Acta Protozool. (2010) 49: 365–372
http://www.eko.uj.edu.pl/ap

ACTA
PROTOZOOLOGICA

Diversity and Distribution of Noctilucoid Dinoflagellates (Noctilucales, Dinophyceae) in the Open Mediterranean Sea

Fernando GÓMEZ

Université Lille Nord de France, Laboratoire d´Océanologie et Géosciences, Station Marine de Wimereux, France

Summary. The noctilucoid dinoflagellates have been investigated in the open waters of the Mediterranean Sea. *Kofoidinium* spp., *Spatulodinium* spp. and *Scaphodinium mirabile* were found in nearly all the stations. The genera *Craspedotella*, *Leptodiscus*, *Petalodinium* and *Pomatodinium* were recorded for the first time in the eastern Mediterranean basin. An undescribed small species of *Kofoidinium* (40–60 µm in diameter) with a pointed extension represented about 1/3 of the genus records. The monotypic character of the genus *Spatulodinium* needs to be reconsidered because numerous specimens differed from the type species. One of these undescribed species showed a distinctive hyposome and an extremely long tentacle (up to 1600 µm long). A leptodiscacean that showed an arrowhead-shaped contour is found for the first time in the Mediterranean Sea. There is a considerable diversity of noctilucaceans yet to be described.

Key words: Biodiversity, *Craspedotella*, Dinophyta, Kofoidiniaceae, Leptodiscaceae, *Kofoidinium*, *Leptodiscus*, *Noctiluca*, *Petalodinium*, *Pomatodinium Scaphodinium*, *Spatulodinium*.

INTRODUCTION

Noctiluca scintillans (Macartney) Kofoid is the first dinoflagellate to be described. This aberrant species lacks, at least in some life stages, typical dinoflagellate characters such as the ribbon-like transversal flagellum or condensed chromosomes. In contrast to *Noctiluca* that is widespread in eutrophic coastal waters, the other noctilucaceans have a predominantly tropical to warm-temperate oceanic distribution (Cachon and Cachon 1967, 1969; Gómez and Furuya 2005, 2007). All the Noctilucales differ markedly from the rest of the dinoflagellates, notably by the presence of contractile muscle-like fibrils involved in cell shape changes and movements. Based on the gene sequences of *N. scintillans*, the phylogenetical position of the noctilucaceans has been controversial. Some studies placed *Noctiluca* within one of the clades of the dinoflagellate order Gymnodiniales (Saldarriaga *et al*. 2004) and other phylogenies have placed it as an early diverging lineage within dinoflagellates, branching after *Oxyrrhis* and before the core dinoflagellates (Liu and Hastings 2007, Fukuda and Endoh 2008, Zhang and Lin 2008, Gómez *et al*. 2009, Ki 2010). Additional sequences of other genera such as *Kofoidinium* Pavillard, *Spatulodinium* J. Cachon et M. Cachon and *Abedinium* Loeblich Jr. et Loeblich III (=*Leptophyllus* J. Cachon et

Address for correspondence: Fernando Gómez, Université Lille Nord de France, Laboratoire d´Océanologie et Géosciences, CNRS UMR 8187, Station Marine de Wimereux, 28 Av. Foch, 62930 Wimereux, France; Fax: +33 321996401; E-mail: fernando.gomez@fitoplancton.com

Cachon-Enjumet) revealed that the Noctilucales emerged before the dinoflagellate core and the weakly supported monophyly of three well-supported noctilucoid clades (Gómez *et al*. 2010). *Noctiluca* branched among species of *Spatulodinium*. This does not support the split of the Noctilucales into the families Noctilucaceae and Kofoidiniaceae, especially in what refers to the conservation of *Noctiluca* as the only representative of its own family (Gómez *et al*. 2010).

Most of the Noctilucales have been described from the NW Mediterranean and knowledge is almost completely restricted to the coastal studies by Cachon and Cachon (1967, 1969). Since their observations, the records of noctilucaceans have been scarce and several genera have never been reported after the initial descriptions. This study describes the diversity and distribution of the noctilucoid dinoflagellates in the open waters of Mediterranean Sea. The results reveal that the species diversity is underestimated. For example, one of the most common species of *Kofodinium* remains to be described, as well as numerous species of the monotypic genus *Spatulodinium* and some leptodiscaceans.

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). 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 solution and stored at 5ºC. 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 composite settling chambers. The sample was examined in Utermöhl chambers at 100 × magnification with a Nikon inverted microscope (Nikon Eclipse TE200) and the specimens were photographed with a digital camera (Nikon Coolpix E995).

RESULTS AND DISCUSSION

Kofoidinium spp.

