NEW RECORDS OF THE DISTINCTIVE BENTHIC DINOFLAGELLATE GENUS Cabra (DINOPHYCEAE)

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ABSTRACT. The benthic dinoflagellate genus Cabra is reported for the first time in the Mediterranean Sea and the South Atlantic Ocean, with additional records in the Caribbean Sea and the eastern Asian coasts. Cabra aremorica is reported for the first time after the original description. However, these records should be considered cautiously because the distinction between Cabra aremorica and C. reticulata is difficult based on routine light microscopy observations. It is uncertain whether there is a high intraspecific morphological variability or several co-occurring undescribed species. Cabra levis, a species recently described, is reported for first time beyond the type locality.

Keywords: benthic Dinophyta, epiphytic Dinoflagellata, microphytobenthos, psammophilic Dinophyceae, sand-dwelling dinoflagellate.

INTRODUCTION

Cabra matta Sh. Murray et D.J. Patterson is the type species of a distinctive genus of marine benthic dinoflagellates described in sand habitats of eastern Australia (Murray & Patterson, 2004). Cabra reticulata Chomérat et Nézan and C. aremorica Chomérat, Couté et Nézan were further described from sandy sediments of the coasts of north-western France (Chomérat & Nezán, 2009; Chomérat et al., 2010). The cells were highly laterally compressed with a more or less pentagonal outline, asymmetrical with different right and left sides. The hypotheca showed one dorsal and three antapical pointed flanges. Beyond the original descriptions in the Australian and French Atlantic coasts, further records were restricted to the Gulf of Mexico (Okolodkov et al., 2007, 2014), Russian coasts of the Japan Sea (Selina & Levchenko, 2011) and Korea (Shah et al., 2013). Recently, a new species, Cabra levis Selina, Chomérat et Hoppenrath has been described from Russian coasts (Selina et al., 2015). It is characterized by a rounded shape and lacking pointed flanges.

This study reports the first records of Cabra in the Mediterranean Sea and the South Atlantic Ocean, and additional records in the Caribbean Sea and the eastern Asian coasts. These observations constitute the first record of C. aremorica beyond the type locality. Specimens with different morphologies co-occurred in the samples from Puerto Rico and Brazil. This raises a question on the delimitation of the known species: is there a high intraspecific morphological variability and/or several undescribed species?: whether there is a high intraspecific morphological variability and/or several undescribed species. The recently described species C. levis is reported for the first time beyond the type locality. These new records are discussed within the context of an increase in the reports of invasive and non-indigenous species.

MATERIALS AND METHODS

The study in the Mediterranean Sea was focused on the planktonic dinoflagellates. However, during surveys near pier walls at Marseille and Valencia numerous epiphytic dinoflagellates were accidentally collected (i.e., Coolia Meunier, Ostreopsis Johannes Schmidt). One specimen of the genus Cabra was collected with a bucket from the pier of the Station Marine d’Endoume at Marseille, France (43° 16’ 48.05” N, 5° 20’ 56.22” E, bottom depth ~3 m) on August 11th, 2008. A strainer of 20 µm mesh size was used to concentrate the sample. The ‘plankton’ concentrate was examined in a composite settling chamber at ×100 with an inverted microscope (Nikon Eclipse TE200, Nikon Inc., Tokyo, Japan).
The cell was photographed at ×200 or ×400 with a digital camera (Nikon Coolpix E995). Another specimen of *Cabra* was collected using a phytoplankton net (20 µm mesh size) at the port of Valencia, Spain (39° 27' 38.13” N, 0° 19’ 21.29’’ W, bottom depth ~4 m) on November 18, 2011. The sample was examined in a composite settling chamber with an inverted microscope (Nikon Eclipse T2000) and photographed with a digital camera (Olympus DP71, Olympus, Tokyo, Japan).

In the coasts of the Caribbean Sea, the epiphytic dinoflagellates attached to the marine plant *Thalassia testudinum* Banks et Sol. ex K.D. Koenig, were examined. Seagrass leaves were collected by snorkeling (2–3 m depth) around the pier of Magüeyes Island (17° 58’ 11.80” N, 67° 2’ 46.56” W) at La Parguera, Puerto Rico, during ten surveys in February and March 2012. The leaves were placed in PVC bottles containing 200 ml of seawater and stirred vigorously to detach the epiphytic cells. The suspended material was sieved through a 100 µm mesh to remove large particles. The samples were examined in a composite settling chamber with an inverted microscope (3030 Accu-scope, Commack, New York) and photographed with a digital camera (Olympus DP71, Olympus, Tokyo, Japan).

