

Diversity and distribution of the dinoflagellates *Brachidinium*, *Asterodinium* and *Microceratium* (Brachidinales, Dinophyceae) in the open Mediterranean Sea

FERNANDO GÓMEZ*

Instituto Cavanilles de Biodiversidad y Biología Evolutiva,
Universidad de Valencia, PO Box 22085, 46071 Valencia, Spain

Abstract – Brachidiniacean dinoflagellates have been investigated in the open waters of the Mediterranean Sea, along a transect from the south of France to the south of Cyprus (20 June–18 July 2008). *Brachidinium* and *Karenia papilionacea* often co-occurred, *B. capitatum* predominating in the surface waters. The highest abundance of *Brachidinium* were found in the upper 25 m in the western Mediterranean with a maximum (24 cells L⁻¹) at a depth of 5 m in the Balearic Sea. *Asterodinium* (up to 4 cells L⁻¹) was recorded below of deep chlorophyll maxima. The genus *Microceratium*, only known from the tropical Indo-Pacific region, is reported for the first time in the Mediterranean Sea. *Microceratium* was found below 100 m in the eastern Mediterranean Sea, with the highest abundance of 8 cells L⁻¹ at 125 m depth, in the Levantine Basin. This study also illustrates for the first time specimens under the division of *Brachidinium* and *Microceratium*. This first occurrence of *Microceratium* in the Mediterranean Sea should be considered an indicator of climate warming. However, it should not be considered a non-indigenous taxon. *Microceratium* is the 'tropical morphotype', the adaptation of a local species (a life stage of *Karenia* – *Brachidinium* – *Asterodinium*) to the tropical environmental conditions that prevail in summer in the open Mediterranean Sea.

Key words: Biodiversity, *Brachyidinium*, Brachyidinales, Dinophyta, introduced species, invasive phytoplankton, *Karenia papilionacea*, non-indigenous taxa.

Introduction

The order Brachidinales encompasses a group of highly flattened unarmoured dinoflagellates with elongate extensions radiating from the episome and hyposome. *Brachidinium* F.J.R. Taylor has four elongate extensions radiating from the hyposome and an apical process on the episome (TAYLOR 1963). *Asterodinium* Sournia differs from *Brachidinium*

* Corresponding author, email: fernando.gomez@fitoplancton.com

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in having two elongate extensions radiating from the hyposome, and one central and other two lateral extensions from the episome. *Microceratium* Sournia has only one central extension in the episome and two extensions from the hyposome (SOURNIA 1972a, b). GÓMEZ et al. (2005) based on light and scanning electron microscopy revealed the morphological similarities between *Brachidinium* and *Asterodinium* and species of the genus *Karenia* G. Hansen et Moestrup, especially with *K. papilionacea* A. J. Haywood et K. A. Steidinger (HAYWOOD et al. 2004). These taxa coincided in distinctive morphological characters such as the straight apical groove, cingulum-sulcus juncture, prominent nucleus in the left hyposome, numerous yellow-green chloroplasts, among other characters. The morphological similarity and the occurrence of intermediate stages between these genera suggested that *Asterodinium*, *Brachidinium*, *Microceratium* and *Karenia papilionacea* constitute a single or several closely related species (GÓMEZ 2006). Species with high morphological versatility are able to project body extensions as an adaptation to environmental conditions. The hypothesis of a phylogenetic relation between these taxa remains to be tested with molecular methods.

All the species of Brachidiniiales were described from the tropical Indian Ocean (TAYLOR 1963, SOURNIA 1972a, b). Within the context of global warming, the expansion of tropical species is of especial interest as biological indicators of the changes in the marine ecosystem. About 700 species dinoflagellates have been reported in the Mediterranean Sea including ca 90% of the dinoflagellate genera of the world's oceans (GÓMEZ 2003, 2005). Despite the historical tradition of taxonomic studies, some genera remain unreported in the Mediterranean basin. This study describes the diversity and distribution of brachidiniacean dinoflagellates in the open waters of Mediterranean Sea. The genus *Microceratium* is reported for the first time, and the first illustration of dividing cells of *Brachidinium* and *Microceratium* is given.

