A new genus of athecate interstitial dinoflagellates, *Togula* gen. nov., previously encompassed within *Amphidinium* sensu lato. Inferred from light and electron microscopy and phylogenetic analyses of partial large subunit ribosomal DNA sequences

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**SUMMARY**

The recent emendation of *Amphidinium* (Dinophyceae), which now only consists of species with minute left-deflected epicone, has left more than 100 species without a clear generic affiliation. In the present study, a strain identified as one of the species with a divergent epicone type, *Amphidinium britannicum* (Herdman) Lebour, and six strains resembling *A. britannicum* but smaller in size were examined by light, scanning and transmission electron microscopy and by sequence analyses of nuclear-encoded partial large subunit ribosomal DNA to establish their phylogeny. *Amphidinium britannicum* was not closely related to other genera included in the molecular phylogenetic analyses, but formed a highly supported clade in Bayesian analysis together with the six small-sized strains. The six strains also formed a highly supported clade, consisting of two closely related, albeit distinct, clades. Light and scanning electron microscopy did not reveal significant differences between the vegetative motile cells; however, cells about to undergo mitosis developed longitudinal grooves on the hypocone in one of the clades but not in the other. Both clades differed substantially from *A. britannicum* in partial large subunit ribosomal DNA as well as in size and shape. Based on morphological similarity and partial large subunit ribosomal DNA evidence, we erect the new genus, *Togula* gen. nov. with the emended type species *Togula britannica* (Herdman) comb. nov. Based on differences in division pattern and partial large subunit ribosomal DNA gene divergence we further describe the species *Togula compacta* (Herdman) comb. nov. and *Togula jolla* sp. nov.

**Key words:** *Amphidinium, Amphidinium britannicum, Dinophyceae, large subunit ribosomal DNA gene, Togula, Togula britannica, Togula compacta, Togula jolla.*

**INTRODUCTION**

Coastal marine environments such as beaches, salt marshes and mudflats are habitats for a wide array of microorganisms including a variety of benthic dinoflagellates (Larsen 1985; Hoppenrath 2000; Al-Qassab et al. 2002; Murray & Patterson 2002; Murray 2003). A significant part of the dinoflagellate species encountered in the interstitial environment belong to the genus *Amphidinium* (Claparède & Lachmann 1859). The genus was defined to include athecate dinoflagellates with a minute epicone (Kofoid & Swezy 1921), more recently specified as an epicone length one-third or less of the total cell length (Steidinger & Tangen 1997). That the definition does not reflect phylogeny has long been expected (e.g. Schiller 1933), but uncertainty regarding identification of the type species, *Amphidinium operculatum* (Claparède & Lachmann 1859), has hampered attempts to redefine *Amphidinium*.

The identity of the type species has only recently been re-established (Flø Jørgensen et al. 2004; Murray et al. 2004). In cladistic analysis based on morphological features and molecular phylogenetic analyses based on large subunit ribosomal DNA (LSU rDNA), *A. operculatum* grouped together with the other *Amphidinium* species with minute left-deflected epicone, but failed to group with any of the *Amphidinium* species with other types of epicone. Therefore, the genus definition of *Amphidinium* was emended as to include only species with minute left-deflected epicone (Flø Jørgensen et al. 2004).

Of the more than 120 species previously placed within *Amphidinium*, only an estimated 20 species have minute left-deflected epicones, leaving approximately
100 species to have their generic relationships re-examined (Murray 2003).

One of the species not falling within the new genus definition is *Amphidinium britannicum* (Herdman) Lebour. This species is generally found in low cell numbers all year round on damp sandy beaches and sand bars (Larsen 1985; Hoppenrath 2000) and it can become abundant at times (Herdman 1913). It is characterized by a highly asymmetrical cingulum course, where the proximal part of the cingulum is continuous with the sulcus, but turns left approximately 0.2 cell lengths from the apex and descends by as much as two-thirds of the total cell length, giving it a distinct appearance.

The first description of a species with a similar cingulum arrangement was by W. Herdman (1913) who observed this species in high numbers at Port Erin, Isle of Man, UK, in the autumn of 1912. As it became abundant following a period with a high concentration of *A. operculatum* (later re-identified as *Amphidinium herdanii*) W. Herdman believed it to be an ecotype of *A. operculatum*. Kofoid and Swezy (1921) were the first to define a new species with a similar cingulum course: *Amphidinium asymmetricum* Kofoid et Swezy, found at La Jolla beach, California, USA. Besides the cingulum course the species was, amongst other characters, defined by faint surface striations similar to those found in *Gyrodinium* Kofoid et Swezy and an asymmetrical epicone with the apex situated left of the mid-ventral plane. The following year, E. C. Herdman (1922) described the variety *A. asymmetricum* var. *britannicum* from a sample taken at Port Erin. Herdman (1922) found the nucleus position, sulcal course and chloroplast arrangement of her specimen to be in accordance with the description of Kofoid and Swezy, but it differed in the lack of surface striae, the centrally positioned apex, as opposed to an apex situated left of the mid-ventral plane, and its dorso-ventral flattening. Herdman (1922) described a smaller variety called *A. asymmetricum* var. *compactum*, which differed from the first in being more compact and darker in color, and having a cingulum descending in a less steep spiral than in *A. asymmetricum* var. *britannicum*. However, in later papers Herdman (1924a,b) described and depicted *A. asymmetricum* var. *compactum* as having a cingulum descending steeply. Lebour (1925) elevated *A. asymmetricum* var. *britannicum* to species rank because of its lack of surface striations, asymmetrically placed apex and its dorso-ventral flattening, arguing that these characters clearly separated it from *A. asymmetricum* (Kofoid & Swezy 1921).

