by Lonza, Levallois–Perret, France). PCRs were done in a volume of 30–50 µl reaction mix containing 10–20 pmol of the eukaryotic-specific SSU rDNA primers EK-42F (5′–CTCAARGAYTAAGCCATGCA–3′) and EK-1520R (5′–CYGCAGGTTCACCTAC–3′) (López-García *et al*., 2001). PCRs were performed under the following conditions: 2 min denaturation at 94 °C; 10 cycles of 'touch-down' PCR (denaturation at 94 °C for 15 s; a 30 s annealing step at decreasing temperature from 65 down to 55°C, employing a 1°C decrease with each cycle, extension at 72 °C for 2 min); 20 additional cycles at 55°C annealing temperature; and a final elongation step of 7 min at 72 °C. A nested PCR was then carried out using 2–5 µl of the first PCR products in a GoTaq (Promega, Lyon, France) polymerase reaction mix containing the eukaryotic-specific primers EK-82F (5′–GAAACTGCGAATGGCTC–3′) and EK-1498R (5′–CACCTACGGAAACCTTGTTA–3′) (López-García *et al*., 2001) and similar PCR conditions as described above. A third, semi-nested PCR was carried out using the dinoflagellate specific primer DIN464F (5′–TAACAATACAGGGCATCCAT–3′) (Gómez *et al*., 2009) and the reverse primer EK-1498R. Negative controls without template DNA were used at all amplification steps. Amplicons of the expected size (~1200 bp) were then sequenced bidirectionally using primers DIN464F and EK-1498R using an automated 96-capillary ABI PRISM 3730xl sequencer (BC Genomics, Takeley, UK).

Phylogenetic analyses

The new SSU rDNA sequences were aligned to a large multiple sequence alignment containing 1100 publicly available complete or nearly complete (>1300 bp) dinoflagellate sequences using the profile alignment option of MUSCLE 3.7 (Edgar, 2004). The resulting alignment was manually inspected using the program ED of the MUST package (Philippe, 1993). Ambiguously aligned regions and gaps were excluded in phylogenetic analyses. Preliminary phylogenetic trees with all sequences were constructed using the Neighbour Joining (NJ) method (Saitou & Nei, 1987) implemented in the MUST package (Philippe, 1993). These trees allowed identification of the closest relatives of our sequences together with a sample of other dinoflagellate species, which were selected to carry out more computationally intensive Maximum Likelihood (ML) analyses. These were done with the program TREEFINDER (Jobb *et al*., 2004) applying a GTR + Γ4 model of nucleotide substitution, taking into account a Γ-shaped distribution of substitution rates with four rate categories. Bootstrap values were calculated using 1000 pseudoreplicates with the same substitution model. Approximately Unbiased (AU) tests (Shimodaira, 2002) were carried out with the AU test tool implemented in TREEFINDER (Jobb *et al*., 2004).

The phylogenetic position of *Heterodinium* was analysed by means of a global alignment of 131 dinoflagellate taxa, including sequences of gonyaulacoid species, with representatives of the Gymnodiniales, Peridiniales, Dinophysales and Prorocentrales, among others. This alignment is available as Supplementary material 1. Our sequences were deposited in DDBJ/EMBL/GenBank under accession numbers JQ446581–JQ446592 (see Table S1).

Results

Species identification

We encountered species of the genus *Heterodinium* sporadically during 2.5 years of sampling along the French Mediterranean coasts at Marseille, Banyuls-sur-Mer and Villefranche-sur-Mer, and also in surface samples collected from the open Mediterranean Sea. Most specimens from surface waters corresponded to the spherical species of the subgenus *Sphaerodinium*, *H. milneri* and *H. globosum*. In contrast, the members of the subgenera *Heterodinium* (*H. scrippsii*, *H. rigdeniae*) and *Platydinium* (*H. pavillardii*) and one species of *Sphaerodinium* (*H. doma*) were found only in material from vertical plankton net hauls in the Bay of Villefranche.

The subgenus *Sphaerodinium* was established for the species with a spherical cell body, usually smaller than members of the other subgenera. This subgenus was divided into two groups: The *kofoidii*-group encompasses the species lacking apical and antapical horns or spines, and the *minutum*-group the species with an apical horn more developed and antapical spines or horns present or not. We obtained the SSU rDNA sequence of *H. doma* (Figs 1–7) as a representative of the *kofoidii*-group. This species was the least differentiated of the genus *Heterodinium*, lacking horns or prominent spines. Our specimens were characterized by a ventrally flattened epitheca and rounded antapex. The cingulum was wide with a prominent precingular rim. The surface of the plates was covered by a heavily marked, fairly regular reticulation, except in the intercalary bands. The cell whose DNA was analysed (isolate FG1527, Figs 1–3) was 80 µm long and 75 µm wide. Several specimens were observed in the Bay of Villefranche in winter of 2010 (e.g. Figs 1–7).