Kofoidinium was the most ubiquitous genus of noctilucaceans in the open waters of the Mediterranean Sea. As a general trend, the abundance of *Kofoidinium* spp. was higher in the upper 25 m depth and slightly increased between 100–125 m depth (Fig. 2B). *Kofoidinium pavillardii* J. Cachon et M. Cachon was the species responsible of the highest abundance (28 cells L^{-1}) found in the easternmost station (Sta. C) at 100 m depth. The second maximum (18 cells L^{-1}) was observed in the surface waters near Sardinia, due to a small species of *Kofoidinium* and *K. pavillardii*. One half of the records of *Kofoidinium* corresponded to *K. pavillardii*, and the other half for the *Kofoidinium* sp. and *K. velelloides* Pavillard (Fig. 3).

All the described species of *Kofoidinium* are larger than 200 µm in diameter (Cachon and Cachon 1967). Forty-one of the 141 records of *Kofoidinium* corresponded to specimens that ranged from 40–80 µm in diameter (Figs 3A–E). This small species showed a pointed extension that arises from the episome towards the ventral side of the cell. The pointed extension was flexible and tended to be in a different plane from the flattened hyposome. The contour of the hyposome was usually rounded and some specimens showed a triangular or irregular contour (Figs 3A–E). Due to the transparency and small size, *Kofoidinium* sp. could be easily overlooked during sample analysis and/or undersampled by net sampling. This species has been also recorded in the Pacific Ocean (Gómez and Furuya 2007). The small subunit ribosomal DNA (SSU rDNA) sequence of a specimen of this undescribed species is available (accession number GU355681). The specimen was isolated from the coastal Mediterranean Sea (Gómez *et al*. 2010).

Kofoidinium pavillardii is the largest species of the genus. The cell diameter is longer than 300 µm and sometimes reached 700 µm (Figs 3F–I). While the identification of *K. pavillardii* and the small *Kofoidinium* sp. is relatively easy, the delimitation between the species *K. velelloides* Pavillard and *K. splendens* J. Cachon et M. Cachon is not simple. The cell diameter of both species were longer than 200 µm (usually 300–400 µm). In this study the specimens have been ascribed to *K. velelloides* (Figs 3J–L) because the diagnostic characters that defined *K. splendens* are unclear. *Spatulodinium* and *Kofoidinium* have a similar life cycle and the immature stages may be confused one each other (Cachon and Cachon 1967). The immature stages of *Kofoidinium* were apochlorotic (Fig. 3M) when compared to those of *Spatulodinium* (Figs 4 J–L).

The specimen of the figure 3N has been ascribed to *Pomatodinium* J. Cachon et M. Cachon. It is uncertain whether *Pomatodinium* may constitute a life stage of *Kofoidinium*. The specimen was collected in the Ionian Sea (Sta. 14) at 25 m depth, and to the best of my