This view was opposed by Baillie (1971), who argued that the characters separating *A. asymmetricum*, *A. britannicum* and *A. britannicum* var. *compactum* were highly variable, based on his observations of specimens in culture. As he observed surface striae in some of his specimens, he suggested that *A. britannicum* and *A. britannicum* var. *compactum* should be considered synonyms of *A. asymmetricum*.

Later reviewers have not adopted this view. Larsen (1985), Hoppenrath (2000) and Murray and Patterson (2002) have all used the name *A. britannicum* for species that they observed to lack surface striae and to be dorso-ventrally flattened. However, they all agree that *A. britannicum* var. *compactum* should be considered a junior synonym of *A. britannicum*. Ono et al. (1999) described two organisms from the Seto Inland Sea, Sagami Bay and Tokyo Bay, Japan, one of which was found to be in accordance with the description of *A. britannicum*, and one similar looking but smaller in size, which they called *A. asymmetricum*, even though no surface striations were depicted or described.

In the present study, one strain considered to be identical to Herdman’s (1922) description of *A. asymmetricum* var. *britannicum* and a further six strains resembling *A. britannicum* but smaller in size, were examined using light microscopy (LM), scanning electron microscopy (SEM), transmission electron microscopy (TEM) and sequence analysis of partial LSU rDNA covering domains D1 to approximately 20 base pairs (bp) downstream D6 (Lenaers et al. 1989). The aim of the present study was to establish the phylogeny of *A. britannicum* and to clarify the taxonomic confusion regarding *A. britannicum* and morphologically similar species.

**MATERIALS AND METHODS**

**Cultures**

Cultures were established by capillary isolation of single cells from Danish sediment samples (SCCAP strain numbers, available from the Scandinavian Culture Collection of Algae and Protozoa), or from water samples taken at Napier, New Zealand (CAWN strain number, available from the Cawthron Collection of Microalgae) and sediment samples from Port Botany Bay, Australia (CS strain number, available from the CSIRO collection of living microalgae) or obtained from the following sources: University of Texas Culture Collection, USA (LB strain number) and the North-east Pacific Culture Collection, University of British Columbia, Canada (NEPCC strain number) (see Table 1). Cultures were maintained at 20°C in TL media (Larsen et al. 1994) adjusted to the salinity of the seawater from the particular sampling area.

**Light microscopy**

Micrographs were taken on an Olympus Provis AX70 microscope mounted either with a Zeiss Axiocam digital camera or a PM20 microphotosystem using Kodak Tech Pan film and with negatives digitalized using a Nikon Cool Scan. Both freshly collected
samples and cultured material were observed. Morphometric measurements were conducted on three cultures in the late exponential growth phase, of K-0658 (Togula britannica), K-0659 (Togula compacta) and LB1562 (Togula jolla) using an Olympus BX60 microscope mounted with a Sony Color Video Camera (3 CCD), and pictures were grabbed using Sony DV-shelf, and saved in JPEG format. A total of 60 pictures were taken of different cells in both the dorsal and ventral plane. Cells were measured using the UTHSCSA Image Tool program (University of Texas Health Science Center, San Antonio, Texas).

Scanning electron microscopy

SEM fixation was done following two different protocols.

Fixation protocol 1. Prior to fixation, subcultures were established in Petri dishes with 5 μm Millipore filters placed at the bottom. When sufficient cell numbers were observed on the filters, these were placed in Microporous Specimen Capsules (SPI supplies) and submerged into a container with a 1% osmium tetroxide solution in seawater adjusted to the salinity of the growth media. The cells were fixed for 15–20 min. Samples were washed twice with distilled water for 20 min.

Fixation protocol 2. 800 μL culture was fixed in a mixture of 1920 μL 2% osmium tetroxide in seawater adjusted to the salinity of the culture media and 480 μL of HgCl saturated solution for 2 hours. Fixed cells were filtered down onto 5 μm Millipore filters using Swinnex holders and rinsed thoroughly with distilled water for 2 h.

All samples were dehydrated in an ethanol series (30%, 50%, 70%, 96% and 100% for 20 min per step and left overnight in 100% ethanol) and critical point dried in liquid carbon dioxide with a Balzer CPD 020 or CPD 030 critical point drying apparatus. Filters were mounted on Cambridge stubs, sputter-coated using a JEOL JFC-2300 HR with a platinum-palladium layer of approximately 20 nm thickness and examined using a JEOL JSM-6335 F field emission microscope.

