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CRYPTOGAMIE

ALGOLOGIE

TOME 15 FASCICULE 2 1994

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OBSERVATIONS ON THE GENUS *PLAGIOSELMIS* (CRYPTOPHYCEAE)

Gianfranco NOVARINO (1, 3), Ian A.N. LUCAS(1) and Susan MORRALL (2, 4)

(1) University of Wales (Bangor), School of Ocean Sciences, Marine Science Laboratories, Menai Bridge, Gwynedd LL59 5EY, U.K.
(2) Imperial College, Botany Department, Prince Consort Road, London SW7, U.K.
(3) Present address: Microbiology Group, Department of Zoology, The Natural History Museum, Cromwell Road, London SW7 5BD, U.K.
(4) Present address: 87 Esmond Road, London W4 IJE, U.K.

ABSTRACT - The description of the genus *Plagioselmis* Butcher (Cryptophyceae) is emended based upon the type species, *Plagioselmis prolonga* sp. nov., *P. prolonga* var. *nordica* var. nov., and *P. nannoplanctica* (Skuja) comb. et stat. nov. The genus belongs to the order Cryptomonadales Novarino et Lucas owing to the fact that the nucleomorph is positioned outside the pyrenoid. The principal feature of the genus is given by the presence of a tail, a posterior appendage lacking the discrete hexagonal periplast areas found on the main portion of the cell body. Taxa of *Plagioselmis* can be distinguished from one another based upon the presence or absence of a ventral sulcus, relative flagellar length, presence or absence of body scales, position of the pyrenoid and nucleus, chloroplast shape, cell size, size of the periplast areas, contents of the tail, and habitat. *Plagioselmis prolonga* contains phycoerythrin I, as does *P. nannoplanctica*. The phycocyanin-containing species *Chroomonas acuta* Utermöhl may belong to the genus *Plagioselmis*, since published SEM micrographs have shown that it possesses the tail typical of the genus. The status of the genus *Isoselmis* Butcher, originally described as being similar to *Plagioselmis*, is uncertain and the generic name *Isoselmis* may represent a nomen dubium which cannot be applied to any taxon.

RÉSUMÉ - Le genre *Plagioselmis* (Cryptophycées) est redécrit à partir d'observations effectuées sur *Plagioselmis prolonga* sp. nov. (espèce-type), *P. prolonga* var. *nordica* var. nov., et *P. nannoplanctica* (Skuja) comb. et stat. nov.. Le genre appartient à l'ordre des Cryptomonadales Novarino et Lucas, caractérisé par la position du nucléomorphe (à l'extérieur du pyrénoïde). La caractéristique principale du genre est donnée par la présence d'une queue postérieure, dont le périplaste est dépourvu des aires hexagonales présentes dans la région principale du corps cellulaire. Les taxons de *Plagioselmis* se distinguent l'un de l'autre par la présence ou absence d'un sulcus ventral, la longueur relative des flagelles, la présence ou absence d'écailles sur le corps cellulaire, la position du pyrénoïde et du noyau, la morphologie du chloroplaste, la taille des cellules et des aires du périplaste, le contenu de la queue, et l'habitat. *Plagioselmis prolonga* et *P. nannoplanctica* contiennent comme pigment accessoire la phycoérythrine I. La cryptomonadine à phycocyanine *Chroomonas acuta* Utermöhl pourrait appartenir au genre *Plagioselmis*, car des micrographies au MEB disponibles dans la littérature ont montré que cette espèce possède la queue typique de *Plagioselmis*. Le genre *Isoselmis* Butcher, décrit par son auteur comme étant semblable à*Plagioselmis*, est à qualifier de 'douteux'.

KEY WORDS: Cryptomonads, Cryptophyceae, fine-structure, *Plagioselmis nannoplanctica* comb. et stat. nov., *Plagioselmis prolonga* sp. nov., *Plagioselmis prolonga* var. *nordica* var. nov., taxonomy.

INTRODUCTION

The genus *Plagioselmis* (Cryptophyceae) was described by Butcher (1967), together with another genus (*Isoselmis*) which Butcher considered as being closely allied to *Plagioselmis*. Butcher based the genus *Plagioselmis* on two phycoerythrin-containing marine species, *P. prolonga* Butcher and *P. punctata* Butcher. He omitted to designate a type species, but this was indicated by Chrétiennot-Dinet (1990) as *P. prolonga*. Since the original description of *Plagioselmis*, there have been only few reports of cryptomonads identified as belonging to that genus (Chang, 1983; Throndsen, 1983; Throndsen & Kristiansen, 1988; Novarino, 1991b; Hill, 1992; Kuylenstierna & Karlson, 1994). Although some information is available on the nucleomorph (Morrall & Greenwood, 1982), the phycoerythrin pigment (Hill & Rowan, 1989), the periplast (Novarino, 1991b; Kuylenstierna & Karlson, 1994), and the morphology of the vestibular region from which the flagella arise (Hill, 1992; Kuylenstierna & Karlson, 1994), *Plagioselmis* is among the least known genera of cryptomonads, and information on its fine-structure and species-level taxonomy is as yet unavailable.

Very few culture strains of *Plagioselmis* are available at present. Butcher (1967) based his *P. punctata* on strain no. 172 from the Plymouth Culture Collection, U. K. (PLY), which had been isolated by Mary Parke in 1957 from St Germains River, U. K.. That strain is no longer listed in the Plymouth collection catalogue, but there is a more recent one which also bears the name *P. punctata* Butcher. This strain (no. 172a) was isolated by R. Jowett in 1969 from the type-locality of *P. punctata*; it was included in fine-structural studies by Morrall (1980) and Morrall & Greenwood (1982). A study by Hill & Rowan (1989) lists a strain named *P. prolonga* Butcher from Melbourne University Culture Collection (MUCC no. Cr0ll).

We have examined strain PLY 172a and carried out further observations on specimens from the North Sea plankton previously identified as *Plagioselmis* sp. (Novarino, 1991b). We have also examined a freshwater strain previously identified as belonging to the genus *Rhodomonas* Karsten. As a result, the diagnosis of the genus *Plagioselmis* is emended; Butcher's diagnosis of *Plagioselmis prolonga* is validated by designating a lectotype; a new variety and a new combination in the genus *Plagioselmis* are described, and the possibility is discussed that *Chroomonas acuta* Utermöhl may belong to the genus *Plagioselmis*.

MATERIAL AND METHODS

Light and electron microscopical observations were carried out on the marine strain no. 172a from the Plymouth Culture Collection, U. K. (PLY), listed in the collection catalogue as *P. punctata* Butcher; and the freshwater strain no. N750301 from the collection of Dr Dag Klaveness, University of Oslo (DK), originally named *Rhodomonas lacustris* (Pascher et Ruttner) Javornicky (Klaveness, 1981). Cultures were maintained as described by Morrall (1980) and Novarino (1991a, b). For light microscopy (LM), it was necessary to fix the rapidly swimming cells with Lugol's iodine. Observations were carried out using bright-field and phase-contrast Zeiss Neofluar and planapochromatic objectives (x40 and x100).

For scanning electron microscopy (SEM), strain PLY 172a was prepared and observed as described by Novarino (1991a), except that the fixation schedule was preceded by a pre-fixation step (3 additions of 10 drops of buffered glutaraldehyde to 15 ml of uncentrifuged culture over a period of 30 mins); strain DK N750301 was prepared as described elsewhere for *Cryptomonas marssonii* (Novarino, 1991b). Measurements of whole cells and periplast areas were taken as described by Novarino (1991a, b) and Novarino & Lucas (1993a).

For transmission electron microscopy (TEM), strain PLY 172a was prepared and observed as described by Morrall & Greenwood (1982); strain DK N750301 could not be fixed satisfactorily for TEM, but information on the internal cell structure of this strain is available from a study by Klaveness (1981).

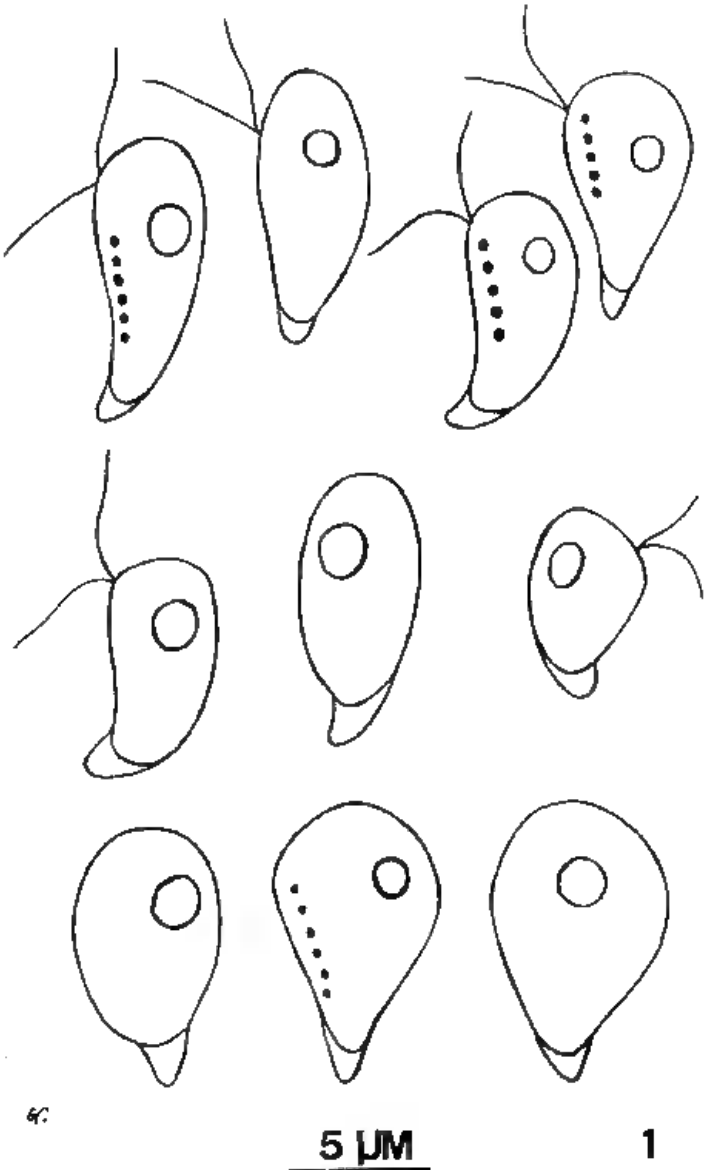


Fig. 1. Lugol-fixed cells of *Plagioselmis prolonga* PLY 172a as seen with the LM. Note the anterior pyrenoid and the posterior hyaline tail.

Further observations were carried out on *Plagioselmis* sp. from the North Sea plankton, prepared and observed as described by Novarino (1991b).

The phycoerythrin pigment of strain PLY 172a was extracted and characterized using a standard procedure (see Novarino & Lucas, 1993a).

ABBREVIATIONS

The following abbreviations are used in the text: CPD = critical point-drying, IPC = internal periplast component, LM = light microscope, PAs = periplast areas, PM = plasma membrane, SEM = scanning electron microscope, TEM = transmission electron microscope.

TAXONOMY

Plagioselmis Butcher emend. Novarino, Lucas et Morrall

Description: Cryptomonadales Novarino & Lucas 1993b (non Cryptomonadales Pascher nec Cryptomonadales auctorum). Cells with apically or subapically attached flagella arising from a vestibular depression with or without a ventral sulcus; with a posterior, often ventrally bent tail variable in shape, length and contents; with a single chloroplast bearing a pyrenoid not traversed by thylakoids. Nucleomorph positioned outside the pyrenoid. Periplast with discrete, hexagonal periplast areas in the main portion of the cell body; discrete periplast areas absent in the region of the tail.

Habitat: marine and freshwater.

Lectotype species: *Plagioselmis prolonga* Butcher ex Novarino, Lucas et Morrall.

Taxa of *Plagioselmis*

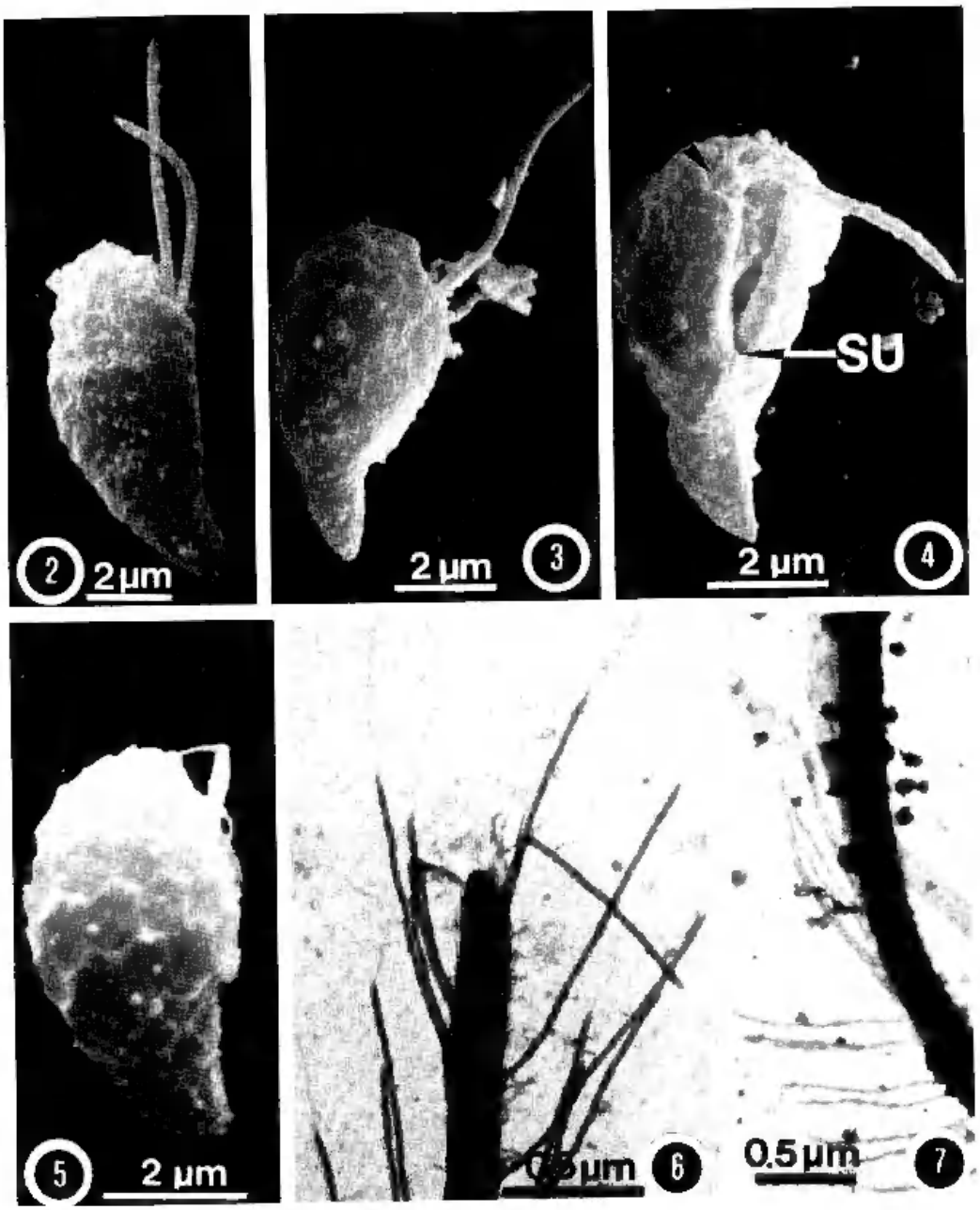
Plagioselmis prolonga Butcher ex Novarino, Lucas et Morrall sp. nov.

(Figs 1 - 18)

Synonym: *Plagioselmis prolonga* Butcher 1967, *Fishery Invest. Lond., Ser. IV*: 18, pl. I fig. 9, pl. XIV fig. 2, typ. non desig.; Hill 1992, p. 165, figs 1 A - P; Kuylenstierna & Karlson 1994, p. 22, figs 8a, b.

Allied taxa: *Plagioselmis prolonga* forma *japonica* Throndsen 1983, p. 5, fig. 11; *Plagioselmis* sp. 'B' Throndsen 1983, p. 5, figs 9, 10; *Plagioselmis punctata* Butcher 1967, p. 19, pl. I fig. 10, pl. XIV fig. 3; *Chroomonas* sp. Andreoli *et al.* 1986, figs 1 - 6; *Chroomonas* sp. Bisalputra *et al.* 1973, fig. 14; *Cryptomonas* sp. Booth *et al.* 1982, fig. 21.

Description: Lugol-fixed, non critical point-dried cells uncompressed, on average 8.6 µm long (SD = 1.15, n = 39) and 4.7 µm thick (SD = 0.77, n = 39). Flagella subequal, 1/2 - 2/2 the cell length. Ventral sulcus present. Tail 1/7 - 1/3 the cell length, usually acute, conical or laterally flattened, containing a mitochondrial profile. Chloroplast dorsal, c-shaped, with ventral margins not extending deeply into the ventral region of the cell, containing phycoerythrin I (535 - 545 nm) as principal accessory pigment. Pyrenoid anterior, small, with a thin starch sheath. Nucleus central. Partially overlapping scales ca. 80 nm in diameter present on the external face of the plasma membrane. Side of the periplast areas

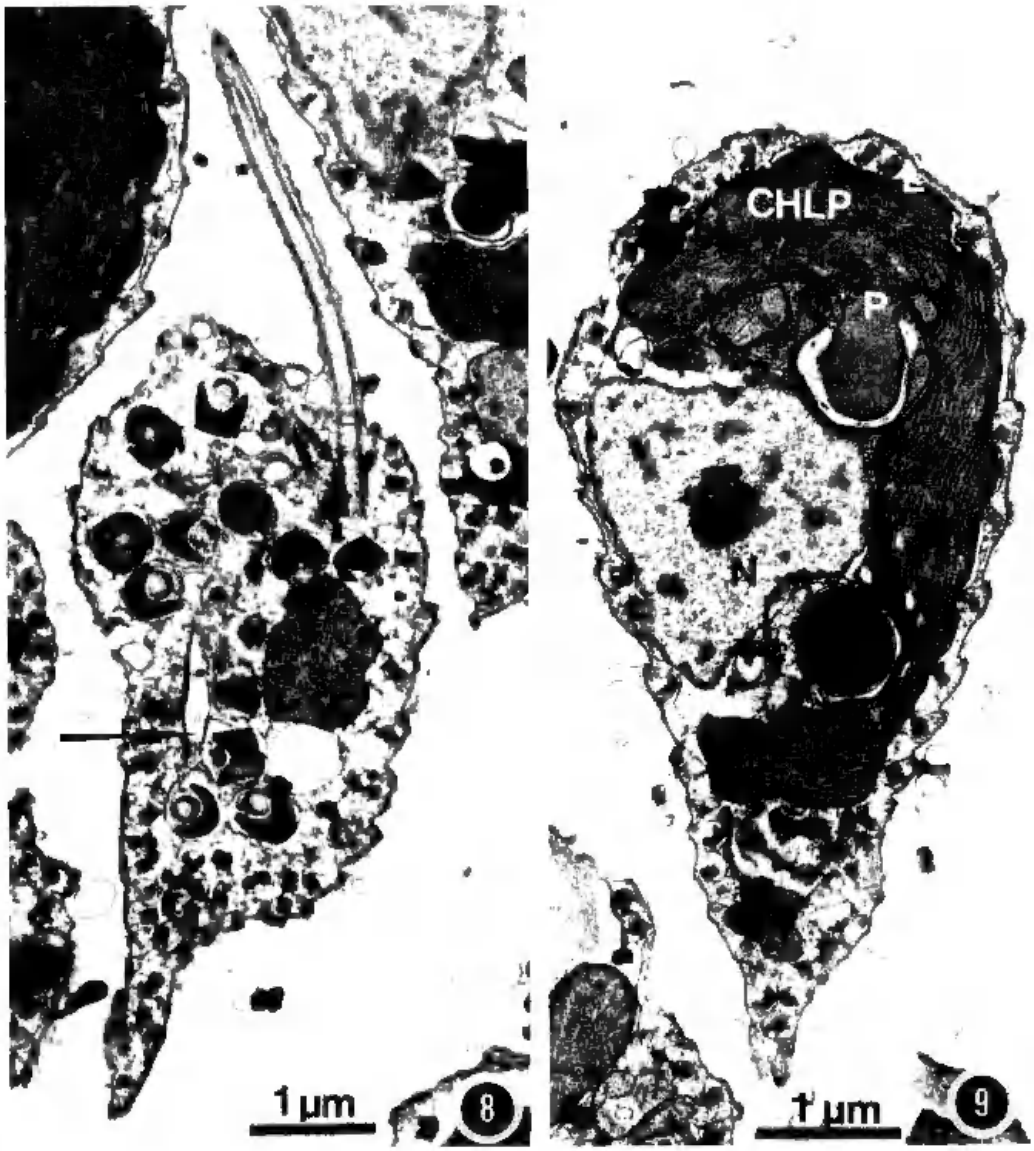


Figs 2 - 7. *Plagioselmis prolonga* PLY 172a, SEM, pre-glutaraldehyde / glutaraldehyde / osmium / CPD, and TEM, uranyl-stained whole-mounts. Fig. 2. Lateral view. Fig. 3. Dorso-lateral view, showing hexagonal periplast areas on the main portion of the cell body only. Fig. 4. Ventral view; note the position of the vestibular depression (arrowhead) with respect to the ventral sulcus (SU). Fig. 5. Dorsal view; note the hexagonal periplast areas on the main portion of the cell body only. Fig. 6. Whole-mount of the dorsal flagellum; note the bilateral array of tubular hairs. Fig. 7. Whole-mount of the ventral flagellum; note the unilateral array of tubular hairs.

on average 0.39 µm long (SD = 0.087, n = 30), in chemically fixed, dehydrated and critical point-dried cells examined by scanning electron microscopy.

