

SHORT COMMUNICATION

# Molecular Phylogeny of the Parasitic Dinoflagellate *Chytriodinium* within the *Gymnodinium* Clade (Gymnodiniales, Dinophyceae)

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## Keywords

Copepod; *Dissodinium*; *Gymnodinium* sensu stricto; parasite Dinophyta; parasitism.

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## ABSTRACT

The dinoflagellate genus *Chytriodinium*, an ectoparasite of copepod eggs, is reported for the first time in the North and South Atlantic Oceans. We provide the first large subunit rDNA (LSU rDNA) and Internal Transcribed Spacer 1 (ITS1) sequences, which were identical in both hemispheres for the Atlantic *Chytriodinium* sp. The first complete small subunit ribosomal DNA (SSU rDNA) of the Atlantic *Chytriodinium* sp. suggests that the specimens belong to an undescribed species. This is the first evidence of the split of the *Gymnodinium* clade: one for the parasitic forms of Chytriodiniaceae (*Chytriodinium*, *Dissodinium*), and other clade for the free-living species.

COPEPODS dominate the zooplankton biomass and are considered to be the most abundant animals in the ocean. The lipid-rich copepod eggs are the target of the specialized parasitic dinoflagellates *Chytriodinium* and *Dissodinium* which dinospores are able to infest crustacean eggs, absorb the host content and form successive cysts that produce colorless gymnodinoid spores (Cachon and Cachon 1968; see Video S1 <http://youtu.be/nwFZQAAm-QaA>).

Our knowledge of the members of the family Chytriodiniaceae is limited. Some stages of the life cycle of *Dissodinium psedolunula*, such as the lunate sporangia, are highly distinctive and commonly recognized by plankton researchers (Gómez and Artigas 2013). In contrast, *Chytriodinium* is absent in identification guides, and it easily goes unnoticed especially in fixed samples. The genus comprises three species. In *Chytriodinium affine*, the numerous dinospores develop in a coiled chain inside a hyaline spherical membrane that was absent in the chain of *C. roseum*. *Chytriodinium parasiticum* parasitizes larger crustacean eggs, and forms a sophisticated stalk apparatus (Cachon and Cachon 1968). These species are only known from the

western Mediterranean Sea, with some recent records of *C. affine* from the Pacific Ocean (Gómez-Gutiérrez et al. 2009; Meave del Castillo et al. 2012). It is clear that abundance and ecological role is being underestimated.

Gómez et al. (2009) provided the first molecular data based on the partial SSU rDNA sequence of the species *Chytriodinium affine* and *C. roseum* from the Mediterranean Sea. *Chytriodinium* branched within the so-called *Gymnodinium* sensu stricto or *Gymnodinium* clade (Daugbjerg et al. 2000). This clade showed a strong diversification in the trophic modes with plastids of different microalgal origins and development of specialized organelles (nematocyst, ocelloid, or piston) that are unknown in other dinoflagellate clades.

The current molecular information on *Chytriodinium* is restricted to partial SSU rDNA sequences of Mediterranean specimens. The complete SSU rDNA sequence and additional molecular markers (LSU rDNA, ITS1) will allow to test whether the heterotrophic *Chytriodinium* and photosynthetic *Dissodinium* were or maybe are derived from a recent common ancestor or the parasitism appeared independently in the *Gymnodinium* clade.

## MATERIALS AND METHODS

Specimens of *Chytriodinium* were isolated from water samples collected at two coastal sites, in the North Atlantic, in the coasts of the Caribbean Sea at Puerto Rico (Bahía Fosforescente, 17°58'19.80"N, 67°0'50.73"W), and in the South Atlantic Ocean, the coasts of São Paulo State, Brazil (São Sebastião Channel, 23°50'4.05"S, 45°24'28.82"W). The Caribbean specimens of *Chytriodinium* were collected from the surface using a phytoplankton net (20 µm mesh size) during the night of March 8, 2012, and they were isolated onboard with a 3030 Accu-scope inverted microscope. The Brazilian specimens of *Chytriodinium* were collected in the São Sebastião Channel on early morning of April 30, 2013, and they were isolated in a coastal laboratory with a Nikon TS-100 inverted microscope. After being photographed, each sporangium of *Chytriodinium* containing tens of immature dinospores was separated from the egg sac. The sporangium was micropipetted individually with a fine capillary into a clean chamber and washed several times in a series of drops of 0.2 µm-filtered and sterilized seawater. Finally, the sporangium of *Chytriodinium* was placed in a 0.2-ml Eppendorf tube filled with several drops of absolute ethanol. The methods of PCR amplification and sequencing, and phylogenetic analysis are detailed in an appendix as supporting information.