Transmission electron microscopy

Five days prior to fixation, new medium was added to the culture to ensure a sufficient number of freshly divided cells. Primary fixation was in most cases in a mixture of 2% glutaraldehyde and 0.3 M sucrose in 0.1 M sodium cacodylate buffer (pH 7.4) for 1 h at room temperature, followed by rinses in a mixture of sucrose and sodium cacodylate buffer with decreasing molarity of sucrose (0.25 M, 0.125 M, 0.0625 M and pure buffer); 20 min in each step. Alternatively, primary fixation was done using a 2% glutaraldehyde solution in growth medium followed by two rinses in pure medium and one in distilled water for 20 min. Secondary fixation was in a 1% osmium tetroxide solution at 4°C for 1 h. Samples were thoroughly washed twice with distilled water, dehydrated in an ethanol series and embedded in Spurr’s resin through either propylene oxide or acetone. The material was sectioned using a LKB (Bromma, Sweden) microtome using a diamond knife, collected on mesh grids and stained with uranyl acetate and lead citrate, half an hour in each solution. Sections were examined in a JEOL 100 SX microscope.

Molecular phylogenetic analyses of partial large subunit ribosomal DNA sequences

DNA extraction, amplification and sequencing were performed as outlined in Flø Jørgensen et al. (2004). Sequences were aligned by incorporating information from the secondary structure of the LSU rDNA molecule (De Rijk et al. 2000). The alignment included as many genera of dinoflagellates as possible in order to increase the possibility of finding the sister group relationship of A. britannicum. Table 2 lists all species and strains with GenBank accession numbers included. The data matrix contained 1392 aligned positions covering domains D1 to approximately 20 bp downstream D6 (Lenaers et al. 1989), but excluding the highly variable domain D2. A total of 666 bp were considered unambiguous and examined using Maximum Parsimony (MP) analysis and Bayesian analysis (BA). MP analysis was performed in PAUP* 4.0b10 (Swoford 2001) using the heuristic search option with random addition of sequences (1000 replicates) and a branch-swapping algorithm (Tree Bisection Reconnection (TBR)). Bootstrap support (BS) values were inferred from re-weighted MP analysis using a rescaled consistency index over an interval of 1–1000 and used to determine the robustness of topologies (Felsenstein

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Table 1. Sampling sites, sample types and year of sampling for the strains of Togula included in the present study

<table>
<thead>
<tr>
<th>Species</th>
<th>Strain number</th>
<th>Sample type, place and year of collection</th>
<th>Collected by</th>
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</thead>
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<tr>
<td>Togula britannica</td>
<td>K-0658</td>
<td>Sediment, Isefjorden, DK, 2001</td>
<td>M. F. Jørgensen</td>
</tr>
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<td>Togula compacta</td>
<td>K-0659</td>
<td>Sediment, Frederikshavn, DK, 2000</td>
<td>M. F. Jørgensen</td>
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<td>Sediment, Hirsholmene, DK, 2000</td>
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<td>CAWDS58</td>
<td>Water sample, Napier, NZ, 1998</td>
<td>L. Rhodes</td>
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<tr>
<td>Togula jolla</td>
<td>CS-742</td>
<td>Sediment, Port Botany Bay, AU, 2001</td>
<td>S. Murray</td>
</tr>
<tr>
<td>Togula jolla</td>
<td>NEPCC725</td>
<td>Unknown, Boundary Bay, CA, 1988</td>
<td>D. Jacobsen</td>
</tr>
<tr>
<td>Togula jolla</td>
<td>LB1562</td>
<td>Sediment, La Jolla Beach, USA, 1966</td>
<td>A. R. Loeblich</td>
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</table>
Table 2. List of dinoflagellates and outgroup alveolate species included in the large subunit ribosomal DNA analyses with corresponding GenBank accession numbers. If available, culture strain numbers are also indicated

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<th>Taxa</th>
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<th>Accession number</th>
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<td>Ceratium lineatum</td>
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<td>K-0010</td>
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RESULTS

Phylogenetic analyses of partial large subunit ribosomal DNA

The results of phylogenetic analyses of partial LSU rDNA using MP and BA are presented in Figure 1. A. britannicum (= T. britannica) formed a sister species to a clade consisting of the six smaller strains resembling A. britannicum, with a PP of 100% and a BS of 68%. This clade will hereafter be referred to as the Togula clade. The six smaller-sized strains formed two separate yet closely related clades, with 100% PP and 100% BS support (Fig. 1).
Fig. 1. Consensus phylogram of 20,000 sampled trees obtained by Bayesian analysis of partial large subunit ribosomal DNA (LSU rDNA) sequences covering domains D1–D6 but excluding the hypervariable domain D2. Posterior probability values above 50% are indicated. Bootstrap values inferred from a reweighted maximum parsimony analysis over an interval of 1–1000 are indicated as the second number, when found to support the same branch pattern as the Bayesian analysis and with a value above 50%. Species belonging to the Togula clade are in bold, and *Amphidinium sensu stricto* is highlighted.
Table 3. Partial large subunit ribosomal DNA sequence comparison (D1–D6 including D2) based on 1349 base pairs including introduced gaps

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</table>

For comparison of *Togula jolla* and *Togula compacta* strains the alignment was minimized to include only 1277 base pairs with no introduced gaps. Absolute differences are given above diagonal. Percentage sequence similarity is given below diagonal. The sequence divergence between these strains with different primer combinations. This region was excluded from the comparison to avoid exaggerating the sequence divergence between the *Togula jolla* strains.