The *minutum*-group was represented by *H. milneri* and *H. globosum* in our samples.

Heterodinium milneri was the most common *Heterodinium* species during our study of material from the French Mediterranean coast. Our two records of *H. milneri* from open waters were the first for the Ionian Sea (Figs 11–17). This species was subspherical, with a low stout apical horn and four stout finned antapical spines (Figs 8–21). The precingular rim was prominent. The thecal wall showed a very coarse reticulation of polygons, each one with a central pit. The cell was 61–66 μm long and 49–56 μm wide. The live specimens showed a brownish pigmentation that resembled that of typical peridinin-containing plastids of dinoflagellates. Unfortunately, we could not determine if chlorophyll *a* was present or not using epifluorescence microscopy.

The second representative of the *minutum*-group, *H. globosum*, was a larger species (length of 110 μm), with a spherical or slightly elongated body divided equally by the cingulum (Figs 22–29). The epitheca was broadly campanulate, with a hemispherical base and flaring rim, and an asymmetric conical horn. The hypotheca showed two sharply pointed, unequal antapical horns. The left horn was larger than the right one. For the same specimen (Figs 22–25), the relative size of the right horn was variable according to the view (Figs 24, 25). The theca was incompletely and very irregularly reticulated, and some large polygons had a marked central pit. The cell was transparent and lacked any pigmentation (Figs 22–29).

The subgenus *Heterodinium* was represented in our molecular phylogeny by *H. rigdeniae* (Figs 30–32) and the type species of the genus, *H. scrippsii* (Figs 33–40). Both taxa are members of the *rigdeniae*-group, characterized by a conical epitheca that is not contracted into an apical horn, whereas the antapical horns are short and stout. Specimens of *H. rigdeniae* were medium-sized with a pentagonal outline (105 μm long, 70 μm wide). The epitheca was conical, with a slightly oblique axis, and the margins were slightly concave. The hypotheca was slightly shorter than the epitheca and had convex margins; it bifurcated into large and quite stout antapical horns, giving a superficial resemblance to some species of *Protoperidinium*. The horns were conical and had acute tips deflected outward from the vertical. The theca was reticulate, with large polygons, especially in the hypotheca in dorsal view, whereas the postcingular region showed coarse square polygons. The entire cell showed a brown to reddish pigmentation (Figs 30–32). *Heterodinium rigdeniae* cells were of similar size to *H. globosum*, but whereas the right horn of *H. globosum* was short and deflected laterally, the antapical horns of *H. rigdeniae* were nearly equal and both projected posteriorly. The theca of *H. rigdeniae* was more reticulated than in *H. globosum* and while *H. rigdeniae* showed a brownish pigmentation, the specimens of *H. globosum* were hyaline (Figs 22–29).