Habitat: marine.

Lectotype: Butcher 1967, pl. I fig. 9.



Figs 8, 9. *Plagioselmis prolonga* PLY 172a, TEM. Fig. 8. Longitudinal section showing the position of the flagella with respect to the gullet (arrow). Fig. 9. Longitudinal section showing chloroplast (CHLP), pyrenoid (P), and nucleus (N).

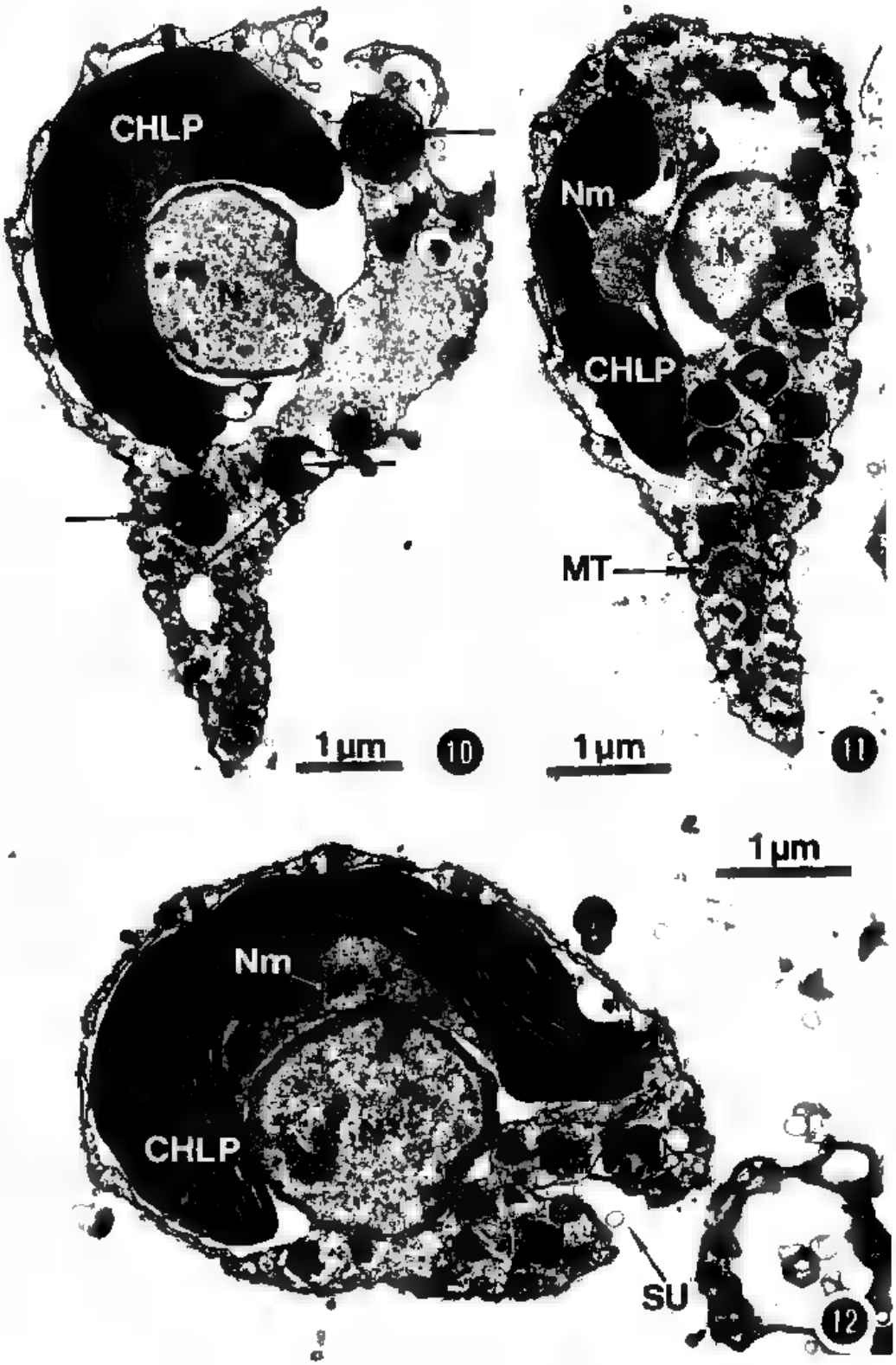
Plagioselmis prolonga var. *nordica* Novarino, Lucas et Morrall var. nov.

(Figs 19, 20)

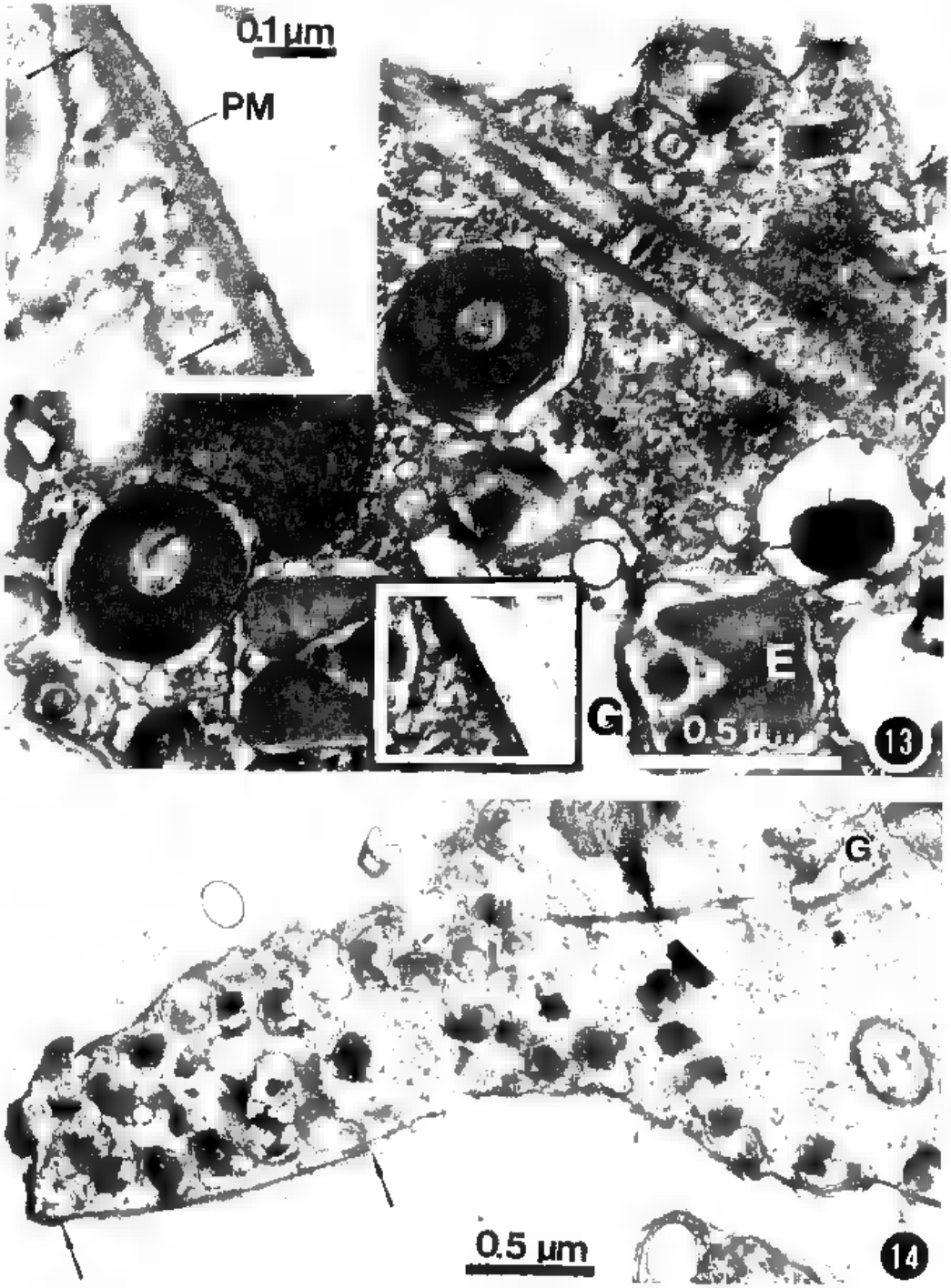
Synonym: *Plagioselmis* sp. Novarino 1991b, p. 602, figs 3, 4.

Allied taxa: *Plagioselmis* sp. 'A' Throndsen 1983, p. 5, figs 7, 8. *Cryptomonas acuta* sensu Chang 1983, fig. 7A.

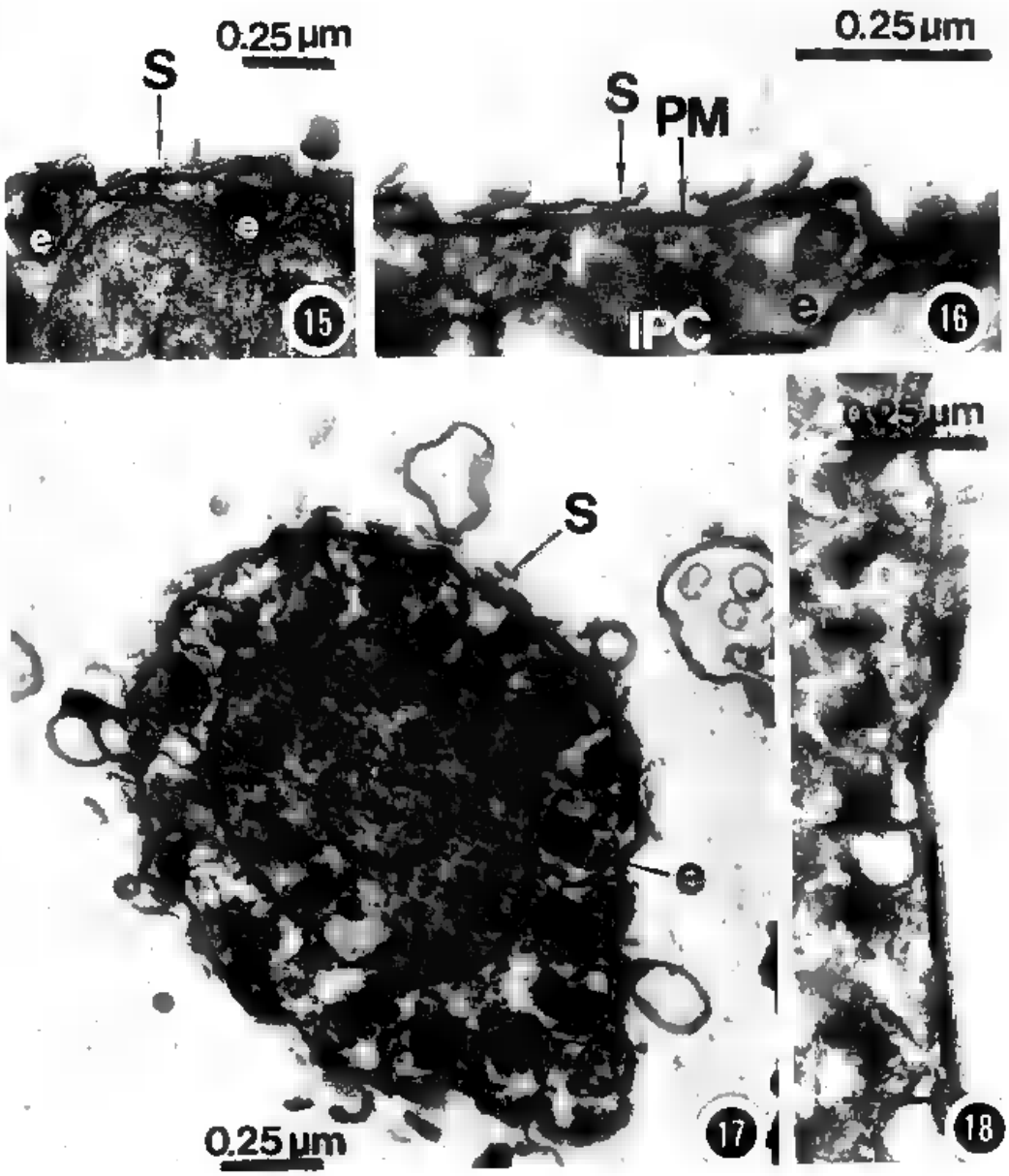
Diagnosis: *Cellulae sine sulco, non compressae, in media 5.8 µm longae (SD = 0.8, n = 6) et 2.6 µm crassae (SD = 0.47, n = 3), in speciminibus fixatis, dehydratatis et ad punctum criticale exsiccatis; flagellis circiter 4/3 longitudinis cellulae; areis periplasti in media 0.31 µm latis (SD = 0.041, n = 15) in speciminibus fixatis, dehydratatis et ad*



Figs 10 - 12. *Plagioselmis prolonga* PLY 172a, TEM. Fig. 10. Longitudinal section showing chloroplast (CHLP), nucleus (N), and circular mitochondrial profiles (arrows). Fig. 11. Longitudinal section showing chloroplast (CHLP), nucleus (N), nucleomorph (Nm), and mitochondrial profile (MT) inside the tail. Fig. 12. Median transverse section showing chloroplast (CHLP), nucleus (N), nucleomorph (Nm) and ventral sulcus (SU).



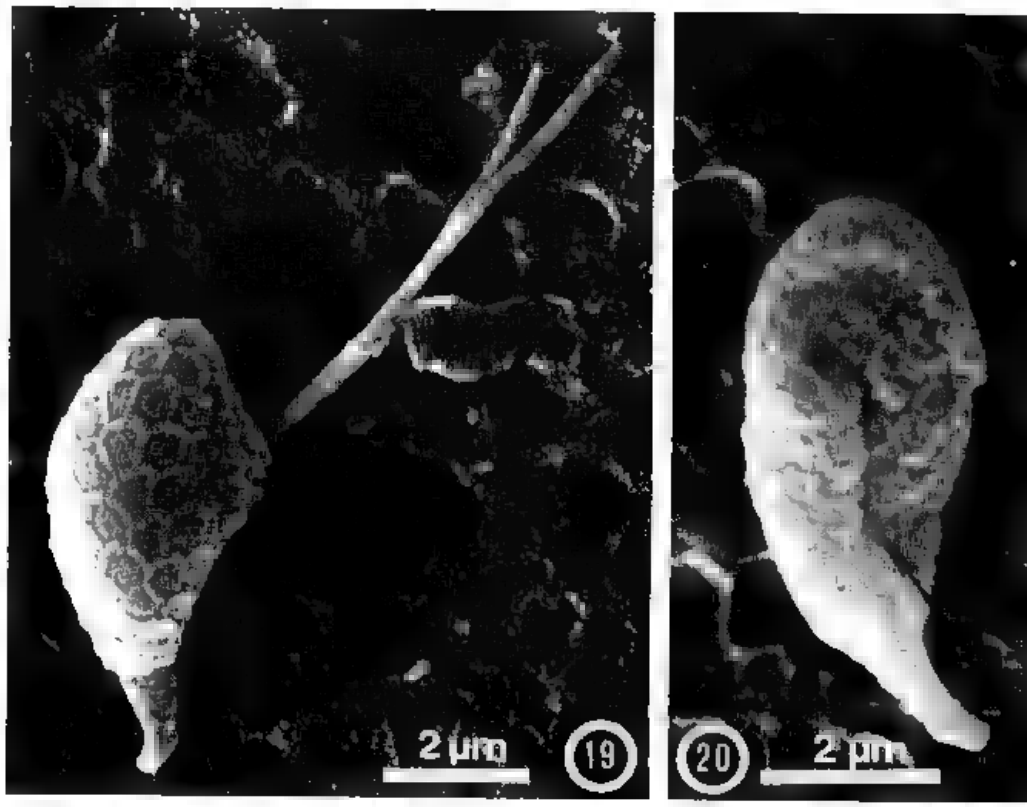
Figs 13, 14. *Plagioselmis prolonga* PLY 172a, TEM. Fig. 13. Longitudinal section in the anterior region of the cell; in the inset, which is a x2 photographic enlargement of the boxed region of the gullet (G), note the electron-opaque band underneath the membrane (PM) on one side of the gullet only (arrows); E = large ejectosome vesicle. Fig. 14. Longitudinal section showing that the periplast of the tail (arrows) is not subdivided into discrete areas; note also the (presumably fibrous) band extending from the gullet (G) towards the tail (arrowhead).



Figs 15 - 18. *Plagioselmis prolonga* PLY 172a, TEM. Figs 15, 16. Sections of the periplast on the main portion of the cell body, showing a single layer of scales (S) on the external face of the plasma membrane (PM), and an electron-dense internal component (IPC), which is subdivided into discrete areas (arrowheads); e = small ejectosome vesicles. Fig. 17. Transverse section of a tail, showing a large mitochondrial profile; note external scales (S) and small ejectosome vesicles (e) bulging towards the cell surface. Fig. 18. Longitudinal section of a tail, showing a continuous internal periplast layer (arrows); note that external scales ▬ absent.

punctum criticale exsiccatis. Cauda circiter 1/5 longitudinis cellulae. Holotypus: Figura 19.

Cells without a sulcus, uncompressed, on average 5.8 µm long (SD = 0.8, n = 6) and 2.6 µm thick (SD = 0.47, n = 3), in chemically fixed, dehydrated and critical point-dried specimens examined by scanning electron microscopy; flagella about 4/3 the cell



Figs 19, 20. *Plagioselmis prolonga* var. *nordica*, North Sea, SEM, Lugol / cold osmium / CPD. Fig. 19. (holotype). Lateral view. Fig. 20. Ventral view; the ventral fold is interpreted as a shrinkage artefact.

