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FIRST RECORD OF THE GENUS *TOGULA* (GYMNODINIALES, DINOPHYCEAE) AND OF THE SPECIES *T. JOLLA* FOR THE SOUTH-WEST ATLANTIC COASTAL WATERS

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Abstract. Sunesen, I.; F. Rodríguez, D. Aguiar Juárez, J. A. Tardivo Kubis & E. A. Sar. 2024. First record of the genus *Togula* (Gymnodiniales, Dinophyceae) and of the species *T. jolla* for the South-West Atlantic coastal waters. *Darwiniana*, nueva serie 12(1): 5-15.

In the framework of a phytoplankton and biotoxin monitoring program implemented since 2008 in marine coastal waters of the Buenos Aires Province (Argentina), a gymnodinioid, photosynthetic dinoflagellate was isolated from a sample collected in Santa Teresita, establishing the strain LPCc007. The strain was identified as belonging to the psammophilic genus *Togula* by light microscopy based on the following distinctive characters: morphology of the cell dorso-ventrally flattened, and course of the cingulum, highly asymmetric, descending, with its ends displaced by about one to two thirds of the total length of the cell and joined by an oblique intercingular groove. The comparative morphological analysis between the motile vegetative cells of the isolated strain and the three species of the genus described to date, did not reveal significant differences that would allow it to be determined at a specific level. Instead, the pattern of development of longitudinal grooves on the hypocone of cells about to undergo mitosis observed in strain LPCc007, perfectly agrees with that described for *T. jolla* as a differential character. The morphological identification was consistent with LSU rDNA (D1-D2) based phylogeny that showed the sequence aggregation in the *T. jolla* clade. This is the first report of the genus *Togula* and the species *T. jolla* from Argentina and the South-West Atlantic Ocean coastal waters.

Keywords. Argentina; cultures; first report; gymnodinioid; morphological and molecular characterization; phylogeny; South-West Atlantic Ocean coastal waters; *Togula.*

Resumen. Sunesen, I.; F. Rodríguez, D. Aguiar Juárez, J. A. Tardivo Kubis & E. A. Sar. 2024. Primer registro del género *Togula* (Gymnodiniales, Dinophyceae) y de la especie *T. jolla* para aguas costeras del Sudoeste del Océano Atlántico. *Darwiniana*, nueva serie 12(1): 5-15.

En el marco de un programa de monitoreo de fitoplancton y biotoxinas implementado desde 2008 en aguas marinas costeras de la Provincia de Buenos Aires (Argentina), un dinoflagelado gimnodinioide, fotosintético, fue aislado de una muestra colectada en Santa Teresita, estableciéndose la cepa LPCc007. La cepa fue identificada como perteneciente al género *Togula* con microscopía óptica con base en los siguientes caracteres distintivos: morfología de la célula dorso-ventralmente aplanada, y curso del cingulum, altamente asimétrico, descendente, con sus finales desplazados por cerca de uno a dos tercios de la longitud total de la célula y unidos por un surco intercingular oblicuo. El análisis morfológico comparativo entre las células vegetativas móviles de la cepa aislada y las tres especies del género descriptas hasta la fecha no reveló diferencias significativas que permitieran determinarla a nivel específico. En cambio, el patrón de desarrollo de surcos longitudinales en el hipocono de células a punto de sufrir mitosis observado en la cepa estudiada, concuerda perfectamente con el descrito para *T. jolla* como un carácter diferencial. La identificación morfológica fue consistente con la filogenia basada en

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LSU ADNr (D1-D2) que mostró la agregación de la secuencia en el clado molecular correspondiente a *T. jolla*. Este es el primer reporte del género *Togula* y de la especie *T. jolla* para Argentina y aguas costeras del Sudoeste del Océano Atlántico.

Palabras clave. aguas costeras del Sudoeste del Océano Atlántico; Argentina; caracterización morfológica y molecular; cultivos; filogenia; gimnodinioides; primer reporte; *Togula*.

INTRODUCTION

Dinoflagellates (Dinophyceae) are a highly diverse group of flagellates that contains 3,962 described species (Guiry & Guiry, 2023), of which about 2,277 (Guiry, 2012) or 2,500 (Hoppenrath et al., 2009) are extant species. Most of living species are both photosynthetic and non-photosynthetic in equal proportions, and 87% are marine (Taylor et al., 2008; Guiry, 2012). The cell covering of the dinoflagellate is called amphiesma, which is formed by a single layer of flattened amphiesmal vesicles placed beneath the cell membrane (Taylor, 1987). These vesicles contain cellulosic plates in the armored/thecate dinoflagellates or lack them in unarmored/athecate dinoflagellates (Fensome et al., 1993).

