PRYMNESIUM FAVEOLATUM SP. NOV. (PRYMNESIOPHYCEAE), A NEW TOXIC SPECIES FROM THE MEDITERRANEAN SEA

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PRYMNESIUM FAVEOLATUM SP. NOV.
PRYMNESIOPHYCEAE
SCALES
ULTRASTRUCTURE
TOXICITY
MEDITERRANEAN

ABSTRACT. – A new marine species of *Prymnesium* is described based on cultured material originating from a sublittoral sample collected on the eastern French Mediterranean coast. *Prymnesium faveolatum* sp. nov. exhibits typical generic characters in terms of cell morphometry, swimming mode and organelle arrangement. Organic body scales, present in several proximal layers, have a narrow inflexed rim and are ornamented with a variably developed cross. Scales in the single distal layer have an upright peripheral rim and an alveolate pattern of ridges. This new species appears to be widely distributed in the Mediterranean Sea. On the basis of scale ornamentation, two groups are recognized within those *Prymnesium* species described by electron microscopy, a separation hypothesized to be related to ploidy level. Preliminary toxicity tests indicate that *P. faveolatum* is a toxic species.

PRYMNESIUM FAVEOLATUM SP. NOV.
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Résumé. – Une nouvelle espèce marine de *Prymnesium* est décrite à partir d'une souche provenant d'un prélèvement réalisé dans la zone supralittorale sur la côte est méditerranéenne française. *Prymnesium faveolatum* sp. nov. présente les caractères du genre en ce qui concerne la forme des cellules, la nage et la disposition des différents organites. Des écailles organiques formant plusieurs couches proximales ont une marge étroite et sont ornementées d'une croix dont les extrémités sont plus ou moins ramifiées. Les écailles distales, en une seule couche, ont un bord vertical périphérique et sont ornées d'alvéoles. Cette nouvelle espèce semble bien répartie dans la Méditerranée. En se basant sur l'ornementation des écailles, il est possible de discerner deux groupes parmi les différentes espèces de *Prymnesium* étudiées au microscope électronique; l'hypothèse d'une séparation en relation avec la ploïdie est émise. Les résultats préliminaires des tests de toxicité indiquent que *P. faveolatum* est une espèce toxique.

INTRODUCTION

The genus *Prymnesium* Massart ex Conrad (Prymnesiophyceae, Haptophyta) is characterized by cells possessing two flagella, a short, non-coiling haptonema, and at least two types of organic scales covering the cell. Intensive interest in this genus has been provoked by the realisation from 1920 onwards that some of its members are the toxic agents responsible for reported fish kills in diverse regions of the world (see reviews by Moestrup 1994, Edvardsen & Paasche 1998, and references therein).

Since different species of *Prymnesium* are practically impossible to distinguish under the light microscope (LM), their identification requires, as is often the case with other haptophytes such as members of the closely related genus *Chrysochromulina* Lackey, transmission electron microscope (TEM) examination of the ornamentation of the organic scales covering the cell. Several authors have recognized the difficulty of confidently designating organisms to species originally described on the basis of LM observations only (Green *et al.* 1982, Billard 1983, Chang & Ryan 1985, Pienaar & Birkhead 1994). At present, only 6 species of *Prymnesium* have been unambiguously described through TEM studies. The first, *P. parvum* N. Carter (Manton & Leedale 1963), was followed nearly 20 years later by *P. patelliferum* Green, Hibberd et Pienaar (as *Prymnesium patellifera*, Green, Hibberd & Pienaar 1982), which has subsequently been shown by flow cytometric analysis to be in fact the alternating haploid phase of *P. parvum* (Larsen & Edvardsen 1998, Larsen 1999). Soon afterwards 3 new species were described: *P. annuliferum* Billard and *P. zebrinum* Billard (Billard 1983) and *P. calathiferum* Chang et Ryan (Chang & Ryan 1985). The last new species to be

discovered, some 10 years later, was the unusual *P. nemamethecum* Pienaar et Birkhead (Pienaar & Birkhead 1994). Excellent schematic summaries of the ornamentation of the scales of these different species are provided by Moestrup & Thomsen (1995) and Larsen (1998).

While the distal scales of each of these species has a different, and hence characteristic pattern, *P. nemamethecum* is the only one in which the proximal (body) scales also possess a specific ornamentation. In this paper we present a new species of *Prymnesium* characterized by the ornamentation of its distal scales, but also, like *P. nemamethecum*, by the pattern of its proximal scales. This new species, isolated from a sublittoral water sample collected on the French "Côte d'Azur", seems to be widely distributed in the Mediterranean Sea.

MATERIALS AND METHODS

The culture examined in this study (Algobank strain HAP79) originated from a single cell isolation from a water sample collected on the 1st May 1996 from the intertidal zone at the beach of Roquebrune Cap Martin on the eastern French Mediterranean coast and subsequently enriched with 50 % Es-TrisII medium (Cosson 1987). Two further strains are maintained in the Algobank collection: Hap79bis was isolated from a sample collected at the same locality shortly afterwards, and Hap79ter isolated by Chrétiennot-Dinet & Puigserver from a sample collected on 23rd June 1998 at 24 m water depth in the bay of Banyuls (western French Mediterranean coast). The cultures are maintained in Es-TrisII medium at ambient temperature with natural illumination from a north facing window.