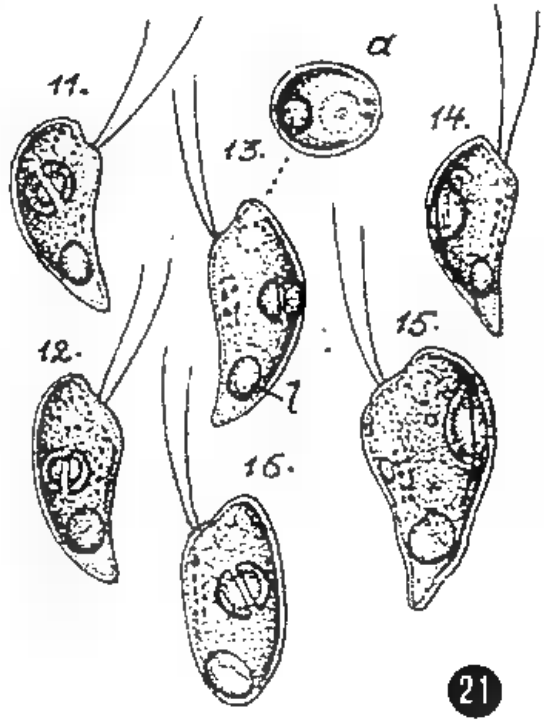


Fig. 21. Reproductions of Skuja's (1948) illustrations of *Rhodomonas minuta* var. *nannoplanctica*, twice the size of the originals.

length; side of the periplast areas on average 0.31 µm long (SD = 0.041, n = 15), in chemically fixed, dehydrated and critical point-dried cells examined by scanning electron microscopy. Tail about 1/5 the cell length.

Holotype: Fig. 19, from a plankton sample from the southern North Sea, collected on 8/8/1988 at 4 m depth from station BG during cruise no. 33 of the U. K. Natural Environment Research Council (NERC) North Sea Community Project 1988/89.

Plagioselmis nannoplanctica (Skuja) Novarino, Lucas et Morrall comb. et stat. nov. (Figs 21 - 26)

Basionym: *Rhodomonas minuta* var. *nannoplanctica* Skuja 1948, *Symbolae Botanicae Upsalienses*, 9: 347, pl. XXXVII, figs 11 - 15 (excl. fig. 16); Kristiansen 1959, p. 22, pl. 5, figs 4, 6; Lund 1962, figs 1-41; Garcia de Emiliani 1973, p. 125, fig. 16.27; Armengol *et al.* 1975, p. 13, fig. 2 B; Munawar & Bistricki 1979, fig. 9 (bottom cell); Sommer 1982, fig. 2 RH M; Caljon 1987, p. 40, pl. 3, fig. 26 (excl. figs 27-30); Dokulil 1988, figs 1.3, 1.4.

Synonyms: *Rhodomonas lacustris* var. *nannoplanctica* (Skuja) Javornicky 1976, p. 103, pl. 24, figs 1-3, 6-8, 10, excl. figs 4, 5, 9, 11, 12 (obligate synonym). *Rhodomonas minuta* sensu Munawar & Bistricki 1979, fig. 11, et sensu Willén *et al.* 1980, fig. 9, non *Rhodomonas minuta* Skuja 1948, p. 346, pl. XXXVII, figs 8-10. *Rhodomonas lacustris* sensu Klaveness 1981, figs 1-15.

Allied taxa: *Rhodomonas pusilla* Bachmann 1923, p. 165, fig. 5. *Cryptomonas curvara* [sic] Guseva 1936, p. 223, fig. 4. *Rhodomonas pusilla* (Bachmann) Javornicky 1967, p. 50, pl. 4, figs 1-6; *Chroomonas pusilla* (Bachmann) Happey-Wood 1976, p. 356. *Rhodomonas minuta* (var. *nannoplanctica*?) sensu Munawar & Bistricki 1979, fig. 14. *Chroomonas acuta* sensu Kugrens & Lee 1988, p. 386, figs 2 et seq..

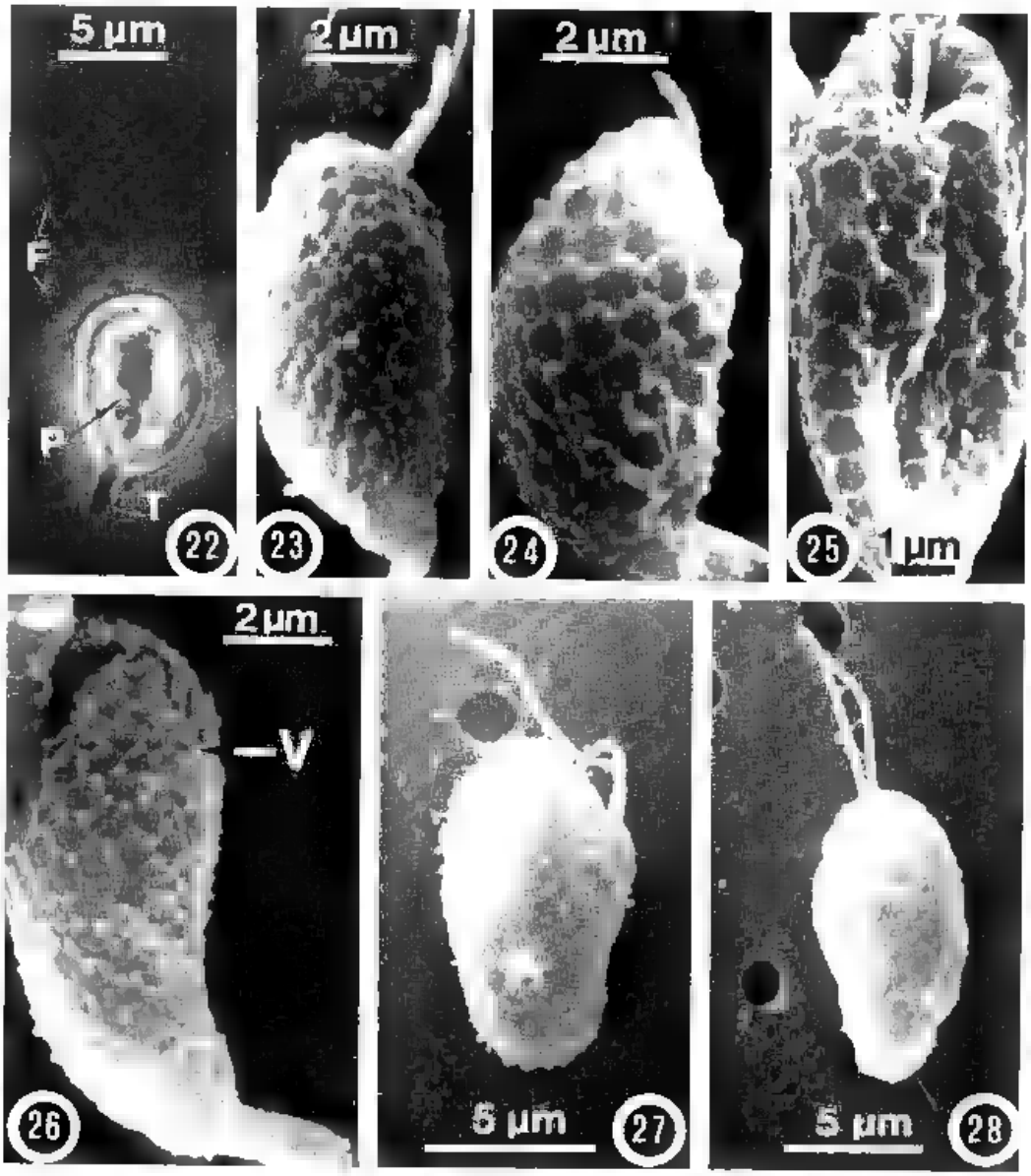
Description: Cells uncompressed, on average 9.3 µm long (SD = 1.20, n = 30), 4.1 µm thick (SD = 0.24, n = 19), and 4.4 µm wide (SD = 0.47, n = 11), in chemically fixed, dehydrated, and critical point-dried specimens examined by scanning electron microscopy. Flagella unequal, long flagellum 3/4 the cell length, short flagellum 1/2 the cell length. Sulcus absent. Tail 1/10 - 1/5 the cell length, usually acute, conical, cylindrical, or blade-like, containing a refringent inclusion ('leucosin granule') not delimited by membranes. Chloroplast dorsal, with ventral margins extending deeply into the ventral region of the cell, containing phycoerythrin I (540 - 545 nm). Pyrenoid central, large, with a thick starch sheath made up of two hemispherical halves. Nucleus posterior. Body scales absent. Side of the periplast areas on average 0.46 µm long (SD = 0.053, n = 30), in chemically fixed, dehydrated, and critical point-dried specimens examined by scanning electron microscopy.

Habitat: freshwater.

Lectotype: Skuja 1948, pl. XXXVII, fig. 13.

OBSERVATIONS

Morphometric features of the taxa of *Plagioselmis* examined here are summarised in Tables I and II. As reported elsewhere for other cryptomonads (Novarino, 1991a, b),



Figs 22 - 28. *Plagioselmis nannoplanctica* DK N750301 and an *'Isoselmis'*-like cryptomonad. Figs 22 - 26. *Plagioselmis nannoplanctica* DK N750301. Fig. 22. Lugol, phase-contrast LM; F = flagella, P = pyrenoid, T = tail. Figs 23 - 26. SEM, glutaraldehyde / osmium / CPD. Fig. 23. Lateral view; tail straight. Fig. 24. Lateral view; tail conical and ventrally bent. Fig. 25. Ventral view of ■ cell showing obvious signs of shrinkage; the long ventral fold is interpreted as ■ shrinkage artefact. Fig. 26. Ventro-lateral view; tail long, cylindrical, and ventrally bent. Note also the vestibular depression (V) and the absence of a ventral fold. Figs 27, 28. An *'Isoselmis'*-like cryptomonad (strain Y/C), SEM, glutaraldehyde / osmium / CPD, in lateral (Fig. 27) and dorsal (Fig. 28) views.

during specimen preparation for SEM cells shrink by a variable amount, whose significance can be tested statistically (Tab. II).

In culture *P. prolonga* and P. *nannoplanctica* behave in a very similar way. Both species are difficult to maintain and must be subcultured frequently (every 2 weeks) in order to minimise the risk that the cells die suddenly. Growth is very slow under the cultural conditions used here. Swimming is usually rapid, but cells under stress may not swim at

all; instead, they may be found rotating rapidly about the perlateral axis, with no net translational movement.

Table I - Morphometric features of three taxa of *Plagioselmis* prepared for SEM according to the glutaraldehyde/osmium/CPD schedule (*P. prolonga, P. nannoplancytica*) or the Lugol/osmium/CPD schedule (*P. prolonga* var. *nordica*). Values are given as mean ± standard deviation, with the number of observations in brackets. CL = cell length, CT = cell thickness (dorso-ventral), CW = cell width (perlateral), PAs = size of the periplast areas (length of the hexagon side).

Taxon	CL (µm)	CT (µm)	CW (µm)	PAs (µm)
P. prolonga PLY 172a	7.3±0.87 (30)	3.4±0.35 (13)	3.4±0.24 (16)	0.39±0.087 (30)
P. prolonga var. *nordica* (*)	5.8±0.80 (6)	2.6±0.47 (3)	2.8±0.26 (3)	0.31±0.041 (15)
P. nannoplanctica DK N750301	9.3±1.20 (30)	4.1±0.24 (19)	4.4±0.47 (11)	0.46±0.053 (30)

(*) data from Novarino (1991b)

Table II - Comparisons of cell length (CL) and cell thickness (dorso-ventral)(CT), measured with the LM (Lugol-fixed cells) and SEM (cells prepared according to the glutaraldehyde/osmium/CPD schedule) in 2 species of *Plagioselmis*. DF = degrees of freedom.

Species and parameter	Mean value in LM (µm)	Mean value in SEM (µm)	Difference (LM - SEM)	t, DF, p
P. prolonga, CL	8.6	7.3	1.3 (15.1%)	4.999 [67]; p<0.001
P. prolonga, CL	4.7	3.5	1.2 (25.5 %)	5.795 [50]; p<0.001
P. nannoplanctica, CL	10.5 (*)	9.3	1.2 (11.4 %)	(**)
P. nannoplanctica, CT	5.7 (*)	4.1	1.6 (28.1 %)	(**)

(*) data from Klaveness (1981)
(**) pooled t-statistic not calculated owing to insufficient data.

In the LM the salient features of *Plagioselmis prolonga* are the anterior pyrenoid, the subequal flagella, and especially the shape of the cells (Fig. 1). The anterior end is broadly rounded, whereas the posterior one is acute. Careful examination shows that the posterior end is hyaline and usually curved in the ventral direction (Fig. 1). The posterior end will be referred to here as the 'tail'. In the SEM the cell shape and the appearance of the flagella match closely those observed with the LM (Figs 2-5). Cells are covered with distinctly hexagonal periplast areas (PAs) (Figs 3, 5), except in the region of the tail, which lacks PAs (Fig. 3). The tail is usually conical in shape, although a blade-like (laterally flattened) form may also occur (Fig. 4). The flagella arise from a small, apical or subapical vestibular depression, which is displaced laterally towards one side of the cell (Fig. 4). Towards the opposite side (i.e., along the median longitudinal axis of the cell), the vestibular depression leads into a wide ventral groove extending roughly halfway along the cell length

(Fig. 4). A total of 40 cells were examined where the ventral face was clearly visible. Since a groove was observed in all of these, and was not characteristically associated with obvious signs of cell shrinkage, it is interpreted as a true, non-artefactual cellular feature. In order to avoid any possible confusion, due to the fact that the term 'furrow' may have been used indiscriminately for artefactual and non-artefactual grooves alike (see Novarino 1991b), the groove of *P. prolonga* will be referred to here as a 'sulcus' sensu Novarino (1991b).

In TEM whole-mounts the dorsal flagellum shows 2 rows of tubular hairs, whereas the ventral one has a single row (Figs 6, 7). In addition, two rows of much shorter fibrillar hairs appear to be present on the dorsal flagellum (Fig. 6; see also Morrall, 1980). Sections confirm the presence of a sulcus (Fig. 12), and suggest that it extends internally into a closed gullet (Fig. 8). On one side of the gullet, the plasma membrane is lined internally with an electron-dense, presumably fibrous band (Fig. 13), which appears to extend towards and possibly reach the tail (Fig. 14). On the other side of the gullet this band is absent, and the limiting membranes of the large ejectosome vesicles may come into close contact with the gullet membrane (Fig. 13). The position of the flagellar bases appears to be displaced relative to the gullet (Figs 8, 13), supporting the SEM observations.

The chloroplast of *P. prolonga* appears c-shaped both in longitudinal (Figs 9 - 11) and transverse (Fig. 12) sections. It does not extend into the tail (Figs 9 - 11), and its ventral margins do not extend deeply into the ventral region of the cell (Fig. 12). In the anterior region it bears a small pyrenoid not traversed by thylakoids and surrounded by a thin starch sheath (Fig. 9). The chloroplast surrounds a central nucleus (Figs 9 - 12). Owing to the fact that the phycobilin extract shows a broad absorbance peak in the region 535 - 545 nm, the chloroplast appears to contain phycoerythrin I (cr-phycoerythrin 545 in the sense of Hill & Rowan 1989) as principal accessory pigment.

In the periplastidial compartment there is a single nucleomorph, closely appressed to the nucleus (Figs 11, 12). Since the nucleomorph is positioned outside the pyrenoid, *P. prolonga* belongs to the order Cryptomonadales Novarino et Lucas (Novarino & Lucas, 1993b) (non Cryptomonadales Pascher nec Cryptomonadales auctorum). Mitochondrial profiles are usually circular (Fig. 10), and one profile always appears to be present inside the tail (Figs 11, 17).

In sections the periplast of *P. prolonga* is made up of an electron-dense component (IPC) on the internal face of the plasma membrane (PM) (Fig. 16). The IPC forms discrete segments, the size of which is comparable to that of the PAs seen with the SEM (Figs 15, 16). Small ejectosome vesicles bulge towards the surface between adjacent PAs (Figs 15, 16). Outside the plasma membrane there is a layer of partially overlapping scales ca. 80 nm in diameter (Figs 15 - 17). The IPC of the tail appears to be sheet-like, i.e. not composed of discrete PAs (Figs 14, 18), although at times the numerous small ejectosome vesicles which bulge towards the surface may give a false impression that the tail periplast is made up of discrete PAs (Fig. 17). Tails have been observed with or without external 80-nm scales (compare Figs 17 and 18).

Plagioselmis prolonga var. *nordica* (Figs 19, 20) is smaller than var. *prolonga*, has longer flagella, and the PAs are slightly smaller. Fig. 20 shows that the ventral cell face may bear a narrow groove. However, the fact that the groove is associated with obvious signs of cell shrinkage strongly suggests that it is a shrinkage artefact.

Apart from the freshwater habitat, *P. nannoplanctica* (Figs 21 - 26) is superficially similar to *P. prolonga*. However the much larger pyrenoid tends to occur in the central region of the cell (Fig. 22), and is covered by a much thicker starch sheath composed of two

halves (Klaveness, 1981, figs 9, 14, 15). The flagella are unequal (Fig. 22). The tail is more variable in length and shape, ranging from acute-conical (Figs 23, 24) to blade-like or cylindrical (Fig. 26). The small circular vestibular depression from which the flagella arise may lead to a long fold on the ventral cell face (Fig. 25). This is regarded here as a shrinkage artefact, owing to the fact that cells lacking obvious signs of shrinkage also lack a ventral fold (Fig. 26). Information available from the TEM study by Klaveness (1981) shows that there are other differences between *P. nannoplanctica* and *P. prolonga*; these are summarised in Tab. III.

DISCUSSION

Strain PLY 172a, identified by its isolator as belonging to *Plagioselmis punctata*, is assigned here to *P. prolonga*. Although the values of cell size are smaller than those given by Butcher (1967) for the latter species, this strain possesses certain key features which warrant its assignment to *P. prolonga*, i.e. the size and position of the pyrenoid, and the usually acute posterior tail. We support Chrétiennot-Dinet's (1990) designation of *Plagioselmis prolonga* as type of the genus *Plagioselmis*. This species should be preferred to *P. punctata* as the type of the genus owing to some contradictions contained in the original description of *P. punctata*. This is described in the Latin diagnosis (Butcher, 1967, p. 19) as having an anterior pyrenoid, but the English description mentions a central pyrenoid, which is also figured in one of the original illustrations (Butcher, 1967, pl. I, fig. 10 left).

The presence of a posterior tail lacking the discrete periplast areas found on the main portion of the cell body, is considered here as a diagnostic feature of the genus *Plagioselmis*. This is in line with current views that major differences in periplast structure are taxonomically significant at the generic level. Minor differences - for instance, the structure of the external periplast component - appear to be significant at or below the specific level (Novarino, 1991a).

Kugrens & Lee (1988) reported that the tail acts as a 'mating structure' between gametes during sexual reproduction. In cultures of *Plagioselmis prolonga* and *P. nannoplanctica* observed in different growth phases over long periods of time, no evidence of sexual reproduction has been found.