The unarmored species are distinguished by size, shape and contour of the cells, relative sizes of epicone and hypocone, position and morphology of the cingulum and sulcus, displacement of the cingulum in relation to the cell length, presence/absence of chloroplasts and pyrenoids, shape and position of the nucleus, presence/absence of surface striations, features observed with light microscopy (LM); presence/absence of apical groove, shape of the apical groove, ultrastructural features observed with scanning electron microscopy (SEM); vesicular chambers in the nuclear envelope, presence/absence of nuclear fibrous connector, among other complex ultrastructural features observed with transmission electron microscopy (TEM) (Daugbjerg et al., 2000; Larsen & Nguyen, 2004; Hoppenrath et al., 2009; Reñé et al., 2011; Escarcega-Bata et al., 2021). This group of dinoflagellates is more delicate than thecate ones and frequently do not maintain their shape, deteriorate or destroy when the sample is preserved with the most frequently used fixatives, thus species only can be detected in live samples. When working with live samples, the most robust species are kept in good condition during the analysis by light microscopy, while other more delicate ones deteriorate during observation (Larsen, 2002). This characteristic explains the very limited knowledge of a great part of the gymnodinoid dinoflagellates (Larsen & Nguyen, 2004; Gómez et al., 2011; Escarcega-Bata et al., 2021, 2022, 2023).

Gymnodinium Stein, *Gyrodinium* Kofoid & Swezy and *Amphidinium* Claparède & Lachmann (Daubjerg et al., 2000) are the main genera of unarmoured dinoflagellates in terms of the number of species, and have long been recognized to be polyphyletic (Hoppenrath et al., 2012). Based on a combination of morphological features and molecular data Daubjerg et al. (2000) amended genera *Gymnodinium* Stein emend. G. Hansen & Moestrup and *Gyrodinium* Kofoid & Swezy emend. G. Hansen & Moestrup, and erected several new genera that differed from the clade of *Gymnodinium* sensu stricto (*Akashiwo* G. Hansen & Moestrup, *Karenia* G. Hansen & Moestrup) and *Gyrodinium* s. str. (*Karlodinium* J. Larsen).

One of the most abundant and diverse sand-dwelling benthic dinoflagellates with short epicone and cingulum distinctly premedian, *Amphidinium* Claparède & Lachmann, was redefined by Flø Jørgensen et al. (2004a) also using a combination of morphological features and molecular data. Additionally, Murray et al. (2004) established the species boundaries in the *Amphidinium operculatum* species complex including identity of the type species of *Amphidinium* s. str. Several species previously encompassed within *Amphidinium* s. l. were analysed and new genera were erected. Flø Jørgensen et al. (2004b), described *Togula* Flø Jørgensen, S. Murray & Daugbjerg; Sparmann et al. (2008) *Apicoporus* Sparmann, Leander & Hoppenrath; Hoppenrath et al. (2012) *Ankistrodinium* M. Hoppenrath, S. Murray, S. F. Sparmann & B. S. Leander; Horiguchi et al. (2012) *Testudodinium* Horiguchi, Tamura, Katsumata & A. Yamaguchi; Yamada et al. (2013) *Bispinodinium* N. Yamada & Horiguchi; Borchhardt et al. (2021) *Bindiferia* Borchhardt, Chomérat, S. Murray & Hoppenrath, among others.

In the framework of a phytoplankton and biotoxins monitoring program in marine coastal waters of the Buenos Aires Province, a gymnodinioid, photosynthetic dinoflagellate, was isolated in 2016 and established as strain. The aim of the present study was to provide the characterization and specific identification of strain LPCc007, using molecular (LSU rDNA sequencing) and morphological analyses.

MATERIAL AND METHODS

Strain isolation and culture

The clonal strain of a gymnodinioid dinoflagellate (LPCc007) was established from a surface sample collected with a 30 µm mesh net hauls in marine coastal waters of Santa Teresita at about 10 to 20 m from the shoreline.

Examined material: ARGENTINA, Buenos Aires Province, de la Costa District, Santa Teresita Locality (36° 32' 30" S; 56° 41' 11" W), 29-VIII-2016, Strain LPCc007, isolated from field material labeled LPC 11455. The water temperature and salinity of Santa Teresita were obtained with a multiparameter probe Hanna HI 9828 (Hanna, USA).

Single cells were isolated by micropipette using a Zeiss Axiovert 40 CFL inverted microscope with phase contrast and differential interference contrast (DIC) (Zeiss Microimaging, Goettingen, Germany). Individual cells were washed several times in local filtered seawater and when free of contaminants they were transferred into 6-well tissue culture plates containing 10 ml natural seawater enriched with Guillard's f/2 medium (Sigma-Aldrich, Saint Louis, USA). Cells were incubated at 16 °C, at salinity of 30, and under light supplied by cool-white fluorescent tubes with irradiance of 100-125 µmol photons m^{-2} s^{-1} on a 12:12 light:dark regime, in a growth chamber (SEMEDIC I-290F, SEMEDIC SRL, CABA, Argentina). After successful isolation, culture was scaled up to 40 ml medium in 100 ml flasks and incubated in the described conditions.