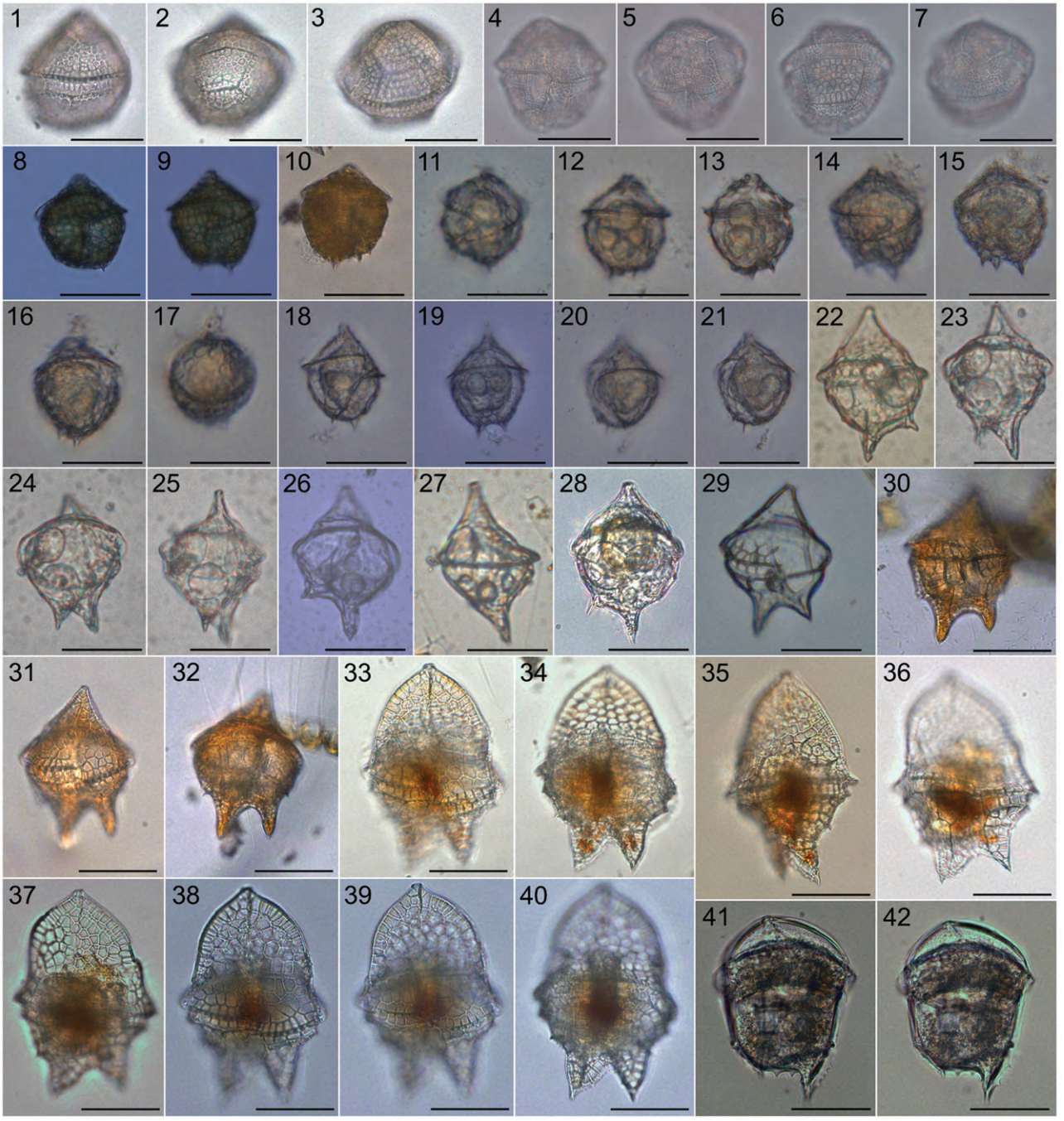
The cell body of *H. scrippsii* was larger and more flattened dorsoventrally than *H. rigdeniae*. The epitheca was pentagonal and considerably larger than the hypotheca; the slightly emergent horn sutures had a ribbed list. The epitheca narrowed towards the truncated apex and showed a well-defined apical horn. Its lateral outlines were marked by symmetrical expansions with rounded shoulders. The conical antapical horns were deflected to the right from the vertical. The left antapical horn was stout, longer and less deflected. The plates were barely marked by a narrow raised rib somewhat heavier than the adjacent mesh. The surface was rather coarsely and heavily reticulated, with porulate polygons. The cell was 130–140 μm long and 95–98 μm wide. It showed a brownish pigmentation in the central body (Figs 33–40).

The subgenus *Platydinium* was represented in our samples by the type of the *pavillardii* group, *H. pavillardii* (= *H. kofoidii* Pavillard, *non H. kofoidii* J. Schiller) (Figs 41, 42). This species was recognizable by its rounded epitheca, lack of reticulation, sharp and unequal incurved antapical horns, and serrated antapical fin. The cell had a length of 85 μm and lacked any pigmentation (Figs 41, 42).

Molecular phylogeny

We examined the phylogenetic position of the *Heterodinium* species using a dataset that included a variety of dinoflagellate SSU rDNA sequences. Trees were rooted using perkinsozoan and syndinean sequences as the outgroup (a similar analysis without these outgroup sequences produced similar results, data not shown). All the *Heterodinium* sequences formed a well-supported clade (bootstrap [BP] of 100%) in maximum-likelihood phylogenetic trees (Fig. 43). However, this clade did not show any particularly close affiliation to other dinoflagellate groups present in public sequence databases. Our *Heterodinium* sequences branched within the large lineage comprising Gymnodiniales, Peridiniales, Dinophysales and Prorocentrales but with poor support, making it difficult to infer the affinity of *Heterodinium* with any of these orders. The long-branched sequences of the typical gonyaulacoid dinoflagellates formed a moderately supported monophyletic group (BP 72%).