Living and fixed cells were observed with a Leitz Orthoplan optical microscope equipped with differential interference contrast optics (DIC). For whole mounts, a cell suspension, after brief exposure to osmium tetroxide vapours, was mounted on 0.5 % formvar coated copper grids, rinsed, and negatively stained with 1 % aqueous uranyl acetate or shadowcasted with gold-palladium. For sectioned material the technique employed was a slight variation of that of Green *et al.* (1982). 9 ml of culture were fixed with 1ml of 25 % glutaraldehyde solution for 1.5 hours. Cells were rinsed 3 times in culture medium using gentle centrifugation and post-fixed in 2 % osmium tetroxide in 0.1M sodium cacodylate (pH 7.2) overnight. After rinsing in the same buffer solution, the pellet of cells was pre-embedded in 0.2 % purified agar, dehydrated through a graded ethanol series and embedded in Spurr's low viscosity resin. Sections, cut with a diamond knife, were double stained for 30 minutes in 2.5 % uranyl acetate in 50 % ethanol, followed by 10 minutes in Reynold's lead citrate. All preparations were examined using either a Siemens 1A or Siemens 102 TEM.

Preliminary toxicity tests were conducted using *Artemia salina* Leach nauplii, the standard reference animal for such tests, following the protocol of the ARC test of the University of Ghent, Belgium. Five 48 h old nauplii were placed in an Eppendorf tube containing 1.5 ml of dense culture of the strain to be tested. Six replicate tubes were inoculated and the experiment conducted twice, giving a test with a total of 60 *Artemia* for each algal species. The mortality of *Artemia* nauplii was visually determined after 24 h of exposure to the algae in the dark. *P. parvum* (Algobank strain Hap45, isolated in 1977 by Billard), a toxic species well known to be the causative agent of fish kills, was used as the positive control species, and *Isochrysis galbana* Parke (Algobank strain Hap34, origin Plymouth collection, 1973) as the negative control. A further control in sterile sea water medium was also conducted.

OBSERVATIONS

Diagnosis

Prymnesium faveolatum sp. nov. Fresnel

Cellulae natantes elongatae vel elongatissimae (8-9 µm × 4-5 µm), parte postica acuminata et parte antica leviter oblique truncata. Appendices, in depressione subapicale insertae. Flagella duo heterodynamica et subaequalia (16-14 µm) ad apices attenuata. Haptonema breve et spiram non formans (2-2.3 µm). Cellulae duarum formarum squamarum tectae. Pluristrata squamae corporis proximales, ellipticae vel ovales (0.38-0.41 µm × 0.28-0.30 µm) cum cristis radiantibus in quattuor quadrantes in aspectu distali et proximali visibilibus (circa 15 per quadrantem); superficies distalis planum, angustum inflatum marginem et ornamentum secondarium cruciforme plus minusve ramiforme limite quadrantium habens. Stratum distale unicum, squamas ellipticas (0.30-0.36 µm × 0.25-0.27 µm) cum cristis radiantibus in quadrantes (circa 15 per quadrantem) in aspectu distali et proximali visibilibus, superficies distalis margine verticale (0.07-0.08 µm alte) et ornamento secondario faveolato cum ramis lateralibus labro conjunctibus. Chloroplasti duo fulvi parietales per longitudinem cellulae quisque pyrenoide lentiforme immersa. Nucleus centralis inter chloroplastos, corpus Golgi parabasale. Vacuola pluries in parte postica prominens. Corpuscula mucifera peripherica. Modus natandi celeriter, cellulis circum axem longitudinem revolventibus.

Motile cells elongate to very elongate (8-9 µm × 4-5 µm), posteriorly tapered, anteriorly slightly obliquely truncated. Appendages subapically inserted. Two subequal heterodynamic flagella (14-16 µm) terminating in a hair tip. Short, non-coiling haptonema (2-2.3 µm). Cells covered by two types of scales. Several layers of proximal body scales, elliptical to oval (0.38-0.41 µm × 0.28-0.30 µm) with a radial fibrillar pattern in quadrants (about 15 lines per quadrant) on both faces, and on the distal face a narrow inflexed rim, and a central thickening forming a variably pronounced 'X' shape along the intersection of the quadrants. The single outer layer consists of elliptical scales (0.30-0.36 µm × 0.25-0.27 µm) with a pattern of radiating fibrils in quadrants (about 15 lines per quadrant) on both