In the South Atlantic Ocean, epiphytic dinoflagellates on macroalgae were investigated from the coasts of São Paulo State, Brazil. At São Sebastião (23° 49’ 34.54” S, 45° 25’ 18.26” W), macroalgae were collected from tidal pools (<1 m depth) during the low tide according to the procedure described above, in sporadic surveys between March and December 2013. Samples were examined in a composite settling chamber at ×200 with an inverted microscope (Nikon Diaphot-300) and photographed with a digital camera mounted on the microscope’s eyepiece (Cyber-shot DSC-W300, Sony, Tokyo, Japan). At Ubatuba (23° 30’ 3.16” S, 45° 7’ 6.78” W), macroalgae were collected from rocky surfaces during the low tide (<1 m depth) according to the procedure described above, in sporadic surveys between January 2014 and December 2014. Samples were examined in a composite settling chamber with an inverted microscope (Nikon Eclipse TS-100F) and photographed with a digital camera mounted on the microscope’s eyepiece (Cyber-shot DSC-W300). In the north-western Pacific Ocean, one macroalgal sample was collected at Dongshan Island, China (23° 35’ 25.64” N, 117° 26’ 4.24’’ W) on August 15th 2014. The sample was examined in a composite settling chamber with an inverted microscope (Nikon TS-100F).

**RESULTS**

The distinction between the type species, *C. matta*, and the other two described species with pointed flanges (*C. aremorica* and *C. reticulata*) was relatively easy because the dorsal margin of the hypotheca of *C. matta* is more or less rounded, while it is polygonal in the other described species. However, it is more difficult to distinguish between *C. reticulata* and *C. aremorica* because the diagnostic characters used for species delimitation (i.e., thecal ornamentation) were not easy to discern based on routine light microscopy observations. The two specimens observed from the Mediterranean Sea corresponded to *Cabra aremorica* or *C. reticulata* (Fig. 1A–B, E–F). The original illustrations of *Cabra aremorica* (Fig. 1C–D) and *C. reticulata* (Fig. 1H–J) were reproduced here in order to facilitate the comparisons. The specimen from Marseille was 42 µm long and 37 µm deep (as dorso-ventral diameter) (Fig. 1E). The specimen from Valencia was 40 µm long and 31 µm deep (Fig. 1F). Consequently, the hypotheca of the latter specimen was relatively more elongated, in agreement with the original description of *C. reticulata* (Fig. 1H). The relative distance from the dorsal margin of cingulum to the pointed flange of the plate 3”’ was higher in the specimen from Marseille. It is assumed that a slight difference in size when compared to the original description is a poor diagnostic character. Based on the cell shape, the specimens from Marseille and Valencia have been designated as *C. aremorica* and *C. reticulata*, respectively. However, we have to be cautious because the delimitation between *C. aremorica* and *C. reticulata* is difficult based on light microscopy. To the best of our knowledge, these are the first records of the genus *Cabra* in the Mediterranean Sea.

While the Mediterranean observations were restricted to two specimens accidentally collected in plankton samples, the surveys in Puerto Rico and Brazil were intended for the study of the epiphytic dinoflagellates. In Puerto Rico, each seagrass sample showed specimens of *Cabra* with different morphologies. A single specimen showed different shapes according to the viewing angle (Fig. 1J–K). The records could be divided into two groups: specimens with a polygonal dorsal margin (Fig. 1J–L) as in *C. aremorica* and *C. reticulata*, and a second group for specimens with a rounded dorsal margin of the hypotheca. The latter group included specimens with pointed flanges, as in *C. matta* (Fig. 1M–O), and rounder specimens lacking the pointed flanges (Fig. 1P–R). The morphology of some specimens did not match with the three described species. Figures J–K show two views of a single specimen. In any view, the relative distance between the dorsal end of the cingulum and the pointed flange of the plate 3”’ was higher in the Puerto Rican specimens (Fig. 1J–K) than in the Mediterranean specimens (Fig. 1E–F) and in the original descriptions (Fig. 1C–D, H–I). The specimens with polygonal hypotheca were 36–41 µm long and 28–35 µm deep. With doubts whether these specimens correspond to *C. aremorica*, *C. reticulata* or undescribed species, we designated the specimens as *C. aremorica* (Fig. 1J–K). Co-occurring with these specimens, the samples contained specimens with a rounded dorsal...
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Margin of the hypotheca that has been identified as *C. matta* (Fig. 1M-O). Other specimens with a round cell shape showed a relative larger epitheca and semi-circular hypotheca. The antapical flanges were lacking or scarcely visible (Fig. 1P-R). The cells were 33–35 µm long and 30–32 µm deep (Fig. 1P-R). These specimens were identified as *Cabra levis*.