We tested the possibility of a relationship between *Heterodinium* and gonyaulacoids using AU tests to compare the tree retrieved with a tree where the monophyly of these two groups was



Figs 1–42. Specimens of *Heterodinium* used for the single-cell PCR analysis (see also Table S1) and some additional specimens; bright field optics. All were live specimens, except for the ethanol-fixed specimens of Figs 4–7, 11–21 and 29. **1–3.** *H. doma* isolate FG1527 (1, 2, dorsal views; 3, ventral view). **4–7.** *H. doma* (4, 5, ventral; 6, dorsal; 7, lateral). **8, 9.** *H. milneri* isolate FG271 (8, dorsal; 9, ventral). **10.** *H. milneri* (ventral view). **11–13.** *H. milneri* isolate FG469 (11, ventral; 12, 13, dorsal). **14–17.** *H. milneri* isolate FG470 (14, ventral; 15, dorsal; 16, lateral; 17, apical). **18–21.** *H. milneri* isolate FG471 (18, ventral; 19, dorsal; 20, 21, lateral). **22–25.** *H. globosum* isolate FG228 (22, ventral; 23, 24, dorsal; 25, lateral). **26, 27.** *H. globosum* isolate FG269 (dorsal view). **28.** *H. globosum* (dorsal view). **29.** *H. globosum* (ventral view). **30–32.** *H. rigdeniae* isolate FG1574 (30, 31, ventral; 32, dorsal). **33–35.** *H. scrippsii* isolate FG1552 (33, dorsal; 34, ventral; 35, lateral). **36, 37.** *H. scrippsii* isolate FG1555 (36, ventral; 37, focus on the epitheca). **38–40.** *H. scrippsii* isolate FG1578 (38, 39, ventral; 40, dorsal). **41, 42.** *H. pavillardii* isolate FG1564 (dorsal views). Scale bars = 50 μm.

constrained and the rest of the tree optimized. The test did not reject this constrained topology ($P = 0.34$). Therefore, although our SSU rDNA phylogeny suggests that the short-branched sequences of the genus *Heterodinium* were not closely related to the clade of gonyaulacoid dinoflagellates (Fig. 43), we lack rigorous statistical evidence for this. A similar AU test did not reject the alternative possibility that *Heterodinium* forms a monophyletic group with the clade containing the