Microscopy

For light microscopy (LM) analyses, live cells of the strain LPCc007 were observed using a Leica DMLA microscope (Leica Microsystems, Wetzlar, Germany) equipped with differential interference contrast (DIC) and UV epifluorescence optics and a Zeiss Axiovert 40 CFL inverted microscope with phase contrast and DIC (Zeiss Microimaging, Goettingen, Germany). Photographs were taken with the digital cameras AxioCam HRc and AxioCam 208 (Carl Zeiss Microscopy GmbH, Jena, Germany), respectively.

The procedures followed for the preparation of the material of strain LPCc007 in order to analyze it using a scanning electron microscope (SEM) were not successful, precluding its examination with this technique.

DNA extraction, amplification, and sequencing

An aliquot of 1.5 ml of late exponential growing culture of strain LPCc007 was taken and concentrated by centrifugation, washed in two drops of milli-Q water, placed in 200 µl microtubes, cold shocked in liquid nitrogen and kept at -20 °C until further analysis. DNA extraction used Chelex® chelating resin (Bio-Rad, Hercules, California, USA), following Richlen & Barber (2005). DNA extracts were kept at -20 °C before PCR analyses. The D1-D2 domain of the LSU rDNA gene regions was amplified using the pair of primers D1R/D2C (5'-ACCCGCTGAATTTAAGCATA-3'/5'-ACGAACGATTTGCACGTCAG-3', Lenaers et al., 1989).

The amplification reaction mixtures (20 µl) were performed using Horse-Power™ Taq DNA Polymerase MasterMix (Canvax, Spain). DNA was amplified in an Eppendorf Mastercycler EP5345 (Eppendorf AG, New York, USA) as follows: 5 min denaturing at 95 °C, followed by 35 cycles of 35 s denaturing at 94 °C, 35 s annealing at 54 °C and 1 min elongation at 72 °C, with an elongation step of 7 min at 72 °C. PCR reactions were checked by agarose gel electrophoresis (1% TAE, 80 V) and GelRed™ nucleic acid gel staining (Biotium, Hayward, CA, USA). PCR products were purified with ExoSAP-IT™ (USB Corporation, Cleveland, Ohio, USA). Sequencing reactions were performed using the Big Dye Terminator v3.1 reaction cycle sequencing kit and migrated in a SeqStudio genetic analyzer (both at Applied Biosystems, Foster City, CA, USA) at the CACTI sequencing facilities (Universidade de Vigo).

Phylogenetic analyses

Partial LSU rDNA gene sequences obtained were inspected and aligned using MEGA X software (Kumar et al., 2018). Sequences from *Akashiwo sanguinea* were used to root the tree. The original alignments for the LSU rDNA phylogeny (including gaps) consisted of 674 bp. Best evolutionary models for maximum likelihood (ML) phylogenetic analyses were estimated using the model selection tool in MEGA X software, and Tamura-Nei model (Tamura & Nei, 1993) was selected. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Joining and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites [5 categories (γ = 0.4670)]. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. All positions with less than 95% site coverage were eliminated, i.e., fewer than 5% alignment gaps, missing data, and ambiguous bases were allowed at any position (partial deletion option). The final dataset contained 354 positions.

Additionally, a Bayesian inference (BI) phylogenetic analysis was carried out by sampling across the entire GTR model space using Mr. Bayes v3.2 (Huelsenbeck & Ronquist, 2001). The program parameters were statefreqpr, dirichlet (1,1,1,1); nst, mixed; and rates, gamma. Phylogenetic analyses involved two parallel analyses, each with four chains. Starting trees for each chain were selected randomly using the default values in Mr. Bayes. The number of generations used in these analyses was 1,000,000. Posterior probabilities were calculated from every 100th tree sampled after log-likelihood stabilization (burn-in phase). All final split frequencies were < 0.02. The two methods rendered similar topologies. Phylogenetic trees used ML/BI for the LSU rDNA, with bootstrap values (indicated as percentages) and posterior probabilities, in each case.

Net mean *p*-distances between clades were calculated using MEGA X. Thus, no corrections for multiple substitutions at the same site, substitution rate biases (e.g. differences in the transitional and transversional rates), or differences in evolutionary rates among sites were considered (Nei & Kumar, 2000).

RESULTS

Morphological analysis

Togula jolla Flø Jørgensen, Murray & Daugbjerg (Fig. 1A-G, Fig. 2A-F)

Reference. Flø Jørgensen et al., 2004b: 293, figs 22-29, 30-35, 38.