*Dinophysis norvegica* formed a sister species to the *Togula* clade with 71% PP support (Fig. 1). However, this branching pattern was not consistently observed, as one out of five analyses found *D. norvegica* to be sister species to the *Amphidinium sensu* stricto clade. The relationship between the *Togula* clade and the other athecate genera included in the phylogenetic analyses was unsupported (Fig. 1). The genetic distances between the six *A. britannicum*-like strains (i.e. *T. compacta* and *T. jolla*) were moderate according to the BA analysis (Fig. 1), whereas there was a substantial difference between the six strains and the sequence of *T. britannica*. A sequence divergence comparison including the variable domain D2 is presented in Table 3.

As a result of the separate divergence between the lineages comprising *Amphidinium sensu* stricto and the clade comprised of *A. britannicum* and the six small-sized *A. britannicum*-like strains we propose a new genus *Togula* for the latter lineage.

**Taxonomic account**


Athecate psammophilic dinoflagellates with irregular chloroplast lobes radiating from the center towards the periphery. Cells dorso-ventrally flattened. Cingulum originates posteriorly to centrally, taking an anterior straight course, turning left approximately 0.2 cell lengths from apex and descending in a sigmoid-straight fashion dorsally, descending in a straight course upon reaching the ventral side. Nucleus situated in the median plane.

Etymology: *Togula* (Latin) = little toga, referring to the course of the cingulum that resembles the outline of a toga.

Type species: *Togula britannica* (Herdman) Flø Jørgensen, Murray et Daugbjerg comb. nov. (Figs 2–12, 36).

Basionym: *Amphidinium asymmetricum var. britannicum* (Herdman 1922, fig. 5, p. 18).


Distribution: Described from Port Erin, Isle of Man, UK (W. Herdman 1913; E. C. Herdman 1922), from sandy beaches around the British Isles (Dodge 1982), the Wadden Sea, Denmark (Larsen 1985), Seto Inland Sea, Sagami Bay and Tokyo Bay, Japan (Ono et al. 1999), German Wadden Sea (Hoppenrath 2000), Ise-fjorden, Denmark (Flø Jørgensen 2002).

Description based on LM and SEM of strain K-0658: *T. britannica* occurs in both a motile and a non-motile stage that differ somewhat in morphology (in culture the non-motile stage was absent). In the motile stage, the greatest width of the cell is found between the apex and the center of the cell in dorso-ventral view (Figs 2, 3), with the cell tapering slightly towards the antapex. Both apex and antapex appear slightly pointed in dorsal view (Fig. 3). In contrast, the non-motile cell outline is ellipsoidal in dorso-ventral view, slightly asymmetrical, as the right side is more convex than the left (Fig. 4). The apex and antapex both are broadly rounded. A hyaline
sheath surrounds these cells (Fig. 4). In lateral view the cell is elongate ellipsoidal as a result of the dorsoventral flattening, with a lateral width/width ratio of approximately 0.7. The average cell length is 51.2 μm ± 7.0 (n = 60), with a length range of 39.5 μm to 69.9 μm. The average cell width is 36.3 μm ± 4.6 (n = 60), with a range of 30.0 μm to 53.9 μm. Non-motile cells tend to be broader than motile cells. The cingulum is highly asymmetrical (Fig. 2). In ventral view, the proximal end originates a little right of the center of the cell and has
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a straight longitudinal to left course towards the apex turning left 0.15–0.2 cell lengths from the apex. Shortly before reaching the left side it begins descending, doing so dorsally in a sigmoid fashion (Fig. 3), but straight after reaching the ventral side (Figs 2–4). The sulcus continues from the proximal part of the cingulum, and is slightly curved toward the right side of the cell. It terminates just right of the antapex. In motile cells the left edge of the sulcus covers the groove, forming a conspicuous sulcal flap (Fig. 2). The longitudinal flagellum has approximately the same length as the cell, and tends to lie within the sulcus for about half its length. Numerous irregular golden-brown chloroplasts radiate from the center towards the cell perimeter (Figs 2–4). The outermost rim of the cell is devoid of chloroplasts, giving it a translucent appearance (Figs 2–4). No pyrenoid is visible in LM. The nucleus is located in the center of the cell. It has a round to ellipsoidal shape, lying with the long axis in the median plane (Fig. 3). Lipid droplets are occasionally observed in freshly collected specimens, but can become numerous in cultured cells.

Prior to cell division an additional groove becomes visible on the ventral side situated left of the sulcus (Figs 5, 6). In motile cells the groove lies parallel to the sulcus (Fig. 6) and specimens with this additional groove were often observed in culture. However, one non-motile cell was observed were the additional groove had a different course (Fig. 5). We were unable to establish whether this groove is analog to the one observed in motile cells.

How the transformation to the next stage takes place we were not able to observe. The cell gets a globular shape, with the two epicoes visible in ventral view (Fig. 7). Dorsally, a conspicuous flap forms (Fig. 8). The actual cell division begins with the splitting of the hypocone, whereby the flap becomes visible before the epicoes split and cell division is complete (Fig. 9). The lifecycle is summarized in Figure 36.