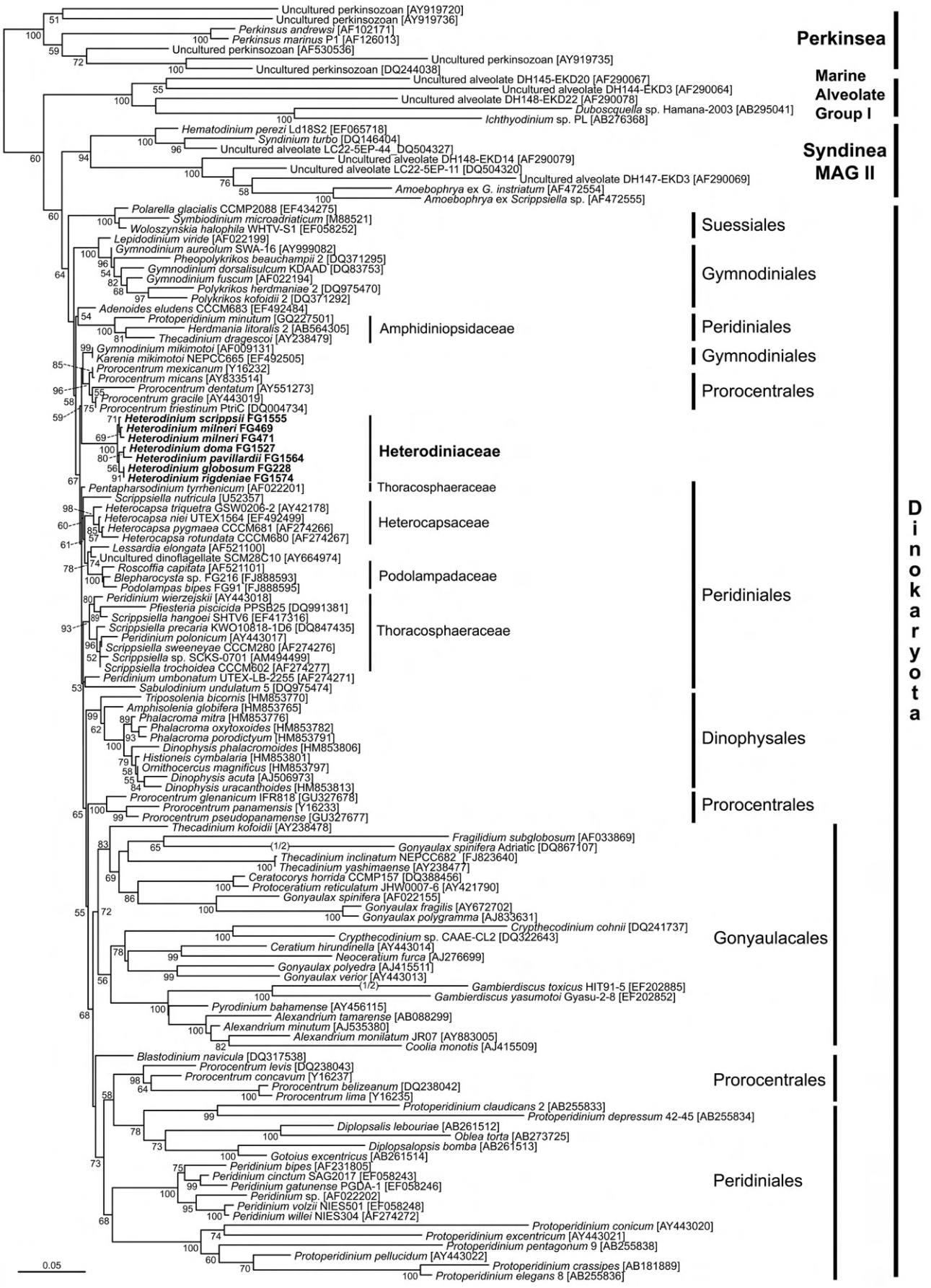


Fig. 43. Maximum likelihood phylogenetic tree of dinoflagellate SSU rDNA sequences, based on 1209 aligned positions. Names in bold represent sequences obtained in this study. Numbers at the nodes are bootstrap proportions (values < 50% are omitted). Accession numbers are provided between brackets. The scale bar represents the number of substitutions for a unit branch length.