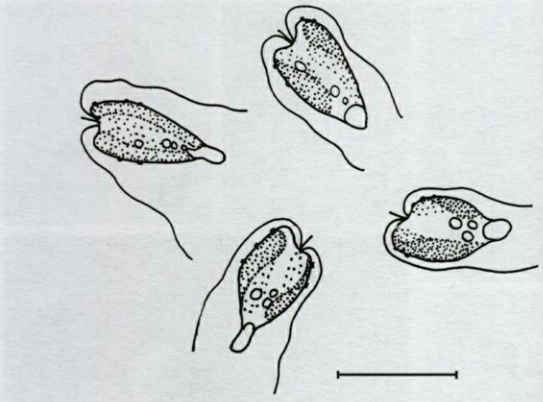

Fig. 1. – *Prymnesium faveolatum* sp. nov.; different aspects of living cells. Scale bar = 10 µm.

faces, the distal face also with a peripheral upright wall (0.07-0.08 µm high) and secondary raised alveolate ornamentation with side branches joining the rim. Two yellowish brown parietal chloroplasts, each with an immersed lenticular pyrenoid, lie along almost the entire length of the cell. Other cell contents include a median nucleus which lies in between the chloroplasts, and a parabasal Golgi body. A vacuole often prominent in the posterior part of the cell. Peripheral muciferous bodies present. Swimming motion rapid, spiralling around the long axis of the cell.

Holotype: Figures 9-17.

Origin: Sublittoral zone, Roquebrune Cap Martin, near Monaco on the French Mediterranean coast. Type material collected in 1996. Type strain: Hap79 in the Algobank collection at the University of Caen.

Etymology: Latin *faveolutus*, *-a*, *-um*, adj. meaning finely honeycombed, reflecting the precise character of the ornamentation of the distal scales.

Light Microscopy

Compared with other *Prymnesium* species, *P. faveolatum* grows extremely rapidly under our culture conditions; shortly after subculturing into fresh medium the cells aggregate into yellowish brown patches at the surface of the liquid and at the walls of the culture flask, and within days form a dense yellowish brown suspension throughout the medium. Observed at low magnification in the LM, cells exhibit a strong tendency to clump together (Fig. 2). Their very elongated, posteriorly tapered shape (Fig. 1) can clearly be seen at low magnification (Fig. 3) as at higher magnifications (Fig. 4, 7). Cells stressed after several minutes viewing under a coverslip usually become more rounded, and in old cultures cells may be spherical and often settle to the bottom of the culture flask, but remain motile (Fig. 6, 8).

Active cells of *P. faveolatum* swim very rapidly, spiralling around their long axis. Cells usually swim straight, but stop and change direction often. Sometimes cells are observed turning in tight circles. In normal swimming, the flagellar pole faces the direction of movement and the flagella trail backward along the sides of the cell (Fig. 5, 6). When, on rare occasions, the cells are observed swimming with the flagellar pole facing backward, the heterodynamic nature of flagellar movement is clearly seen; one flagellum undulates slowly, while the other beats rapidly, propulsing the cell forwards. Cells possess 2 large parietal yellowish brown to golden brown chloroplasts. A vacuole is situated in the posterior region of the cell along with droplets of reserve metabolite. In young cultures with active cells, the chloroplasts are often separated at the flagellar pole and the vacuole and metabolite droplets form a posterior prolongation to the cell (Fig. 4, 7). In older cultures, the numerous accumulations of reserves are brightly birefringent.

Normal elongated cells typically measure 8-9 × 4-5 µm. The two subequal flagella, 14-16 µm in length, and the short, non-coiling haptonema, measuring only 2-2.3 µm, are subapically inserted in an anterior groove. The ratio of flagellar length to cell length is 1.76. Encysted stages have never been observed in this species.

Electron microscopy

In TEM whole mounts, the hair tip of each flagellum and the short, non-coiling haptonema are clearly observed (Fig. 9). The scales covering the cell are of two types (Fig. 10-17). The proximal (body) scales, present in several layers, are elliptical to oval (0.38-0.41 × 0.28-0.30 µm) with a radial fibrillar pattern in quadrants (ca. 15 lines per quadrant) on both faces, and on the distal face a narrow inflexed rim (Fig. 11) and a central thickening forming an elongated "X" shape along the intersection of the quadrants (Fig. 14-16). Most often simple (Fig. 14), this central ornamentation shows considerable variability in form between scales. The points of the "X" may each be extended into two or three branches (Fig. 15), and the branches from two different points may occasionally join up to form irregular circles (Fig. 16). While not possessing a differentiated haptonematal scale as such (i.e. with a different pattern as in *P. nemamethecum*, Pienaar & Birkhead 1994), the body scales of *P. faveolatum* are typically smaller (0.30 × 0.18 µm) in the region of the flagellar insertion than on the rest of the cell (Fig. 10 and 19). Scales in the single outer layer are elliptical (0.30-0.36 × 0.25-0.27 µm) with a pattern of radiating microfibrils in quadrants on both faces (Fig. 10), the distal face also with a peripheral upright wall (0.07-0.08 µm high) which collapses in specimens viewed flat on a grid, but can clearly be seen in sections (Fig. 12), and secondary raised alveolate