Materials and methods

Samples were collected during the BOUM (Biogeochemistry from the Oligotrophic to the Ultra-oligotrophic Mediterranean) cruise on board R/V *L'Atalante* from the south of France to the south of Cyprus (20 June–18 July 2008) (Fig. 1) (for details see: http://www.biogeosciences-discuss.net/special_issue63.html). Seawater samples were collected by Niskin bottles from 30 stations. At each station 6 depths were sampled between 5 and 125 m and an additional sample at 250 m depth. These were preserved with acid Lugol's solu-



Fig. 1. Map of the station locations in the Mediterranean Sea during the BOUM cruise in June–July 2008.

tion and stored at 5 °C. Samples of 500 mL were concentrated via sedimentation in glass cylinders. The top 450 mL of sample was slowly siphoned off with small-bore tubing during 6 days. The remaining 50 mL of concentrate, representing 500 mL whole water, was then settled in a composite settling chamber during one day. The sample was examined at 100 × magnification with a Nikon inverted microscope (Nikon Eclipse TE200) and the specimens were photographed with a digital camera (Nikon Coolpix E995).

Results

A total of 50 specimens of *Brachidinium* were observed from the 212 samples analysed. The highest abundance was recorded in the surface waters (0–25 m depth) of the Balearic (up 24 cells L⁻¹) and Tyrrhenian Seas. The hydrological and biochemical conditions during the cruise are detailed at: http://www.biogeosciences-discuss.net/special_issue63.html. *Brachidinium* and *Karenia* coincided in the distribution, with a wider vertical distribution of *Karenia* (0–50 m depth) (Figs. 2, 3). The figure 3O illustrates for the first time dividing cells of *Brachidinium* that were collected from the Tyrrhenian Sea at a depth of 5 m. In all the known brachidiniaceans, including *K. papilionacea*, the nucleus (dark oval area in the Lugol-fixed specimens) was invariably located in the left hyposome. In the recently divided specimens, the right extension of the hyposome of one of the daughter cells contacted with the hyposome of the other daughter cell. The ventral side of the daughter cell was opposed to the dorsal side of another cell (Fig. 3O).

Ten specimens of *Asterodinium* were recorded (Figs. 4A–G). The highest abundance (4 cells L⁻¹) was recorded at 100 m depth in the Tyrrhenian Sea. The records were higher in the western Mediterranean Sea. All the records were below 100 m depth, with the exception of one specimen collected at 50 m depth (Fig. 2).

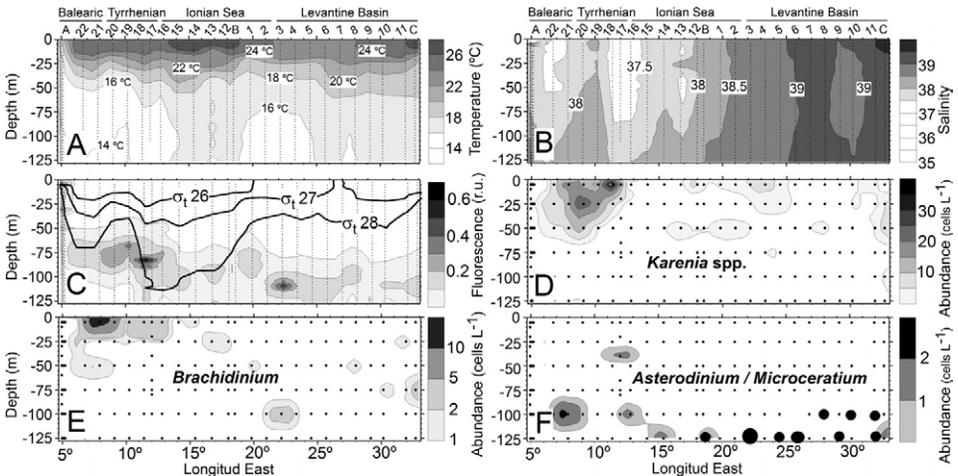


Fig. 2. Distribution of temperature, salinity, density, fluorescence and abundance of brachidiniaceans in the open Mediterranean Sea. **A** – temperature (°C), **B** – salinity, **C** – Fluorescence (relative units) and density, σ_t , **D** – *Karenia* spp., **E** – *Brachidinium*, **F** – *Asterodinium* and *Microceratium*, the latter indicated by filled circles.