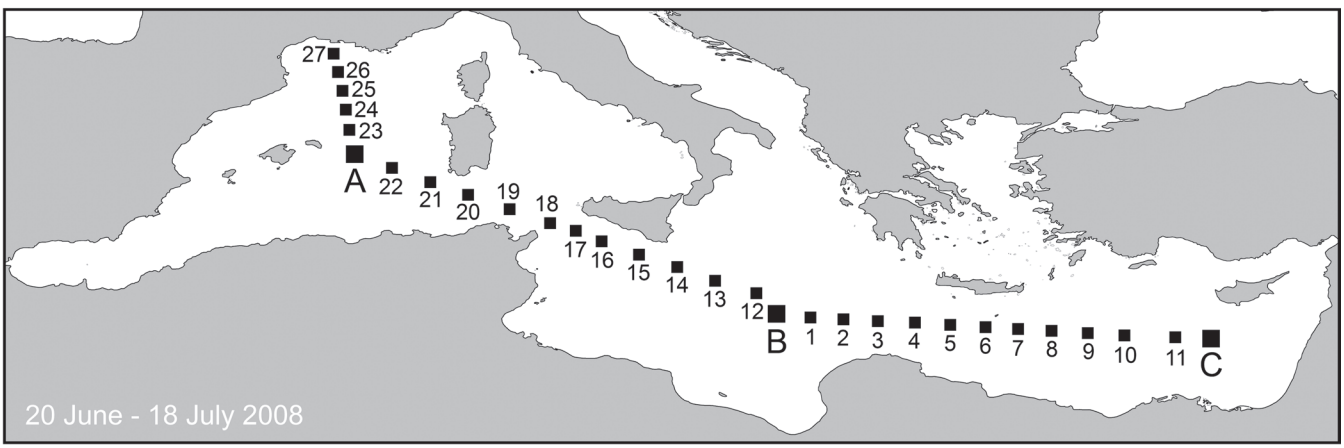
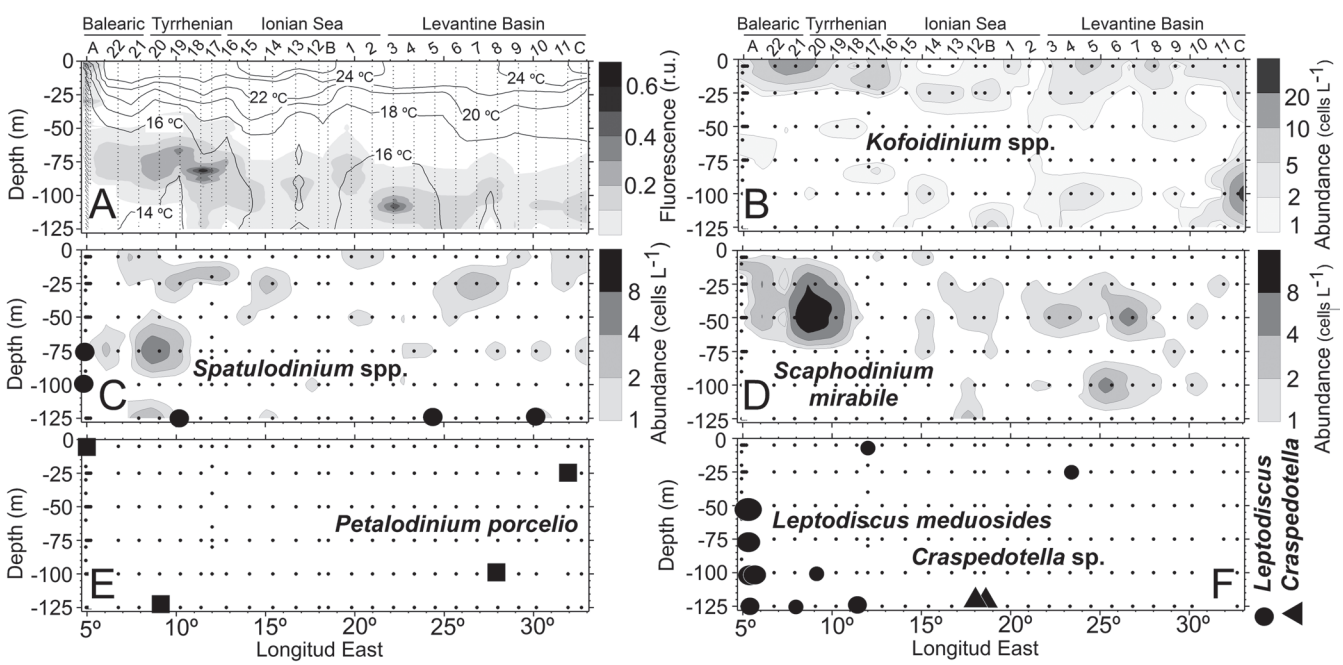


Fig. 1. Map of the station locations in the Mediterranean Sea during the BOUM cruise in June–July 2008.