## RESULTS AND DISCUSSION

In the North Atlantic Ocean, detached copepod egg sacs infected with *Chytriodinium* were observed in one survey carried out during the night on March 8, 2012 in Bahía Fosforescente, Puerto Rico. The egg sac typically contained six copepod eggs of about 50–60 µm in diameter. The infected eggs showed one or several sporangia of *Chytriodinium* that reached a diameter slightly larger (~60–70 µm) than the infected egg. A chain of dinospores was coiled within a fine hyaline membrane of the sporangium. The sporangium remained attached to the copepod egg and more than 60 dinospores were released when the membrane was lysed (see Video S2 as supporting information, [http://youtu.be/JA\\_Gu57WkXQ](http://youtu.be/JA_Gu57WkXQ)). The epicone and hypocone of the recently released dinospores were hemispherical with a deep constriction at the cingulum level (Fig. S1).

In the South Atlantic Ocean, the plankton observations were carried out during 6 mo in São Sebastião Channel. *Chytriodinium* infecting copepod eggs was observed only on April 30, May 20, June 7 and July 5, 2013. Nearly all the observations of *Chytriodinium* corresponded to detached sacs of six or more eggs. In a few cases, the infected eggs were observed in sacs that still remained attached to the copepod such as *Oithona* cf. *robusta*. The dinospores of about 8–9 µm long were attached to the egg, and multi-infections were frequent with different degrees of sporangia development. The copepod eggs were 40–50 µm in diam, and the sporangium reached a diameter of 50–65 µm. The dinospore was attached to the

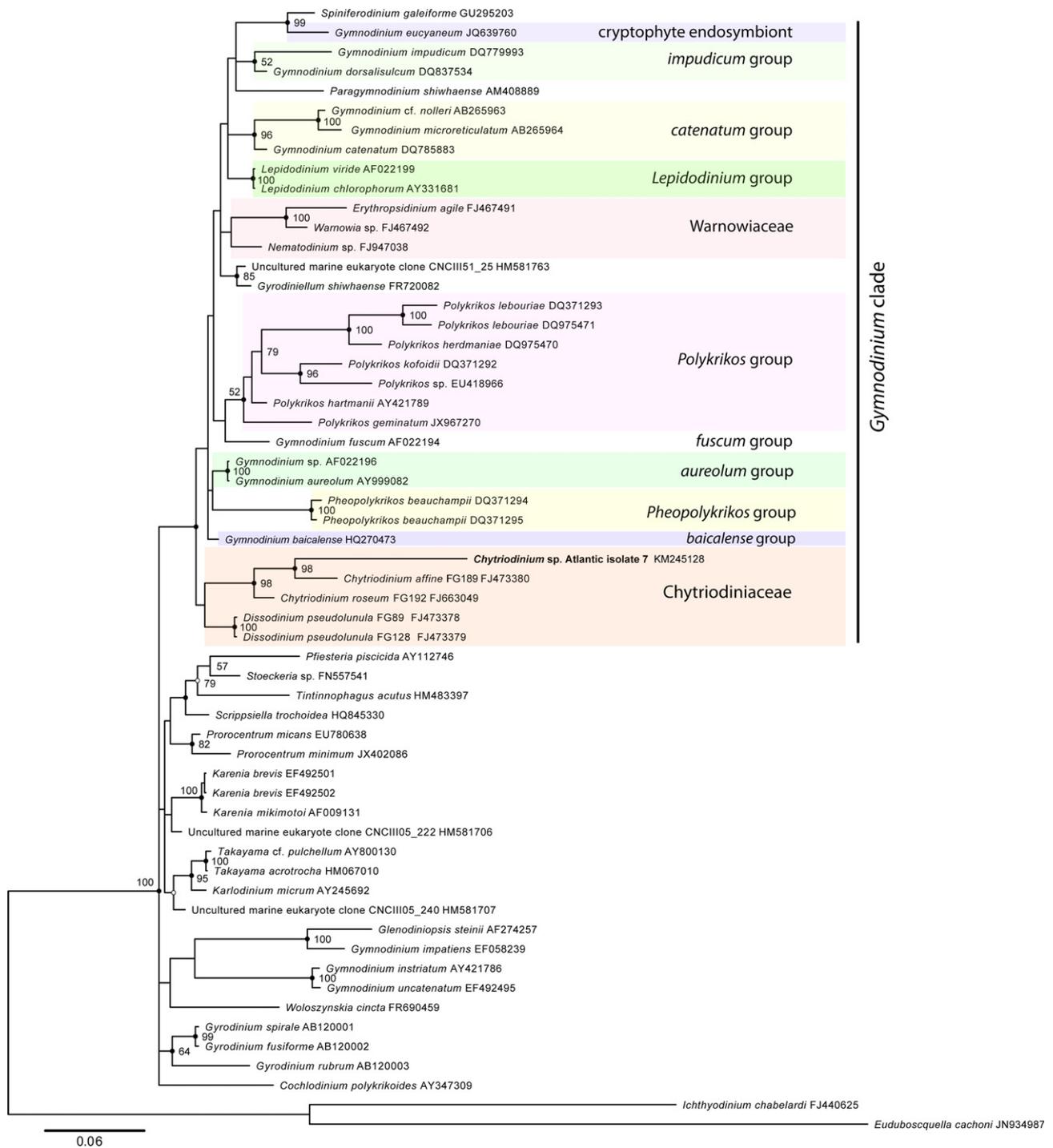
host by means of a feeding tube, enlarged at its base. An orange ampulla with one large trophic vacuole formed at the end of the peduncular disk that gradually absorbed the egg cytoplasm. The recently released dinospores showed a deep constriction at the cingulum level. The sporangia used for PCR analysis were isolated on April 30, and the sporangia with successful PCR products are illustrated in the Fig. S2.