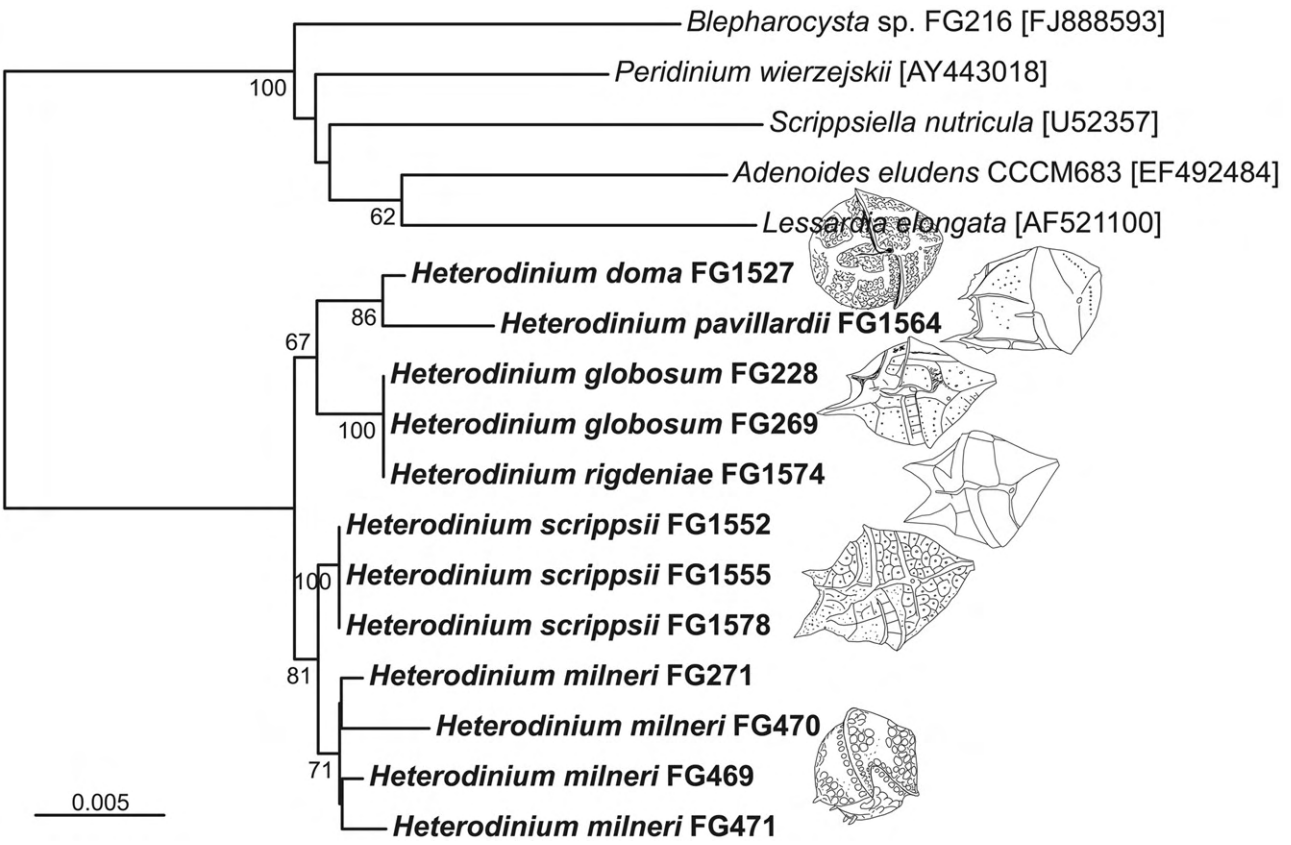


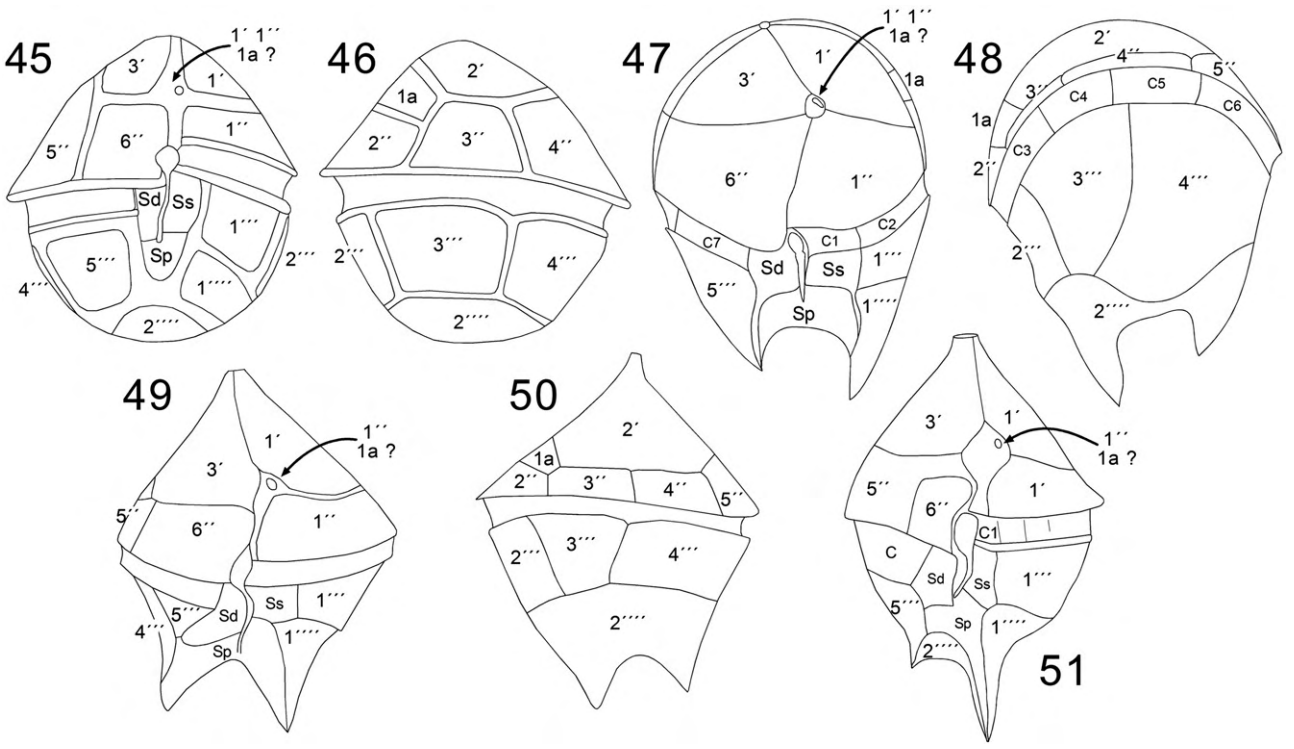
Fig. 44. Maximum likelihood phylogenetic tree of heterodiniaceans rooted on five short-branching dinoflagellate SSU rDNA sequences, based on 1210 aligned positions. Names in bold represent sequences obtained in this study. Numbers at nodes are bootstrap proportions (values < 50% are omitted). Accession numbers are provided between brackets. The scale bar represents the number of substitutions for a unit branch length.

type of *Peridinium*, *P. cinctum* (O.F. Müller) Ehrenberg ($P = 0.27$).