The presence of a tail typical of *Plagioselmis* in several cryptomonads illustrated with the SEM in the literature, has led us to include these as synonyms or 'allied taxa' of some of the taxa described here. The tail typical of *Plagioselmis* is also present in a freshwater cryptomonad (strain DK N 750301), named *Rhodomonas lacustris* sensu Javornicky (1976) by Klaveness (1981). This is a common and ecologically important cryptomonad which, contrary to the views of Javornicky (1976), is most often referred to as *Rhodomonas minuta* var. *nannoplanctica* Skuja (Reynolds, 1978, and references in Stewart & Wetzel, 1986). In the original description of that variety by Skuja (1948, p. 347) there are detailed comments on the tail and its refringent granule ('Leukosinballe'), which are absent in *Rhodomonas minuta* var. *minuta* Skuja (1948, p. 346). A comparison with strain DK N 750301 shows that this strain can effectively be identified as *Rhodomonas minuta* var. *nannoplanctica*. However, our observations show that strain DK N 750301 possesses the distinctive features of the genus *Plagioselmis*. It is necessary, therefore, to recombine Skuja's *Rhodomonas minuta* var. *nannoplanctica* under *Plagioselmis*. In doing so the rank of Skuja's variety is raised to that of a species, since there are considerable differences

between it and *P. prolonga* (Tab. III). The most obvious difference is given by the freshwater habitat of *P. nannoplanctica*. In this respect it is interesting to note that the generic name *Plagioselmis* has already been used in the ecological literature for a freshwater bloom-forming cryptomonad (Nishijima *et al.*, 1990, no figs)

Table III - A comparison of some non-morphometric characters in three taxa of *Plagioselmis*. CVM = chloroplast ventral margin, F = flagella, H = habitat, LG = 'leucosin granule', MT = mitochondrion, N = nucleus, P = pyrenoid, S = body scales, SU = sulcus, T = tail. Missing data are listed as '?,' absence of feature as '0'.

Taxon	T	SU	F	S	P	N	CVM	H
P. prolonga PLY 172a	with MT	+	subequal 1/2-2/2 cell length	+	anterior	central	not extending deeply into ventral cell region	marine
P. prolonga var. *nordica*	?	0	subequal 4/3 cell length	?	anterior	?	?	marine
P. nannoplanctica DK N750301 (*)	with LG	0	unequal (3/4 and 1/2 cell length)	0	central	posterior	extending deeply into ventral cell region	fresh water

Under the International Code of Botanical Nomenclature (ICBN), the type of a new combination is the type of the basionym (art. 7.12). The holotype of the basionym of *Plagioselmis nannoplanctica* is unknown since Skuja (1948) did not designate it. Although this does not contravene the requirements of the ICBN at Skuja's time of writing (art. 37.1), in order to adequately typify *Plagioselmis nannoplanctica* a lectotype chosen from among Skuja's original illustrations of *Rhodomonas minuta* var. *nannoplanctica* is proposed (Skuja, 1948, pl. XXXVII, fig. 13; reproduced here in Fig. 21 along with Skuja's other illustrations).

Arvola *et al.* (1991, figs 5, 6), have illustrated with the SEM a cryptomonad from the Culture Collection of Algae and Protozoa, U. K. (strain no. 995/3), identified as *Rhodomonas minuta* var. *nannoplanctica*. It is unclear whether or not that strain can be assigned to the genus *Plagioselmis*, since it is unknown whether the tail possesses a sheet-like periplast or a periplast subdivided into discrete areas. Similarly, a cryptomonad illustrated with the SEM and identified as *Cryptomonas cryophila* Taylor et Lee by McMinn & Hodgson (1993, fig. 5) shows an overall resemblance to *Plagioselmis prolonga*, but the periplast type in the region of the tail is also unknown. In contrast, a cryptomonad identified as *Rhodomonas lacustris* by Basualto (1992, p. 27, figs 1, 4-6) was described as having hexagonal periplast areas on the main portion of the cell body, but no such areas on the posterior tail. Therefore, it can be considered as a member of the genus *Plagioselmis*, and probably a new taxon since it combines some of the features typical of *P. prolonga* var. *prolonga* (presence of a non-artefactual sulcus and flagella arising from a laterally displaced vestibule: see the SEM micrograph of Basualto, 1992, fig. 6, and p. 29) with other features characteristic of *P. nannoplanctica* (presence of a refringent inclusion inside the tail and a large pyrenoid: see Basualto, 1992, p. 28).

The tail of *Plagioselmis* may have been observed as early as in the 1920s or 1930s in some freshwater cryptomonads assigned by their authors to the genus *Cryptomonas*

Ehrenberg, e.g. *Cryptomonas caudata* Massart (1920), *C. pusilla* Bachmann (1923), and *C. curvara* [sic] Guseva (1936). The names of those species, together with some later recombinations, are considered here as names of taxa allied to *Plagioselmis nannoplanctica*, but it is also possible that some or all of those species effectively correspond to *P. nannoplanctica*. If this were indeed to be the case, then the correct (earliest available) basionym for the species bearing the name *Plagioselmis nannoplanctica* would have to be chosen from among *Cryptomonas caudata*, *C. pusilla*, and *C. curvara*. However, owing to the insufficient descriptions of *Cryptomonas caudata*, *C. pusilla* and *C. curvara*, the basionym *nannoplanctica* is retained here. Skuja's diagnosis of *Rhodomonas minuta* var. *nannoplanctica* contains an unequivocal reference to the posterior tail typical of *Plagioselmis* (*parte posteriore acutius attenuata deorsum plerumque plus incurva:* Skuja, 1948, p. 347). In addition, Skuja's illustrations (reproduced here, Fig. 21) show a large pyrenoid bearing a starch sheath composed of two halves, as is also the case with strain DK N750301 on which our description of *Plagioselmis nannoplanctica* is based (Klaveness, 1981, figs 9, 14, 15).

In the early literature several other cryptomonads have been described which appear to possess a posterior tail and, therefore, are possible members of the genus *Plagioselmis*. Most are 'red' (phycoerythrin-containing); some examples can be found in Schiller (1957), e.g. *Cryptomonas vindobonensis* Schiller on p. 36, pl. XII figs 54 a - c. *Chroomonas acuta* Utermöhl (1925, p. 399, fig. 34), on the other hand, is an example of a tail-bearing cryptomonad described as being olive-green or 'dirty green' in colour; as such, it probably contains phycocyanin. (Cells of *Chroomonas acuta* sensu Kugrens & Lee (1988) were described as being golden-brown in colour. This may reflect the presence of phycoerythrin, and therefore suggests that *Chroomonas acuta* sensu Kugrens & Lee is closely allied to *Plagioselmis nannoplanctica*). Hill (1991) listed *Chroomonas acuta* as a synonym of *Komma caudata* (Geitler) Hill, and provided an SEM micrograph (Hill, 1991, fig. 28) showing that the tail of *Komma caudata* bears hexagonal periplast areas, as does the main portion of the cell body. However, SEM micrographs of *Chroomonas acuta* (Hickel, 1975, fig. 1; Cronberg, 1982, fig. 173), show that the hexagonal periplast areas of the cell body do not extend onto the tail. This supports the idea that *Chroomonas acuta* and *Komma caudata* are distinct, non-congeneric species, and suggests that *C. acuta* may belong to the genus *Plagioselmis*. If *Plagioselmis* effectively includes both phycoerythrin- and phycocyanin-containing species, it would be unlike most cryptomonad genera. The only genus known to include both phycoerythrin- and phycocyanin-containing species is *Hemiselmis* Parke, which is subdivided into two subgenera based on the phycobilin type (Butcher 1967; Hill & Rowan 1989; Novarino & Lucas 1993b).

In redescribing the genus *Plagioselmis* an attempt has been made to establish which characters are taxonomically significant at the generic level, and which ones can be used for delimiting species and varieties. Characters at and below the species level (Tab. III) include the 80-nm scales covering the cell body of *P. prolonga*. Scales of that size are unusual since they are only about 1/2 the size found in other cryptomonad genera. However, they do not seem to occur in species of *Plagioselmis* other than *P. prolonga* and, therefore, they do not appear to be taxonomically significant at the generic level.

The set of specific characters used in the genus *Plagioselmis* is comparable to that used in other genera of cryptomonads (Novarino, 1991a, b; Novarino & Lucas, 1993a), since it includes the size of the periplast areas and the presence of a sulcus. These characters appear of general usefulness for delimiting cryptomonad species. Some of the specific characters in the genus *Plagioselmis* are visible with the light microscope, i.e. relative fla-

gellar length and the position of the pyrenoid. Those characters could be useful during routine identifications of specimens in natural samples. If other taxonomically important characters in the Cryptophyceae may be observed with the light microscope, as suggested elsewhere for the periplast areas and the nucleomorph (Novarino, 1993), then there is great potential in trying to correlate light and electron microscopical observations for taxonomic and identification purposes.

The status of the genus *Isoselmis* is unclear. This was described by Butcher (1967, p. 19) based on a single species, *I. obconica* Butcher (1967, p. 20, pl. I fig. 11, pl. XII fig. 1, pl. XIV fig. 4). *Isoselmis* was distinguished from *Plagioselmis* 'with some hesitation' based on the behaviour of the flagella and the number of large ejectosomes. Both of these characters are difficult to observe with certainty, the behaviour of the flagella in particular owing to the cells' rapid swimming. Specimens which could be identified by light microscopy as possible members of the genus *Isoselmis* (Fig. 29) were isolated from aquarium tanks at the School of Ocean Sciences, Menai Bridge, U. K.. A posterior refringent granule was a prominent feature of all of the cells examined, as described for *I. obconica*. In the SEM (Figs 27, 28) the periplast appeared sheet-like, as in the 'diplomorphs' of the genus *Proteomonas* Hill et Wetherbee (Hill & Wetherbee, 1986; Novarino, 1991b). It is impossible to know with certainty if the specimens effectively belong to the genus *Isoselmis*, since the original culture of *I. obconica* (PLY no. 9) is no longer available, and no other strains bearing the generic name *Isoselmis* are available from culture collections. As is often the case with names of insufficiently characterized taxa on which little information is available (Jeffrey, 1977), it appears that the generic name *Isoselmis* may represent a nomen dubium which cannot be applied to any taxon.

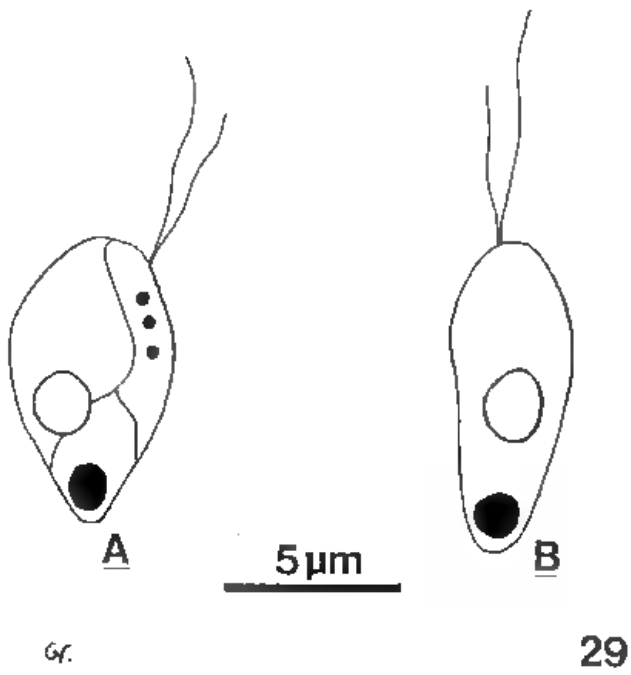


Fig. 29. An *'Isoselmis'*-like cryptomonad (strain Y/C), LM, in lateral (a) and dorsal (b) views.

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Source : MNHN, Paris

SOME BIOLOGICAL AND ECOLOGICAL OBSERVATIONS ON *SPHAEROPLEA ANNULINA* (ROTH) AG. (CHLOROPHYCEAE) IN NORWAY

Anders LANGANGEN
Hallagerbakken 82b, 1256 Oslo, Norway

ABSTRACT - *Sphaeroplea annulina* (Roth.) Ag. has been found in three small rainwater pools near the sea in southern Norway. Observations on the growth and developement of the alga were made in 1992 and 1993. In general this alga develop very quickly and complete its life-cycle in 3-4 weeks in its natural environment. These figures are confirmed in culture experiments. The alga has been found growing under optimal conditions in fresh water and slightly brackish water (0,02 - 1,3 g l^{-1} salt). These are observations made both in nature and under laboratory experiments. Due to evaporation of the water in these pools the alga must stand a higher variation of salt content. In nature I observed wellgrowing specimens in salt content to 2,12 ■ l^{-1}. In the culture experiments the oospores germinated in water of 3,6 g l^{-1} salt content. The germination in this type of water was slower and the development of the alga was prolonged. In the experiment with filaments they survived only in water with a salt content below 2,7 g l^{-1}. The Norwegian specimens fit the general description of the alga, but the variation in the two characters width of the cells and diameter of the oospores was less than in material procured elsewhere in Europe. *Sphaeroplea annulina* is also found in eutrophic lakes, but the special adaptations like fast life-cycle and massproduction of oospores support the idea that this alga has specialized to survive in temporary waters, where there is little competition from other plants.

RÉSUMÉ - *Sphaeroplea annulina* (Roth) Ag. ■ été trouvé dans trois petites flaques d'eau de pluie à proximité de la mer dans le sud de la Norvège. Des observations sur la croissance et le développement de cette algue ont été réalisées en 1992 et 1993. Elle se développe en général très rapidement et accomplit son cycle de vie en 3 à 4 semaines dans son environnement naturel, ce qui ■ été confirmé dans des cultures expérimentales. Elle trouve les conditions optimales de sa croissance dans l'eau douce ou faiblement saumâtre (0,02-1,3 g l^{-1} de sel). Ces observations ont été réalisées à la fois dans la nature et en expérimentation ■ laboratoire. L'évaporation de l'eau de ces flaques provoque une concentration plus élevée de la salinité à laquelle l'algue doit s'adapter. Dans la nature j'ai observé des spécimens ■ croissance active dans des eaux contenant 2,12 g l^{-1} de sel. En culture des oospores ont germé dans de l'eau salée à 3,6 g l^{-1}; la germination dans ce type d'eau était plus lente et le développement de l'algue était prolongé; les filaments eux ne survivaient que dans de l'eau avec une concentration en sel inférieure à 2,7 g l^{-1}. Les spécimens de Norvège sont conformes à la description générale de cette espèce, mais la variation de deux caractères, longueur des cellules et diamètre des oospores, était plus faible que dans le matériel provenant d'autres régions d'Europe. *Sphaeroplea annulina* se rencontre aussi dans des lacs eutrophiques mais certaines particularités, comme un cycle de vie rapide et une production en masse d'oospores, permettent de supposer que cette algue est adaptée à la survie dans des eaux temporaires, là où il y a peu de compétition avec d'autres plantes (traduit par la rédaction).

KEY WORDS : Chlorophyta, *Sphaeroplea annulina*, ecology, Norway.

INTRODUCTION

In 1991 I found *Sphaeroplea annulina* in Langesund in South-Norway (Langangen, 1992). Both in 1992 and 1993 I visited the localities several times.

Ecological and biological observations on *Sphaeroplea* in Norway are found in Langangen (1992). General information on biology and especially reproduction are found in many classical works on this alga (Fresenius, 1851; Cohn, 1856; Heinricher, 1883; Rauwenhoff, 1888; Klebahn, 1899; Fritsch, 1929 and Palik, 1950).

Sphaeroplea annulina is found in fresh water and slightly brackish water (Skuja, 1927; Palik, 1950; Christensen, 1954, 1971). The alga also appear frequently in fountains and springs in different botanical gardens throughout Europe (Fritsch, 1906; Fritsch, 1929; Palik, 1950). Some ecological information on the species are given by Lowe (1924) and by Cambra & Couté (1988).

DESCRIPTION OF THE LOCALITIES

The localities of *Sphaeroplea* can be found in Krogshavn situated in the small coastal town of Langesund in southern Norway. Krogshavn was earlier a brackish water area which now has been filled out and redeveloped into a local recreation ground. Here I found *Sphaeroplea* for the first time on 06.07.1991 (Langangen, 1992).

The bedrock here is cambro-silurian limestone with small rainwater pools scattered all over the area. These pools are most often found in small hollows in the limestone, probably of glacial age. All the pools are in varying degrees influenced by the nearby sea. The content of salt in the pools will therefore vary from brackish to fresh water. In summer most of the pools normally contain fresh water from rainfall, if they are not dried out. In the autumn the content of salt is higher, as a result of the storms normally taking place at this time of the year. In this period the pools near the sea often contain marine algae thrown up from the sea.

On 27.07.92 I found *Sphaeroplea annulina* in three small rainwater pools located along a line 5-30 meters from the sea and 0,5-1,0 meters above sea level.

Loc. 1 The locality from 1991. 2-3 m long, 0,5-1,0 m broad and 20-25 cm deep.

Loc. 2 Closer to the sea. 2-3 m long, 0,5 m broad and 20 cm deep. At a lower water level this pool divides in two smaller pools.

Loc. 3 Close to the sea in an area 1-2 m long, 0,5 m broad and 15-20 cm deep.

The localities are shown in figure 1.

MATERIAL AND METHODS

During the field studies water samples were taken in bottles for water analyses. pH, conductivity and salt content were measured at the spot. Calcium and chloride were measured later. pH was measured with a Hellige Comparator; conductivity and salt content with a Hach Conductivitymeter Model 44600/CND/TDS Meter. Chloride

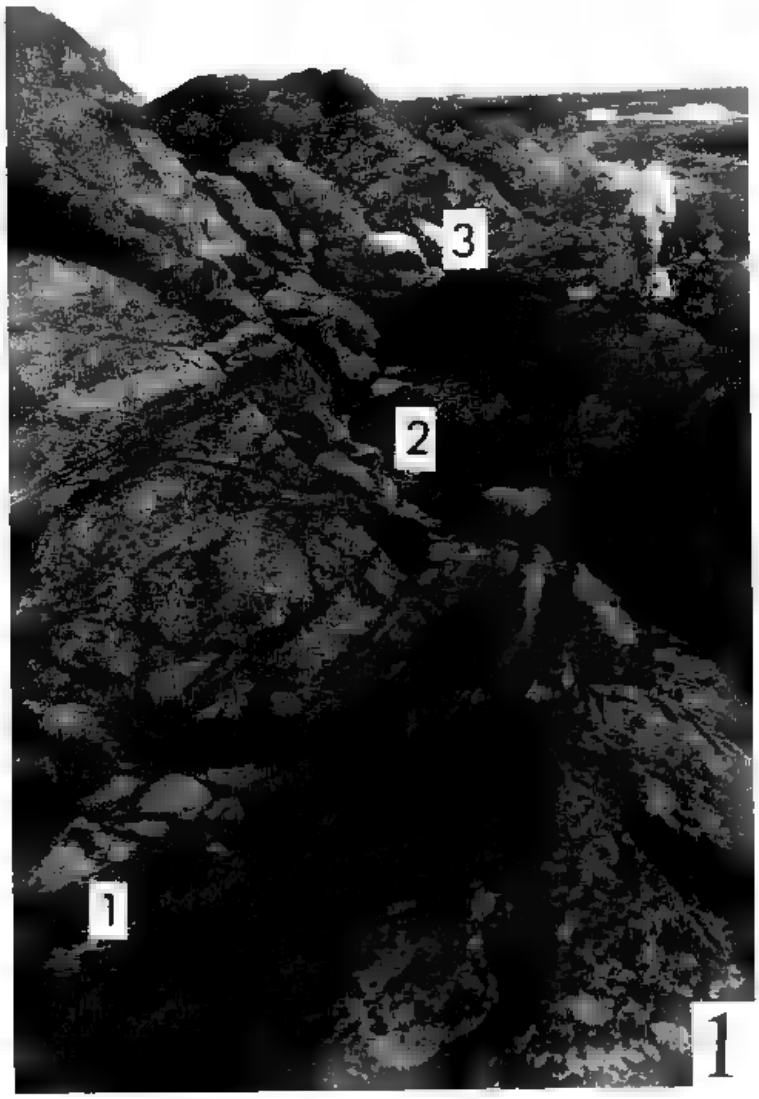
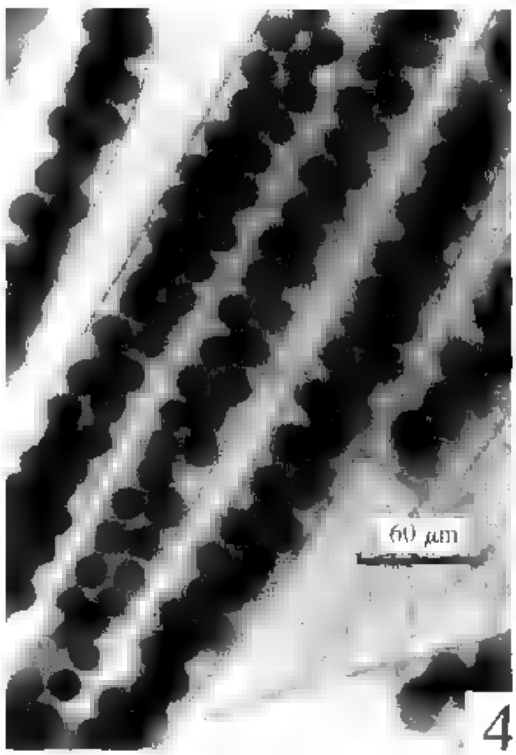


Figure 1. The localities with *Sphaeroplea annulina* by Langes und.The three localities are marked by a number. 1. is loc. 1, in the left corner with green tufts of the alga. 2. is loc. 2, in the middle of the photo, now divided in two parts. 3. is loc. 3, hidden behind the white stone. *Photo 29.07.1992.*

and calcium were measured with Aquamerck 11106 Chlorid and Calcium with Aquamerck 11110 Calcium.