We obtained the complete SSU rDNA sequence (1,797 base pairs) of the isolate #7 of *Chytriodinium* sp. from Brazil (Fig. S2). A BLAST search revealed that the closest species based on the entire SSU rDNA sequence was *Gymnodinium aureolum* with an identity of 93%. Only a partial sequence *Chytriodinium affine* from the Mediterranean Sea is available (1,206 base pairs, #FJ473380). If the first 550 base pairs of our new sequence are removed, the closest BLAST match was the Mediterranean *C. affine*. The percentage of identity between the sequences of the Mediterranean and Atlantic specimens was 96%.

In the Bayesian consensus tree, the *Chytriodinium* sequences branched within a sister group to the *Gymnodinium* clade with maximum support (albeit unsupported in the ML analysis). The *Gymnodinium* clade subdivided into four weakly supported groups. The first group included the sequences of *G. impudicum*, species with cryptophyte endosymbionts, *Lepidodinium*, *Paragymnodinium*, *G. catenatum*, warnowiids and *Gyrodiniellum*; the second group included *Polykrikos*, with *Gymnodinium fuscum* in a basal position; the third group comprised *Gymnodinium aureolum* and *Pheopolykrikos*, and the fourth group for *Gymnodinium baicalense*. As a sister group of the major *Gymnodinium* clade, the sequence of the Atlantic *Chytriodinium* sp. branched in a distal position of a group with *Chytriodinium affine* and *C. roseum*, and *Dissodinium pseudolunula* (Fig. 1).

The first LSU rDNA sequences of the genus *Chytriodinium* were obtained from one sporangium (isolate #2; Fig. S1) from Puerto Rico and the other one from Brazil (isolate #7, Fig. S2). The LSU rDNA and ITS1 sequences of the Caribbean and Brazilian isolates were identical (100%). The closest BLAST match of the LSU rDNA sequence was *Gymnodinium aureolum* (85%). *Dissodinium pseudolunula* showed an identity of 88%, although with a lower coverage. In the Bayesian consensus tree, the new LSU rDNA sequences of *Chytriodinium* branched in the *Gymnodinium* clade (Fig. S3). This clade subdivided into 13 subclades, with the Atlantic *Chytriodinium* branching in a separate lineage than *Dissodinium pseudolunula*. The latter branched with negligible support with the benthic pseudocolonial species *Polykrikos lebouriae* (Fig. S3).

This study provides the first observations of *Chytriodinium* in the Atlantic Ocean. The limited records of *Chytriodinium* do not seem to reflect the actual abundance of this parasite in the world oceans. The partial SSU rDNA sequence of the Mediterranean *Chytriodinium affine* differs from that of the Atlantic *Chytriodinium* (identity of 96%). This suggests that the Atlantic specimens belong to an undescribed species. The genus *Chytriodinium* currently comprises three species described in 1906. The



**Figure 1** Phylogeny tree inferred from the SSU rDNA sequences using Bayesian inference method. The species newly sequenced in this study are bold. Accession numbers are provided. Posterior probability of 1 is denoted with a black circle; white circles denote posterior probability of 0.95–0.99. ML bootstrap support (when above 50%) is given near nodes. The scale bar represents the number of substitutions per site. Numbers at the end of each taxon name are GenBank accession numbers.

morphology of the dinospores of the Mediterranean *Chytridinium affine* has not been examined in detail in order to establish differences with other tentative species. No obvious differences exist between the Mediterranean *Chytri-*