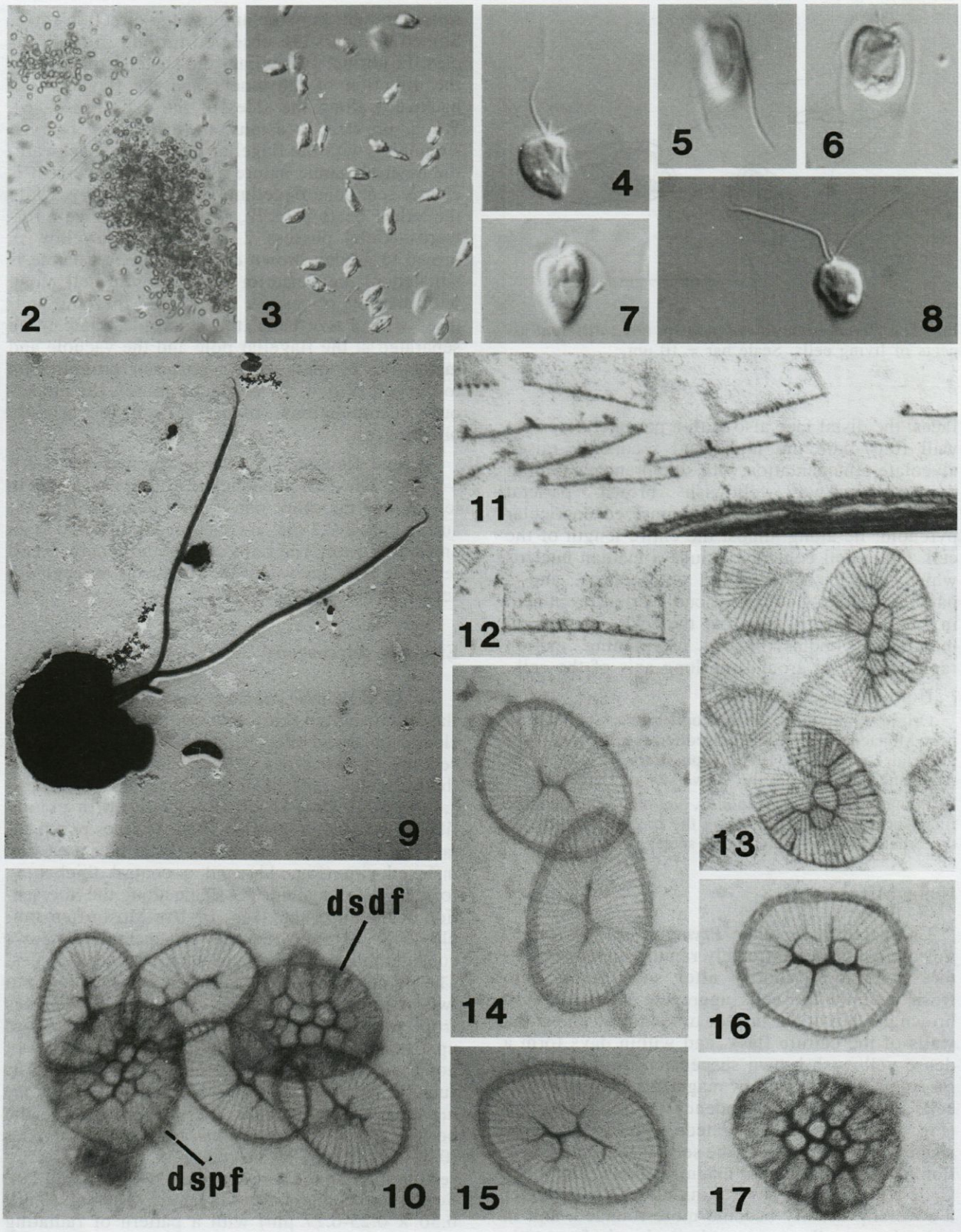

Fig. 2-17. – *Prymnesium faveolatum* sp. nov. 2-8, Light micrographs: 2, cells aggregating (× 160); 3, elongated shape of cells (× 400); 4-8, detail of living cells (× 1600). 9-17, transmission electron micrographs: 9, shadowcast (Gold/Palladium) of whole cell showing detail of flagella and haptonema (× 4400); 10-17, all × 84000: 10, 14-17 negative stained, 11-13 sections; 10, group of scales: two distal scales showing distal face (dsdf) and proximal face (dspf), and small body scales from the haptonematal region; 11-12, cross sections of two scale types; 13, tangential section of scales; 14-16, body scales showing increasing complexity of central thickening; 17, distal scale distal face with alveolate ridges and collapsed rim.