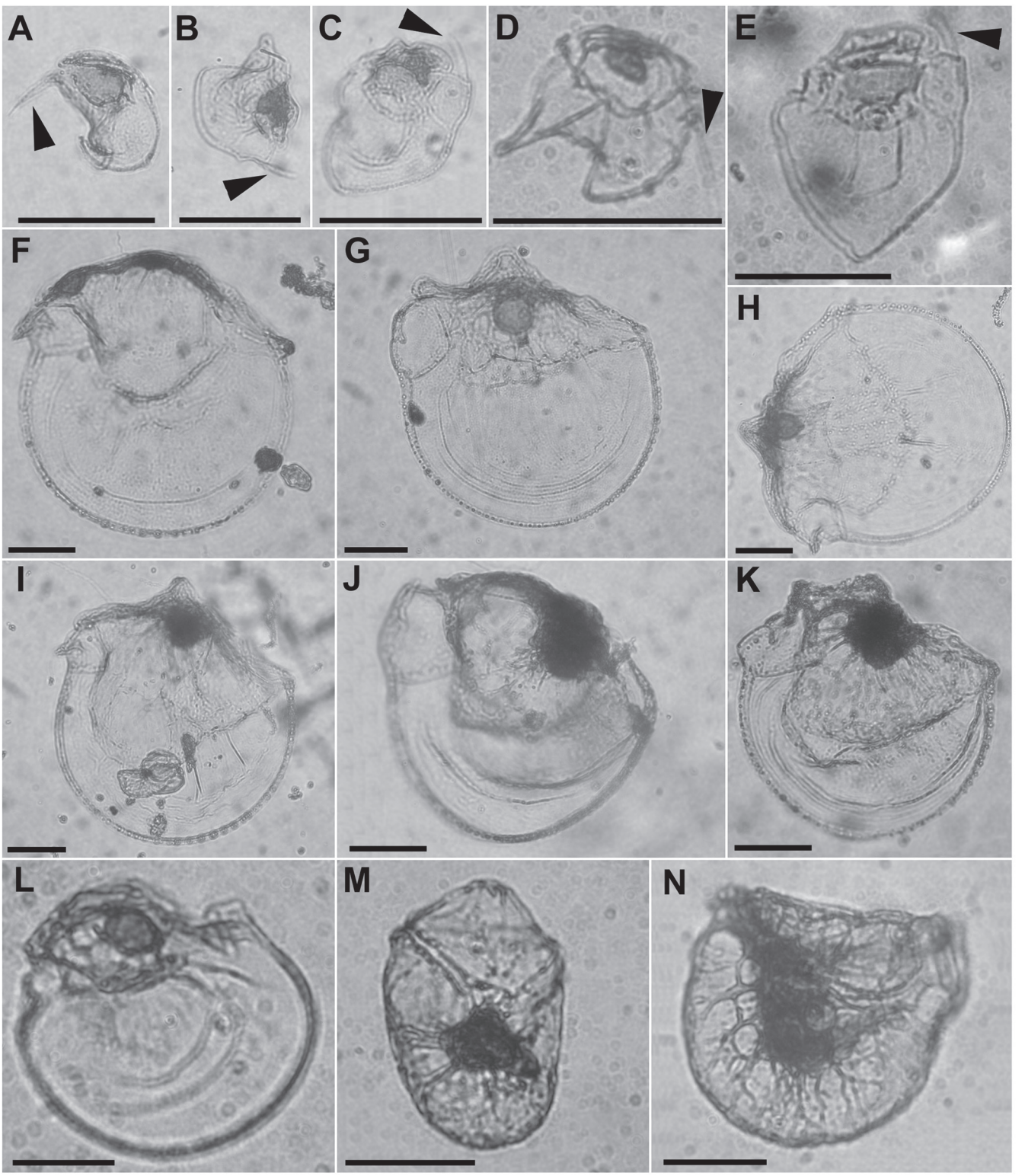


Figs 2A–F. Section plots of the temperature, fluorescence, distribution and abundance of noctilucaceans in the open Mediterranean Sea. **A –** temperature (ºC) and fluorescence (relative units); **B –** *Kofoidinium* spp., **C –** *Spatulodinium* spp. Records of an undescribed species with a long tentacle indicated by filled circles; **D –** *Scaphodinium mirabile*; **E –** records of *Petalodinium porcelio* indicated by filled squares; **F –** records of *Leptodiscus medusoides* and *Craspedotella* sp. indicated by filled circles and triangles, respectively.

knowledge this is the first record in the Eastern Mediterranean Sea (Gómez 2003).

Spatulodinium spp.

The species of *Spatulodinium* were present in most of stations and preferentially in the upper 75 m depth (Fig. 2C). The abundance was low with a maximum 12 individuals L^{-1} in the Balearic Sea (Sta. A) at 75 m depth. The genus *Spatulodinium* is currently restricted to one species, *S. pseudonoctiluca* (Pouchet) J. Cachon et M. Cachon, known from the coastal waters of the northern hemisphere (Cachon and Cachon 1967, Gómez and Souissi 2007). In the open waters of the Mediterranean Sea, none of the 52 specimens of *Spatulodinium*



Figs 3A–N. Photomicrographs of *Kofoidinium* spp. and *Pomatodinium* sp., bright field optics. **A–E –** *Kofoidinium* sp. (small form with a pointed extension). The arrowhead indicated the extension. **F–I –** *Kofoidinium pavillardii*; **J–L –** *Kofoidinium velelloides*; **M –** immature stage of *Kofoidinium*, Sta. 6, 100 m depth; **N –** *Pomatodinium* sp., Sta. 14, 25 m. Scale bar: 50 µm.

corresponded to the morphology that usually presented the type species. In order to facilitate the comparison, a Lugol-fixed specimen from the coastal Mediterranean Sea is here illustrated (Fig. 4A). While the morphology of the Lugol-fixed specimens of *S. pseudonoctiluca* from coastal waters is quite constant, there was a high diversity of size, shape of the hyposome and position and length of the tentacle in the specimens collected in open waters (Figs 4B–I). Some specimens corresponded to the life stage known as *Gymnodinium lebouriae* with the tentacle (Fig. 4I) or lacking the tentacle (Figs 4K–L). The life stage precursor of *G. lebouriae* may appear in pairs of smaller cells joined at the elongate episome (Fig. 4J).