Cells roughly oval in shape, strongly to slightly asymmetrical, with the right side more convex than the left (Fig. 1A-G) and dorso-ventrally flattened (Fig. 2A, B). Cell length 30.8-49.8 µm (37.8 ± 4.8), cell width 20.0-40.0 µm (29.1 ± 5.3) and length/width ratio 1.2-1.6 (1.3 ± 0.1) (n = 32). Cingulum very asymmetric, starting at a distance

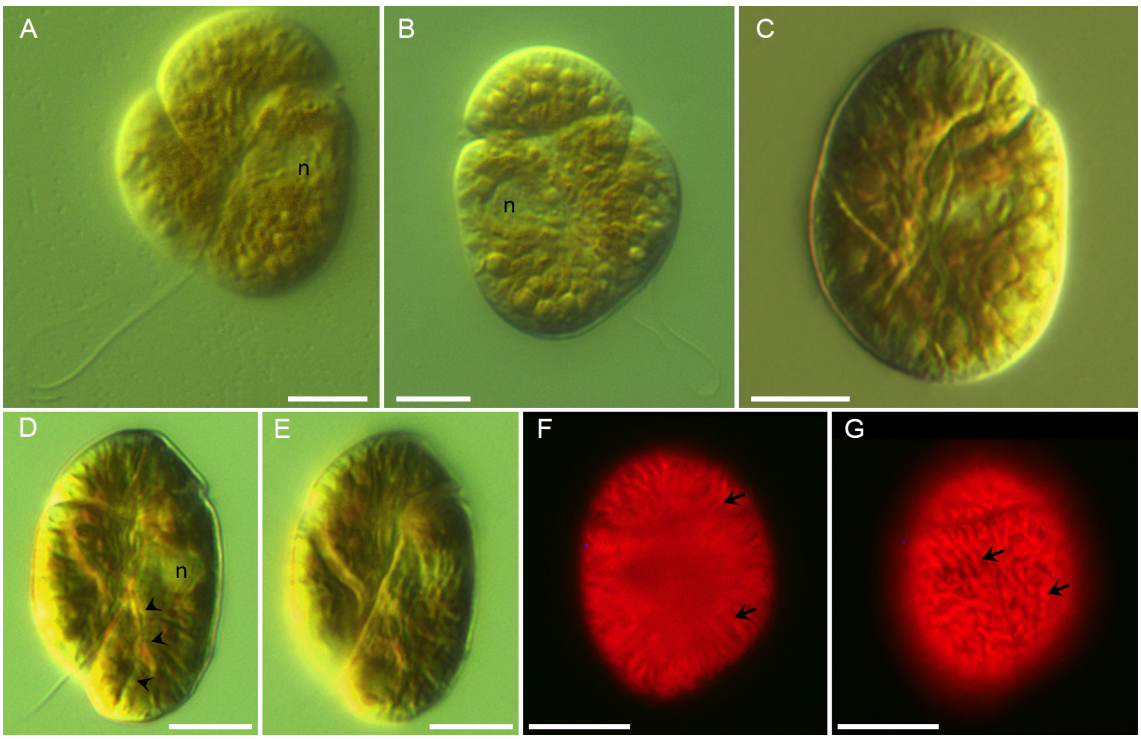

Fig. 1. Light and fluorescence microscopy of *Togula jolla.* Strain LPCc007. Scale bars = 10 µm. **A,** cell in ventral view showing cingulum asymmetric, descendent, displaced, with both ends joined by an intercingular groove (n = nucleus). **B,** same cell than Fig. A in different focus showing sigmoid cingulum dorsally (n = nucleus). **C,** cell in ventral view, less asymmetric than those in A, B. **D,** cell showing the nucleus (n) in mid ventral plane and a longitudinal groove ventrally bent towards the right side of the sulcus (marked by arrowheads). **E,** same cell with the focus in the terminal end of the cingulum. **F-G,** fluorescent micrographs of the same cell in different foci, showing the chloroplasts (arrows).

of 20-30% of the cell length from the apex, with the proximal end displaced towards left ventral side (Fig. 1D, E), sigmoid dorsally (Fig. 1B, D, F), and descending obliquely towards the sulcus ventrally (Fig. 1A, C, E). Cingulum displacement 25-48% of the cell length, intercingular region crossed by a more or less oblique groove located between the two ends of the cingulum (Fig. 1A, C, E). Sulcus short, curved towards the antapex and bent to the right (Fig. 1A, D), with a longitudinal flagellum longer than the cell (Fig. 1A, B). Chloroplasts greenish to golden brown, numerous, irregular and elongated in shape, radiating from the centre towards the periphery (Fig. 1D-G, arrows show two individual chloroplasts). Nucleus roundish, situated at left of the mid-ventral plane (Fig. 1A, B, D, n). The initial phase of the cell division started with the formation of a groove running between the intercingular region and the antapex, directed towards the right side of the cell, as the sulcus, in the ventral side of the hypocone (Fig. 1D, arrowheads), and continued with sequential formation of longitudinal parallel grooves running between the cingulum and the antapex, in the dorsal side of the hypocone (Fig. 2C, D, black arrowheads). After the formation of the longitudinal grooves (black arrowheads), the cell swelled, the hypocone protruding between the grooves, and the epicone formed a groove starting the cell division (Fig. 2E white arrowhead). Previous to completed the cell division the daughter-cells remained attached and slightly twisted relative to each other (Fig. 2A, B, F).

