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Molecular phylogeny of the parasitic dinoflagellate *Syltodinium listii* (Gymnodiniales, Dinophyceae) and generic transfer of *Syltodinium undulans* comb. nov. (= *Gyrodinium undulans*)

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Abstract

The parasitic dinoflagellate *Syltodinium listii* was investigated from the open waters of the English Channel, southern North Sea and the NW Mediterranean Sea.

Syltodium listii has been unreported since its original description in the North Sea. Cells of *S. listii* were able to infect copepod eggs of different species, and even nauplii, and after each infection formed up to 32 cells embedded in a mucous envelope. Infection of the same host by more than one dinoflagellate was frequent; although overall, the progeny were reduced in number. Molecular phylogeny based on the small subunit ribosomal RNA (SSU rRNA) gene revealed that *S. listii* clusters with a group of environmental sequences from the cold North Atlantic region as a sister group of *Gymnodinium aureolum*. The large subunit ribosomal RNA (LSU rRNA) gene sequences of *S. listii* from the English Channel and cf. *Gyrodinium undulans* from the Mediterranean Sea were identical. Thus, we propose *Syltodium undulans* comb. nov. for *Gyrodinium undulans*. The first internal transcribed spacer (ITS) and complete SSU rRNA gene sequences of *Dissodinium pseudolunula* are provided. The parasitic species of *Chytriodinium*, *Dissodinium* and *Syltodium* cluster together within the family Chytriodiniaceae, including the free-living species *Gymnodinium aureolum*, *G. corollarium* and *G. plasticum*.

Keywords: *Chytriodinium*; Copepod parasite; *Dissodinium*; *Gymnodinium*; *Gyrodinium*; Parasitic Dinoflagellata

Introduction

Copepods are the most abundant animal group in the ocean. The lipid-rich copepod eggs are the target of parasitic species of the dinoflagellate genera

Chytriodinium, *Schizochytriodinium* and *Dissodinium*, whose dinospores infest the eggs, absorb the host contents and form successive sporangia that release gymnodinioid infective spores (Apstein 1906; Cachon and Cachon 1968; Dogiel 1906; Drebes 1969, 1978, 1981; Elbrächter 1988; Gómez and Artigas 2013; Gönnert 1936; Lebour 1925; Pouchet 1885). Molecular phylogenies reveal that *Chytriodinium* spp. and *Dissodinium pseudolunula* cluster within the *Gymnodinium* clade (Gómez et al. 2009; Gómez and Skovgaard 2015; Kim et al. 2008).

According to Drebes (1988), the parasitic dinoflagellate *Syltodium listii* appeared by chance as a contaminant in a culture of *Dissodinium* fed with copepod eggs, and once recognized, it was identified from the summer plankton of the North Sea. Cells of *S. listii* developed a peduncle that sucked out the contents of copepod and rotifer eggs. After food uptake, the trophont detached and began successive binary divisions to form up to 16 or 32 gymnodinioid cells inside of a mucous envelope (Drebes 1988). In a clonal culture of the diatom *Odontella aurita*, Drebes and Schnepf (1998) detected the contamination of a dinoflagellate that fed on the diatom, and was able to feed on copepod eggs. Drebes and Schnepf (1998) identified it as *Gyrodinium undulans* that was described as a free-living species from the North American coasts at Great Pond, Woods Hole (Hulburt 1957). The morphology and life cycle was similar to *Syltodium listii*, and Drebes and Schnepf (1998) suggested that *G. undulans* and *S. listii* may be the winter and summer forms, respectively, of the same species.