Among the high species diversity of *Spatulodinium*, six individuals showed a very distinctive morphology that differed from the other species (Figs 4M–V). The most distinctive character was an extremely long extension that seems to be analogue to the tentacle of the other species of *Spatulodinium*. The cell diameter was 80 µm and the tentacle that represented about 20 × times the cell diameter (up 1600 µm) is the longest known extension among the dinoflagellates (Fig. 4M). All the specimens were collected below 75 m depth and one of them at 250 m depth (Fig. 2C). While the other species of *Spatulodinium* showed a shallower distribution and the cell body was pigmented due to the occurrence of chlorophyll *a* (Gómez and Souissi 2007, Gómez *et al.* 2010), this undescribed species was apochlorotic. The hyposome showed concentric rings and was bordered by a differentiated band with septae (Figs 4O–P). The lack of plastids and the hyposomal bands resembled *Kofoidinium*. At least one species of *Kofoidinium* showed a short extension (Figs 3A–E) that may be analogous with the tentacle of *Spatulodinium*. Despite these common features with *Kofoidinium*, the specimens have been tentatively ascribed to the genus *Spatulodinium*.

Spatulodinium contains one species in current taxonomic schemes. Molecular data are available for *S. pseudonoctiluca* from live specimens collected in the type locality, English Channel, and the coastal NW Mediterranean Sea (Gómez *et al.* 2010). Other sequence was obtained from a distorted ethanol-fixed specimen collected from the open Balearic Sea. The general appearance and size was close to some specimens here illustrated (Figs 4D, G). The phylogenetical distance between both species of *Spatulodinium* was high and *Noctiluca* branched between them (Gómez *et al.* 2010). This confirmed that among these Mediterranean specimens there is, at least, a second species for the genus *Spatulodinium*.

Leptodiscaceans

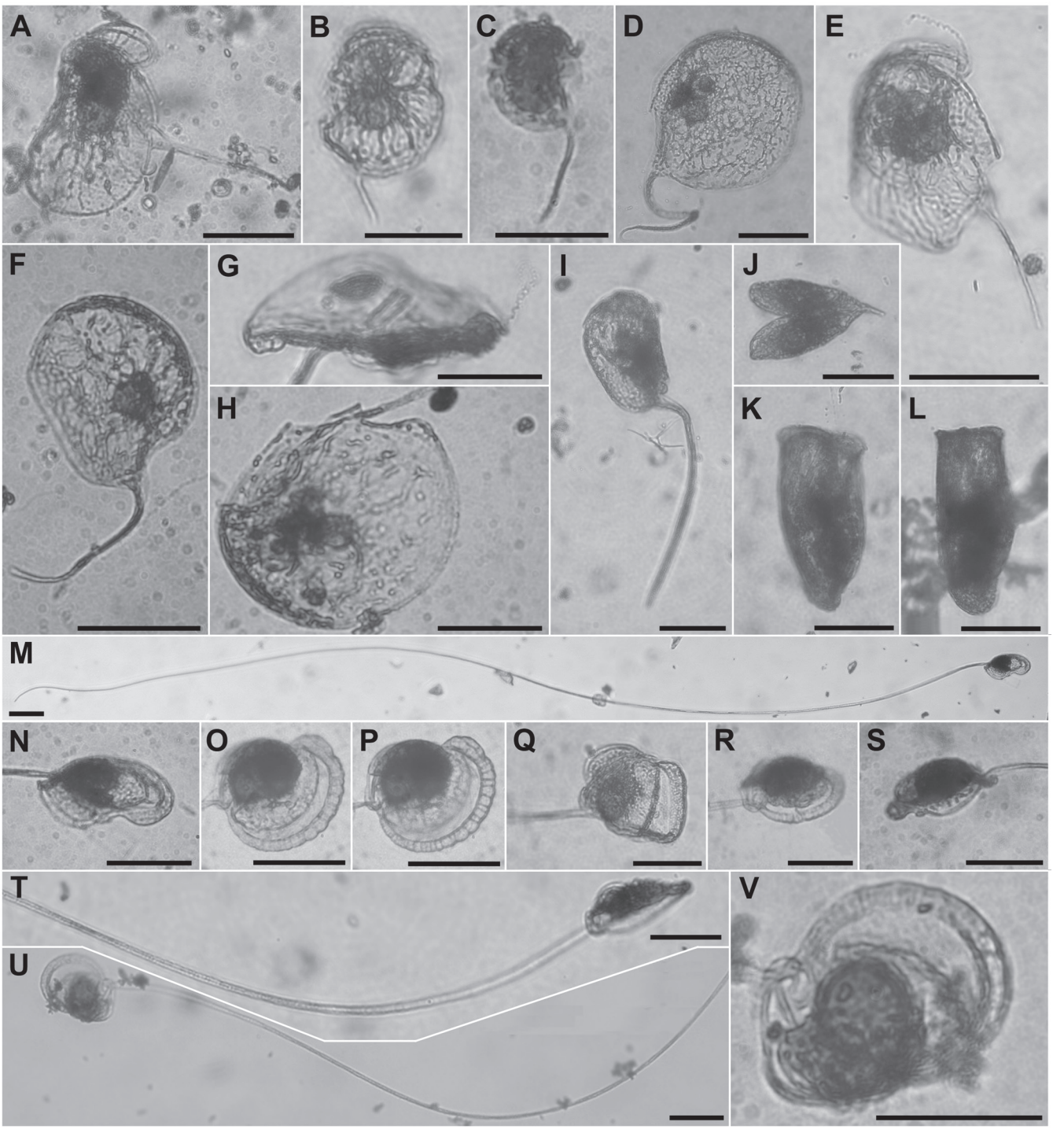
The leptodicaceans are the less known of the noctilucaceans. After *Kofoidinium pavillardii*, *Scaphodinium mirabile* Margalef (71 individuals) was the most common noctilucacean in the open Mediterranean Sea. The highest abundance (28 cells L^{-1}) was recorded in the Balearic Sea (Sta. 20) at 50 m depth (Fig. 2D). As previously illustrated by Gómez and Furuya (2004), most of the Lugol-fixed specimens appeared folded (Figs 5A–E). Observations of live specimens revealed that the specimens fold as a response to environmental stress such as the fixation.