**Figure 1.** Light micrographs of *Cabra* spp.: A–B, E–G. Specimens from the western Mediterranean Sea; J–R. Specimens from the Caribbean Sea; S–AD. Specimens from the South Atlantic Ocean; A–B. Right lateral view of *C. aremorica* from Marseille, France; C–D. Line drawings of *C. aremorica* redrawn from Chómerat et al. (2010); E. Left lateral view of *C. aremorica* from Marseille, France; F–G. Left lateral view of *C. reticulata* from Valencia, Spain; H–I. Line drawings of *C. reticulata* redrawn from Chómerat et al. (2010); J–L. *C. aremorica* from La Parguera, Puerto Rico; M–O. *C. matta* from La Parguera, Puerto Rico; P–R. *Cabra levis* from La Parguera; S–U. Left lateral view of *C. aremorica* from São Sebastião, Brazil; V–Y. *C. matta* from São Sebastião; Z–AB. *Cabra levis* from São Sebastião; AC–AD. Left lateral view of *C. reticulata* from Ubatuba, Brazil. Note the yellow-brownish food bodies in the middle of the cells. Scale bars, 20 µm.
**Cabra** morphological diversity was similar on macroalgal epiphytes from Brazil. A group of specimens with a polygonal dorsal margin of the hypotheca has been tentatively designated as *C. aremorica* (Fig. 1S–U) and *C. reticulata* (Fig. 1AC–AD). Other group of specimens showed a rounder hypotheca (Fig. 1V–AB). This group included specimens with a rounded dorsal margin, and the dorsal margin with an irregular outline, more or less dentate (Fig. 1V–W), and other specimens with an elipsoidal hypotheca and smooth dorsal outline (Fig. 1X–Y). The cells were 32-36 µm long, and about 27 µm deep. These specimens were designated as *C. matta* (Fig. 1V–Y). Other specimens showed a rounded cell shape with a diameter of about 31 µm, slightly longer than deep. In some angles of view, at least two short antapical flanges were visible (Fig. 1Z–AA); while in other specimens the flanges were not observed (Fig. 1AB). These specimens were identified as *Cabra levis*. These are the first records of the genus *Cabra* in the South Atlantic Ocean.

In the Chinese coasts at Dongshan Island, we observed three specimens from a single sample that corresponded to the morphology of *C. matta*.

**DISCUSSION**

**Historical records of Cabra**

The morphology of the known species of *Cabra* is very distinctive, and here we show that they are a relatively common epiphyte on macrophytes in different biogeographic regions. However, the records are scarce and surprisingly the genus had not been described until the last decade (Murray & Patterson, 2004) (Fig. 2). Benthic dinoflagellates have been undersampled for decades until Japanese researchers discovered that some epiphytic dinoflagellates (i.e., *Gambierdiscus* Adachi et Fukuyo) were responsible for the ciguatera disease (Yasumoto et al., 1977). Carlson (1984) investigated the epiphytic dinoflagellate assemblage in a ciguatera endemic area in the U.S. Virgin Islands, near Puerto Rico. He illustrated a specimen as a new genus, *Thecadinium* sp. (Carlson, 1984). However, he did not provide a formal description and Chomérat & Nézan (2009) considered that Carlson’s *Thecadinium* sp. corresponded to *C. reticulata*. Ballantine et al. (1988) investigated the epiphytic dinoflagellates at La Parguera, Puerto Rico. They did not report *Cabra* probably because their study was exclusively focused on the potentially toxigenic species (*Gambierdiscus*, *Ostreopsis*, *Coolia*, *Procoreanum* Ehrenb.). The present study, based on only ten surveys from the same location, revealed that *Cabra* spp. are common epiphytes on the seagrass *Thalassia testudinum*. Okolodkov et al. (2007, 2014) reported *T. testudinum* as substrate for epiphytic dinoflagellates. It should be noted that the leaves of *Thalassia* are also substrate for algal turfs. Other studies in the Caribbean Sea that focused on epiphytic dinoflagellates on *T. testudinum* have not recorded species of *Cabra* (i.e., Rodriguez et al., 2010). Jacobson (1999, p. 378) illustrated a specimen of *Cabra*, but with no information on the figure legend or the text. According to this author, the specimens were collected in Vineyard Sound, near Woods Hole, USA. He did not describe them due to the paucity of specimens and the difficulties to interpret the thecal plate pattern (D.M. Jacobson, personal communication). The shape of his specimens resembles *C. aremorica*, with a dentate dorsal margin of the hypotheca with 3-4 projections or spines (Jacobson, 1999, p. 378). These spines are lacking in the original description of the known species of *Cabra*. In the present study, an irregular outline of the dorsal margin of the hypotheca was observed in some specimens (Fig. 1V).