The smooth cell surface of *Gyrodinium undulans* does not fit with the current circumscription of the genus *Gyrodinium* that is restricted, among other characters, to cell coverings with longitudinal striae (Hansen and Daugbjerg 2004; Takano and Horiguchi 2004). *Gyrodinium undulans* and *Syltodium listii* are characterized by a distinctive sigmoid anterior extension of the sulcus (Drebes 1988; Hulburt 1957). A

sigmoid apical groove is a diagnostic character of the genus *Takayama* (de Salas et al. 2003). All the known species of *Takayama*, and other closely related genera (*Brachidinium*, *Karenia*, *Karlodinium*) are photosynthetic species with fucoxanthin as an accessory pigment, while *G. undulans* and *S. listii* are heterotrophic species. From the NW Mediterranean Sea, Reñé et al. (2015) reported a micrograph of a cell identified as cf. *G. undulans* with the distinctive sigmoid anterior sulcus. The partial LSU rRNA gene sequence (GenBank accession number KP790206) clustered within the *Gymnodinium* clade with sequences of *Gymnodinium aureolum* as the closer relative. Reñé et al. (2015, p. 256) concluded “Since a close phylogenetic relationship between *G. undulans* and *S. listii* can be expected (if not representing the same species), *G. undulans* can be rejected as a member of the *Gyrodinium* genus because it is included within the Gymnodiniales s.s. clade. However, since our specimen could not be precisely identified, any systematic change would be premature”.

In this study, we investigate the molecular phylogeny, life cycle and host specificity of *Syltodium listii* from the English Channel. We provide the first molecular data of the genus *Syltodium* (SSU-, LSU rRNA and ITS gene sequences), and the first ITS and complete SSU rRNA gene sequences of *Dissodinium pseudolunula*.

Material and Methods

Sampling, isolation and cultures

Syltodium listii was occasionally observed in the live samples from the NW Mediterranean Sea at Marseilles (43°16'48"N, 5°20'57"E) in June 2009, and in coastal monitoring in the NE English Channel following the methods described in Gómez et al. (2009) and Gómez and Artigas (2013), respectively. Recent observations were carried out during the research cruise ECOPEL Manche-Leg 2 on board R/V *Antea* (IRD,

Institut de Recherche pour le Développement). Fifty-one stations were sampled from Dunkirk (North Sea) to Brest (South English Channel) on 16–31 July 2018. Plankton samples were collected from surface waters with a phytoplankton net (20- μm mesh size). Aliquots were left to settle in a composite settling chamber, examined on-board with an inverted microscope (Eclipse TS-100, Nikon Inc., Tokyo, Japan) and photographed with a digital camera (Nikon D5000). In order to establish cultures, detached trophonts of *S. listii* were isolated using a micropipette, and placed in a 6-well tissue culture plate with 0.2 μm -filtered seawater. The surface seawater temperature was 18–19 °C, and the culture was maintained at a room temperature of 22 °C. Freshly collected copepod eggs were daily added to the cultures. In addition, primary (spherical) and secondary (lunate) sporangia of *Dissodinium pseudolunula* were isolated and cultured following the same procedure.

For molecular analyses, 40–60 cells of *Syltodinium listii* were micropipetted with a fine capillary into a 0.2 mL microcentrifuge tube filled with absolute ethanol. Cells of *Dissodinium pseudolunula* were isolated from lunate sporangia before the release of the dinospores. About 80–100 cells of immature infective spores were placed into a 0.2 mL microcentrifuge tube filled with absolute ethanol. The samples were kept at room temperature and in darkness until the molecular analyses could be performed.

PCR amplification and sequencing

The 0.2 mL microcentrifuge tubes were briefly centrifuged and then opened to allow the ethanol to evaporate overnight on the benchtop in a covered container. Cells were resuspended in 20 μL extraction buffer (final concentrations: 1 mg mL⁻¹ bovine serum albumin, 10 mM Tris pH 7.4, 100 mM KCl, 1 mM EDTA, 50% glycerol). A negative extraction control was 20 μL of extraction buffer in a sterile 0.2 mL microcentrifuge tube. The tubes were frozen at –80 °C for 20 min followed by rapid

warming to room temperature for 20 min. A 2 μ L aliquot of the extracted product was used as DNA template for polymerase chain reaction (PCR) amplification. To amplify SSU rRNA gene fragments, the primers EukA1 and EukB2 (Table 1) were used in a reaction with GoTaq polymerase (Promega Corp., Madison, WI, USA). For this reaction, the following thermocycler program was performed: initial denaturation at 94 °C for 5 min; 40 cycles of denaturation at 94 °C for 30 s, annealing at 54 °C for 30 s, and extension at 72 °C for 2 min; then final extension at 72 °C for 7 min. The *Syltodium* sample yielded a product, but the *Dissodium* sample did not. A nested PCR was carried out for *Dissodium* using 1 μ L of the first reaction mix as DNA template and the primer pairs EukA1/1200R and 373F/EukB2 (Table 1). Conditions for the second round of PCR were the same as the first, except that the cycling extension time was shortened to 1 min.