Four specimens of *Petalodinium porcelio* J. Cachon et M. Cachon were observed (Fig. 2E). As occurred in *Scaphodinium*, the specimens of *Petalodinium* appeared folded in the fixed material (Figs 5F–J). Two of the specimens collected from the Levantine Basin constituted the first records in the eastern Mediterranean Sea. These scarce records do not allow establishing any trend on the distribution.

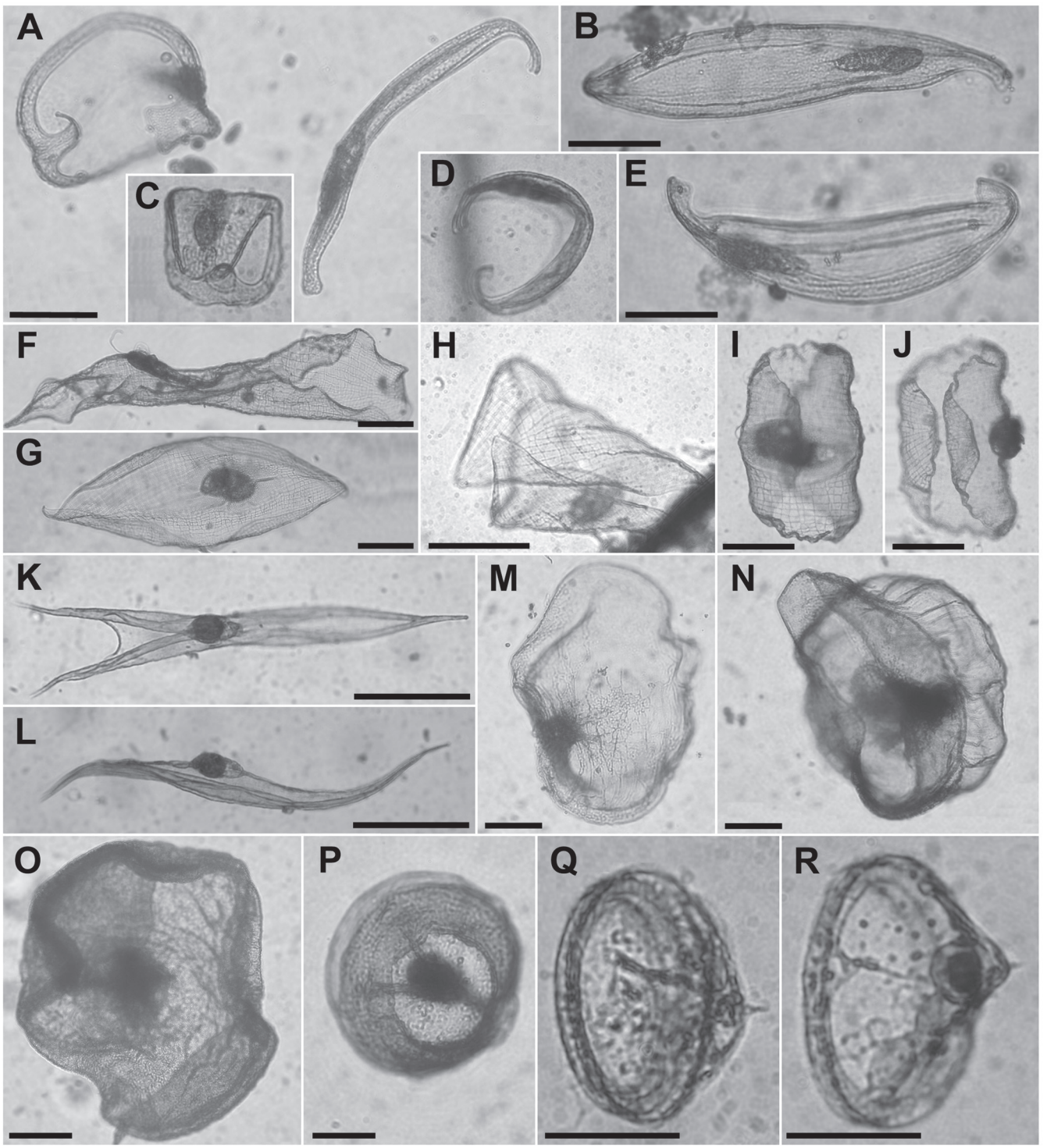
The distinctive reticulation of myo-fibrils that characterized *Petalodinium* was also observed in an undescribed leptodiscacean collected at 250 m depth in the Levantine basin (Sta. 8). The specimen (130 µm long) showed an arrowhead-shaped outline and the proximal extremity bifurcated. The margins of the cell appeared folded as far as the region of the nucleus (Figs 5K–L). This undescribed species was also recorded at 100 m depth from the South China Sea as illustrated by Gómez and Furuya (2005).