In the case of the heterotrophic genus *Cabra*, the paucity of specimens represents a challenge for studies on intraspecific morphological variability or even molecular analysis. *Cabra* is one of the few benthic genera described in the last decade with no molecular information. Carlson (1984) realized that it was a new genus, but he did not describe his specimens. Jacobson (1999) did not describe the genus due to the difficulties to interpret the tabulation (D.M. Jacobson, personal communication). The interpretation of the tabulation is challenging and varied between Murray & Patterson (2004) and Chomérat et al. (2010). For these reasons, *Cabra* was not described until recently from sandy sediments in Australia (Murray & Patterson, 2004).

The original descriptions of the flange-bearing species were based on specimens collected in sandy sediments (Murray & Patterson, 2004; Chomérat & Nézan, 2009; Chomérat et al., 2010). However, most records of *Cabra* are as epiphyte on macroalgae (Carlson, 1984; Okolodkov et al., 2007, 2014; Selina & Levchenko, 2011; Shah et al., 2013). Selina & Levchenko (2011) reported that *C. matta* was found fairly often on macrophytes (with occurrence up to 35% of the examined samples). *Cabra* did not show a clear preference for a substrate, being found as epiphyte of several species of Rhodophyta, Phaeophyta and Chlorophyta in the cold waters of the western Pacific (Selina & Levchenko, 2011). We have not observed *Cabra* as a sand-dwelling dinoflagellate (Gómez & Artigas, 2014). However, according to our observations *Cabra* is relatively common as epiphyte on macrophytes. Although the flange-bearing species of *Cabra* were described as sand-dwelling dinoflagellates, we should consider that macrophyte surfaces are the preferential habitat.

There is a high tradition of taxonomical studies on dinoflagellates in the Mediterranean Sea. That basin represents a small percentage of the world’s oceans (<1%). However, about 88% of the dinoflagellate genera have been reported in the Mediterranean Sea (Gómez, 2006). In the last decade, the Mediterranean Sea has been subjected to intensive
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monitoring of epiphytic dinoflagellates, especially after reports of human health problems attributed to Ostreopsis proliferations (e.g., Del Favero et al., 2012). In the present study, Ostreopsis, Coolia, Amphidinium Clap. et J. Lach., and some benthic species of Prorocenrum were the most common dinoflagellates co-occurring with Cabra. The Mediterranean records of Cabra corresponded to specimens accidentally detached from the macroalgae, collected from the water column near piers. It is expected that dedicated studies on the epiphytic dinoflagellates, including heterotrophic species, will provide a more realistic estimate of Cabra distribution and abundance in the Mediterranean Sea, and other ocean basins.

Dinoflagellates reported as new records in Mediterranean Sea are often categorized as newcomers, invasive species or biological indicators of global warming (Gómez, 2008). These first Mediterranean records of Cabra are explained because this genus has been overlooked in the past. One Mediterranean specimen was observed in November, when water temperature was low, suggesting that Cabra is not a biological indicator of warming. Despite its common occurrence in the tropical waters of the Caribbean Sea and Brazil, the warm-water affinity of the genus Cabra is unclear. Three of the four species were first described from the cold waters of northwestern France (Chomérat et al., 2010) or the cold Pacific waters (Selina et al., 2015). In the South Atlantic Ocean, the studies on the benthic dinoflagellates in the South American and African coasts are almost inexistent, and any observation reveals new records of benthic dinoflagellates. We also observed three specimens of C. matta from a single sample of macroalgae epiphytes near Xiamen, China. Cabra matta has been recently cited in the eastern coasts of Asia (Selina & Levchenko, 2011; Shah et al., 2013; Selina et al., 2015) (Fig. 2). Consequently, we have to be cautious on the consideration of the species of Cabra as a newcomer, invasive species or as a biological indicator of warming. Obviously, the species of Cabra have been overlooked in the past and its biogeographic range has been underestimated.