To amplify the ITS/5'-LSU rRNA gene region, primers 1200F and NL1fR (Table 1) were initially used with the same conditions as the first round SSU rRNA gene amplification. A product was obtained for *Dissodium*, but not for *Syltodium*, so a nested amplification was accomplished using the primer pair ITS1F/NL1fR (Table 1) using the same cycling parameters and 1 μ l of the prior PCR reaction as template. To obtain more of the LSU region, a nested amplification strategy was employed. For *Dissodium*, the first round primer set was 1200F/TW14, followed by a second round using CTB6/TW14 (Table 1). For *Syltodium*, the first round primer set was 1200F/TW15 (Table 1), followed by ITS1F/TW14, and then by CTB6/TW13. The amplification conditions were the same as the first round of SSU rRNA gene amplification, and for the nested reactions the extension time was reduced to 1 min. PCR products were cleaned up using the MinElute PCR Purification Kit (Qiagen Inc. Germantown, MD, USA) and directly sequenced at GENEWIZ Inc. (South Plainfield,

NJ, USA). Chromatograms were checked and assembled using Sequencher v.5.4.6 (Gene Codes Corp., Ann Arbor, MI, USA), and the contig exported as a FASTA file. The sequences were deposited in DDBJ/EMBL/GenBank under accession numbers MK626523 (SSU, ITS, LSU; *Dissodinium*), MK629450 (SSU; *Syltodium*) and MK629451 (ITS, LSU; *Syltodium*). Due to the nested amplification strategy employed for *Syltodium* ITS/LSU, it was not possible to obtain sequence overlap between the 3' end of the SSU rRNA gene and the ITS region to form a single contig.

Phylogenetic analyses

The small and large subunit rRNA gene sequences of *Dissodinium pseudolunula* and *Syltodium listii* were analysed using Basic Local Search Tool (BLAST, <http://blast.ncbi.nlm.nih.gov/Blast.cgi>) against the GenBank database. The closest matches of the SSU rRNA gene sequence of *S. listii* (MK629451) were environmental sequences, and the first identified sequences belonged to *Gymnodinium aureolum*. Based on these results, SSU rRNA gene sequence alignments were constructed from the closest environmental sequences and sequences representatives of nearly all the species of the *Gymnodinium* clade, the genera *Gyrodinium*, *Takayama*, *Karlodinium*, *Karenia*, *Brachidinium* and other dinoflagellates. A first tree was built including short sequences from gymnodinioid parasites of copepod eggs: *Chytriodinium roseum* (FJ663049, 1216 bp, base pairs), *C. affine* (FJ473380, 1206 bp), and *Dissodinium pseudolunula* (FJ473378, 1217 bp; FJ473379, 1143 bp). The sequences of the Chytridiaceae was also represented by the complete SSU rRNA gene sequence of *Chytriodinium* sp. (KM245128, 1800 bp). Other environmental sequences closely related to the sequence of *Syltodium* were included: one sequence from a Bay in Greenland [EF100288, 1346 bp, (Stoeck et al. 2007)], and two sequences from a Norwegian fjord [EF526797 and EF526804, 1343 bp (Behnke et al. 2010)]. Two environmental sequences from a Danish