To establish whether any surface structures are present in *T. britannica*, cells were examined by SEM. No surface striae or other ornamentations were observed in either ventral or dorsal view (Figs 10, 11) and no apical groove (or acrobe sensu Biecheler (1934)) was found on the epicone (Fig. 12).

TEM: Successful fixation of cells of *T. britannica* has proven very difficult. Both freshly sampled specimens and specimens kept in culture were fixed using glutaraldehyde, a mix of glutaraldehyde and formaldehyde or a mix of glutaraldehyde and osmium tetroxide as primary fixatives in either cacodylate buffer with different molarities of sucrose, phosphate buffer or in medium and with variations in fixation times. Regardless of protocol, no well-preserved cells were recovered on the grids examined.

Remarks: Strain K-0658 is identical to the description of *A. asymmetricum var. britannicum* by Herdman (1922) in regard to the following characters: general cingulum course, cell length of approx. 50 μm (the length of 150 μm stated in Herdman (1922) was corrected in Herdman (1924a) to 50 μm), the dorso-ventral flattening (depicted in Herdman 1921), left epicone length/total cell length ratio of approx. 0.15 and right epicone length/total cell length ratio of approx. 0.5, nucleus position and golden-brown chloroplasts radiating from the nucleus towards the perimeter. The average length/width ratio is 1.42 and not 1.7 as described by Herdman; however, we consider this difference to be of minor importance, as the length/width ratio is likely to be influenced by the number of non-motile to motile cells observed and therefore variable.

Strain K-0658 differs from the description of *A. asymmetricum* by Kofoid and Swezy (1921) by the lack of surface striaions, by the apex being situated in the mid-ventral plane and not left of it, and by its dorso-ventral flattening.
Togula compacta (Herdman) Flø Jørgensen, Murray et Daugbjerg comb. nov. Figures 13–21,37.
Basionym: Amphidinium asymmetricum var. compactum (Herdman 1922, p. 22, Fig. 6(p). 18).

Descriptions we consider to represent Togula compacta: E. C. Herdman (1924a), as A. asymmetricum var. compactum, E. C. Herdman (1924b), as A. britannicum var. compactum, Lebour (1925), as A. britannicum var. compactum, Larsen (1985), as A. britannicum.

Distribution: Port Erin, Isle of Man, UK (Herdman 1922), the islands of Hirsholmene and beaches near Frederikshavn, Denmark (this study).

Description based on LM of strain K-0659: The motile cell is broadly ellipsoidal in dorso-ventral view, with the width of the epicone a little less than the width of the hypocone (Fig. 13) and golden-brown to dark-brown in color. The apex and antapex are broadly rounded (Figs 13,14), but the antapex can appear slightly pointed in dorsal view (Fig. 14). The separation between epicone and hypocone created by the cingulum is deeply incised laterally (Fig. 13). In lateral view, the cells are elongate ellipsoidal because of the dorso-ventral flattening, with a lateral width/width ratio of 0.65. However, the dorso-ventral plane is somewhat twisted, making the posterior right part of the cell appear as a bulge when seen in ventral view (Fig. 13). This is most easily observed in swimming cells. The average cell length of strain K-0659 is 32.4 μm ± 3.4 (n = 60) with a length range of 25.4–38.8 μm. The average cell width is 25.1 μm ± 3.1 (n = 60) with a width range of 18–35 μm.

The cingulum is highly asymmetrical (Fig. 13). The proximal end originates below and to the right of the center of the cell, and has a straight longitudinal left course towards the apex, turning left 0.2–0.25 cell lengths from the apex (Fig. 13). Dorsally, it takes a nearly horizontal course that is slightly sigmoid (Figs 14,20) with the distal part of the cingulum taking a straight left-vertical course (Figs 13,19). The sulcus is inconspicuous but has a curved course towards the...
antapex, with a right bend. The longitudinal flagellum is approximately the same length as the cell. Multiple irregular golden-brown chloroplasts radiate from the center towards the cell perimeter (Fig. 14). No pyrenoid is visible in LM. The nucleus is situated in the median plane, and has a variable shape. It varies from elongate oval to roundish (Figs 13, 14). Nuclei with the elongate shape span most the entire width of the cell (Fig. 14), whereas more rounded nuclei are normally situated left of the mid-ventral plane (Fig. 13). Oil droplets are commonly observed, and tend to become more numerous in specimens kept in culture.

Cell division is similar to that of *T. britannica* but there are some differences. Cells with additional grooves were not observed in culture, but small sized *Togula* cells with additional grooves have been observed in natural samples from Australia (Murray and Patterson 2002). Based on morphology alone, it cannot be ruled out that these cells are specimens of *T. compacta*. Prior to division, the cell shape becomes rounded and ventrally two new epicones become visible (Fig. 15). Dorsally, one conspicuous groove is formed (Fig. 16) along which the cell divides (Fig. 17). However, no flap is formed as is the case with *T. britannica*. Cell division begins at the antapex and the last parts to split are the two epicones (Fig. 17). The life cycle is illustrated in Figure 37.