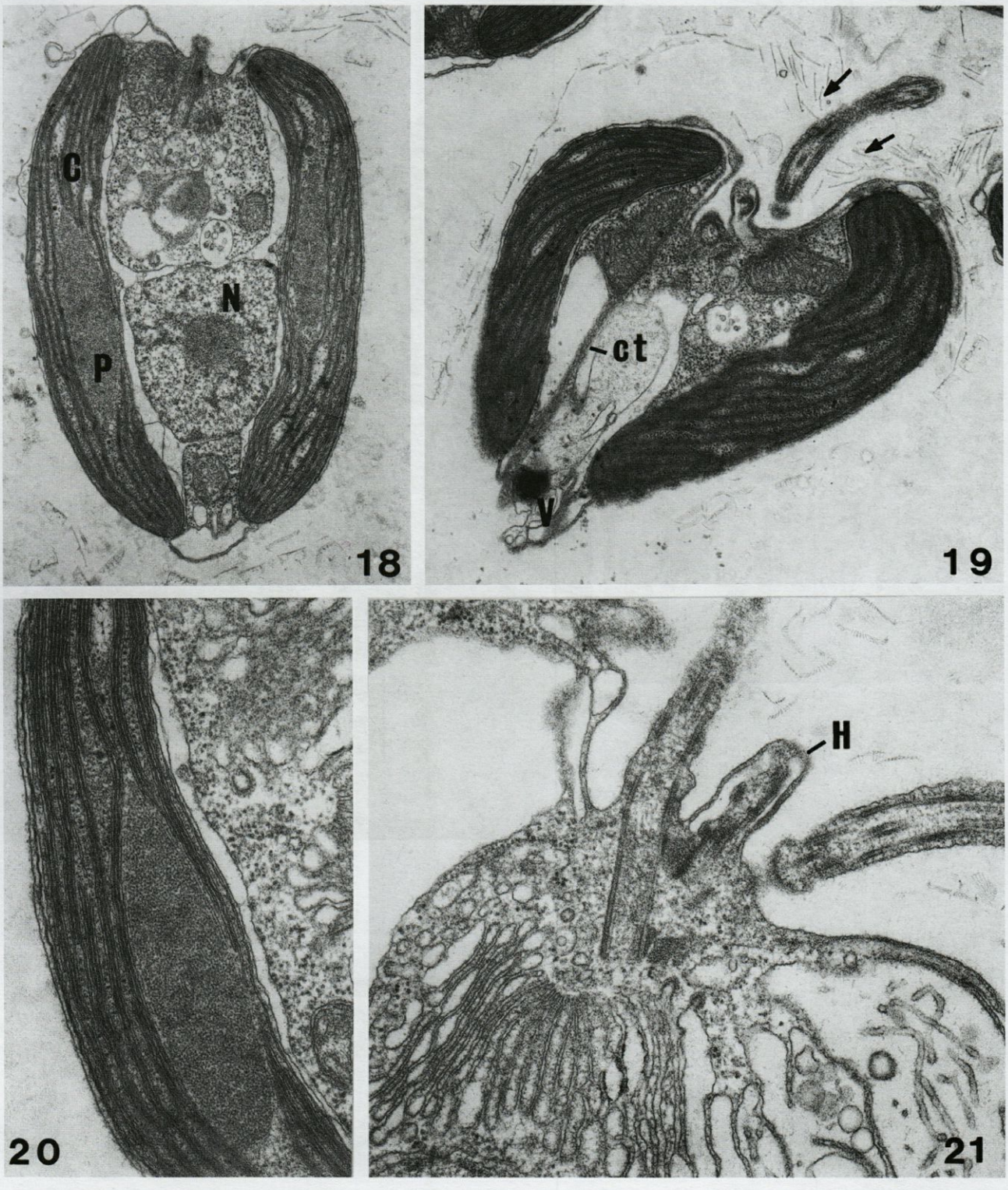

Fig. 18-21. – *Prymnesium faveolatum* sp. nov. 18, longitudinal section of whole cell showing the nucleo-plastidial complex: median nucleus (N) and two parietal chloroplasts (C) with immersed pyrenoid (P)(× 15500); 19, oblique section showing flagellar apparatus in apical groove with several layers of small scales (arrows) surrounding emergent flagella, posterior vacuole (V). Note the cytoplasmic tongue (ct) which extends through the cell (× 15500); 20, detail of immersed pyrenoid (× 42000); 21, section of apical zone showing Golgi body surrounding the flagellar bases, and emergent haptonema (H) (× 38000).