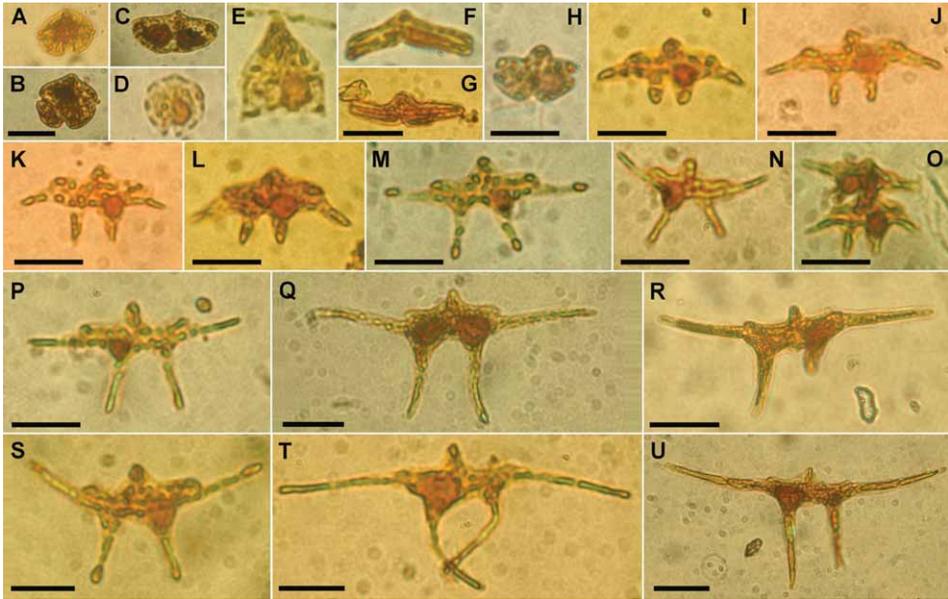


Fig. 3. Photomicrographs of *Karenia* spp. (A–H), and *Brachidinium* (I–U), bright field optics. A–E – Specimens of *Karenia*, D – *Karenia bidigitata*, E – an unknown species. F–G – *Karenia papilionacea*, O – *Brachidinium* after cell division. Scale bar: 20 μm .

A total of 13 specimens of *Microceratium* were found (Figs. 4H–R). These records can be grouped in two forms, one with shorter extensions (Fig. 4H–L) and other forms with longer extensions (Figs. 4M–R). All the specimens were encountered in the eastern Mediterranean (Fig. 2). The highest abundance (8 cells L^{-1}) was recorded at a depth 125 m in the Levantine Basin. A couple of recently divided specimens were observed (Fig. 4K). One of the daughter cells lacked the apical extension. This shows that the central extension is formed after the cell division. The position of the nucleus was similar that in the divided cells of *Brachidinium*. The dorsal side of one daughter cell was opposed to the ventral side of the other daughter cell (Fig. 4K).

Discussion

Previous studies based on ultrastructural similarities (GÓMEZ et al. 2005) and the occurrence of intermediate stages (GÓMEZ 2006) suggested a close relation, if not the conspecificity, of *Karenia papilionacea* and the genera *Brachidinium* and *Asterodinium*. In this study, we found specimens of *Asterodinium* lacking one of the antapical extensions (Fig. 4A–B) or specimens of *Microceratium* with a short left antapical extension (Fig. 4O). These observations supported the view of the retraction or regeneration of the body extensions. In contrast to the antapical or lateral extensions, the central extension of the episome has a different morphology because it harbours the straight apical groove (GÓMEZ et al. 2005). Figure 4K shows two daughter cells after a recent division, with one the daughter cells lacking the apical extension. This evidences the change of the cell contour from *Karenia* into the morphologies of *Asterodinium*/*Microceratium*. The number of body ex-