We carried out a more detailed analysis of the internal phylogeny of the heterodiniaceans using several short-branching sequences of the Gymnodiniales–Peridiniales–Prorocentrales lineage as outgroup (GPP, Fig. 44). In agreement with the result of the more general phylogenetic tree, this analysis showed the *Heterodinium* sequences were subdivided into two subclades: one comprised the type species *H. scrippsii*, and *H. milneri* (BP 81%), while the second was a moderately supported subclade (BP 67%) containing *H. doma*, *H. pavillardii*, *H. globosum* and *H. rigdeniae*. Each subclade was divided into two groups. In the first subclade, the three identical sequences of *H. scrippsii* formed the sister group of the four specimens of *H. milneri*, which showed slightly different sequences. In the second subclade, *H. doma* and *H. pavillardii* were closely related (BP 86%) and formed the sister group of *H. globosum* and *H. rigdeniae*. The two latter species had identical SSU rDNA sequences (Fig. 44).

Discussion

Heterodinium species, a group which displays a remarkable range of morphologies, formed a highly supported monophyletic clade that correlates with common tabulation features. This finding supports the view that tabulation is of greater diagnostic value compared with other morphological characters, such as the degree of flattening, spines, reticulation, pigmentation and ornamentation. As pointed out long ago (Kofoid & Adamson, 1933), identifying close relatives of *Heterodinium* based on the plate formula as a synapomorphy is problematic because no other dinoflagellates coincide in the number of plates. Balech (1980) considered the number of cingular and postcingular plates to be a very conservative character. The number of the precingular, cingular and postcingular plates of *Heterodinium*, 6″, 6c, 6‴, coincides with that of typical gonyaulacoids and has not been reported in peridinioids (Steidinger & Tangen, 1997, p. 412). However, our SSU rDNA molecular phylogenies show the genus *Heterodinium* to be divergent from the clade of Gonyaulacales. This opens the possibility that the tabulation 6″, 6c, 6‴ might not be limited to the monophyletic lineage of typical gonyaulacoid dinoflagellates and may perhaps be a symplesiomorphy, or that the interpretation of the tabulation needs to be revised.



Figs 45–51. Line drawings of *Heterodinium* spp. **45, 46.** Ventral and dorsal views of *H. doma*, redrawn and modified from Kofoid & Adamson (1933, plate 1). **47, 48.** Ventral and dorsal views of *H. laticinctum* Kofoid, redrawn and modified from Kofoid & Adamson (1933, plate 18). **49, 50.** Ventral and dorsal views of *H. rigdeniae*, redrawn and modified from Kofoid & Adamson (1933, plate 17). **51.** Ventral view of *H. globosum* redrawn and modified from Kofoid & Adamson (1933, plate 4). The tabulation has been re-interpreted as explained in the text.

The peridinioids and gonyaulacoids have typically two antapical or perisulcal plates *sensu* Balech (1980). The three antapical plates reported for *Heterodinium* are quite exceptional among the dinoflagellates and found otherwise only in *Crypthecodinium*, *Lessardia* and *Pyrophacus*. The occurrence of a third antapical plate could be considered either as an independently acquired character for each of those genera or as a misinterpretation of the hypothecal plate formula. Balech (1962) modified the tabulation proposed by Kofoid, and interpreted the seventh precingular plate (7″) as a sulcal plate. The hypotheca of *Heterodinium* has remained with the atypical plate formula 6‴, 3⁗. Balech (1962, 1988) illustrated the tabulation of several species, and Dodge (1985) showed the plates of *H. whittingiae* Kofoid (often misspelled as 'whittingae', it should be corrected according to ICBN Article 60.11, Recommendation 60C.1, and Article 32.7: see McNeill *et al.*, 2006) using scanning electron microscopy. We have reproduced the line drawings of several species of the genus *Heterodinium* by Kofoid & Adamson (1933), with a re–interpretation of the tabulation (Figs 45–51). In species such as *H. doma*, the first antapical plate seems to belong to the sulcus and it could be interpreted as the posterior sulcal plate. Similarly, the first postcingular plate could be interpreted as a left sulcal plate (Fig. 45). The new interpretation of the plate formula (5‴, 2⁗) corresponds to the most typical tabulation of peridinioid or gonyaulacoid dinoflagellates. However, we cannot establish a relationship of *Heterodinium* to any of these groups on the basis of the plates, because species with the same tabulation can be found in different clades of the dinoflagellate core (Saldarriaga *et al.*, 2004; Logares *et al.*, 2007; Moestrup & Daugbjerg, 2007; Fig. 43).