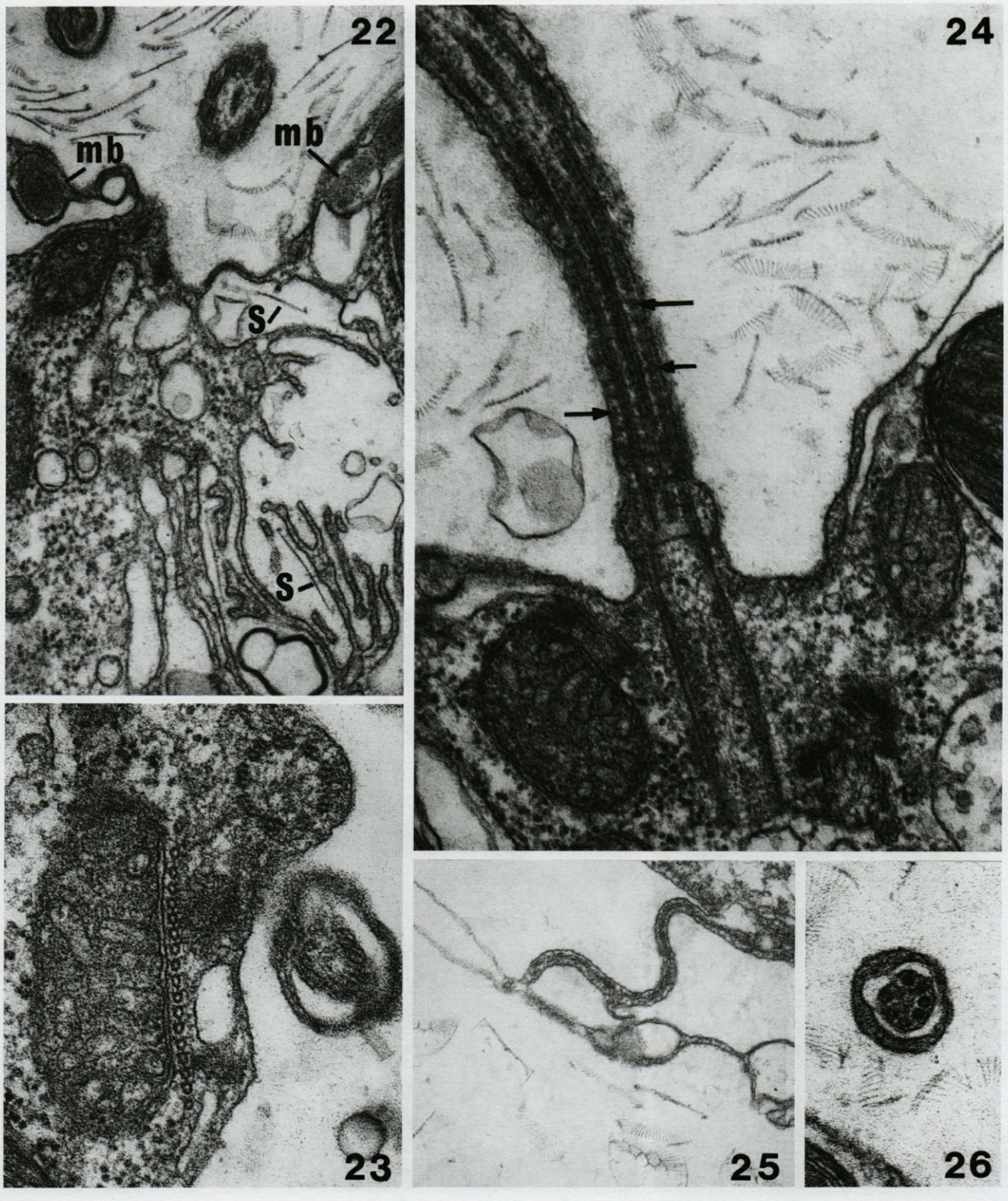

Fig. 22-26. – *Prymnesium faveolatum* sp. nov. 22, Forming scales (S) in Golgi vesicles in the region of flagellar insertion, and two muciferous bodies (mb) (× 47000); 23, cross section of sheet of microtubules of root 1 passing over the surface of a mitochondrion (× 65000); 24, longitudinal section of a flagellum showing tubular rings under the flagellar membrane (arrows), above the transitional region (× 58800); 25, part of the cytoplasmic tongue attached to the plasma membrane (× 42000); 26, cross section of emergent haptonema showing 7 microtubules surrounded by a sheath of the endoplasmic reticulum (× 82000).

ornamentation with side branches joining the rim (Fig. 17). This honeycomb pattern of the distal scales is the main distinguishing feature of the species and is the derivative of its name.

Thin sections reveal ultrastructural features typical of the Haptophyta and very similar to that of other *Prymnesium* species (Manton & Leedale 1963, Green *et al.* 1982, Billard 1983, Chang & Ryan 1985). The two chloroplasts each contain an immersed lenticular pyrenoid (Fig. 18, 20) in which fragments of traversing thylakoids are occasionally observed. The chloroplast membrane is continuous with that of the median nucleus (Fig. 18), the cell also containing numerous mitochondrial profiles. The Golgi body, which possesses intercalary dilations typical of haptophytes, is polarized around the flagellar bases (Fig. 21). Scales are formed in Golgi cisternae and released in the region of the flagellar insertion (Fig. 22). Few muciferous bodies are present under the surface of the cell membrane (Fig. 22). The flagella and haptonema emerge from an anterior subapical groove (Fig. 19, 21). The flagellar root 1 (R1) is simple, consisting of a sheet of ca. 18 microtubules which pass over the surface of a mitochondrial profile (Fig. 23), with no associated crystalline root. A continuation of this root, the cytoplasmic tongue (Gayral & Fresnel 1983), extends deep into the cell (Fig. 19), emerging far from the flagellar bases where it may be observed in contact with the plasma membrane (Fig. 25). In longitudinal sections of flagella, tubular rings are observed above the transitional region between the outer doublets and the flagellar membrane (Fig. 24), as described by Birkhead & Pienaar (1994). The emergent haptonema consists of 7 microtubules surrounded by a sheath of endoplasmic reticulum (Fig. 26).