Distribution. *T. jolla* was found in marine coastal waters of Santa Teresita (Buenos Aires Province) at temperature 12.01 °C and salinity 33.36. This is the first record of the species from Argentina and from South-western Atlantic waters.

Molecular analysis

The phylogenetic analysis of strain LPCc007 included 44 partial LSU rDNA sequences (Fig. 3). The trees obtained by the ML (shown) and BI (not

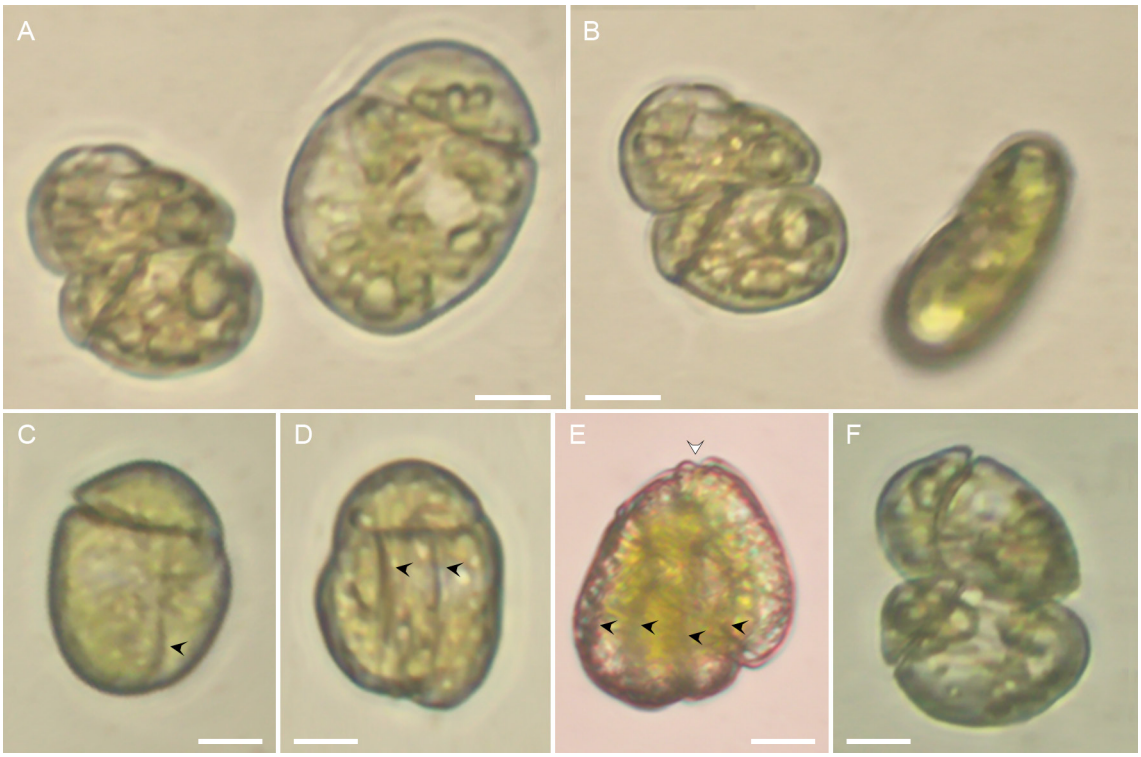

Fig. 2. Light microscopy of *Togula jolla*. Strain LPCc007. Scale bars = 20 µm. **A,** Motile cell in dorsal view during the swimming, accompanied by two connected daughter-cells before cell division is completed. **B,** Lateral view of the same cell than in Fig. A, rotated during the swimming. **C,** Dorsal view of a cell with one longitudinal groove in the hypocone (black arrowhead). **D,** Dorsal view of a cell with three longitudinal grooves in the hypocone (black arrowheads). **E,** Ventral view of a swelled cell with several longitudinal grooves on the hypocone (black arrowheads) and with an initial groove on the epicone (white arrowhead). **F,** Two connected daughter-cells, slightly twisted to one another, before cell division is completed.