On rare occasions, cells of *T. compacta* were observed altering their shape in a manner resembling amoeboid movements. Such cells were all trapped by soil extract residues in the medium or squeezed by the coverslip and they extended their epicones in a proboscis-like fashion. Once through the gap in the obstacle, the cell contents seemed to flow into this prolonged epicone, enabling the cell to escape. The cell would regain its original shape immediately afterwards. Amoeboid cells were observed twice in what may be a sexual conjugation (Fig. 18).

To examine whether surface striations were present, SEM micrographs were taken of vegetative motile cells in dorsal and ventral view. No surface striations were observed (Figs 19, 20) and no apical groove was present (Fig. 21).

TEM: As was the case for *T. britannica*, fixation of *T. compacta* proved difficult. Only a few organelles were preserved, including the chloroplasts and an associated pyrenoid structure (not shown), which resembles the pyrenoid found in *T. jolla* (Fig. 35).

Remarks: As in *T. britannica*, non-motile cells of *T. compacta* do not occur in culture under stable conditions. As cells were only rarely encountered in wild samples, it is not known whether *T. compacta* has a non-motile stage. However, non-motile small-sized *Togula* species have been recorded from wild samples (Fig. 24) (Patterson et al. 1989; Murray & Patterson 2002), that could represent a non-motile stage of *T. compacta*. But as these non-motile forms have been recorded from Australia where only *T. jolla* has been recorded as yet, we consider it more likely that these forms represent non-motile stages of *T. jolla*.

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Pyrenoides composita. Series LSU rDNA partialis. (GenBank AY455680).

Cells 25–43 μm long, 19–35 μm wide, dorso-ventrally flattened, depth 0.6–0.7 of cell width. Cingulum originating just below the cell center, with an anterior-left course before turning left 0.2–0.3 cell lengths from apex, and descending in a sigmoid fashion dorsally. Cingulum displacement 0.4–0.5 times the cell length. Numerous irregular elongated chloroplasts present, radiating from the center towards the periphery. Nucleus oval, situated left of the mid-ventral plane. The position of the nucleus in this species is variable. Arrowheads point to the surrounding hyaline sheath and additional ventral division groove. 25. Dorsal view of predivison cell, with longitudinal grooves on the hypocone. 26. Swollen predivison cell. 27. Initial cell division. An additional groove forms on the epicone (arrowhead). 28. Cell division. The initial division is at the apex, with the epicones being the first part to divide. 29. Cell division. Just before cell division is completed, the two daughter cells are connected by a small band-like structure (arrowhead). Notice how the two cells are twisted relative to one another. Scale bars 10 μm.

Figs 22–29. *Togula jolla* sp. nov. 22. Ventral view of motile cell. Notice the rounded nucleus situated left of the mid-ventral plane. The position of the nucleus in this species is variable. 23. Dorsal view of motile cell. 24. Ventral view of a non-motile cell from wild sample, likely to be a *T. jolla* (see remarks about *T. jolla*, result chapter). Arrowheads point to the surrounding hyaline sheath and additional ventral division groove. 25. Dorsal view of predivison cell, with longitudinal grooves on the hypocone. 26. Swollen predivison cell. 27. Initial cell division. An additional groove forms on the epicone (arrowhead). 28. Cell division. The initial division is at the apex, with the epicones being the first part to divide. 29. Cell division. Just before cell division is completed, the two daughter cells are connected by a small band-like structure (arrowhead). Notice how the two cells are twisted relative to one another. Scale bars 10 μm.
in the mid-ventral plane. Cell division preceded by the formation of 6–8 longitudinal grooves on the hypocone.

Cell division in the longitudinal plane, heralded by a cleavage of the epicone into two equal parts. Pyrenoid compound. Partial LSU rDNA sequence (GenBank AY455680).

Type material: Platinum-palladium coated critical point dried cells mounted on a Cambridge stub have been submitted to the Botanical Museum, Copenhagen (C) database under accession number A 2086.

Etymology: Named after the location where it was first isolated, jolla also means jewel in Spanish.


Description: The morphology of the vegetative motile cell is very similar to that of \textit{T. compacta} (Figs 22,23), but there are a few subtle differences. The cell color tends to be olive-green rather than golden-brown and the twisting of the cell in the dorso-ventral plane is less pronounced than observed in \textit{T. compacta}. However, as both of these characters are somewhat variable, separating these species on vegetative cell morphology alone is extremely difficult.

The cell size of \textit{T. jolla} is also very similar to \textit{T. compacta}, except for strain CS-742, which is somewhat smaller. The average cell length for strain LB1562 is 32.7 μm ± 3.6 (n = 60) with a length range of 25.4–42.7 μm and the average cell length is 25.0 μm ± 3.2 (n = 18) for strain CS-742 with a length range of 20–31 μm (Murray 2003). The average cell width for strain LB1562 is 25 μm ± 2.8 (n = 60) with a width range of 18.9–35.4 μm and the average cell width is 18 μm ± 2.5 for strain CS-742 (n = 18) with a width range of 12–20 μm (Murray 2003).