Toxicity test

The mortality rates of *Artemia* (60 nauplii) after 24 h exposure to cultures of the different microalgal species were: 96.6 % for *P. parvum* (positive control), 75 % for *P. faveolatum*, 3.3 % for *I. galbana* (negative control), and 0 % in medium only.

Geographic distribution

First seen in a sample from the coast of Malta in 1986 (collected by JFs mother), then in 1987 from the Island of Spetsae in eastern Greece (collected by P. Griveau, friend). Observed for the first time at Roquebrune Cap Martin, France in 1989 (mixed with *P. zebrinum*) and the type culture isolated from a sample from the same location taken in 1996. Subsequently identified in samples from the bay of Banyuls, France in 1998 (by M.-J. Chrétiennot-Dinet), and from Blanes, northeastern Spain, also in 1998 and in 2000. *P. faveolatum* is thus found in diverse regions of the Mediterranean Sea. Having had the opportunity to collect sea water samples several times each year since 1980 at Roquebrune Cap Martin, it is noteworthy that after the first observation of this species at this locality in 1989, it has systematically been present only since 1996. *P. calathiferum* and *Prymnesium* sp. (=Algobank strain Hap53) are common in this part of the Mediterranean, and infrequently we have also observed *P. zebrinum* and *P. annuliferum*. Our observations of *P. faveolatum* over the last 15 years suggest a progressive westward spreading of this species in the Mediterranean (Aegean Sea/ French coast/Spanish coast).

DISCUSSION

This new species clearly belongs to the genus *Prymnesium*, the generic characters easily discernible under both the LM (cell shape, short and non-coiling haptonema, swimming mode, and absence of a dominant non-motile life stage) and EM (general arrangement and fine structure of organelles). The ornamentation of the distal scales of *P. faveolatum*, finely alveolated on their distal face, characterizes this new species, and is sufficient to differentiate it from the 6 other species described by TEM studies. The ornamentation of both proximal and distal scales of *P. faveolatum* is schematically summarized in Fig. 27. Of the species described before the advent of electron microscopy, cell size and shape distinguish *P. faveolatum* from the much smaller *P. minutum* N. Carter and the continental species *P. czosnowskii* (Czosnowski) Starmach, the latter also being globose (Green *et al.* 1982). Differences from the remaining (and type) species, *P. saltans* Massart ex Conrad, are less clear, the swimming mode providing the most obvious distinguishing character, that of *P. saltans* having been described as typically erratic and jerky with flagella extended obliquely forward (Conrad 1941, Heynig 1978). In addition, the haptonema of *P. faveolatum* is apparently markedly shorter than that of *P. saltans*, and the latter species has been reported from brackish waters, whereas *P. faveolatum* is in our experience exclusively marine.

Although *P. faveolatum* can be considered a typical *Prymnesium* species in terms of possessing only two types of organic scales compared to *P. nemamethecum* which has three types, it resembles the latter species in that these are the only two *Prymnesium* species described to date which have a specific pattern on the body proximal scales as well as the distal scales. In this respect, it is worth noting that while this body scale pattern is typically significantly different between these two species,

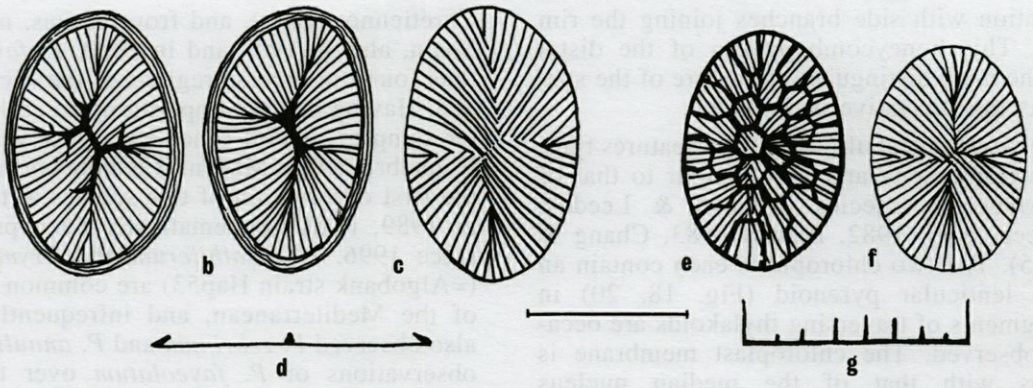

Fig. 27. – *Prymnesium faveolatum* sp. nov. Schematic drawing of scale ornamentation: (a-d) proximal (body) scales: (a,b) varying ornamentation of distal face (c) proximal face (d) transverse section showing narrow inflexed rim and central thickening. (e-g) distal scales: (e) distal face showing alveolate pattern (f) proximal face (g) transverse section showing upright peripheral rim and ridges of alveolate ornamentation. Scale bar = 0.25 μm.