shown) methods presented differences in their topologies, due to the low statistical significance in the connection of certain clades. The LSU rDNA-based phylogeny confirmed that the studied strain belonged to a strongly supported clade of *Togula jolla* (ML: 99%, BI: 1), the sequences included in this clade being identical. *Togula jolla* sequences grouped as a sister clade with *T. compacta* with strong statistical support (ML: 86%, BI: 1; phylogenetic *p* distance: 0.017) and were more

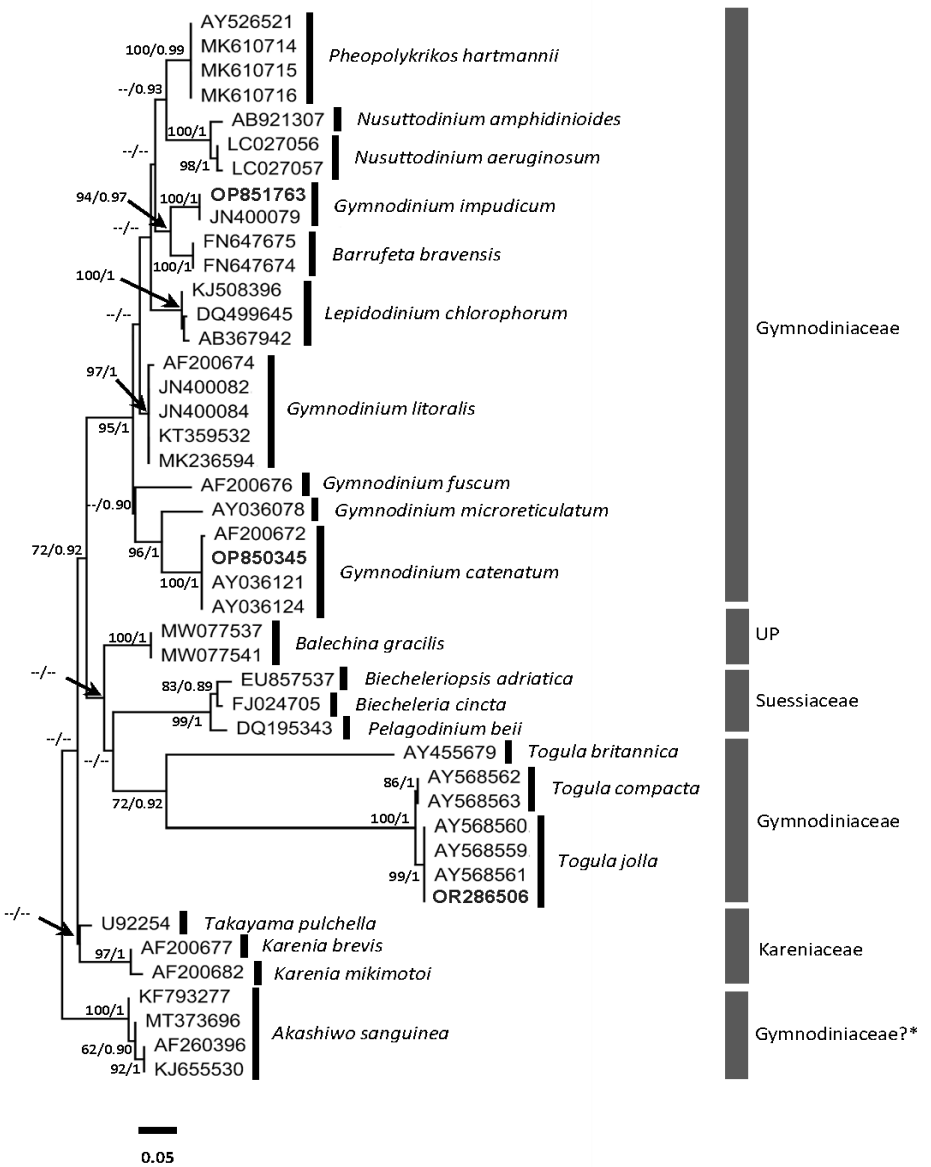

Fig. 3. Phylogenetic tree of the D1-D2 LSU rDNA obtained by ML model showing the relationships between *Togula jolla* strain LPCc007, Genbank accession number OR286506, from the coastal waters of Buenos Aires Province and strains from other places around the world. The tree includes sequences from *Gymnodinium catenatum* LPCc043, Genbank accession number OP850345, and *G. impudicum* LPCc044, Genbank accession number OP851763, both from coastal waters of Buenos Aires Province. Numbers on branches represent the bootstrap percentages (n = 1,000) and posterior probabilities (n = 1,000,000) after ML and BI analyses, respectively. Values lower than 60%/0.60 or unrepresentative in one of the runs are not shown or are shown with dashes, respectively. UP, uncertain position; S, Suessiales; ?*, the belonging of *Akashiwo sanguinea* to the Fam. Gymnodiniaceae was questioned by Escarcega-Bata et al. (2022).