A total of 17 specimens of the medusoid leptodiscacean *Leptodiscus* were observed (Fig. 2F). The highest abundance (10 individuals L^{-1}) was recorded at 50 m depth in the Balearic Sea (Sta. A) (Figs 2F, 5M–P). One specimen collected from the Ionian Sea constituted the first record of this genus in the eastern Mediterranean Sea.

Four specimens of *Craspedotella* were observed from two stations in the Ionian Sea at 125 m depth (Figs 2F, 5Q–R). *Craspedotella pileous* was described from the tropical Pacific Ocean (Kofoid 1905). Cachon and Cachon (1969) found *C. pileous* in coastal waters and also in very deep waters (4300 m depth) in the Mediterranean Sea. The specimens observed in this study were similar to those observed in the Pacific Ocean by Gómez (2007).



Figs 4A–V. Photomicrographs of *Spatulodinium* spp., bright field optics. **A –** *Spatulodinium pseudonoctiluca* from the Berre lagoon, coastal NW Mediterranean Sea; **B–L –** other species of *Spatulodinium*; **B –** Sta. 3, 175 m depth; **C –** Sta. 19, 25 m; **D –** Sta. 1, 25 m; **E –** Sta. 7, 25 m; **F –** Sta. 1, 5 m; **G–H –** Sta. 6, 25 m; **I–L –** immature stages of *Spatulodinium*; **I –** *Gymnodinium lebouriae* with tentacle, Sta. 21, 75 m; **J –** Sta. 24, 100 m; **K–L –** Sta. A, 25 m; **M–V –** undescribed *Spatulodinium* species with a long tentacle; **M–N –** Sta. 20, 125 m; **O–P –** Sta. 10, 125 m; **Q –** Sta. 25, 250 m; **R –** Sta. 25, 75 m; **S–T –** Sta. A, 100 m; **U–V –** Sta. 5, 125 m. Scale bar: 50 µm.



Figs 5A–R. Photomicrographs of leptodiscaceans, bright field optics. **A**–**E** – several specimens of *Scaphodinium mirabile*; **F**–**J** – specimens of *Petalodinium porcelio*; **F** – Sta. 20, 125 m depth; **G** – Sta. 24, 5 m; **H** – Sta. 11, 25 m; **I**–**J** – Sta. 8, 100 m; **K**–**L** – undescribed arrowhead-shaped leptodiscacean, Sta. 8, 250 m; **M**–**P** – specimens of *Leptodiscus medusoides*; **M** – Sta. 20, 74 m; **N** – Sta. 7, 100 m; **O** – Sta. 15, 50 m; **P** – Sta. 4, 100 m; **Q**–**R** – *Craspedotella* sp.; **Q** – Sta. 12, 125 m; **R** – Sta. B, 125 m. Scale bar: 50 µm.