*odinium affine* and *Chytridinium* sp. from the tropical Atlantic Ocean. The size, shape and general appearance of the infective spores of the Mediterranean and Atlantic species is similar. The life cycle and appearance of the

sporangium is also similar. All the reported observations of *Chytriodinium affine* in the Mediterranean Sea concern infections of individual eggs of free-spawning copepod species (Cachon and Cachon 1968; Gómez et al. 2009). In contrast, the Atlantic specimens of *Chytriodinium* of both hemispheres were exclusively observed infecting egg sacs of brood-carrying copepod species (Fig. S1–S2). To the best of our knowledge this feature is reported for the first time in the genus *Chytriodinium*. At the present, difference in host species is the main character distinguishing the Atlantic species and *Chytriodinium affine*. However, it is necessary to perform infection experiments to verify whether this apparent host specificity is exclusively due to the type of egg available.

Similar to the presence or absence of plastids, parasitism is known for numerous groups of dinoflagellates (Coats et al. 2010). *Gymnodinium aureolum* ingests only small microalgal preys through a peduncle and divides by binary division (Jeong et al. 2010). This mechanism of feeding may be essentially the same as in members of Chytriodiniaceae that possess a more sophisticated peduncle (i.e., *C. parasiticum*, see Video S1 as supporting information, <http://youtu.be/nwFZQAAMQaA>). *Dissodinium*, *Chytriodinium* and *Myxodinium* are able to feed on larger preys, and this allows producing a high number of dinospores after each infection. This study provides the first evidence of the split of the *Gymnodinium* clade: one for the parasitic forms of Chytriodiniaceae (*Chytriodinium*, *Dissodinium*), and other clade for the free-living species.

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## LITERATURE CITED

Cachon, J. & Cachon, M. 1968. Cytologie et cycle évolutif des *Chytriodinium* (Chatton). *Protistologica*, 4:249–262.

- Coats, D. W., Kim, S., Bachvaroff, T. R., Handy, S. M. & Delwiche, C. F. 2010. *Tintinnophagus acutus* n. g., n. sp. (Phylum Dinoflagellata), an ectoparasite of the ciliate *Tintinnopsis cylindrica* Daday 1887, and its relationship to *Duboscquodinium collini* Grassé 1952. *J. Eukaryot. Microbiol.*, 57:468–482.
- Daugbjerg, N., Hansen, G., Larsen, J. & Moestrup, Ø. 2000. Phylogeny of some of the major genera of dinoflagellates based on ultrastructure and partial LSU rDNA sequence data, including the erection of three new genera of unarmoured dinoflagellates. *Phycologia*, 39:302–317.
- Gómez, F. & Artigas, L. F. 2013. The formation of the twin resting cysts in the dinoflagellate *Dissodinium pseudolunula*, a parasite of copepod eggs. *J. Plankton Res.*, 35:1167–1171.
- Gómez, F., Moreira, D. & López-García, P. 2009. Life cycle and molecular phylogeny of the dinoflagellates *Chytriodinium* and *Dissodinium*, ectoparasites of copepod eggs. *Eur. J. Protistol.*, 45:260–270.
- Gómez-Gutiérrez, J., Kawaguchi, S. & Nicol, S. 2009. Epibiotic suctorians and enigmatic ecto- and endoparasitoid dinoflagellates of euphausiid eggs (Euphausiacea) off Oregon, USA. *J. Plankton Res.*, 31:777–785.
- Jeong, H. J., Yoo, Y. D., Kang, N. S., Rho, J. R., Seong, K. A., Park, J. W., Nam, G. S. & Yih, W. 2010. Ecology of *Gymnodinium aureolum*. I. Feeding in western Korean waters. *Aquat. Microb. Ecol.*, 59:239–255.
- Meave del Castillo, M. E., Zamudio-Reséndiz, M. E. & Castillo-Rivera, M. 2012. Riqueza fitoplanctónica de la Bahía de Acapulco y zona costera aledaña, Guerrero, México. *Acta Bot. Mex.*, 100:405–487.

## SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

**Figure S1.** Light micrographs of *Chytriodinium* sp. from Bahía Fosforescente, Puerto Rico.

**Figure S2.** Light micrographs of *Chytriodinium* sp. from São Sebastião Channel, Brazil.

**Figure S3.** Phylogeny tree inferred from LSU rDNA sequences using Bayesian inference method.

**Data S1.** Materials and methods.

**Video S1.** The parasitic dinoflagellate *Chytriodinium* from Villefranche sur Mer, France, by J. Cachon and M. Cachon, <http://youtu.be/nwFZQAAMQaA>.

**Video S2.** The parasitic dinoflagellate *Chytriodinium* from Bahía Fosforescente, Puerto Rico, [http://youtu.be/JA\\_Gu57WkXQ](http://youtu.be/JA_Gu57WkXQ).