fjord [DQ103845, 1648 bp; DQ103846, 1645 bp, (Zuendorf et al. 2006)] were included, together with other two longer sequences closely related from the same location (DQ103860, 1801 bp; DQ103871, 1788 bp). In order to improve the statistical support, the SSU rRNA gene sequences smaller than 1660 bp were excluded in a second alignment. The closest matches of the D1-D2 LSU rRNA gene sequence of *S. listii* (MK629450) were sequences of *Gyrodinium undulans* (KP790206), followed by *Gymnodinium corollarium*, *G. aureolum* and *Dissodinium pseudolunula*. Based on these results, LSU rDNA sequence data were compiled from the sequences of these species, and sequences representatives of nearly all the genera of *Gymnodinium* clade, the genera *Gyrodinium*, *Takayama*, *Karlodinium*, *Karenia*, *Brachidinium* and other dinoflagellates. SSU- and LSU rRNA gene sequence alignments were accomplished by ClustalW (Larkin et al., 2007) and the evolutionary history was inferred by using the Maximum Likelihood method based on the General Time Reversible model with the default settings (uniform rates, complete deletion of gaps/missing data) in MEGA7 software (Kumar et al. 2016). Bootstrap values were obtained after 1000 replications. The sequences of the syndinean *Syndinium turbo* and the perkinsid *Perkinsus marinus* were used for rooting the SSU- and LSU rRNA gene phylogenetic trees, respectively. A phylogenetic tree based on the ITS marker was not built due to the scarce number of sequences of other members of the *Gymnodinium* clade.

Results

Observations of *Syltodinium listii* Drebes, 1988 (Fig. 1a–s, 2a–q)

A cell was observed sucking the contents of a copepod egg at the beginning of the research cruise in a sampling station off Boulogne sur Mer, France (50°41'14"N, 1°26'01"E). The parasitic cell was colourless, differing from the cells *D. pseudolunula* that contain chloroplasts, and larger (~30 µm long, ~25 µm wide) than the infective

spores of *Chytriodinium* or *Dissodinium*. Once recognized as different from *Dissodinium* and *Chytriodinium*, the cell was isolated to establish a first culture. Throughout the cruise, additional cultures were established from new parasite isolates.

The infective cells showed an almost conical to hemispherical episome, and the hyposome showed convex sides when the feeding peduncle penetrated into the host (Fig. 1a). The hyposome was hemispherical when the food uptake began (Fig. 1b, c). The feeding peduncle of the infective cells formed a tear-shaped structure that may show orange pigmentation when encountering the egg contents (Fig. 1b). The infective cell progressively inflated to form an ellipsoidal (Fig. 1d–f) to almost spherical trophont (Fig. 1g–k). The trophonts had a variable size (50–90 μm long), were motile and detached immediately after the food uptake. The recently detached trophont showed numerous refractive lipid bodies (Fig. 1d–m). The cingulum was median, with a displacement of about one cingular width. The sulcus extended from the antapex to the apex, and almost straight in the hyposome. The anterior sulcus in the episome extended from the upper cingulum to the apex with a sigmoid curvature to the right (Fig. 1d–e, h–k). It is uncertain if the sulcus connected with an apical groove because the optical resources on-board did not allow the observation. In some cells, it was visible as a short longitudinal intrusion of the cingulum into the episome (Fig. 1g–k). The nucleus was located in the episome, above the cingulum, and it did not show a capsule or any special covering. The trophont divided successively by binary fission (Fig. 1n–q) forming a cluster of 16–32 cells (Fig. 1r). The dividing cells were surrounded by a mucous envelope with an amorphous outer layer with attached debris (Fig. 1q–r). At any time, the cells swam actively and dispersed if the envelope was disrupted.

Under culture conditions, the recently released infective cells were able to attack newly added copepod eggs. Copepod eggs that fell near the border of the culture well