Cell division in \textit{T. jolla} differs substantially from that observed in the other \textit{Togula} species. As in \textit{T. britannica}, the initial phase includes the formation of a new longitudinal groove ventrally, running parallel to the sulcus and proximal part of the cingulum (Fig. 30). Dorsally, two parallel longitudinal grooves are...
formed in close succession (Fig. 32). This is followed by the formation of additional longitudinal grooves, resulting in the hypocone being indented all way round by 6–8 grooves (Figs 25,26,33). No grooves divide the epicone (Figs 26,33). Following the formation of the longitudinal grooves, the cell swells (Fig. 26), resulting in the hypocone bulging slightly between the grooves in a conspicuous manner (Figs 26–28). A groove is now formed on the epicone (Fig. 27) and the epicone is the first part to divide (Fig. 28). During division of the hypocone, the two daughter-cells are twisted relative to one another (Fig. 29) and just before separation the two daughter cells are only connected by a small band-like structure (Fig. 29). The life cycle is summarized in Figure 38.

SEM was used to examine whether surface striations were present in *T. jolla*. As the culture of *T. jolla* grows rapidly under stable conditions, most cells were in some state of cell division, and we did not succeed in finding a cell in ventral view that had not initiated cell division. No surface striations were observed in ventral or dorsal view in the cells observed (Figs 30,31) and no apical groove was found (Fig. 34).

TEM: Fixation of *T. jolla* was slightly more successful than in the other *Togula* species, but again not all organelles were well preserved. Organelles preserved included a simple type of pusule with numerous vesicles opening directly into the flagellar canal, trichocysts lining the amphiesma and chloroplasts, some of which formed part of a compound pyrenoid (Fig. 35). The compound pyrenoid was formed by ends of chloroplast lobes lying closely against each other, still enclosed by the outer membranes of the chloroplasts (Fig. 35). These ends are devoid of lamellae, and have a fine granular appearance.

Remarks: Two strains considered by us to be *T. jolla*, LB1562 and NEPCC725, have previously been included in a phylogenetic study of small subunit (SSU) rDNA sequences (Saldarriaga et al. 2001) as *Amphidinium corpulentum* Kofoid et Swezy and...
**Togula gen. nov.** A. asymmetricum Kofoid et Swezy, respectively. They formed a well-supported clade, but SSU rDNA sequences were only 88% identical. In the present study we found the partial LSU rDNA sequence divergence between the same two strains to be <0.2%. As the D1–D6 part of the LSU rDNA gene normally exhibits a greater variation than the SSU rDNA gene (Daugbjerg et al. 2000), this is highly surprising. We have no explanation for the SSU rDNA divergence observed.

Strain LB1562 differs from the original description of *A. corpulentum* (Kofoid & Swezy 1921) by the lack of a sulcal extension onto the epicone, by having a steeply descending cingulum lacking in *A. corpulentum*, and by its smaller size. Strain NEPCC725 differs from the description of *A. asymmetricum* in the lack of surface striae, the lack of a very asymmetrical epicone and in its smaller size (Kofoid & Swezy 1921). In our view, these strains represent *T. jolla*.

**DISCUSSION**

Phylogenetic results

The present study has shown that *A. britannicum* (= *T. britannica*), based on phylogenetic analyses of partial LSU rDNA sequences, is closely related to two species with a similar cingular course and chloroplast arrangement. No other genera included in the analyses were closely related to this clade (*Dinophysis norvegica* did not group consistently with the clade) including *Amphidinium sensu* stricto. Hence, a new genus, *Togula*, is described to include the species of this clade.

The partial LSU rDNA sequence divergence observed is surprisingly high between *T. britannica* and the two smaller species, *T. jolla* and *T. compacta* (Table 3). In studies of the genus *Amphidinium*, a high amount of intragenic sequence divergence between morphological similar species was also found (Flø Jørgensen et al. 2004). This could indicate that *T. britannica* is so divergent that it should be placed in a genus of its own. In our view the high degree of morphological similarity between *T. britannica* and the other two species, in the cingular course and the chloroplast arrangement, indicates that they are closely related. Furthermore, the sequence divergence could be the result of differences in mutation rates, which would exaggerate the divergence time between the *Togula* species.

Species identification

The phylogenetic analyses of partial LSU rDNA clearly support that *T. britannica* is a separate species. It can be identified by its larger size, and by its slimmer appearance when compared to the smaller species *T. compacta* and *T. jolla*.

It has been debated whether the name *A. britannicum* should be considered a junior synonym of *A. asymmetricum* (Kofoid and Swezy 1921). Baillie (1971) argued that the characters used to separate the two species (dorso-ventral flattening and surface striation) were extremely variable in his cultures, and that the difference should be considered variations of the same species. Our observations do not support this view. We were not able to observe any surface striations with LM (Figs 2–5) or with SEM (Figs 10–12). Both specimens from natural samples that we have observed previously (M. F. Jørgensen, unpubl. data 2002) and the cultures examined in the present study were consistently found to be dorso-ventrally flattened, with a lateral diameter approximately 0.7 of the cell width. Furthermore, cells did not have an apex deflected to the left of the mid-ventral line, as depicted by Kofoid and Swezy (1921). Based on our observations we do not consider *A. britannicum* to be synonymous with *A. asymmetricum*, and the appropriate combination for the type species is, therefore, *T. britannica*.