**Difficulties in species delimitation**

While the genus Cabra is highly distinctive, identification at the species level is problematic due to difficulties in the distinction between C. reticulata and C. aremorica based on routine light microscopy observations. In addition, we cannot discard that records attributed to these species corresponded to undescribed taxa. The three flange-bearing species of the genus Cabra were described based on detailed observations of the thecal plate pattern and ornamentation by scanning electron microscopy (Murray & Patterson, 2004; Chomérat & Nezán, 2009; Chomérat et al., 2010). However, these studies included few light microscopy pictures and there is no information on the intraspecific morphological variability. The records of Cabra in the Gulf of Mexico are an example of the confusion. Before the descriptions of C. reticulata and C. aremorica, Okolodkov et al. (2007) provided a micrograph of Cabra, which they identified tentatively as C. matta. More recently, Okolodkov et al. (2014) reported the species as Cabra cf. aremorica. In a recent book by the authorities who described the flange-bearing species of Cabra, the Mexican records have been attributed to C. reticulata (Hoppenrath et al., 2014, p. 74). This evidences the difficulties to discern between C. aremorica and C. reticulata. Both species more or less overlay in size, and we can observe that the length or shape of the dorsal and antapical...
flanges are variable, as well as the relative distance between the cingulum and the dorsal pointed flange.

Other difficulty is that some specimens cannot be assigned to a known species. Selina & Levchenko (2011) reported rounded cells lacking the pointed flanges, which they designated as Cabra cf. matta, and recently described as C. levis (Selina et al., 2015). Their pictures corresponded to the same morphology here illustrated from Puerto Rico and Brazil (Figs 1P–R, 1Z–AB). This implies a wide distribution of C. levis which co-occurred with C. matta and C. aremorica/reticulata. The co-occurrence of congeneric species is a common feature in other heterotrophic benthic dinoflagellates, such as Amphidiniopsis Wolosz. (Gómez et al., 2011; Gómez & Artigas, 2014). The absence or reduced extension of the flanges and the rounded cell shape are distinctive features for congeneric species. However, the pointed flanges and the polygonal shape are distinctive characters of the genus Cabra. Consequently, the less distinctive member of Cabra species such as C. levis could be mistaken with other benthic dinoflagellates, such as large specimens of Adncodinium glandula (Herdman) N.S. Kang, H.J. Jeong et Moestrup, or Durinsksia agilis (Kof. et Swezy) Saburova, Chomérat et Hoppenrath.

In the last decade, almost all new species of benthic dinoflagellates have been described including their molecular data. This is not the case of the four species of Cabra. The study of the intraspecific morphological variability, life stages from cultures, or single-cell PCR could help to discern whether there are several species or a single species with a high intraspecific morphological variability. We are unable to demonstrate an affinity of Cabra to any other known dinoflagellate. In the species descriptions, Cabra has been related to the family Podolampadaceae (i.e., Podolampas F. Stein; Blepharocysta Ehrenb.) (Murray & Patterson, 2004; Chomérat et al., 2010). A close relation between benthic and plankton species has been demonstrated in species such as Amphidiniopsis or Herdmannia J.D. Dodge (Gómez et al., 2011; Yamaguchi et al., 2011). The sand-dwelling genus Roscoffia Balech has been placed in Podolampadaceae (Saldarriaga et al., 2003; Gómez et al., 2010). The phylogenetic relation between Cabra and Podolampadaceae remains unresolved.

**Concluding remarks**

The records of species of Cabra indicate that the genus is relatively common as epiphyte on macrophytes in the examined locations. The genus Cabra is a good example that: 1) despite the distinctive morphology, a genus could remain undescribed due to the difficulties in the interpretation of the tabulation and paucity of specimens for the standard requirements for benthic dinoflagellate descriptions. In fact, Cabra was too rare to be described before. Cabra was first described as a sand-dwelling dinoflagellate when it is really more common as an epiphytic dinoflagellate. Taxonomists working on epiphytic dinoflagellates are more focused on the photosynthetic cultivable species, especially the toxigenic genera, and the surrounding assemblage of heterotrophic dinoflagellates did not receive attention. In contrast, taxonomists working on sand-dwelling dinoflagellates have more expertise on species descriptions based on fewer specimens and taxa with difficult interpretations of the plate pattern; 2) the delimitation of the species Cabra aremorica and C. reticulata is unclear based on routine light microscopy observations; and 3) the records of Cabra will spread to other ocean regions in the next years. Benthic dinoflagellates, especially the heterotrophic species, were undersampled and overlooked in the past. This should be taken into account when categorizing benthic dinoflagellates as newcomers, invasive species or biological indicators of warming.

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