The tabulation of the epitheca is usually considered more variable than that of the hypotheca (Balech, 1980). Kofoid illustrated an intercalary plate (1a) in the dorsal epitheca of *Heterodinium* (Figs 47, 50) and, in ventral view, he showed the mid-ventral plate to have a pore that was omitted in the plate formula (3′, 1a, 6″). Later, Balech (1962, p. 150) reported the epithecal plate formula as 3′, 2a, 6″. The occurrence of anterior intercalary plates, usually located on the dorsal face, is a typical feature of Peridiniales, and it can be also found in some gonyaulacoids such as *Gonyaulax* and *Lingulodinium*. For *Heterodinium*, the small left-dorsal and the mid-ventral intercalary plates are not in contact. This contrasts with peridinioid and gonyaulacoid dinoflagellates, which have adjacent anterior intercalary plates. Fensome *et al.*

(1993, p. 114) considered that the ventral anterior intercalary plate of *Heterodinium* may be homologous with the standard gonyaulacoid first apical plate.

Dodge (1985) illustrated the mid-ventral intercalary plate of *H. whittingiae* using scanning electron microscopy. This plate differs in shape between species. In *H. doma*, the thick suture between the plates makes it difficult to distinguish whether the mid-ventral plate is in contact with the apex or with the cingulum. Kofoid & Adamson (1933) represented some species with the mid-ventral plate in contact with the cingulum (Figs 49, 51). In this case, the mid-ventral plate is interpreted as the first precingular plate. The possession of seven precingular plates is typical of most peridinioids, while this is rare in gonyaulacoids. If the mid-ventral intercalary plate (1a) of *Heterodinium* is interpreted as the first apical plate, the tabulation would be 4′, 6″, typical of several gonyaulacoid genera.

A typical feature of gonyaulacoid dinoflagellates is a pore on or near the right anterior margin of the first apical plate; no equivalent pore is known amongst peridinioids (Fensome *et al*., 1993, p. 62). Species of *Heterodinium* have a pore in the mid-ventral intercalary plate (Dodge, 1985) and Fensome *et al*. (1993, p. 114) defined the family Heterodiniaceae as gonyaulacaleans in which the ventral pore is situated on a small platelet on the ventral epitheca. They considered that this platelet may be homologous with the standard gonyaulacoid first apical plate, which bears the ventral pore in that group. Our SSU rDNA phylogeny placed *Heterodinium* among the poorly resolved short-branching sequences of the lineage comprising groups of Gymnodiniales, Peridiniales, Dinophysales and Prorocentrales. Although a relationship with Gonyaulacales cannot be discarded, our results suggest that *Heterodinium* species are not typical gonyaulacoids and our results are in disagreement with the classification of *Heterodinium* in the order Gonyaulacales. We propose to interpret the hypothecal tabulation of the genus *Heterodinium* as 5‴, 2⁗.

The current classification of *Heterodinium* into subgenera, and even into groups sharing similar morphologies (Kofoid & Adamson, 1933), is also not supported by the molecular data, with a close relationship indicated between *H. scrippsii* (subgenus *Heterodinium*) and *H. milneri* (subgenus *Sphaerodinium*), instead of the *H. scrippsii–H. rigdeniae* or *H. milneri–H. globosum* kinships that might have been expected. Moreover, SSU rDNA did not discriminate between *H. globosum* (subgenus *Sphaerodinium*) and *H. rigdeniae* (subgenus *Heterodinium*). Clearly, much further sequencing effort will be necessary to establish a natural classification within *Heterodinium*, although the genus itself may be monophyletic.

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Supplementary material

The following supplementary material is available for this article, accessible via the Supplementary Content tab on the article's online page at http://dx.doi.org/10.1080/09670262.2012.662722.

Supplementary material 1. Multiple sequence alignment of SSU rDNA sequences (Nexus format) used to reconstruct the phylogenetic tree shown in Fig. 43.

Table S1. List of new SSU rDNA sequences of *Heterodinium* used for the phylogenetic analysis. Accession numbers, geographical origin and collection dates are provided.

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