were infected before eggs in the centre. Multi-infection was a frequent feature in the cultures (Fig. 2a–d). It was common to observe eggs infected by more than 10 cells, while other eggs were intact (Fig. 1s). During the uptake of the egg contents, the egg chorion split from the eggshell with the exception of the area near the feeding peduncle (Fig. 2e–h). In case of multi-infection, the peduncle of the parasites that arrived later were able to penetrate the eggshell, but the peduncle might not be long enough to reach the retracted egg chorion [due to the consumption by other parasite(s)]. The unsuccessful infective cell retracted the peduncle, detached and searched for a new host. The resulting trophonts were of smaller sizes when two or three cells competed for the same egg contents (Fig. 2b–d). In case of multi-infection, the less satiated trophonts produced only four or eight new cells. *Syltodium listii* infected individual eggs of different sizes of free-spawning copepod species (Fig. 2a–h), and the egg sacs of brood-carrying copepods (Fig. 2i–j). In some cases, the cells of *Syltodium* attacked an immature nauplius inside the eggshell (Fig. 2k), and hatched nauplii were also infected (Fig. 2l–m). Infections of eggs of appendicularians were not observed. Infective cells and other life stages of *Syltodium listii* were also previously observed from live samples of the NW Mediterranean Sea (Fig. 2n–q).

Molecular phylogeny of *Syltodium listii* and *Dissodinium pseudolunula*

In the SSU rRNA gene sequence phylogeny, *Syltodium listii* clustered with strong support (99%) with two environmental sequences from the Mariager fjord, Denmark, Kattegat Sea (Fig. 3). The SSU rDNA sequence of *S. listii* (MK629451) from the English Channel is 99.6% similar to an environmental sequence from the Kattegat (DQ103845). If the shorter environmental sequences were included, *S. listii* also clustered with other environmental sequences from the Mariager fjord, the Framvaren fjord in Norway, North Sea, and one sequence from the coast of Greenland (see inset in

Fig. 3). *Syltodium listii* is the first characterized member of this group of sequences from the cold North Atlantic region. The sequences of *Dissodinium pseudolunula* clustered with *Gymnodinium aureolum* as a sister group of *Syltodium listii* and the environmental sequences from the cold North Atlantic region. The sequences of the parasite *Chytriodinium* sp. and the free-living species *Gymnodinium plasticum* clustered in a more basal position (Fig. 3).

The LSU rRNA gene sequence of *Syltodium listii* from the NE English Channel (MK629450) was identical (100%) to the sequence of cf. *Gyrodinium undulans* (KP790206) from the Mediterranean Sea. In the LSU rRNA gene phylogeny, the sequence of *Syltodium listii* clustered with the sequence of cf. *Gyrodinium undulans* (KP790206) with strong support, as a sister group of the sequences of *Gymnodinium corollarium* and *G. aureolum*. All these sequences clustered as a sister group of the sequences of *Dissodinium pseudolunula* and *Gymnodinium plasticum* without support (Fig. 4). The sequences of *Chytriodinium*, characterized by very long branches, clustered as an independent lineage within the *Gymnodinium* clade (Fig. 4).

Discussion

The spherical (primary) and lunate (secondary) sporangia of *Dissodinium pseudolunula* are large, tough, and immotile, and they lasted for about two days before the release of the dinospores (Drebes 1978; Gómez et al. 2009). This facilitates the detection of *D. pseudolunula* in plankton samples (see Fig. A1 as Supplementary data). In contrast, *Syltodium listii* is easily overlooked in live material, as well as in preserved samples, because it lacks cysts or other distinctive life stages. Cell division of *S. listii* occurred inside of a fragile envelope that was easily disrupted due to the sample collection and manipulation, and the spores swam and dispersed after any perturbation. The clusters of dividing cells surrounded by an envelope were also not different from

the life stages of other parasites (i.e., *Amyloodinium*, Gómez and Gast 2018). Therefore, the infective cells could be easily mistaken for other gymnodinioid heterotrophic dinoflagellates or even described as free-living forms (tentatively as *Gyrodinium undulans*). All these features suggest that the distribution and abundance of *S. listii* have been underestimated in both live and preserved samples.