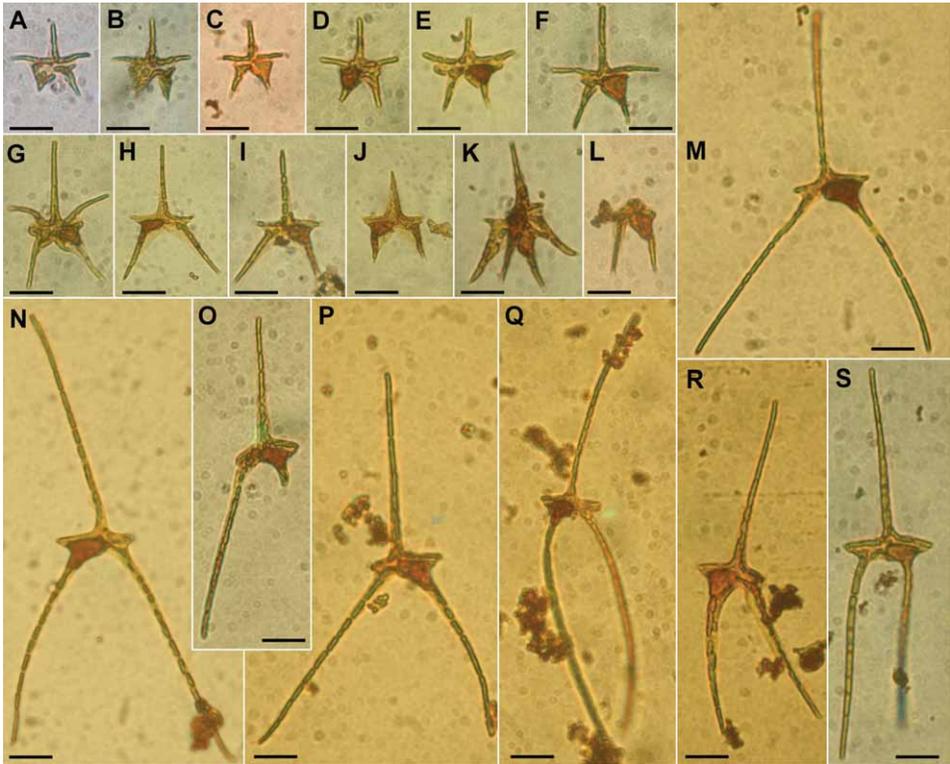


Fig. 4. Photomicrographs of *Asterodinium* (A–G) and *Microceratium* (H–S), bright field optics. Note the lack of the left antapical extensions in *Asterodinium* (A–B). H–L – Specimens of *Microceratium* with short extensions. K – Two cells after the division. Note the lack of the apical extension in one of the daughter cells. M–S – Specimens of *Microceratium* with long extensions. O – Note the scarcely developed left antapical extension. Scale bar: 20 μ m.

tensions, which has been used for the generic separation of the Brachidinales, has a rare diagnostic value.

The western Mediterranean Sea has been historically one of the most investigated regions for phytoplankton studies. The first record of *Asterodinium* in the Mediterranean Sea was considered to be a biological indicator of the progressive warming of the Mediterranean Sea (GÓMEZ and CLAUSTRE 2003, LEJEUSNE et al. 2009). *Asterodinium* was described from the tropical Indian Ocean (SOURNIA 1972a, b). For this reason, *Asterodinium* was further listed as an alien or invasive species in the Mediterranean Sea (ZENETOS et al. 2006). As it happened with *Asterodinium*, this first occurrence of *Microceratium* in the Mediterranean Sea could be considered an indicator of global warming. However, it should not be considered as a non-indigenous or exotic taxon. *Microceratium* is the 'tropical morphotype' of a local species that appears in the Mediterranean when environmental conditions resemble those in tropical waters.

Microceratium is another of the life stages of the morphotypes *Karenia* – *Brachidinium* – *Asterodinium* that appears under warm and highly stratified conditions. *Microceratium* is

a biological indicator of 'tropicalization', an adaptation of a local species to the near tropical environmental conditions that prevail in summer in the open Mediterranean Sea.

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