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# Diversity and Phylogeny of Gymnodiniales (Dinophyceae) from the NW Mediterranean Sea Revealed by a Morphological and Molecular Approach

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**The diversity and phylogeny of dinoflagellates belonging to the Gymnodiniales were studied during a 3-year period at several coastal stations along the Catalan coast (NW Mediterranean) by combining analyses of their morphological features with rDNA sequencing. This approach resulted in the detection of 59 different morphospecies, 13 of which were observed for the first time in the Mediterranean Sea. Fifteen of the detected species were HAB producers; four represented novel detections on the Catalan coast and two in the Mediterranean Sea. Partial rDNA sequences were obtained for 50 different morphospecies, including novel LSU rDNA sequences for 27 species, highlighting the current scarcity of molecular information for this group of dinoflagellates. The combination of morphology and genetics allowed the first determinations of the phylogenetic position of several genera, i.e., *Torodinium* and many *Gyrodinium* and Warnowiacean species. The results also suggested that among the specimens belonging to the genera *Gymnodinium*, *Apicoporus*, and *Cochlodinium* were those representing as yet undescribed species. Furthermore, the phylogenetic data suggested taxonomic incongruences for some species, i.e., *Gyrodinium undulans* and *Gymnodinium agaricoides*. Although a species complex related to *G. spirale* was detected, the partial LSU rDNA sequences lacked sufficient resolution to discriminate between various other *Gyrodinium* morphospecies.**

**Key words:** Dinoflagellates; HAB; naked; phylogeny; rDNA; single-cell PCR.

## Introduction

The diversity of living marine dinoflagellates is estimated at 2,500 species (Gómez 2005; Sournia 1995). Traditionally, dinoflagellate taxonomy has been based on the morphological features of the different groups, with the major orders established accordingly (Fensome et al. 1993; Taylor 1987). However, the interrelationships among the different lineages remain unresolved and many orders have proved to be polyphyletic (Moestrup and Daugbjerg 2007; Murray et al. 2005; Saldarriaga et al. 2004). The coupling of morphological features with molecular phylogenetic data has enabled analyses of the relationships between many genera and species (Handy et al. 2009; Murray et al. 2009; Taylor 2004). Unfortunately, most of the organisms that

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have been sequenced are cultivable photosynthetic species whereas there is little information for a large number of genera, such as those that are mixo- and heterotrophic. One method to avoid cell culture bias is single-cell PCR (Ruiz-Sebastián and O'Ryan 2001), which has been successfully and recurrently applied to dinoflagellates (Lynn and Pinheiro 2009).

Most dinoflagellates that lack a theca are included in the order Gymnodiniales Apstein. These "unarmoured" or "naked" dinoflagellates, which comprise about 600 species (about 25% of the described dinoflagellate species), belong mainly to the genera *Amphidinium*, *Cochlodinium*, *Gyrodinium*, and the highly diverse *Gymnodinium*, for which about 250 species have been described thus far (Gómez 2005, 2012; Guiry and Guiry 2013). However, the identification of unarmoured species is challenging because several of their key characters are difficult to observe. In addition, they show a high phenotypic plasticity, their life cycle consists of morphologically different stages, and the lack of a theca often results in their deformation when fixed for microscopy studies. Therefore, routine samplings using fixatives are not suitable for species identifications, which instead must rely on live specimens. Moreover, the original descriptions of some species are incomplete and doubtful, with a large number of species never observed again since their original description. This includes 40% of the species belonging to the genus *Gymnodinium* (Thessen et al. 2012). Some of these unarmoured taxa have been studied in detail, especially harmful algal bloom producers, whereas studies on the diversity and distribution of many other unarmoured species and genera are scarce and incomplete, in addition to being hindered by a lack of molecular data (Gómez 2014).

Molecular sequences of unarmoured dinoflagellates provide highly valuable information, as they usually allow both the discrimination of similar morphospecies and the characterization of specimens that cannot be easily assigned to a genus based on morphology alone, together with a determination of their phylogenetic position. Because many species of unarmoured dinoflagellates are heterotrophic or mixotrophic, efforts to obtain viable and dense cultures are time consuming but often unsuccessful. The available phylogenetic information is therefore scarce and generally restricted to photosynthetic cultured species. Early phylogenetic studies noted the polyphyletic nature of unarmoured dinoflagellates (Fensome et al. 1999; Lenaers et al. 1991). Subsequently, the taxonomy of this group underwent major revisions, beginning with the work of Daugbjerg et al. (2000), who redefined the genera *Gymnodinium* and *Gyrodinium* and erected the genera *Akashiwo*, *Karenia*, and *Karlodinium* by combining the morphological features, mainly the shape of the apical groove, with the ultrastructural features of these organisms with their phylogeny. The phylogenetic clade containing *Gymnodinium fuscum* (Ehrenberg) Stein, 1878, the type species of the genus, was called *Gymnodinium* sensu stricto (s.s.). Further phylogenetic studies led to the inclusion of other unarmoured genera within this clade: *Lepidodinium* Watanabe, Suda, Inouye, Sawaguchi et Chihara (Saunders et al. 1997); *Barrufeta* Sampedro et Fraga (Sampedro et al. 2011); *Paragymnodinium* Kang, Jeong, Moestrup et Shin (Kang et al. 2010); and *Gyrodiniellum* Kang, Jeong et Moestrup (Kang et al. 2011). Furthermore, non-*Gymnodinium*-like families, such as Polykrikaceae (Hoppenrath and Leander 2007a), Chytriodiniaceae (Gómez et al. 2009b; Kim et al. 2008), and Warnowiaceae (Gómez et al. 2009a; Hoppenrath et al. 2009a), also cluster within this clade. Accordingly, as this cluster contains *G. fuscum*, the first unarmoured species described, but also many other genera and families, it is considered as the Order Gymnodiniales sensu stricto clade (Gómez et al. 2009a; Hoppenrath and Leander 2007a, 2010; Yamaguchi et al. 2011). In parallel, the phylogenetic relationships of many other unarmoured genera and species have been studied, resulting in the erection of more than ten new genera (e.g. de Salas et al. 2003, Flø Jørgensen et al. 2004b, Sparmann et al. 2008). Using single-cell PCR, a powerful tool to study organisms that are difficult to culture because of their heterotrophic requirements or because they are usually found in their natural environment at low abundances, several authors have obtained phylogenetic data on Gymnodiniales, increasing the available molecular data for heterotrophic and not easily cultivable species (Hansen and Daugbjerg 2004; Hoppenrath et al. 2009a; Reñé et al. 2014; Takano and Horiguchi 2004). Based on this new phylogenetic information, the order Gymnodiniales has been recognized as either polyphyletic, and thus artificial with respect to single rRNA genes (Daugbjerg et al. 2000; Gómez et al. 2011; Saldarriaga et al. 2001), or paraphyletic when multiple genes have been used to reconstruct phylogenies (Orr et al. 2012). In light of the polyphyly or paraphyly of the order, genera and clades that do not cluster within the Gymnodiniales s.s. clade are still included in the order Gymnodiniales, but since they are not phylogenetically related they are considered as Gymnodiniales sensu lato members.