The molecular data place the sequences of *Syltodinium listii* as a member of the *Gymnodinium* clade (Figs 3–4). This is a morphologically and ecologically diverse clade that contains marine and freshwater species (*G. fuscum*), solitary, colonial (*G. catenatum*) and pseudo-colonial (*Polykrikos*) species, heterotrophic, mixotrophic and photosynthetic species with chloroplasts of different origins (*Lepidodinium*, *Nusuttodinium*), species with highly elaborated organelles (*Erythrospidinium*), and free-living, mutualistic symbiotic (*Gymnoxanthella*) or parasitic forms (*Chytriodinium*, *Dissodinium*). Gómez (2012) classified *Chytriodinium*, *Dissodinium*, *Myxodinium*, *Schizochytriodinium*, and *Syltodinium* within the family Chytriodiniaceae in the Gymnodiniales sensu stricto. *Syltodinium listii* is the first characterized member of a group of environmental sequences related to the sequences of the parasitic species of *Dissodinium* and *Chytriodinium*, and several free-living species (Fig. 3).

Based on the SSU- and LSU rRNA gene molecular data, one of the closest morphologically identified relative of *S. listii* is *Gymnodinium aureolum* (Figs 3, 4). This ichthyotoxic species is predominantly associated with blooms in cold waters. It was first described as *Gyrodinium aureolum* because the high cingular displacement was used in the past to split *Gymnodinium* and *Gyrodinium* (Hulburt 1957). *Gymnodinium aureolum* possesses an anterior extension of the sulcus (Hansen et al. 2000) as reported in *S. listii* and *G. undulans*, but it is straight while sigmoid in the two latter taxa (Drebes 1988; Hulburt 1957). The mixotrophic *G. aureolum* is able to

protrude a posterior peduncle to feed on microalgae (Hansen 2001; Jeong et al. 2010). This peduncle or phagopod has also been reported in *S. listii* and *G. undulans* when feeding in crustacean eggs or diatoms (Drebes 1988; Drebes and Schnepf 1998). The cysts of *G. aureolum* are surrounded by mucus (Hansen et al. 2000; Tang et al. 2008), similar to the mucous envelope surrounding the dividing cells of *S. listii* and *G. undulans* (Fig. 1q–r; Drebes 1988; Drebes and Schnepf 1998). Other close relatives of the sequences of *Syltodinium* are *Gymnodinium corollarium* and *G. plasticum* that are photosynthetic brackish and freshwater taxa, respectively, with a sulcal extension in the episome (Sundstrom et al. 2009; Wang et al. 2017).

In the original description, Drebes (1988) did not compare *Syltodinium listii* and *Gyrodinium undulans* despite them sharing a distinctive sigmoid anterior extension of the sulcus. Hulburt (1957) described *G. undulans* in high latitudes in the North American coasts at Great Pond, Woods Hole, from samples collected in January–February. In that season, a high abundance of copepod eggs is not expected, leading to speculation that *G. undulans* was feeding on diatoms. Hulburt (1957) described *G. undulans* with three illustrations showing the variability of the cell shape and size, and he also remarked the presence of "assimilate bodies sometimes in form of large blocks" that shows similarities to the refractive lipid bodies of *S. listii* (Fig. 1d–n) or *G. undulans* as described in Drebes and Schnepf (1998). We have identified our isolates as *Syltodinium listii* because the morphology and life cycle fully agree with the original description by Drebes (1988), and the samples were collected from the NE English Channel, close to the North Sea. The SSU rRNA gene sequences of *S. listii* clusters with environmental sequences from waters of the North Atlantic region (Denmark, Norway, Greenland) (Fig. 3; Behnke et al. 2010; Stoeck et al. 2007; Zuendorf et al. 2006). *Gyrodinium undulans* has been illustrated from the North Sea (Drebes and Schnepf

1998), and the Kattegat Sea (Hansen and Larsen 1992). Records of *G. undulans* have been also reported in Brittany (Paulmier 1994) and Australia (Sonneman and Hill 1997). Paulmier (1997) reported as *G. cf. undulans* a cell that was significantly smaller (19 μm) than the previous observations. Sonneman and Hill (1997, p. 172) described one cell that germinated from a cyst as resembling *G. undulans*. They reported red pigmented bodies distributed throughout the cell, which are unknown in that species.