The growth experiments took place in the spring and summer of 1993. The main purpose of the experiments was to find out how this alga reacted to different contents of salt in the water. In order to obtain these differences in salt content I used water from the localities and mixed this with salt (NaCl) or distilled water.

Specimens of the alga were taken from the localities, and grown in small culture-glasses of 500 cm^3. These were placed in a window facing north to northeast without any extra source of light. Here they were exposed to sun early in the morning and late in the evening. Observations on the growth and development were made



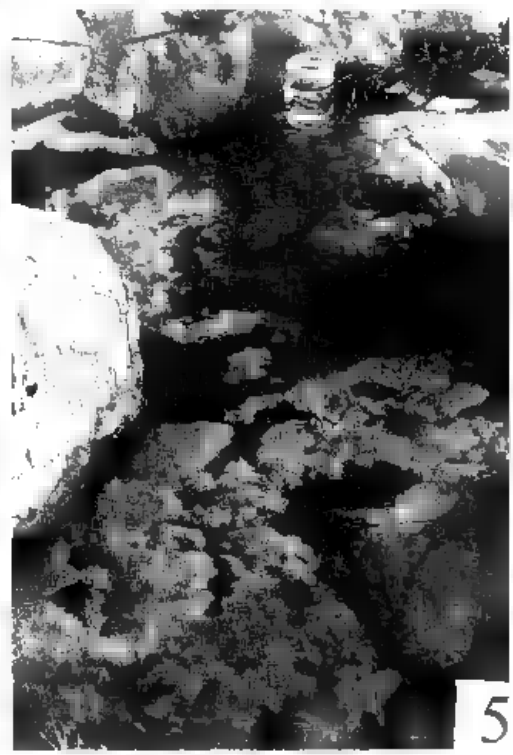


Figure 2. *Sphaeroplea annulina.* Vegetative filaments. From loc.1. *Photo 27.07.1992.* - Figure 3. *Sphaeroplea annulina.* Vegetative filaments with cells tranformed to oogonia. The oogonia are filled with ova. From loc. 2. *Photo 27.07.1992.* - Figure 4. *Sphaeroplea annulina.* Oogonia filled with red oospores. Loc. 1. *Photo 04.08.1992.* - Figure 5. Part of loc. 3. Green masses of *Sphaeroplea annulina. Photo 29.07.1992*

regularly. All normal developed filaments of *Sphaeroplea annulina* were brought back to the original pool after the completion of the experiment.

RESULTS

Field observations in 1992

When I visited the area in early July 1992, most of the pools were dried out as a result of the extremely dry and warm weather in May and June that year. During the first weeks of July rain filled up the pools, but later they dried out again. From the middle of July the rainfall was sufficiently high to give persistent water content in the pools. Most probably this happened around 18-21 July when the precipitation was 24,5 mm according to measurements made at Porsgrunn Brannstasjon (Firestation), situated 10 km north of the localities.

When I visited Krogshavn on 26.07.92 the locality studied in 1991 contained *Sphaeroplea*. Later the water level varied a lot. A rainfall the next day (27.07 / 11 mm) filled the pools with water, but two days later the water level was low again (because of the outflow and evaporation). The observed variations in chemical parameters was due to the differences between evaporation and rainfall.

The development from oospores (c. 20 July) to mature status took about three weeks. The results of my observations are given in table I.

b. Field observations in 1993

The situation in 1993 was more complicated, since the weather was more fluctuating. This year the growth of *Sphaeroplea annulina* presumably started during the last weeks of June. When I visited the localities on 05.07.93, the pools had already or had nearly dried out. After a rainfall 10.07.93 the alga started again, in loc. 1 and loc. 2 some filaments must have survived, but in loc. 3 the old oospores germinated to form new filaments. This year I did not observe any red filaments in the pools.

The results of my observations are given in table II.

c. Growth experiments.

These experiments are qualitative only, and gives no answer on the nutritional demands of the alga.

1. Growth of oospores

The experiment started on 30.05.1993 and ended on 29.06.93. I used bottom sediments and water from loc. no. 2.

The salt content in the 13 culture glasses varied between 0,05 g l^{-1} - 6,15 g l^{-1} (CND 107 µS cm^{-1} - 12300 µS cm^{-1}).

Only oospores in water with a salt content below 3,6 g l^{-1} germinated. In distilled water oospores did not germinate. It seems that a high salt content prolonged the germination and the development of the alga. In these environments the alga was also poorly developed, and contained fewer oospores.

Table I. Some physical/chemical parameters and biological status for loc. 1-3 in 1992.

Loc. 1

Date	Rain	CND µS cm^{-1}	Salt g l^{-1}	pH	Ca mg l^{-1}	Cl mg l^{-1}	t^{0} C
27.07.92	11mm	350	0,18	8,8	6,0	47,5	20,7
29.07.92	-	470	0,24	8,8	12,0	45,0	22,6
31.07.92	-	470	0,24	8,8	10,0	60,0	21,2
04.08.92	7,7mm	447	0,22	>8,8	6,0	75,0	-
10.08.92	10,5mm	447	0,22	8,0	6,0	70,0	-

Date	Short description of the biological conditions
27.07.92	Vegetative filaments only. Fig. 2.
29.07.92	Vegetative filaments. In many filaments the cells have been changed and are acting as oogonia or antheridia. Fig. 3. The sexual process could now be followed in detail. The water had a brown colour. Fertile *Oedogonium* is mixed with *Sphaeroplea*.
31.07.92	The quantity of the alga in the small pool has increased considerably. The filaments contain mostly cells with ova or empty antheridia. The number of vegetative filaments are low. Spermatozoids swim around in the water and inside the oogonia. Some ova have been fertilized and have changed to green, verrucose oospores. But most of the contents in oogonia are still round, fertilized (most probably) ova.
04.08.92	The alga are now reddish in contrast to earlier green.This is due to the change in colour of the oospores, which now are reddish. Many cells are now filled up with oospores. Vegetative filaments are not found any longer. Threads with empty cells or antheridia are common. Filaments 30-45 µm broad. Oospores 15-20 µm in diameter in two rows. Fertile *Oedogonium*.
10.08.92	The alga in the pool are now red. The filaments contained only ripe, red oospores which filled up the cells (Fig. 4) and many empty antheridia.
05.09.92	There was no visible filaments of *Sphaeroplea* in the water now. In the bottom sediment there were masses of red oospores.

Loc. 2

Date	CND µS cm^{-1}	Salt g l^{-1}	pH	Ca mg l^{-1}	Cl mg l^{-1}	t^{0} C
27.07.92	180	0,09	8,4	3,0	17,5	19,5
29.07.92	400	0,20	8,8	8,0	25,0	24,0
31.07.92	460	0,23	8,8	8,0	30,0	20,8

Date	Short description of the biological conditions
27.07.92	Few vegetative filaments. Most cells have been converted to oogonia or antheridia. Fig. 3.
29.07.92	Almost only filaments with oogonia with ova or empty antheridia. Only few vegetative threads.
31.07.92	Only oospores and empty antheridia. The oospores are green, verrucose, and they fill up the cells. Cell diameter is in average 45 µm. A few vegetative cells are still found. The locality have nearly dried out.

Loc. 3

Date	CND µS cm^{-1}	Salt g l^{-1}	pH	Ca mg l^{-1}	Cl mg l^{-1}	t^{0} C
27.07.92	280	0,14	7,6	2,0	27,5	18,5
29.07.92	450	0,23	8,8	8,0	40,0	20,7
31.07.92	580	0,29	8,8	8,0	55,0	19,8

Date	Short description of the biological conditions
27.07.92	The filaments have cells with ova or empty antheridia. Big quantities of spermatozoids are swimming around in the water.
29.07.92	Filaments with oogonia and antheridia are dominating. Only a few vegetative threads are found at this point Fig. 5.
31.07.92	Still few vegetative threads. Cells with oospores and empty antheridia dominate. The oogonial cells contain a mixture of round (fertilized ova) and verrucose oospores. The colour of these oospores are green or yellowgreen.

Germination with optimal growth of the alga was obtained in the concentrations of salt 0,05 g l^{-1} - 0,20 g l^{-1}.

In the optimal cultures the steps in the development of the alga were as follows:

1. From oospore to vegetative filaments ♦ 10 days
2. The commencement of differentiation in vegetative filaments to green oospores ♦ 13 days
3. From green oospores to ripe, red oospores ■ 7 days

The total number of days needed to complete one cycle was here c. 30 days. In the optimal salt area the alga was well developed. The values for some important characters were:

1. Width of cells 20 - 37,5 µm
2. Oospore diameter 17 - 20 µm
3. Number of oospores in each cell 1 - 54

In each cell the oospores were arranged in 1- 2 rows.

2. Growth of filaments

The experiment started on 06.07.1993 and ended on 27.07.93. Material used contained filaments with vegetative cells and cells with masses of ova (from loc. 1).

The salt content in the 21 culture glasses varied between 0,02 g l^{-1} -3,84 g l^{-1} (CND 50 µS cm^{-1} - 7680 µS cm^{-1}).

Only filaments in water below salt content 2,7 g l^{-1} survived. The alga did not survive in distilled water .

Optimal growth of the alga was in the salt area 0,02 g l^{-1} - 1,30 g l^{-1}. In higher salt concentrations only a small part of the cells content ripened to become red oospores. In water with a 0,5 g l^{-1} salt content only some filaments developed into ripe, red oospores. These filaments were narrow and filled only one row of red oospores (Fig. 6). In the optimal area (0,3 g l^{-1}) there were many cells filled with red oospores in double rows (Fig. 7).

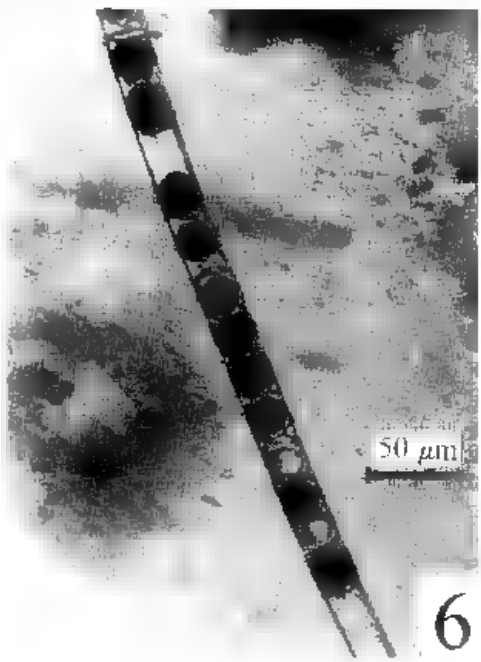
In the optimal cultures the steps in the development of the alga were:

1. From cells with ova to green oospores ♦ 2-6 days
2. From green oospores to red oospores ■ 8-9 days

In the optimal salt area the alga developed well. The values for some important characters are:

1. Width of cells 15 - 40 µm
2. Oospore diameter 20 - 25 µm
3. Number of oospores in each cell 6 - 75

In each cell the oospores develop in 1 - 2 rows.



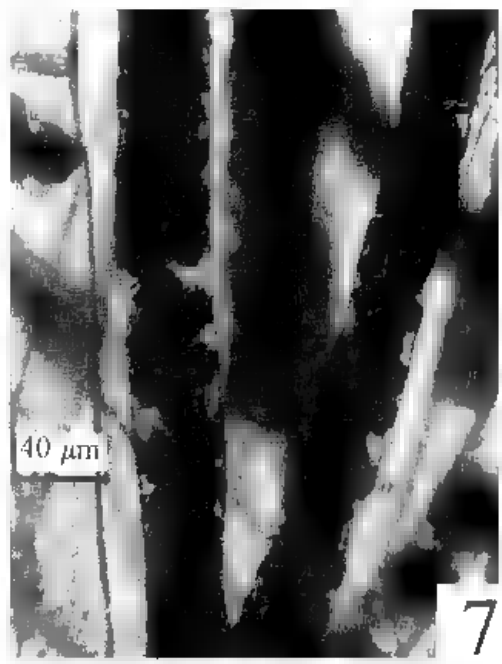


Figure 6. *Sphaeroplea annulina*. From experiment no. 2., water with 0,5 g l^{-1} salt. In cells with red oospores in ▬ row. *Photo 13.08.93*. - Figure 7. *Sphaeroplea annulina*. From experiment no. 2. Salt content 0,27 g l^{-1}. The cells are filled up with red oospores in 1-2 rows. *Photo 24.07.93*.

DISCUSSION

Sphaeroplea annulina is found on all continents, except Australia (Gauthier-Lièvre, 1941). In Europe the species is found scattered, and it occurs only rarely (Palik, 1950). The known Scandinavian distribution is given in Langangen (1992). In addition there are three old finds in Sweden, two in Uppsala in 1877, and one in Jönköping (see exsiccatae).

Sphaeroplea is found in different kind of waters, but often reported from temporary pools near the sea. It is also found in eutrophic lakes (Christensen, 1971): i.e. one high mountain lake in India (Randhawa, 1958) and in artificial waters in different botanical gardens (Palik, 1950). Pascher (1939) states that *Sphaeroplea* prefers limerich waters, and that it has never been found in dystrophic lakes.

Table II. Some chemical parameters and biological status for loc. 1-3 in 1993.

Loc. 1

Date	CND µScm^{-1}	Salt g l^{-1}	Biological status
05.07.93	4230	2,12	The pool is nearly dried out. Green threads of *Sphaeroplea annulina*. Both vegetative cells and cells with masses of ova and antheridia. Cells 30-35 µm broad, length of oogonia 375-600 µm, number of ova 21-80 per cell.
10.07.93	1600	0,80	Rainfall 10.07 filled all the pools with water. Some green filaments of *Sphaeroplea*.
11.07.93	1600	0,80	Some vegetative filaments. Most cells with verrucose, green to brown oospores.
20.07.93	1120	0,56	No algae. t = 19,2°C.
28.07.93	-	-	No algae.

Loc.2

Date	CND µScm^{-1}	Salt g l^{-1}	Biological status
05.07.93	-	-	The pool is dried out, except for two deeper parts where there was a wet cover of *Sphaeroplea*. The material consisted of vegetative cells and cells with masses of ova. Also plenty of antheridia which were filled with spermatozoids. Cells 20-25 µm broad, length of oogonia 350-900 µm, length of antheridia 300-550 µm, number of ova 30-38 per cell. A few slightly verrucose, green oospores.
10.07.93	-	-	Rainfall 10.07 filled all the pools with water.
11.07.93	380	0,19	Only vegetative filaments. In some cells differentiation had started.
20.07.93	460	0.23	Few threads only. Most are vegetative, but cells with verrucose, green oospores are also found. Some of this material was grown in a glass. These algae had already on 24.07 masses of ova and green,verrucose oospores. On 24.09 masses of red oospores. t = 20,7°C.
28.07.93	-	-	No algae found.

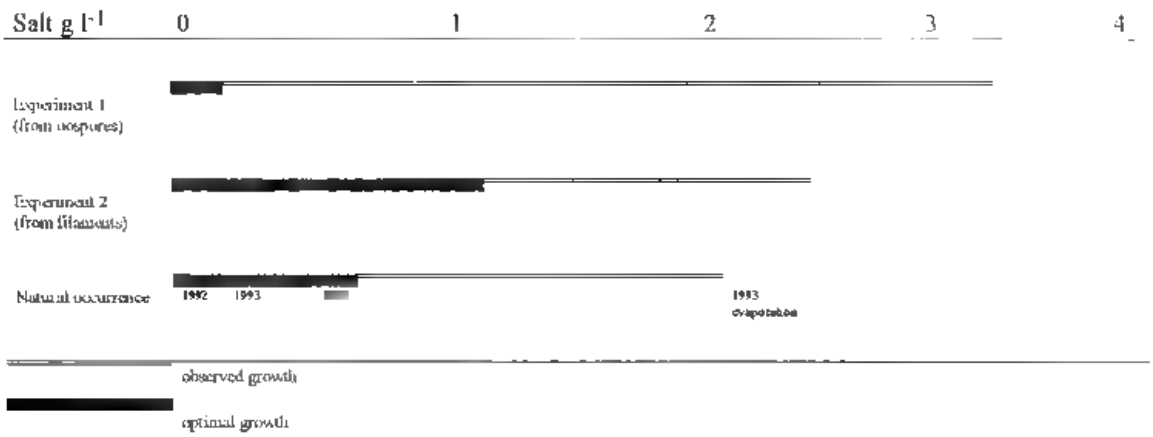
Loc.3

Date	CND µS cm^{-1}	Salt g l^{-1}	Biological status
05.07.93	-	-	The pool is dried out.
10.07.93	480	0,24	Rainfall 10.07 filled all the pools with water. No algae.
20.07.93	460	0,23	No visible algae. t = 20,9°C.
24.07.93	-	-	Green masses of filaments in the water. Only vegetative cells.
28.07.93	-	-	Many green filaments, much vegetative filaments. Many cells with ova and some with green oospores.

Most commonly the species has been reported from fresh water and slightly brackish water. This is supported by my own observations and experiments.

These results are given in table III.

Table III. Summary of the observed tolerance of *Sphaeroplea annulina* for salt (g l^{-1}) in two experiments and in natural occurrence. Most observed values for salt are relatively low, and indicate optimal growth in fresh water (< 0,5 g l^{-1} salt) or slightly brackish water.



Germination of oospores was best in water with low salt content, but took place up to a salt content of around 3,6 g l^{-1}. Optimal growth of filaments was found to be up to 1,3 g l^{-1} of salt. In localities near the coast this property is probably necessary for survival, as the salt content here can vary considerably due to evaporation and spray from the sea. This is also observed in loc. no. 1 on 05.07.1993, where the salt content was 2,12 g l^{-1} due to evaporation , and where the alga grew very well.

In their investigation of the species in Spain, Cambra & Couté (1988) found *Sphaeroplea* in a rice-field with slightly brackish water with a conductivity of 2650 µS cm^{-1}. In my field observations I found the conductivity to vary between 180 - 4230 µS cm^{-1}.