Table 1. Morphological comparison among *Togula jolla* from Buenos Aires coastal waters and the species described by Flø Jørgensen et al. (2004b). nd: no data

	Togula jolla strain LPCc007	***Togula jolla***	***Togula britannica***	***Togula compacta***
Cell length (µm)	30.8-49.8 37.8 ± 4.8 (n = 32)	25.4-42.7 32.7 ± 3.6 (strain LB1562, n = 60) 20.0-31.0 25.0 ± 2.8 (strain CS-742, n = 18)	39.5-69.9 51.2 ± 7.0 (n = 60)	25.4-38.8 32.4 ± 3.4 (n = 60)
Cell width (µm)	20.0-40.0 29.1 ± 5.3 (n = 32)	19.0-35.0 25.0 ± 2.8 (strain LB1562, n = 60) 12.0-20.0 18.0 ± 2.5 (strain CS-742, n = 18)	30.0-53.9 36.3 ± 4.6 (n = 60)	18.0-35.0 25.1 ± 3.1 (n = 60)
Length / width ratio	1.2-1.6 1.3 ± 0.1 (n = 32)	1.3-1.4 1.3 ± 0.0	1.4-1.7 1.5 ± 0.1 (n = 60)	1.3-1.4 1.3 ± 0.0 (n = 60)
Cell shape in dorso-ventral view	oval, strongly to slightly asymmetrical, with the right side more convex than the left	oval, asymmetrical, with the right side barely more convex than the left	ovoid, asymmetrical, with the right side strongly more convex than the left	broadly ellipsoidal, asymmetrical, with the right side more convex than the left
Flattening type, (% cell width)	dorso-ventral, nd	dorso-ventral, 0.60-0.70 of cell width	dorso-ventral, 0.70 of cell width	dorso-ventral, 0.65 of cell width
Distance between the proximal end of the cingulum and the apex (% cell length)	0.20-0.30 20-30 (n = 19)	0.20-0.30 20-30	0.15-0.20 15-20	0.20-0.25 20-25
Cingulum displacement (% cell length)	0.3-0.5 30-50 (n = 20)	0.4-0.5 40-50	nd	nd
Cell color	greenish brown golden-brown	olive-green	golden-brown	golden-brown
Chloroplasts, shape and number	irregular, elongated, numerous radiating from the centre towards the periphery	irregular elongated, numerous, radiating from the centre towards the periphery	irregular, numerous, radiating from the centre towards the periphery	numerous, radiating from the centre towards the periphery
Nucleus shape and position	roundish, situated left of the mid-ventral plane	oval, situated mid-ventral plane, variable	round to ellipsoidal, lying in the median plane at the long axis	elongate oval, occupying most the entire width of the cell, to roundish, situated left of the mid-ventral plane
Prior of cell division in ventral side of hypocone.	a groove situated left of the sulcus	a groove situated left of the sulcus	a groove situated left of the sulcus	groove situated left of the sulcus not detected
Prior of cell division in dorsal side of hypocone	formation of longitudinal grooves on the hypocone	formation of longitudinal grooves on the hypocone	longitudinal grooves on the hypocone not observed	longitudinal grooves on the hypocone not observed

distantly related to *T. britannica* (ML: 72%, BI: 0.92; $p = 0.497$).

The phylogenetic relationships between *Togula* sequences and related genera from the Gymnodiniaceae and Suessiaceae varied in the ML and BI trees, with different results for *Togula* clade and the closest ones.

GenBank accession number of strain LPCc007: OR286506

DISCUSSION

Comparison of morphometric and morphologic features

The gymnodinioid, psammophilic, photosynthetic genus *Togula* was characterized with light microscopy based on the following morphological striking features: dorso-ventrally flattened cell and highly asymmetric cingulum, descending, with ends displaced by about one to two thirds of the total length of the cell and joined by an oblique intercingular groove. The course of the cingulum and the intercingular groove is what gives the cell its characteristic toga-like appearance in ventral view described by Flø Jørgensen et al. (2004b) in the protologue.

Currently, the genus includes three recognized species, *Togula britannica* (Herdman) Flø Jørgensen, Murray & Daugbjerg (Basionym: *Amphidinium asymmetricum* var. *britannicum*), *T. compacta* (Herdman) Flø Jørgensen, Murray & Daugbjerg (Basionym: *Amphidinium asymmetricum* var. *compactum*) and *T. jolla* Flø Jørgensen, Murray & Daugbjerg.