This study also reports life stages of *S. listii* from the coastal NW Mediterranean Sea at Marseilles (Fig. 2n–q), co-occurring with *Dissodinium pseudolunula* and *Chytriodinium* spp. (Gómez et al. 2009). In the case of *Dissodinium pseudolunula*, the SSU rRNA gene sequence from the English Channel (MK626523) was 99.6% similar the sequences from the Mediterranean Sea (FJ473378, FJ473379), and the LSU rRNA gene sequence (MK626523) was 99.3% and 99.4% similar to the sequences from Korea and Brittany (AY526523, KJ508391), respectively. From the NW Mediterranean Sea, Reñé et al. (2015) reported a micrograph of a cell identified as cf. *G. undulans* with the distinctive sigmoid anterior extension of the sulcus. The LSU rRNA gene sequence of the Mediterranean isolate of cf. *G. undulans* (KP790206) is identical (100%) to our sequence of *S. listii* from the NE English Channel (MK629450). The molecular data suggest that *S. listii* is present in the Mediterranean Sea, NE English Channel, and tentatively in the Kattegat. Based on the molecular data, parasites of copepod eggs such as *D. pseudolunula* and *S. listii* have a wide range of distribution in temperate and cold waters. This supports the synonymy of *G. undulans* and *S. listii* as suggested by Drebes and Schnepf (1998). On the other hand, the genetic divergence of short environmental sequences (<1347 bp) from Norway (EF526804, EF526797) and Greenland (EF100288) suggests a second species within the genus *Syltodium* (see inset in Fig. 3). The doubt

is whether *G. undulans* described near Woods Hole (Hulburt 1957) is *S. listii* (= cf. *G. undulans* sensu Reñé et al. 2015) or they are two closely related species.

Gyrodinium undulans does not belong to the genus *Gyrodinium* because in the molecular phylogenies it is distantly related to the type species, *G. spirale* (Fig. 4; Reñé et al. 2015), and it does not fit with the emended diagnosis of the genus *Gyrodinium* (Hansen and Daugbjerg 2004; Takano and Horiguchi 2004). The name *Syltodinium* appears as the first available generic name. If *Syltodinium listii* and *Gyrodinium undulans* are identical, then *G. undulans* is the senior synonym of *S. listii*, and the accepted name for the type species of *Syltodinium* should be *S. undulans* (Hulburt, 1957) F. Gómez, Artigas & Gast, comb. nov. If they are not synonyms, *G. undulans* also needs to be transferred into *Syltodinium* as a second species within the genus. We propose the transfer of *Gyrodinium undulans* to *Syltodinium*: *Syltodinium undulans* (Hulburt, 1957) F. Gómez, Artigas & Gast, comb. nov.

Basionym: *Gyrodinium undulans* Hulburt, 1957, Biol. Bull. 112: p. 218, pl. 3, figs 7–9.

The SSU rRNA gene molecular phylogeny reveals that the members of the Chytriodiniaceae (*Chytriodinium*, *Dissodinium*, *Syltodinium*) cluster with several free-living species of the genus *Gymnodinium* (Fig. 3). *Gymnodinium aureolum*, *G. corollarium* and *G. plasticum* are more closely related to *Dissodinium pseudolunula* and *Syltodinium listii* than to the type species of *Gymnodinium*, *G. fuscum* (Figs 3, 4). The Chytriodiniaceae, until now restricted to parasitic forms, needs to extend to free-living species.

Author contributions

Fernando Gómez (FG) and Luis F. Artigas collected, isolated and cultured the cells. Rebecca J. Gast obtained the DNA sequences. The three authors wrote the manuscript.

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Supplementary data

Fig. A1. Life stages of *Dissodinium pseudolunula* from the English Channel in July 2018.