the more complex crosses of *P. faveolatum* (this character shows considerable variability in both species) do resemble the simplest pattern in *P. nemamethecum* (compare our Fig. 15 with Fig. 15 in Pienaar & Birkhead 1994). The number of microfibrils per quadrant is, however, lower on both proximal and distal scales in *P. faveolatum* compared with *P. nemamethecum* (ca. 15 compared with ca. 30 respectively). These two species also differ in an important ultrastructural feature; the R1 of *P. faveolatum*, like that of *P. patelliferum* (Green & Hori 1990) is simple, while that of *P. nemamethecum* has an associated crystalline root (Birkhead & Pienaar 1994).

With the addition of this new species, two groups of *Prymnesium* can clearly be defined in terms of body scale ornamentation: (1) species having body scales with very narrow rims and radiating microfibrils on both faces; (2) species having body scales with a relatively wide inflexed rim, radial microfibrils on the proximal face, but also with a pattern of concentric ridges on the distal face (see schematic drawings of Larsen 1998). The first group includes *P. patelliferum* (=*P. parvum*, *patelliferum* stage), *P. nemamethecum*, and *P. faveolatum* (the latter two species also having secondary ornamentation on the distal surface of body scales). The second group comprises *P. parvum*, *P. annuliferum*, *P. zebrinum*, and *P. calathiferum*.

The pattern of the body scales of members of the closely related genus *Platychrysis* Geitler also fit into these groups, with *P. pigra* Geitler (Chrétiennot 1973) and *P. pienaarii* Gayral et Fresnel being the *P. parvum* type (group 2) and *P. simplex* Gayral et Fresnel the *P. patelliferum* type (group 1) (Gayral & Fresnel 1983). We have suspected for some time a digenetic cycle involving the latter two *Platychrysis* species, and the demonstration of such a cycle with *P. parvum* alternating with, and diploid relative to, the haploid *P. patelliferum* (Larsen 1999), obviously lends indirect support to this idea. Further evidence comes from the body scale ornamentation of two further new *Prymnesium* species isolated from Mediterranean waters (Fresnel *et al.* in prep.). It seems very plausible that, as hypothesized for the coccolithophores (Fresnel 1994), scale ornamentation may be an indicator of ploidy level. We predict that *P. faveolatum* may thus eventually be joined in a life cycle with another group 2 (diploid) species.

In our experience the appearance of cysts in *Prymnesium* remains mysterious. Over several decades we have observed this phenomenon only twice; in *P. nemamethecum*, in which these stages were not reported by Pienaar & Birkhead (1994), and in Algobank strain Hap64, *Prymnesium* sp. In each case, the cysts appeared only briefly and on one occasion only. We have not seen cysts in cultures of other *Prymnesium* species, not even *P. patelliferum*, where these stages have been reported, but may be enhanced by higher salinities (Green *et al.* 1982).

Molecular genetic analyses of the small subunit 18SrRNA gene of a limited number of species within the family Prymnesiaceae indicate that *Chrysochromulina* is a polyphyletic genus, one sub-group forming a clade with *Prymnesium*, separate from another sub-group (Edvardsen *et al.* 2000). Detailed ultrastructural studies, including particularly reconstructions of the flagellar root system, have been undertaken for only relatively few members of this family, e.g. *Chrysochromulina apheles* Moestrup et Thomsen (Moestrup & Thomsen 1986); *C. scutellum* Eikrem et Moestrup (Eikrem & Moestrup 1998); *C. ahrengotii* M. Ø. Jensen et Moestrup (Jensen & Moestrup 1999); *P. patelliferum* (Green & Hori 1990), *P. nemamethecum* (Birkhead & Pienaar 1994). Since we currently maintain cultures of all described *Prymnesium* species except *P. nemamethecum* (our culture was lost, but this species was examined in detail by Birkhead & Pienaar 1994), as well as cer-

tain undescribed *Prymnesium* and all three described *Platychrysis* species, further molecular genetic and detailed ultrastructural examinations seem worthwhile in order to attempt to elucidate phylogenetic relationships within this group.

Toxicity tests previously carried out in our laboratory on cultures of various *Prymnesium* species (Lhuissier, Poncet unpubl results) have shown that the slow growing *P. zebrinum* is toxic to *Artemia*, while *P. annuliferum*, which grows very rapidly and produces dense cultures, shows no signs of toxicity. *P. faveolatum* is also a toxic species, the level of toxicity in our tests being somewhat lower than that of *P. parvum*, a species which has been reported on several occasions to be responsible for fish kills (see review by Edvardsen & Paasche 1998). To our knowledge, *P. faveolatum* has not been linked with any natural toxic events.

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