The content of chloride indicates some influence from the nearby sea. Skuja (1927) found *Sphaeroplea annulina* near the coast in Lettland. In his paper he says that the water in one of the pools, where the species grew, tasted salty. Cambra & Couté (1988) found *Sphaeroplea* in a pool with slightly brackish water with a content of 730 mg Cl l^{-1}. My own measurement of chloride are incomplete. They are all made in fresh water (value below 100 mg Cl l^{-1}).

pH is normaly reported in the alcaline area, from 7,6 - 8,8 in Norway. Christensen (1954) gives 7,9 and Cambra & Couté (1988) gives 9,7 (high photo-synthetic rate).

Sphaeroplea annulina is a species well adapted to variations in the ionic content of water. The optimal growth of *Sphaeroplea annulina* occurs in fresh water to slightly brackish water. According to Økland (1983) the limit between these two types is 0,5 g l^{-1} salt.

The species has also been found in eutrophic lakes in Denmark (Christensen, 1971) and in a high mountain lake in India, also eutrophic (Randhawa, 1958). Such localities are very different from the temporary pools, where the chances for drying out are considerable.

The specimens of *Sphaeroplea annulina* found in Norway fit in very well with the general description given for the species. Both width of filaments and diameter of oospores fit with Skuja (1927) and Gauthier-Lièvre (1941). Both Palik (1950) and Cambra & Couté (1988) give a bigger variation in the two characters.

Temperature is an important ecological factor affecting the growth of algae in general. In my field observations the temperature variation was 18,5° C - 24,0° C, and in the experiments c. 19° C - 25° C. In Budapest Palik (1950) measured 22 - 23° C in the watertanks there. Cambra & Couté (1988) give 29,2° C from their locality in Spain.

In the Norwegian localities *Sphaeroplea* is the dominating species (Fig. 5) only found together with *Oedogonium* sp. and *Scenedesmus* sp. Skuja (1927) reported *Sphaeroplea* mixed with *Ulothrix* sp., *Oedogonium lautumnarium* Wittr., together with a few species of *Scenedesmus*, *Ankistrodesmus falcatus* (Corda) Ralfs, *Nodularia spumigena* Mertens and *Pandorina morum* (Müller) Bory. In eutrophic lakes *Sphaeroplea* are found in vegetation mixed with phanerogames and other algae (Randhawa, 1958; Christensen, 1971; Cambra & Couté, 1988).

Sphaeroplea annulina complete its life-cycle in a relatively short time. In 1992 I found only vegetative filaments on 26.07 and only filaments with red oospores on 10. 08. In my culture experiments the total life-cycle was completed in about four weeks. Lowe (1924) states that "the algae which thrive here complete their life-cycle and produce resting spores in six to eight weeks at low temperatures".

In scientific papers *Sphaeroplea* is reported as a spring alga in Europe, where it normally can be found from April to June (Roth, 1806; Palik, 1950; Cambra & Couté, 1988). In Northern Europe most observations of the alga are from June-August (Skuja, 1927, Christensen, 1954; Langangen, 1992). In Norway red oospores were found in August both in 1992 and 1993.

It seems that *Sphaeroplea annulina* is well adapted to survive in small temporary pools, both inland but most commonly near the sea. The fast life-cycle and the massproduction of oospores are both adaptations to survive in such localities.

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COMPOSICIÓN QUÍMICA DE DOS MICROALGAS MARINAS UTILIZADAS COMO ALIMENTO EN MARICULTURA

R.O. CAÑIZARES, G. MOLINA y A.R. DOMÍNGUEZ

Departamento de Biotecnología y Bioingeniería
Centro de Investigación y de Estudios Avanzados del Insituto Politécnico Nacional
Apartado Postal 14-740. 07300 México, D.F.

RESUMEN - En el presente trabajo se determinó la composición química de las microalgas *Isochrysis* sp. (Chrysophyta) y *Tetraselmis chui* (Chlorophyta) cultivadas bajo condiciones controladas de laboratorio, utilizando agua de mar natural enriquecida de acuerdo con las formulaciones de Miquel-Matue y f/2 de Guillard.

En análisis químico de sus biomasas, reveló que el principal constituyente orgánico fueron los carbohidratos para ambas algas, independientemente del medio de cultivo utilizado, siendo la ramnosa y xilosa los azúcares predominentes. El contenido de proteínas de ambas algas, se vió influído por la formulación del medio de cultivo y estuvo caracterizado principalmente por los aminoácidos alanina, leucina y por los ácidos glutámico y aspártico. Los lípidos variaron de acuerdo con la composición del medio de cultivo, siendo mayor su concentración cuando se utilizó la formulación del Miquel-Matue. El principal ácido de 18 carbones, presente en la biomasa de ambas algas fue el ácido oleico, seguido de linoleico y linolénico.

ABSTRACT - The chemical composition of *Isochrysis* sp. (Chrysophyta) and *Tetraselmis chui* (Chlorophyta) grown in two types of enriched seawater media (Miquel-Matue and f/2 Guillard) under controlled conditions, was determined.

The chemical analysis of the biomass revealed that carbohydrates were the main constituents in both algae, and that these was not affected by the formulation of the culture media. The predominant sugars were xylose and rhamnose. Protein and lipid content in the biomass varied according to the formulation used. Algal proteins were mainly characterized by alanine, leucine, glutamic and aspartic acids. In relation to lipids, oleic acid was the main contituent followed by linoleic and linolenic acids.

RÉSUMÉ - La composition chimique de *Isochrysis* sp. (Chrysophyta) et de *Tetraselmis chui* (Chlorophyta), cultivés en conditions contrôlées dans l'eau de mer enrichie (milieu de Miquel-Matue, et f/2 de Guillard), a été déterminée.

Pour les deux microalgues, l'analyse quantitative de biomasse montre que les principaux composants sont les carbohydrates indépendamment du milieu employé. Par contre le taux des acides aminés (essentiellement alanine, leucine, acides glutamique et aspartique) diffère selon le milieu utilisé. La concentration la plus élevée en lipides a été obtenue avec le milieu de Miquel-Matue. Pour les deux microalgues, l'acide oléique est le principal constituant.

KEY WORDS - Microalgae, *Isochrysis* sp., chemical composition, culture.

INTRODUCCIÓN

El cultivo de las algas verdes se ha estudiado desde principios de siglo en investigaciones fundamentales de fisiología y bioquímica vegetal (Bold, 1942), pero

fue en las décadas de los anos 30 y 40, cuando se concibió la idea de que las algas microscópicas podían cultivarse masivamente para ser utilizadas como fuente de alimento, siempre y cuando se observaran las condiciones óptimas de cultivo. Estas técnicas se han difundido considerablemente en los último 20 años, haciendo del estudio del fitoplancton en ambientes controlados, un método útil en diversas investigaciones (Richmond, 1980; Sakschaug, 1980).

El enfoque que actualmente recibe atención por parte de muchos investigadores en diferentes partes del mundo, es la utilización de las algas planctónicas como alimento en el cultivo de organismos marinos comerciales, larvas o adultos, principalmente filtradores que actualmente son de importancia en maricultura. Las algas también se utilizan para el cultivo masivo de zooplancton (rotíferos, copépodos y artemia salina) en donde su valor en esta cadena alimentaria es crítico ya que los nutrimentos esenciales contenidos en las microalgas pasan vía el cultivo intermediario de zooplancton a los organismos bajo cultivo. El valor nutricional de las microalgas depende principalmente de su composición química y de los requerimientos nutricionales específicos de los animales que serán alimentados con ellas.

Existen estudios que demuestran que dietas elaboradas con especies seleccionadas de microalgas, pueden favorecer el crecimiento y desarrollo normal de formas juveniles de moluscos bivalvos y que cuando se alimentan estos organismos con dietas que no contienen microalgas, los resultados que se obtienen son poco relevantes (Loosanof & Davis, 1963; Flaak & Epifanio, 1978; Enright *et al.*, 1986; Hotsman, 1985).

Se han utilizado diferentes especies de microalgas como alimento vivo para invertebrados y vertebrados acuáticos. Así por ejemplo se ha demostrado que *Isochrysis galbana* favorece el crecimiento de larvas de bivalvos. *Chlorella* es más conveniente para el cultivo de *Brachionus*, pero no para ostión ni camarón (Epifanio, 1979a, 1979b). Recientemente, se sugirió que la concentración de aminoácidos y su composición en la biomasa de microalgas tiene un papel importante en el crecimiento y desarrollo de moluscos bivalvos (De Pauw, 1981; Aldana, 1986). Lo mismo se puede decir sobre los lípidos, cuya calidad importa más que su cantidad.

Las variaciones en el crecimiento y la composición química de las células de microalgas dependen de numerosos factores, entre los que se encuentran la luz, salinidad, pH, temperatura y concentración de nutrientes, mismos que guardan relación con las condiciones de cultivo.

Debido a la amplia variedad de aplicaciones que tienen los sistemas de algas, existe una gran cantidad de medios de cultivo, y modificaciones de ellos reportados en la literatura (Nichols, 1973). Por lo que antes de elegir uno de ellos, es importante tomar en cuenta la proporción de N:P, el pH, los macro y micronutrimentos además del conocimiento básico de la fisiología y nutrición del organismo bajo cultivo. Entre los medios de uso generalizado que cumplen con estas características encontramos al medio f/2 de Guillard (Guillard & Ryther, 1962) entre otros y el de Miquel-Matue (Alfonso & Leal, 1981), no tan utilizado como el anterior, pero que permite el crecimiento de ciertas algas en forma espicífica.

Algunos estudios sobre los diferentes factores que afectan el crecimiento de cultivos de *Isochrysis* y de *Tetraselmis* son los de Walne (1970) que analizó la relación N:P de *Tetraselmis chui* y *T. suecica;* Ukeles (1961) que estableció los intervalos de temperatura para el crecimiento de *Isochrysis galbana* y *Platymonas* sp.; Kain & Fogg (1958) quienes trabajaron sobre el pH y la saturación de luz para *I. galbana;* Laing &

Utting (1980) quienes estudiaron la influencia de la salinidad sobre *I. galbana* y *T. suecica* y Laing & Helm (1981) sobre los factores que afectan la producción de *T. suecica* (Kylin) Butch en recipientes de 200 l. Estos trabajos se vieron complementados por Fábregas *et al.* (1984, 1985) quienes analizaron la respuesta de cultivos estáticos de *I. galbana* y *T. suecica* a diferentes salinidades y concentración de nutrimentos. Uno de los primeros trabajos sobre composición química de biomasa de microalgas en función de las condiciones de cultivo y su relación con el medio ambiente, fue llevado a cabo por Spoehr & Milner (1949) con *Chlorella pyrenoidosa*, quienes encontraron que células desarrolladas en condiciones de limitación de nitrógeno, contienen aproximadamente 86 % del peso celulas seco como lípidos, comparadas con células normales que tienen 4.5 %. Parsons *et al.* (1961) cultivaron y determinaron la composición química de once microalgas marinas de diversas clases, durante su fase exponencial de crecimiento y reportaron un contenido de proteínas en el rango de 17.6% a 57% en base seca, carbohidratos de 4.1% a 37%, lípidos de 2.9 a 18% y cenizas de 7.6% a 57%. Fábregas & Herrero (1985) reportaron la concentración y composición de amínoácidos de cuatro especies de microalgas marinas, cuyo contenido de proteínas fluctuó entre 39.13% y 54.20% en base seca, mientras que el contenido de lisina disponible estuvo entre 3.67 y 4.52 g/100 g de proteína.

En la literatura se citan también algunos informes sobre la composición química de diatomeas (Myklestad, 1974).

MATERIALES Y MÉTODOS

Microorganismos.- Las cepas de *Isochrysis* sp. (clona CCMP, T-Iso o NEPCC 601) y *Tetraselmis chui* fueron proporcionadas por el laboratorio de microalgas del Departamento de Recursos del Mar, del Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional, Unidad Mérida, Yucatán México. Cultivos "Stock" de estos organismos fueron conservados en medio líquido f/2-Guillard (Guillard & Ryther, 1962), preparado con agua de mar natural. Se preparon dos series de estos cultivos los cuales se mantenían en anaqueles con iluminación horizontal (ca. 3500 luxes) proporcionada por dos tubos de luz flourescente marca Solar de 30 watts y de 95 centímetros de longitud cada uno, a una temperatura de 20 a 22°C. Una vez que se observaba crecimiento en los tubos (después de 2 días), una serie se trasladaba a un cuarto frío (4°C) en obscuridad y la otro permanecía en el mismo anaquel iluminado, hasta su posterior resiembra (cada mes).

Agua de mar. - En todos los ensayos se utilizó agua de mar natural colectada en Puerto Progreso, Yucatán y enviada al laboratorio. Antes de su envío, el agua se hizo pasar primero por filtros de concha y arena y porteriormente por una batería de cartuchos Millipore, siendo de 0.45 micras el poro más pequeño; por último fue irradiada con luz UV y envasada en recipientes de plástico opaco desinfectados con cloro. Ya en el laboratorio fue almacenada en refriferación (4°C) en ausencia de luz.

Medios de cultivo. - Se prepararon enriqueciendo agua de mar natural, según las formulaciones de Miguel-Matue citado por Alfonso & Leal (1981) (composición en la Tabla I) y el f/2-Guillard ya mencionado. El medio de cultivo Miquel-Matue no se utiliza estéril, solo se somete a ebullición, por lo que los garrafones para los experimentos de producción de biomasa, se esterilizaron vacíos en autoclave horizontal ■ 121°C durante 15 minutos. Una vez a temperatura ambiente se les adicionó el medio

de Miquel-Matue y se dejó reposar por 48 horas. El medio f/2-Guillard contenido en los garrafones se esterilizó en la misma autoclave y bajo las condiciones descritas. Las condiciones de cultivo de las microalgas fueron para *Isochrysis* sp.: medio de cultivo Miquel-Matue, pH inicial de 8.2, 27±2°C de temperatura y 32‰ de salinidad. Para *Tetraselmis chui* fueron las mismas condiciones anteriores pero a la salinidad de 30‰.

TABLA I
COMPOSICIÓN DEL MEDIO DE CULTIVO DE MIQUEL-MATUE MODIFICADO
(Alfonso & Leal, 1981)

	SUSTANCIA	
A	KNO_3	4.04 g ■ 100 g de agua destilada
B	$Na_2HPO_4\,12H_2O$	0.40 g " " " " "
C	K_2SiO_3	0.80 g " " " " "
D	Agar-agar	1.0 g " " " " "
E	$FeCl_3$	0.315 (quelada con 0.436 g de EDTA-Na_2).

Estas soluciones son añadidas a un litro de agua de mar en las siguientes proporciones: 10 ml de A, B y D, 3 ml de C, 1 ml de E.
Hervir y dejar durante 48 horas antes de utilizar.
Nota: Tanto las vitaminas (en el caso de *Isochrysis*) como la solucion E, se adicionaron en la misma concentración que para el medio f/2-Guillard.

Preparación de inóculos. - Se tomaron alícuotas de un mililitro de los cultivos "Stock" y se pasaron a tubos de vidrio Pyrex de 15 x 150 mm, con 10 ml del medio de cultivo bajo ensayo. Para los experimentos con *Isochrysis* sp., se hizo necesario añadir vitaminas al medio. La incubación se llevó a cabo en los anaqueles ya mencionados. Después de 72 horas, cuando los cultivoss se encontraban en fase exponencial, se procedió a ■ propagación mediante pases sucesivos a matraces Erlenmeyer de mayor capacidad cada vez. Finalmente a partir de un matraz Erlenmeyer de 500 ml se tomó una alicuota de 70 ml y se inoculó un matraz Fernbach de 2.8 l de capacidad total con 700 ml de medio de cultivo. La incubación de los matraces se hizo a una temperatura de 25 ± 2°C, con iluminación y aereación contínuas. Estos cultivos sirvieron como inóculos para los experimentos de producción de biomasa.

Obtención de biomasa - Se llevó ■ cabo en garrafones de vidrio Pyrex de 20 litros de capacidad total con 7 litros de medio de cultivo y una relación de inóculo de 10% (v/v). Cada garrafón tiene un tapón de neopreno con cuatro orificios que permiten la introducción simultánea de cuatro tubos de vidrio, con las funciones de toma de muestra (y al mismo tiempo para efectuar la cosecha), difusor de aire, puerta de inoculación y venteo del sistema. Los garrafones se colocaron en una unidad de cultivo con un sistema de iluminación con apagadores independientes para 10 lámparas flourescentes de 20 watts y 57 cm de longitud, dispuestas ocho de manera vertical ■ los lados de la unidad, con una separación de 15 cm entre sí y 10 cm distantes de los garrafones. Dos lámparas estan colocadas horizontalmente con la misma separación entre sí pero 20 cm distantes de los garrafones. En la parte superior de la unidad se encuentra una conexión de cobre con dos llaves de paso para regular el aire que se introduce al cultivo. Los cultivos se mantuvieron con aereación (1.5 vvm para *Isochrysis* sp. y 1.0 vvm para *T. chui*) e iluminación contínuas (ca. 3500 luxes). Antes de introducirse al garrafón, el aire se hace pasar a través de filtros de vidrio empacados

con fibra de vidrio. Todo el sistema se manejó de manera estéril. La aereación impidió la sedimentación de las células de algas y su depósito en la paredes del garrafón, exponiéndolas al mismo tiempo a períodos iguales de luz.

Sistema de cultivo. - utilizó el sistema de cultivo semi-contínuo (Cáceres *et al.*, 1980), con una dilucion del 50%. El reemplazo de medio de cultivo se hizo con la ayuda de una bomba peristáltica.

Recuperación y secado de la biomasa celular. - La biomasa se separó por centrifugación, en una centrífuga semicontínua Westfalia a 10,000 rpm, con una velocidad de flujo de alimentación de 1 litro por minuto. La torta resultante de la primera centrifugación se lavó 3 veces consecutivas, con una solución de formiato de amonio al 1% p/v para eliminar las sales, centrifugándose cada vez en las mismas condiciones. Posteriormente, se congeló y liofilizó en una liofilizadora de piso New Brunswick Scientific Co., Inc. modelo V-13, el polvo obtenido se almacenó en viales con tapón de baquelita colocándose en un desecador a temperatura ambiente.

Determinaciones Analíticas. - A la biomasa se le efectuaron los siguientes análisis por triplicado. Contenido de cenizas (Aldana, 1986), proteína verdadera por el método de Dorsey *et al.* (1978) utilizando el reactivo de Folin. Para la determinación de lípidos, se hizo una extracción según la técnica de Davis *et al.* (1969), previa hidrólisis de la muestra con 3 ml de HCl 6N en ampolletas selladas al vacío. La muestra hidrolizada se trató con 3 ml de hexano para extraer los lípidos, repitiendo la extracción 3 veces. Se obtuvieron dos fracciones, la fase orgánica se colocó en vasos de precipitado previamente puestos a peso constante y se evaporó, por último se pesaron. La fase acuosa se utilizó para la determinación de azúcares por cromatografía de gases.

Los carbohidratos totales se determinaron por el método de Dubois *et al.* (1956). Para el análisis de la fracción de azúcares se utilizó la fase acuosa obtenida durante la extracción de lípidos, la que pasó por una columna de intercambio iónico en fase OH^- eluyendo con 15 ml de metanol. La fracción eluída se evaporó a sequedad en un evaporador al vacío. En esta fracción se analizaron los azúcares por cromatografía de gases, manteniéndose las siguientes condiciones: nitrógeno, flujo 30 ml mn^{-1} columna OV-17 al 3% supelcoport 80/100 mesh 1.80 m 2 mm DI, las temperaturas de operación fueron de 350 °C para el detector de 300 °C para el inyector y para la columna fue de 140 °C manteniéndose así por cuatro minutos. Los carbohidratos deshidratados se pasaron con metanol a un frasco de reacción, lavando varias veces el recipiente que los contenía para evitar pérdidas de material y se evaporó a sequedad en corriente de nitrógeno (N_2). A partir de esta fracción se formaron los derivados trimetilsilil que fueron analizados siguiendo el método de Sweeley *et al.* (1963), con ligeras variantes. A la fracción deshidratada se le adicionaron: piridina, hexametildisilizano y trimetil clorosilano (1:6:3) grado analítico (Merck). Los frascos de reacción se protegieron de la humedad y se colocaron en baño de agua a 65 °C durante 40 min. Las muestras así preparadas se inyectaron en el cromatógrafo de gases aplicando las condiciones antes mencionadas.