Supplementary data associated with this article can be found in the online version, at

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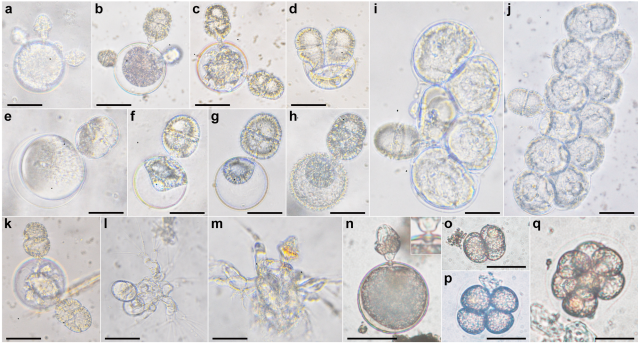
Figure legends

Fig. 1. (a–s) *Syltodium listii* from the English Channel in July 2018. (a–c) Infective cells on copepod eggs. (b) The inset shows the feeding peduncle. (d–f) Recently detached trophont in different focal planes. (g–k) Another recently detached trophont in different focal planes. (l, m) Recently detached trophonts. (n–p) Different stages of the binary fission. (q, r) Clusters of dividing cells surrounded by a mucous envelope. (s) The arrows and the insets show copepod eggs infected numerous cells. The arrowheads point uninfected copepod eggs. ac: anterior cingulum; as: anterior sulcus; ci: cingulum; su: sulcus. Scale bars: 50 μm .

Fig. 2. (a–q) Life stages of *Syltodium listii* from the English Channel in July 2018 (a–m), and the NW Mediterranean at Marseilles, in June 2008 (n–q). (a–h) Infection of individual eggs from different species of free-spawning copepod species. (i, j) Infection of egg sacs of brood-carrying copepod species. (k) Two cells infecting a nauplius inside the eggshell. (l, m) Cells infecting nauplii. (n–q) Life stages of Mediterranean cells. (n) The inset shows the feeding peduncle. Scale bars: 50 μ m.

Fig. 3. Phylogenetic tree based on SSU rRNA gene sequences, showing the position of the sequences of *Syltodium listii* and *Dissodinium pseudolunula* by Maximum Likelihood (ML). Numbers near branches denote ML bootstrap probability value. Bootstrap values <70 are omitted. The inset shows the environmental sequences closer to *Syltodium*. The geographic origin is placed between parentheses. Scale bar denotes 0.01 substitutions per site.

Fig. 4. Phylogenetic tree based on LSU rRNA gene sequences, showing the position of the sequences of *Syltodium listii* and *Dissodinium pseudolunula* by Maximum Likelihood (ML). Numbers near branches denote ML bootstrap probability value. Bootstrap values <70 are omitted. Scale bar denotes 0.02 substitutions per site.



Gymnodinium clade

Chytridiaceae





Table 1. List of primers used for initial amplification, nested PCR, and sequencing of the isolates of *Syltodium listii* (MK629450–1) and *Dissodinium pseudolunula* (MK626523) from the English Channel.

Primer name	Sequence (5'–3')	Use	Reference
EukA1	AAY CTG GTT GAT YCT GCC AG	nested PCR, sequencing	Modified from Medlin et al. (1988)
EukB2	GAT CCT KCT GCA GG TTC ACC TA	Initial amplification, nested PCR, sequencing	Modified from Medlin et al. (1988)
373F	GAT TCC GGA GAG GGA GCC T	Nested PCR, sequencing	Weekers et al. (1994)
1200F	CAG GTC TGT GAT GCC C	Initial amplification	Weekers et al. (1994)
1200R	GGG CAT CAC AGA CCT G	Nested PCR	Weekers et al. (1994)
CTB6	GCA TAT CAA TAA GCG GAG G	Nested PCR	https://nature.berkeley.edu/brunslab/our/primers.html
ITS1	TCC GTA GGT GAA CCT GCG G	Nested PCR	https://nature.berkeley.edu/brunslab/our/primers.html
TW13	GGT CCG TGT TTC AAG ACG	Nested PCR	https://nature.berkeley.edu/brunslab/our/primers.html
TW14	GCT ATC CTG AGG GAA ACT TC	Nested PCR	https://nature.berkeley.edu/brunslab/our/primers.html
TW15	CTT GGA GAC CTG CTG CGG	Initial amplification	https://nature.berkeley.edu/brunslab/our/primers.html