The fragility, transparency and polymorphism of the leptodiscaceans are responsible for the scarce records, going unnoticed in the world's oceans. It is not easy to identify noctilucaceans to species due to the high morphological variability during their life cycle and the difficulties to delimit the species from preserved specimens. This study illustrated some species for the first time since the studies by Cachon and Cachon (1967, 1969) and other undescribed species that preferentially inhabit in deep waters. There is a considerable diversity of noctilucoid dinoflagellates yet to be described.

Acknowledgements. This is a contribution to the project DIVERPLAN-MED supported by a post-doctoral grant to F.G. of the Ministerio Español de Educación y Ciencia #2007-0213. I thank K. Leblanc and V. Barthaux for the BOUM samples. I acknowledge financial support from the ANR Biodiversity program (ANR BDIV 07 004-02 'Aquaparadox'). This is a contribution of the BOUM (Biogeochemistry from the Oligotrophic to the Ultraoligotrophic Mediterranean) project of the French national LEFE-CYBER program and of the European IP SESAME.

REFERENCES

Cachon J., Cachon M. (1967) Contribution a l'étude des Noctilucidae Saville-Kent, I. Les Kofoidininae Cachon J. et M. Évolution, morphologique et systématique. *Protistologica* **3:** 427–444

Cachon J., Cachon M. (1969) Contribution a l'étude des Noctilucidae Saville-Kent. Évolution, morphologique, cytologie, systématique. II. Les Leptodiscinae Cachon J. et M. *Protistologica* **5:** 11–33

Fukuda Y., Endoh H. (2008) Phylogenetic analyses of the dinoflagellate *Noctiluca scintillans* based on β-tubulin and Hsp90 genes. *Eur. J. Protistol*. **44:** 27–33

Gómez F. (2003) Checklist of Mediterranean free-living dinoflagellates. *Bot. Mar.* **46:** 215–242

Gómez F., Furuya K. (2004) New records of *Scaphodinium mirabile* (Dinophyceae), an unnoticed dinoflagellate in the Pacific Ocean. *Phycol. Res.* **52:** 13–16

Gómez F., Furuya K. (2005) Leptodiscaceans (Noctilucales, Dinophyceae) from the Pacific Ocean: First records of *Petalodinium* and *Leptodiscus* beyond the Mediterranean Sea. *Eur. J. Protistol.* **41:** 231–239

Gómez F. (2007) Nuevos registros del dinoflagelado *Craspedotella* Kofoid, 1905 (Dinophyceae: Noctilucales) en el Océano Pacífico. *Rev. Biol. Mar. Oceanogr.* **42:** 83–87

Gómez F., Furuya K. (2007) *Kofoidinium*, *Spatulodinium* and other kofoidiniaceans (Noctilucales, Dinophyceae) in the Pacific Ocean. *Eur. J. Protistol.* **43:** 115–124

Gómez F., Souissi S. (2007) The distribution and life cycle of the dinoflagellate *Spatulodinium pseudonoctiluca* (Dinophyceae, Noctilucales) in the northeastern English Channel. *C. R. Biol.* **330:** 231–236

Gómez F., López-García P., Nowaczyk A., Moreira D. (2009) The crustacean parasites *Ellobiopsis* Caullery, 1910 and *Thalassomyces* Niezabitowski, 1913 form a monophyletic divergent clade within the Alveolata. *Syst. Parasitol.* **74:** 65–74

Gómez F., Moreira D., López-García P. (2010) Molecular phylogeny of noctilucoid dinoflagellates (Noctilucales, Dinophyta). *Protist* **161:** 466–478

Ki J.-C. (2010) Nuclear 28S rDNA phylogeny supports the basal placement of *Noctiluca scintillans* (Dinophyceae; Noctilucales) in dinoflagellates. *Eur. J. Protistol.* **46:** 111–120

Kofoid C. A. (1905) *Craspedotella*, a new genus of the Cystoflagellata, an example of convergence. *Bull. Mus. Comp. Zool. Harv.* **46:** 163–165

Liu L., Hastings J. W. (2007) Two different domains of the luciferase gene in the heterotrophic dinoflagellate *Noctiluca scintillans* occur as two separate genes in photosynthetic species. *Proc. Nat. Acad. Sci. U.S.A.* **104:** 696–701

Saldarriaga J. F., Taylor F. J. R., Cavalier-Smith T., Menden-Deuer S., Keeling P. J. (2004) Molecular data and the evolutionary history of dinoflagellates. *Eur. J. Protistol.* **40:** 85–111

Zhang H., Lin S. (2008) mRNA editing and spliced-leader RNA trans-splicing groups *Oxyrrhis*, *Noctiluca*, *Heterocapsa*, and *Amphidinium* as basal lineages of dinoflagellates. *J. Phycol*. **44:** 703–711

Received on 17th June, 2010; revised on 23rd August, 2010; accepted on 23rd August, 2010