La composición de ácidos grasos, se determinó extrayendo la fracción de lípidos de acuerdo al método de Bligh & Dyer (1959) modificado por Mayzaud & Martin (1957). La cuantificación de ácidos grasos se realizó en un cromatógrafo de gases Hewlett Packard 5710 con detector de ionización de flama bajo las siguientes condiciones: columna FFAP 15% supelcoport 80/100 mesh, las temperaturas de operación fueron de 300 °C para el detector, de 250 °C para el inyector y para la columna fue de 180 °C, manteniéndose así por ocho minutos y con aumento gradual de

2 °C por minuto, hasta alcanzar los 240 °C, temperatura que se sostuvo durante 16 min. La fracción de lípidos se trató previamente según el método de Metcalf (1969) para obtener los metil ésteres. El residuo de cloroformo se evaporó a sequedad, se adicionaron 20 ml de etanol-eter (3:1 v/v) y 0.5 ml, de KOH 10 N, se llevó a ebullición durante 2 h cubriendo con vidrio de reloj y controlando que el contenido de etanol permaneciera constante. Se dejó enfriar, adicionándose 20 ml de agua destilada y 30 ml de éter. Después de agitar y sedimentar, se formaron dos fases: la fase acuosa se trató con 3 ml de HCl 1.5 N, adicionándose 30 ml de éter y las fracciones estéreas fueron evaporadas a sequedad. El residuo fue tratado con 1 ml de solución de trifloruro de boro-metanol al 14%, colocándose en agua hirviendo por 20 minutos, una vez frío se extrajo con 3 ml éter y se lavó dos veces más con 2 ml de éter, dejándose evaporar hasta un volumen de 1 ml. Una alícuota de esta solución fue inyectada en el cromatógrafo de gases.

Para la análisis de aminoácidos, se utilizo un analizador Beckman 118 CL. Se hidrolizaron 2 mg de muestra en 1 ml de HCl mercaptoetanol 6 N, en ampolletas selladas al vacío durante 20 h. La muestra hidrolizada se redisolvió en una solución buffer pH 3.5, previa evaporación del ácido y se filtró utilizando una membrana Millipore de 0.45 micrómetros de diámetros de poro. De este filtrado se tomaron 100 microlitros y se inyectaron al analizador de aminoácidos.

RESULTADOS Y DISCUSION

En Tabla II se observa que el contenido de cenizas de *Isochrysis* sp. fue similar en ambos medios de cultivo. Estos datos concuerdan con los reportados por Whyte (1987) para *I. galbana* (10.78%) crecida en medio f/2-Guillard. Para *T. chui* en los medios de Miquel-Matue y f/2-Guillard el contenido de cenizas fue elevado (20 y 22% respectivamente). Whyte (1987), informó sobre valores similares para *T. chui*. Parsons *et al.* (1961) trabajando con *T. maculata* encontraron un alto porcentaje de cenizas y lo atribuyeron a los cloruros.

Con relación al contenido de proteínas su concentración fue mayor para *Isochrysis* en el medio f/2-Guillard (24% base seca) que en Miquel-Matue (19% base seca). Fábregas *et al.* (1985), determinaron para *I. galbana* 209 g de proteinas ml^{-1} cuando se desarrolló a una concentración de 4 mM de $NaNO_3$ y 30‰ de salinidad, en el presente trabajo encontramos para *Isochrysis* sp. 182 g de proteínas ml^{-1} en el medio Miquel-Matue con la misma concentración de nitratos (KNO_3) y a una salinidad de 32‰. Por el contrario para una concentración de nitratos menor (como la del f/2-Guillard), el contenido de proteínas fue mayor (325 g ml^{-1}).

TABLA II
COMPOSICIÓN QUÍMICA DE LAS BIOMASAS DE MICROALGAS

		% EN BASE SECA			
Microalga	Medio de cultivo	Proteína	Lípidos	Carboh.	Cenizas
Isochrysis sp.	Miquel-Matue	18	12	54	12
Isochrysis sp.	f/2-Guillard	24	32	20	15
T. chui	Miquel-Matue	19	22	36	20
T. chui	f/2-Guillard	19	25	36	22

Para *T. chui* el valor de proteínas se mantuvo constante (19% base seca) en ambos medios. Esta cantidad es superior a la reportada por Wikfors *et al.* (1984) para *T. maculata* (15.6%) y *Dunaliella salina* (17.2%) en un medio conteniendo 77.5 mg l^{-1} de $NaNO_3$ y 5 ml de KH_2PO_4. Probablemente lo que aquí influyó fue la concentración de los oligolementos, que en la formulación de Miquel-Matue es menor que en la de f/2-Guillard. Cuando Wikfors *et al.* (1984) cultivaron a *T. maculata* y *D. salina* en concentraciones superiores a 300 mg l^{-1} de $NaNO_3$ y 20 mg l^{-1} de KH_2PO_4 manteniendo la misma concentración de oligoelementos que la presente en f/2-Guillard, obtuvieron 31 y 39% proteínas respectivamente. Por otra parte, la formulación de Miquel-Matue resultó más adecuada para la obtención de una mayor densidad celular de *Isochrysis* sp. pero no para la cantidad de proteinas; mismas que fue superior en f/2-Guillard.

TABLA III
PERFIL DE AMINOÁCIDOS DE LA BIOMASAS DE MICROALGAS
(g de aminoácido por 100 g de proteina)

	Isochrysis sp. Miquel-Matue	*Isochrysis* sp. f/2-Guillard	*T. chui* Miquel-Matue	*T. chui* f/2-Guillard
Lis	2.24	5.20	5.25	4.68
His	2.24	2.28	2.19	1.78
Arg	6.09	4.71	5.41	4.94
Asp	9.18	4.71	10.03	9.26
Tre	3.93	4.64	5.56	5.15
Ser	4.59	4.99	5.64	5.42
Glu	11.34	10.05	12.54	11.05
Pro	5.90	4.36	4.94	5.0
Gli	6.46	6.24	7.29	6.84
Ala	9.84	6.74	10.19	8.94
Cis	-	-	-	-
Val	5.99	5.06	6.11	5.10
Met	0.56	0.62	-	0.42
Ile	4.12	3.60	3.52	3.94
Leu	9.18	9.15	9.41	9.94
Tir	6.09	3.5	2.50	3.05
Fen	5.62	5.13	5.25	5.94
Amonia	6.56	13.10	4.0	9.0

- : No se detecto.
Lis: Lisina, His: Histidina, Arg: Arginina, Asp: Aspártico, Tre: Treonina, Ser: Serina, Glu: Glutámico, Pro: Prolina, Gli: Glicina, Ala: Alanina, Cis: Cistina, Val: Valina, Met: Metionina, Ile: Isoleucina, Leu: Leucina, Tir: Tirosina, Fen: Fenilalanina.

En general, los valores obtenidos son bajos comparados con los reportados por Parsons *et al.* (1961) y Fábregas & Herrero (1985) entre otros; sin embargo para los fines de alimentación larvaria se pueden considerar aceptables (Andrews *et al.*, 1972).

En la Tabla III se observa que la proteína de las dos microalgas estuvo caracterizada principalmente por ácido glutámico, aspártico, alanina y leucina. Estos resultados están de acuerdo con los reportes de Parsons *et al.* (1961). Para los aminoácidos restantes su concentración fue menor (en un rango de 3-6 g aa para 100g de proteínas). Se detectaron trazas de histidina y aminoácidos azufrados en las

biomasas analizadas. No hubo resolución para cisteína en ninguna de las muestras. Por lo que se refiere a metionina, no hubo resolución en la muestra de *T. chui* crecida en el medio de Miguel-Matue. La concentración de los aminoácidos azufrados fue inferior al patrón de referencia de la FAO (1973) lo que es común para otros microorganismos (bacterias, levaduras, mohos y otros tipos de algas) (Fisher & Little, 1953; De la Fuente *et al.*, 1977). El perfil de otros aminoácidos (tre, lis, val, ile, leu y fen) compara favorablemente con el patrón de referencia de la FAO (1973). Chau *et al.* (1967) informaron que en las microalgas estudiadas por ellos (entre las que se encuentra *Monochrysis lutheri*), los aminoácidos predominantes fueron leucina (10.2%), alanina (9.7%) y los ácidos glutámico y aspártico, en concentraciones que resultaron similares a las reportadas en el presente trabajo para *Isochrysis* sp. *crecida* en el medio Miquel-Matue (leu 9%, ala 9.8%). Los valores para los ácidos aspártico y glutámico fueron ligeramente superiores a los determinados, por Chau *et al.* (1967).

T. chui prácticamente no presentó diferencias en su composición de aminoácidos al crecer en ambos medios de cultivo, siendo similar a la de *Isochrysis* sp. En la literatura se reportan para *T. suecica* (Fabregas & Herrero, 1985a, 1985b) concentraciones de leucina de 9.26% y valina 5.6%. En este trabajo, los valores fueron 5 y 6% desarroladas en f/2-Guillard y Miquel-Matue respectivamente. De manera general, en la literatura se informa de bajos niveles de cisteína y mentionina para todas las especies de microalgas, así como de abundancia en alanina, ácidos aspártico y glutámico y de leucina (Fábregas & Herrero, 1985; De la Fuente *et al.*, 1977). Es importante hacer notar que en las biomasas de *Isochrysis* sp. y *T. chiu* aquí estudiadas, se identificaron los once aminoácidos clasificados como esenciales para *Mytilus californianus* (Harrison, 1975).

El contenido de lípidos totales varió entre 10 y 32% (base seca) para las dos microalgas. Para *Isochrysis* sp. crecida en Miquel-Matue el contenido de lípidos fue de 12%. Este valor es inferior al encontrado por autores como Whyte (1987), quien determinó 19 % para *I. galbana*, Enright (1986) determinó 17% para la misma especie y Parsons *et al.* (1961) reportaron 11.6% para *Monochrysis lutheri*.

Cuando *Isochrysis* sp. se cultivó en el medio f/2-Guillard, la concentración de lípidos aumentó comparada con la obtenida en el medio Miquel-Matue y con relación a otras especies de fitoplancton. Esto no debe resultar extraño, ya que las microalgas están consideradas como una fuente alternativa de aceites naturales y de grasas (Zuñiga, 1983; Dubinsky *et al.*, 1978; Berner, 1982). Por otra parte, se sabe que el contenido de lípidos así como cualquier otro constituyente celular, puede variar de acuerdo a las condiciones de cultivo y que específicamente la concentración de nitrógeno en el medio así como la temperatura, influyen directamente sobre las proporciones intracelulares de lípidos. Medios de cultivo deficientes en nitrógeno estimulan la acumulación de lípidos. En el medio f/2-Guillard, la fuente de nitrógeno en forma de nitratos ($NaNO_3$) es menor (100 mg l^{-1}) que la presente en el medio Miquel-Matue (400 mg l^{-1}), lo que pudiera explicar el incremento en el contenido de lípidos o bien que este valor fuera característico de la especie.

Por el contrario *T. chui* en ambos medios de cultivo mantuvo su contenido de lípidos prácticamente sin cambio, 25% en f/2-Guillard y 22% en Miquel-Matue, valores que concuerdan con lo señalado por Wikfors (1986) para *T. maculata* (20%). El porcentaje de lípidos totales fue elevado para las dos especies.

En general para las dos microalgas la concentración de ácidos grasos fue mayor cuando crecieron en el medio de Miquel-Matue (Figuras 1 y 2). Es evidente que los

ácidos de 16 y 18 carbones predominaron, siendo los principales el palmítico y el oleíco. La concentración de ácido oleico, para *Isochrysis* sp. en medio Miquel-Matue fue 39% y en f/2-Guillard 30%. Para *T. chui* fue 25% en el medio de Miquel-Matue y 12% en f/2-Guillard. Yamayuchi (1987) determinó una concentración elevada de ácido oleico (57%) en el alga verde *Botriococcus braunii*. En *T. chui* el contenido de ácido

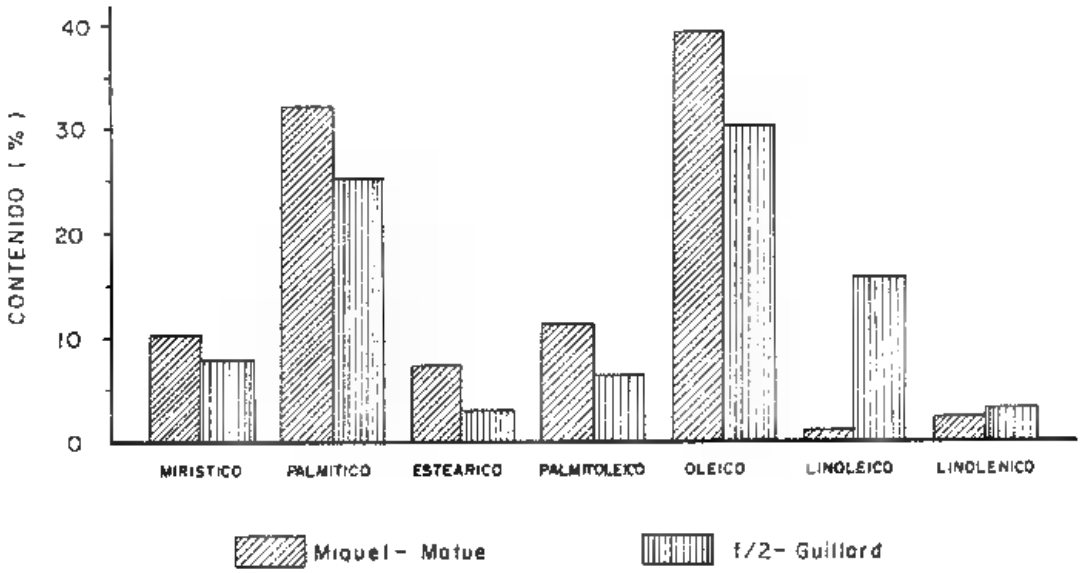

Figura 1. - Ácidos grasos ▬ la fraccion de lípidos de *Isochrysis* sp.

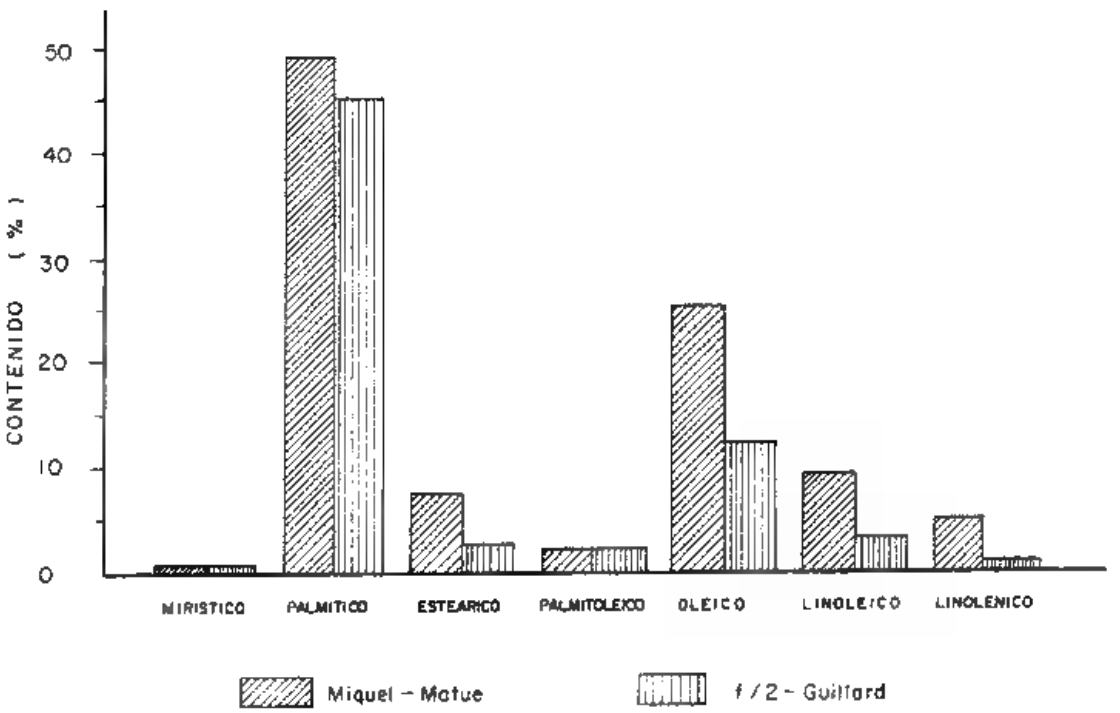

Figura 2. - Ácidos grasos ▬ la fraccion de lípidos de *Tetraselmis chui*.

palmítico fue superior al del oleico en ambos medios, al contrario de *Isochrysis* sp. En f/2-Guillard el contenido de ácido linoleico para *Isochrysis* sp. fue de 15.52%, mientras que en el medio de Miquel-Matue fue de 1.02%. Esto concuerda con lo que Enright *et al.* (1986) encontraron para el alga *I.* aff. *galbana* clona T-iso, donde los principales ácidos grasos saturados fueron los de 14:0 y 16:0 carbones y los insaturados de 18:1 y 18:2 carbones.

En la biomasa de *T. chui* la concentración de ácido linoleico fue de 9.23% cuando creció en Miquel-Matue y 3% en f/2-Guillard. Es importante destacar que las dos microalgas tuvieron una cantitad elevada de ácido linoleico (9.23% para *T. chui* y 15.52% para *Isochrysis* sp.) y de ácido linolénico (4.5% y 2.93% respectivamente). Este último es un ácido graso esencial específicamente para peces y crustáceos (Kanazawa *et al.*, 1978; Shewbart *et al.*, 1973, citado por New, 1976). El hecho de que en general exista mayor concentración de ácidos grasos en las biomasas obtenidas en el medio de Miquel-Matue, con respecto al f/2-Guillard, puede atribuirse a que la concentración de nitratos (KNO_3) es más elevada en el primer medio de cultivo y según observaciones hechas por De Paschke & Wheiler (1954), Manguld & Shlenk (1957) y Schlen *et al.* (1960), citados por Pugh (1971), un nivel elevado de nitrógeno en el medio de cultivo de *Chlorella pyrenoidosa* está asociado con altas concentraciones de ácidos grasos de 16 y 18 carbones. Chuecas & Riley (1969) puntualizaron que los efectos del estado nutritivo del medio de cultivo sobre la composición de ácidos grasos de la célula, varía de un clase de alga a otra.