The situation in regard to the two smaller species is less clear. The divergence of less than 2% observed in partial LSU rDNA and the morphological similarity between *T. compacta* and *T. jolla* naturally raises the question of whether they should be considered varieties of the same species or separate species. In our view, the subtle differences in motile vegetative cell morphology alone would be insufficient to justify the erection of an additional species. However, the difference in cell division is substantial and indicates that the two forms are not conspecific. Therefore, we have designated these two forms as separate species, even though they are difficult to separate based solely on LM observations of the motile vegetative cell.

It may be argued that a partial LSU rDNA sequence divergence of less than 2% does not support the erection of two separate species. However, it is problematic to use percentage sequence divergence as a direct measure of whether two taxa should be designated separate species. One insertion or deletion of a series of basepairs can result in a relatively high percentage difference even though only one mutational event occurred. Furthermore, variation in mutation rates might exaggerate differences while very slow mutation rates might do the opposite. An example of the latter is the two species *Amphidinium hermanii* and *Amphidinium mootonorum*, which also differ by only approximately 2% in partial LSU rDNA sequence divergence, but differ substantially in size, nucleus position, chloroplast arrangement and the shape of the sulcus, and, therefore, clearly constitute two separate species (Flø Jørgensen et al. 2004; Murray et al. 2004).

The morphological similarity between the two smaller species has also made the taxonomic revision difficult. Herdman (1922) originally defined the
variety A. asymmetricum var. compactum as being more compact, slightly darker in color, usually smaller and with a less evident cingulum and sulcus than A. asymmetricum (= T. britannica). The course of the cingulum is not clear in Herdman’s descriptions (1922, 1924a, b). In Herdman (1922), the cingulum course is described and depicted as descending less steeply in A. asymmetricum var. compactum (= T. compacta) than in A. asymmetricum var. britannicum (= T. britannica), but Herdman (1924a) describes and depicts A. asymmetricum var. compactum as having a steeply descending cingulum. Finally, Herdman (1924b) depicts A. asymmetricum var. compactum with a cingulum descending steeply but with the distal part running almost horizontally.

Based on size and the cingulum course described in Herdman (1924a,b) all of the six small sized Togula strains are in accordance with her description, but only strain K-0659 and K-0660 are darker in color and possess a sulcus that is less evident than the one of T. britannica (Figs 13,19). Therefore, we consider strains K-0659 and K-0660 to agree with the description of A. britannicum var. compactum.

A. asymmetricum was described by Kofoid and Swezy (1921) from La Jolla Beach, USA. This is the same area from which the T. jolla strain LB1562 was originally isolated, raising the question whether the description of A. asymmetricum could in fact be a description of a predision cell of T. jolla (Figs 25, 26,33). If so, the appropriate combination would be T. asymmetrica. However, T. jolla differs from A. asymmetricum (Kofoid and Swezy 1921) in its smaller size, its pronounced dorso-ventral flattening and the presence of grooves on the hypocone, as opposed to striations, described by Kofoid and Swezy as being similar to those observed in Gyrodinium. Furthermore, the striations were described and depicted as present on both the epicone and hypocone. Hence, strain LB 1562 is not in accordance with A. asymmetricum Kofoid et Swezy.

Baillie (1971) and Ono et al. (1999) have both used the name A. asymmetricum for a small sized species. The T. jolla strain, NEPCC 725, was isolated from the area where Baillie conducted his study, raising the question whether his A. asymmetricum is identical to the species we here describe as T. jolla. Baillie did not describe whether the faint surface striations he observed were similar to those described by Kofoid and Swezy (1921) and, therefore, it is impossible to determine whether the species he observed could have been T. jolla. The description of A. asymmetricum by Ono et al. (1999) is within the size range of the small-sized Togula species and does not mention or depict surface striations. Therefore, it is not in accordance with the original description by Kofoid and Swezy (1921).

Biogeography

The relatively few studies of benthic dinoflagellates and the morphological similarity between T. jolla and T. compacta make it difficult to estimate the biogeographic distribution of Togula species. The absence of records from subtropical and tropical regions of T. britannica suggests that this species is restricted to temperate regions. Because of its size and conspicuous slow-rotating swimming behavior it is unlikely that it should be overlooked in samples if present. Small-sized species of Togula have been recorded from France (Paulmier 1992), Japan (Ono et al. 1999), Australia (Murray & Patterson 2002) and Greenland (Daugbjerg, unpubl. data 2002), but as these records could represent either T. jolla or T. compacta, the biogeography of these species can only be assessed when cultures are established. The four strains of T. jolla included in the present study all originated from the Pacific area, covering both temperate and subtropical regions, and the biogeographic range of T. jolla in our view is likely to exceed that of T. britannica. The two Danish strains of T. compacta have been sampled from localities lying in close proximity to each other, and could represent specimens of the same population. Therefore, it is impossible at present to assess the biogeographic distribution of this species.

The recent more restrictive definition of Amphidinium (Flø Jørgensen et al. 2004) has resulted in more than 100 described species being in need of a taxonomic re-evaluation. Some species are likely to group together with other well-established genera, while other descriptions are of such a quality as to make re-identification virtually impossible. However, judging by the morphological variation of species of Amphidinium sensu lato, it is likely that more new genera will be described in the future.

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