The strain under study of *T. jolla* was compared with the material described in the protologue of this species and the descriptions of the other two species given by Flø Jørgensen et al. (2004b) (Table 1). The analysis with light microscopy allowed to determine that the cell length and width average of Argentinian strain of *T. jolla* is somewhat greater than those of strains from USA and Australia, and that of strains from Denmark of *T. compacta*. Additionally, the cell size average of all strains of *T. jolla* and *T. compacta* is considerably smaller than that of the strain of *T. britannica*. The cell shape is similar in all the compared species, however the Argentinian strain of *T. jolla* showed a wider range of asymmetry (Fig. 2A-E) than that described and illustrated by Flø Jørgensen et al. (2004b). The fact that the analysis was not carried out with scanning electron microscopy did not have any consequences for the evaluation of the differential characters of the species, since according to Flø Jørgensen et al. (2014b), this also does not reveal significant differences between vegetative motile cells.

Several details about the pattern of development of longitudinal grooves on the hypocone of cells about to undergo mitosis observed in Argentinian strain of *T. jolla* perfectly agree with those described by Flø Jørgensen et al. (2004b) in the protologue. In both cases the early phase of cell division started with the formation of a groove in the ventral side of the hypocone and continued with formation of longitudinal parallel grooves in the dorsal side of the hypocone. This characteristic pattern of cell division is a differential feature that allows to distinguish *T. jolla* from *T. britannica* and *T. compacta* using LM.

T. jolla was described based on cultures isolated from La Jolla Beach, USA (type locality, sediment samples); Boundary Bay, Canada; Napier, New Zealand, North Island (plankton sample) and Port Botany Bay, Australia (sediment samples). Subsequently, the distribution was extended to Awaroa, New Zealand, South Island (sediment samples) by Borchhardt et al. (2021) and Santa Teresita, Buenos Aires Province, Argentina (this study, plankton samples). Considering the opinion of Flø Jørgensen et al. (2004b) about the fact that determination of species of *Togula* is reliable if it is carried out on previously established cultures, the record given by Shah et al. (2013) for the Jeju Island, Korea, was not considered for extending the distribution of *T. jolla*.

Molecular comparison

The molecular results in the present study confirmed the identity of strain LPCc007 as *T. jolla*. Single gene phylogenies based on rDNA genes, like the D1-D2 LSU rDNA regions, are widely used in the literature given their utility for distinguishing closely-related dinoflagellate species (e.g. Nézan et al., 2014; Nishimura et al., 2020). In turn, they fail to provide reliable branching patterns among deeper lineages (Ott et al., 2022). The genus *Togula* represents an exemplary case, with contradictory results in the literature depending on the implemented approach. First, the original description by Flø Jørgensen et al. (2004b) found that no other genera were closely related to *Togula* in their LSU rDNA phylogeny (that encompassed D1-D6 regions and 1392 aligned positions). Then, the phylogenetic relationships of *Togula* were fully resolved and characterized as a member of the Gymnodiniaceae family, based on more extensive analyses including multiprotein phylogeny (Janouškovec et al., 2016) and transcriptome sequence data (Price & Bhattacharya, 2017). In fact, these two studies reported *T. jolla* as a sister group to *Gymnodinium catenatum* H.W. Graham. In our work, same as in Borchhardt et al. (2021), the *Togula* clade emerged separate to the Gymnodiniaceae and more closely related to Suessiales. However,

the low statistical support in ML analyses did not allow to delineate these relationships, which yielded distinct topologies by ML or BI methods. Borchhardt et al. (2021) suggested that their results (based on a concatenated alignment of SSU, ITS1, 5.8S, ITS2 and LSU rRNA gene regions) could arise either from the use of rRNA genes instead of large multi-gene datasets or from a previously overlooked relationship with Suessiales. The first hypothesis seems more likely considering the athecate nature of *Togula* and the monophyletic origin of theca in dinoflagellates derived from multi-gene analyses, first shown by Orr et al. (2012), using concatenated analyses from eight molecular markers. Finally, the large genetic distance observed between *T. jolla* and *T. britannica,* already pointed out by Flø Jørgensen et al. (2004b), led these authors to suggest that *T. britannica* could represent a new genus.

In conclusion, the molecular phylogenetic results obtained and those related to the pattern of cell division for strain LPCc007 in this study, showed to be perfectly consistent with those stated in the protologue of *T. jolla* (Flø Jørgensen et al., 2004b). The present study was the first of a series focused on the unarmoured dinoflagellate of the Order Gymnodiniales. Several species of this group are harmful algal bloom (HAB) formers, known to produce toxins that can cause human fatalities and extensive fish kills (Glibert et al., 2005; Iwataki, 2023). In the framework of a monitoring of harmful algae and toxins in cultured material and shellfish, the isolated strains belonging to the Gymnodiniales will be characterized in the near future based on morphology, phylogeny, toxinology and citotoxicity.

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