La concentración de carbohidratos totales fue mayor en *Isochrysis* sp. cultivada en el medio Miquel-Matue (54%) que la obtenida en el medio f/2-Guillard (20%). Esto podría sugerir que esta microalga es más sensible a cambios en la composición del medio de cultivo, ya que en igualdad de condiciones *T. chui*, tuvo la misma concentración de carbohidratos totales en ambos medios. Autores como Mayzaud & Martin (1957), y Marshal & Or (1962), determinaron valores superiores a 50% en peso seco como carbohidratos para fitoplancton natural. En fitoplancton cultivado, la proporción relativa de carbohidratos a otros componentes orgánicos varía a través del ciclo de vida, aunado a las condiciones de cultivo (Walne, 1974). Por su parte Pillsbury (1975) determinó para *Dunaliella tertiolecta* (Butch) clona, un contenido de carbohidratos de 34% y para *Prorocentrum minimum* (Parvillard) Schuller clona EXUV uno de 43%. El mismo autor reportó para *I.* aff. *galbana* clona T-iso 18% de carbohidratos, similar a lo reportado para *I. galbana* clona Iso (21%) por Enright *et al.* (1986). Estos datos están cercanos a los obtenidos en el presente trabajo en el medio f/2-Guillard (20%). Es probable que el alto contenido de carbohidratos encontrado para *T. chui*, se deba a la naturaleza de su envoltura celular, que está compuesta por un glucano celulósico (Dodge, 1973). Wikfors (1986) reportó para *T. maculata* un contenido de carbohidratos de 37.8% base seca, en un medio con 77.5% mg l^{-1} de $NaNO_3$ y 5 mg l^{-1} de KH_2PO_4, relación que es similar a la del medio f/2-Guillard aquí utilizado, no así para el medio de Miquel-Matue, que teniendo una concentración mayor de KNO_3 y Na_2HPO_4 estimuló el mismo nivel de producción de carbohidratos.

Con respecto al perfil de carbohidratos, los azúcares predominantes fueron la ramnosa y xilosa para ambas algas crecidas tanto en el medio f/2-Guillard como en Miquel-Matue. Algunos autores han reportado la presencia de ramnosa y xilosa, en diatomeas y algas verdes (Handa & Yanagi, 1969; Parsons *et al.*, 1961). Whyte (1987) encontró estos dos azúcares en *I. galbana*, *Isochrysis* sp. clona T-Iso, *T. suecica* y en

algunas diatomeas. Chu *et al.* (1982) determinó ramnosa en la biomasa de *P. lutheri*. En la literatura se reporta que el perfil de azúcares varía ampliamente entre las microalgas.

CONCLUSIONES

Los medios de cultivo ensayados, tuvieron efecto sobre la composición química de la biomasa de *Isochrysis* sp., no así sobre la de *Tetraselmis chui*, que no presentó cambios en ninguna de las condiciones estudiadas, encontrándose como su principal componente ■ los carbohidratos. Los medios de cultivo utilizados en los experimentos, no son adecuados para la obtención de biomasas con alto contenido de proteínas, de ninguna de las dos microalgas estudiadas. El efecto de la composición del medio de cultivo, sobre los perfiles de aminoácidos y de ácidos grasos de las algas estudiadas, fue más evidente en *Isochrysis* sp. que en *Tetraselmis chui*. El medio de cultivo f/2-Guillard estimuló la síntesis de lípidos en *Isochrysis* sp. mientras que el de Miquel-Matue, la de carbohidratos en la misma alga. El análisis químico de las biomasas de *Isochrysis* sp. y *T. chui*, crecidas en cualquiera de los dos medios de cultivo y bajo las condiciones del presente estudio, indica que es posible su utilización para alimentar juveniles de bivalvos y otras especies.

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Source : MNHN, Paris

A CHECK-LIST OF THE *COCCONEIS* SPECIES (BACILLARIOPHYCEAE) IN ANTARCTIC AND SUBANTARCTIC AREAS, WITH SPECIAL FOCUS ON KERGUELEN ISLANDS.

Catherine RIAUX-GOBIN

Laboratoire Arago, CNRS, URA 117
66650 Banyuls / mer -France-

ABSTRACT - The high diversity and abundance of the genus *Cocconeis* in subtidal marine sediments in the Kerguelen area, especialy under the *Macrocystis* canopy, prompted a bibliographic review concerning this genus in Antarctic and Subantarctic areas. The major oceanographic expeditions favoured the discovery and description of numerous new species and varieties, but also a lot of dubious taxa: of the 38 new taxa mentioned, only 23 are recognized as valid by VanLandingham (1968). A few species, or their synonyms, are regularly mentioned by authors and may characterize these Southern polar habitats, the others seem to be rare or geographically restricted. Freshwater species are also listed.

RÉSUMÉ - La très grande diversité du genre *Cocconeis* (Bacillariophyceae) dans les sédiments subtidaux marins de Kerguelen ainsi que l'abondance numérique de ces diatomées ont amené à effectuer une revue bibliographique de ce genre en zones Antarcticques et Subantarctiques. Les premières grandes expéditions océanographiques permirent la découverte et la description de très nombreuses espèces et variétés mais également de nombreux taxons douteux: sur les 38 taxons mentionnés comme nouveaux dans cette revue, seulement 23 sont considérés comme étant valides par VanLandingham (1968). Quelques espèces ou leurs synonymes, régulièrement mentionnés par les auteurs, caractérisent ces zones polaires; les autres espèces sont rares ou ont une répartition géographique limitée. Les espèces d'eau douce sont également mentionnées.

Keywords - Southern Polar *Cocconeis*, Kerguelen, review.

INTRODUCTION

Preliminary studies on marine microphytobenthos from various sediments of the Kerguelen Islands, in relation to the algal pigment diversity (Klein & Riaux-Gobin 1991), bear witness to the great richness of these diatom assemblages. On subtidal sediments under the *Macrocystis* canopy, the genus *Cocconeis* is particularly well represented. However, some are very small and difficult to identify (Riaux-Gobin, 1992, 1993; Riaux-Gobin & Compère, *in prep.*). In addition to the fact that it is difficult to obtain the oldest documents and those of limited distribution (De Toni,

1891-94; Karsten, 1905 or Larson, 1974 *in* Prescott, 1979), bibliographic references mention species that are presently recognized as synonyms, or moreover that have been assigned to other genera. Incomplete descriptions or drawings which are too simplistic make some comparisons difficult. Some recent authors still use older synonyms or still separate two forms or varieties that are regrouped by VanLandingham (1968-1979).

All these taxonomic difficulties prompted the present synthesis of published work on the genus *Cocconeis* (marine and fresh water); special attention is paid to the species discovered or encountered during the major oceanographic expeditions (Table I-IV). Comments are added when changes to the names have subsequently occurred. Special focus is given to species mentioned by authors as pertaining to the diatom flora of the Kerguelen Islands. The nomenclature set up by VanLandingham (1968-1979) has been followed here to standardize all the names used successively by authors for a given taxa (keepping in mind that this author is not specialized on the *Cocconeis* species).

Northern polar regions are not included here, in spite of the interest of pointing out differences between the floras and distinguishing species which are ubiquitous, cold adapted and / or geographically restricted.

RESULTS AND DISCUSSION

Some authors, such as Van Heurck (1909), Peragallo (1921) and Heiden & Kolbe (1928), have contributed more than others to the discovery and description of the Southern diatom species (Tables I-IV). On the other hand, Castracane (1886), working particularly on marine muds from Kerguelen, did not mention *Cocconeis*; however, Heiden & Kolbe (1928) working on samples from several Southern areas (particularly Kerguelen and the St Paul Islands) mentioned 48 taxa (including 4 new taxa, 3 of which were from Kerguelen). The oldest works seem to be far from exhaustive perhaps because the samples were not taken specifically for diatom investigation (deep mud, sludge samples for sedimentology and not adapted to the observation of real microphytobenthic assemblages); this may explain why most of the works (Table I-II) mention only a few species. Furthermore, less importance was perhaps given to common and ubiquitous diatoms so that new species were proportionally abundant (Van Heurck, 1909: 10 taxa mentioned of which 8 were new; Peragallo, 1921: 22, 6 new; Peragallo, 1924: 21, 2 new; Heiden & Kolbe, 1928: 47, 4 new).

Van Heurck (1909) carried out a recollection and a comparison with previous works, particularly 10 works from Antarctic areas (plankton, ice and coastal samples; see Table I-II); 35 *Cocconeis* taxa were mentioned, of which 15 are not accepted by VanLandingham (1968). More recently Prescott (1979) established a check-list of freshwater taxa reported up to 1977 (17 *Cocconeis* taxa). The present work is a continuation of these approaches, but restricted to the *Cocconeis* genus.

Since the earliest descriptions, a great deal of changes have occurred: some taxa described as *Cocconeis* have now been returned to other genera (they are not incorporated in the Tables since they are not refered as in the VanLandingham nomenclature, 1968): for example *C. regalis* Greville (O'Meara, 1875) became *Campyloneis grevillei* var. *regalis* (Greville) Cleve; *C. wrightii* O'Meara (Petit, 1888)

became *Mastogloia barbadensis* (Greville) Cleve (present in Kerguelen, pers. obs.); *C. splendida* Gregory (Petit, 1888) became *Mastogloia splendida* (Gregory) Cleve; *C. coelata* Walker-Arnot (Petit, 1877) became *Diploneis campylodiscus* (Grunow) Cleve. *C. glacialis* Cleve was later described as *Navicula kerguelensis* (*in* Castracane, 1886) and then *Navicula glacialis* (Cleve) Grunow. More recently *C. kerguelenensis* Manguin (*in* Bourrelly & Manguin, 1954) mentioned by Hirano (1965, *in* Prescott, 1979) and Larson (1974, *in* Prescott, 1979) has been transferred to *Achnanthes saxonica* Krasske (Le Cohu & Maillard, 1983), and became *A. oblongella* Ostrup (Krammer & Lange-Bertalot, 1991). *C. sancti-pauli* has been transferred to *Achnanthes* as *A. sancti-pauli* (Heiden) Kobayasi & Sawatari (1986).

Difficulties with species determinations arise due to the distinction by different authors of two synonyms of the same species (*sensu* VanLandingham nomenclature): Heiden & Kolbe (1928) and Hustedt (1958) mentioned *C. costata* var. *pacifica* along with *C. imperatrix* that are regrouped by VanLandingham as *C. fasciolata* (Ehr.) Brown. Fukushima (1965), working in South Georgia, also mentioned *C. imperatrix*. More recently Zhu (1989) mentioned *C. costata* var. *pacifica* along with *C. fasciolata*. Even more surprisingly: Gilbert (1991) mentioned *C. fasciolata* along with *C. imperatrix*. Therefore, and in contradiction with Brown (1920), *C. imperatrix* and *C. costata* var. *pacifica* may, perhaps, be considered as independent taxa, differing from each other and from *C. fasciolata*. The same bibliographic difficulties exist for the distinction of some varieties of the species *C. gautieri*, *C. antiqua* and *C. schuettii*. Another problem is the validity of some varieties: for example Heiden & Kolbe (1928) mentioned *C. californica* along with *C. californica* var. *kerguelensis* (that are regrouped in the VanLandingham nomenclature), so that the validity of this variety may be reconsidered (Riaux-Gobin, 1992 and Riaux-Gobin & Compère, *in prep.*). Moreover, some species have been discovered by two authors at the same time and their descriptions are incomplete and contradictory (cf. *C. curiosa* Hustedt and *C. infirmata* Manguin, that may also have some affinities with *C. californica* var. *kerguelensis* Heiden; see remarks by Simonsen, 1992). Furthermore some species have been described and drawn but not named, for example *Cocconeis* sp. *in* Heiden & Kolbe (1928; description p. 584 and drawing fig. 107; Simonsen, 1992, did not mention this species).

In order to solve these taxonomic uncertainties, it will be necessary to seriously examine the types of original material (when still existing) and compare the oldest descriptions and drawings. Furthermore, studies using electron microscopy should provide valuable information, especially about the usefulness of separating, or not, some varieties.

Some species reported from the Southern Polar regions are characteristic, such as *C. imperatrix* (*C. fasciolata in* the VanLandingham nomenclature) and their synonyms (Manguin, 1960) or *C. gautieri* (Peragallo, 1921). In addition, *C. schuettii*. and *C. costata* var. *kerguelensis* are also frequently mentioned; the ubiquitous species *C. costata*, *C. pinnata* or *C. scutellum* are also regularly recorded. The other species (Tables II and IV) seem to be rare or geographycally restricted. Some species seem to be restricted to the Antarctic region (*C. adeliae* Manguin, *C. antarctica* Van Heurck and also *C. schuettii* Van Heurck or *C. gautieri* Van Heurck).

Upon the diatoms from Kerguelen Islands, the fresh water diatom flora is well documented (Table IV). Many localities and different communities have been investigated (see references in Table III and also: Germain, 1937; Le Cohu, 1982 and Le Cohu & Maillard, 1986). The marine diatom flora of Kerguelen have been less studied although its species richness appears higher (see asterisks on Table II). The important contribution of Heiden & Kolbe (1928) however, is representative of only one area (Observatory Bay). Hustedt (1958) examined the stomach contents of *Euphausia* (krill) from the Kerguelen region. Van Heurck (1909) working with the "Janisch collection" from Kerguelen does not mention the origin of the samples. A recent work on the nannoplankton of Kerguelen (Hédoin & Couté, 1992) mentioned six *Cocconeis* species (see Table I-II). Our investigations on subtidal muds under the macroalgae belts seems to be promising. Epiphytic assemblages (especially on *Macrocystis pyrifera*) will also give more information about the diversity of this genus in the Subantarctic area.

The genus *Cocconeis*, pertaining to the *Monoraphidineae*, is epiphytic, epilithic, kryotic (sea ice) or epipsammic and occasionally epipelic. The samples from which material for the oldest descriptions mentioned here were drawn (muds of various dephts, plankton samples or stomach contents of zooplankton) were often not adapted for the specific study of these diatoms. A large part of the taxa are reported by one author only, so that the question remains whether these taxa are rare, restricted to a small area, or simply unadequately sampled. Further investigations must pay attention to the sampling (for example sediments must be sampled with corers that do not disturb the interface) and also pay attention to the diversified smaller forms (nannophytobenthos, which dimensions are smaller than 20 µm).

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Table I: References of the works corresponding to the numbers quoted in Table 2.

N°	Area	Reference
	Marine	
1	Kerguelen	(O'Meara 1875)
2	Campbell island & New Zeland	(Petit 1877)
3	Cap Horn	Petit 1888)
4	Subantarctic	(De Toni 1891-94; Van Heurck 1909)
5	Antarctic phytoplankton	(Karsten 1905; Van Heurck 1909)
6	Exped. "J. Charcot" (1903-1905)	(Petit 1908)
7	Exped. "Belgica"(1897-1899)	(Van Heurck 1909)
8	Kerguelen (Janisch collection)	(Van Heurck 1909)
9	Schwedische Südpolar Exped.	(Carlson 1919)
10	Adelie Land	(Brown 1920)
11	Exped. "J. Charcot" (1910)	(Peragallo 1921)
12	Exped. "J. Charcot" (1903-1905)	(Peragallo 1924)
13	Exped. "Pourquoi Pas"	(Mangin 1915)
14	Deutsche Südpolar Exped.	(Heiden & Kolbe 1928)
15	Discovery reports	(Hendey 1937).
16	Orcades del Sur, plankton	(Frenguelli 1943)
17	Heard Island	(Manguin 1954)
18	Exped. "Mundus"	(Hustedt 1958)
19	(South Am.) Antarctica	(Frenguelli & Orlando 1958)
20	Terre Adélie	(Manguin 1957, 1960)
21	Terre Adélie (Exped. "P.E. Victor")	(Frenguelli 1960)
22	Arthur Harbor	(Krebs 1983)
23	King George Island, plankton	(Ligowski 1986)
24	Deception Island, Antarctica	(Zhu 1989)
25	South Orkney Islands	(Gilbert 1991)
26	Kerguelen, nannoplankton	(Hédoin & Couté 1992)

Table II: References of *Cocconeis* in marine water Antarctic and Subantarctic areas: Species are listed following VanLandingham (1968, 1979) nomenclature.
In bold italics: valid name in VanLandingham (1968)
In brackets: species not referred to in VanLandingham (1968)
In italics: species quoted ■ uncertain or badly defined in VanLandingham (1968)
In italics and indented: species quoted as synonyms in VanLandingham (1968).
+ = present; O = quoted as new by the author; * = Kerguelen Islands
?= annotation of the author

see next page →

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
Cocconeis adeliae Manguin																				O				x		
C. antarctica Brown										O																
C. antarctica (V. Heurck) Frenguelli & Orlando																			x		x			x		
C. antiqua Tempère & Brun															x									x		
C. antiqua var. ***tenuistriata*** V. Heurck							O					x											x	x		
C. gautieri var. *inornata* V. Heurck							O																			
C. gautieri var. *ornata* M. Peragallo											x	x														
**C. arraniensis* Gr. var. (*tenera* A. Schmidt)								x																		
C. arraniensis Greville																										
C. regina Johnston			x																							
C. arctica Cleve																										
**C. inflexa* A. Schmidt														x												
C. australis Petit		O																								
C. balatonis Pantocsek																							x?			
**C. biflexa* A. Schmidt														x												
C. britannica Nägeli														x												
C. californica Grunow *in* Cleve														x								x		x		
**C. californica* var. *kerguelensis* Heiden														O												x
C. californica var. ***antarctica*** Frenguelli & Orlando																			O							
C. ceticola Nelson															■			x								
**C. charcotii* M. Peragallo											O															
**C. costata* Gregory			x				x				x	x	x						x		x		x			
C. costata var. *typica* Cleve																										
C. costata var. *antarctica* Manguin																				O						
C. costata var. ***hexagona*** Grunow *in* V.Heurck																							x		x	
C. costata* var. ***kerguelensis* (Petit) Cleve				x	x			x						x								x	x			
C. extravagans Janisch					x			x		■	x			x						x						
**C. kerguelensis* Petit			O								x															
**C. imperatrix* var. *kerguelensis* (Petit) M. Peragallo											x?															
C. crux Ehrenberg		x?																								
(*C. curvirostrata* Tempère & Brun)														x												
C. cyclophora Grunow			x	x																						
C. cyclophora* var. ***kerguelensis* Cleve														x												

C. debenedettii Frenguelli & Orlando																			O								
C. decipiens Cleve	x	x																									
**C. fulgur* Brun														x													
C. sigma Pantocsek														x													
C. sparsipunctata Brun																		O									
****C. diminuta*** Pantocsek																										x	
C. dirupta Gregory		x												x													
****C. dirupta*** var. ***antarctica*** Grunow														x													
C. dirupta var. ? ***heitmeyeri*** (Janisch) Cleve																											
C. heitmeyeri Janisch														x													
C. dirupta var. ***dubia*** Grunow				x																							
C. dirupta var. ***flexella*** (Janisch & Rabenhorst) Grunow														x													
C. discrepans A. Schmidt														x													
C. distans Gregory		x												x													
C. granulifera Greville														x													
C. distans var. ***bahusiensis*** Cleve-Euler																								x			
C. fasciolata (Ehrenberg) Brown									x													x		x	x		
**C. costata* var. *pacifica* (Grunow) Cleve			x	x		x								x				x	x								
**C. imperatrix* A. Schmidt										x		x	x	x	x	x		x	x	x	x		x		x		
**C. janischii* A. Schmidt													x														
**C. pacifica* Grunow	x	x																									
C. fluminensis (Grunow) H. & M. Peragallo													x														
C. fluminensis var. ***subimpleta*** H. & M. Peragallo													x														
C. formosa Brun										x																	
C. formosa var. ***antarctica*** M. Peragallo										O																	
****C. gaussii*** Heiden													O				x										
****C. gautieri*** V. Heurck							O			x									x		x						
C. gautieri var. *craticula* M. Peragallo										O																	
C. schuettii var. *craticula* (M. Peragallo) Manguin																				x							
C. gautieri var. *maxima* M. Peragallo										O																	
C. gautieri var. *minor* M. Peragallo										O																	
C. heteroidea Hantzsch		x	x										x														
****C. hevdrichii*** V. Heurck							O					x															
C. illustris A. Schmidt																						x					

C. imperatrix f. *minor* M. Peragallo																										
C. imperatrix f. *plena* (M. Peragallo) Frenguelli																										
C. pinnata var. *plena* M. Peragallo															x											
C. imperatrix var. *acuta* M. Peragallo																										
C. imperatrix var. *opposita* M. Peragallo										O																
C. infirmata Manguin																			O							
C. curiosa Hustedt																	O					x		x?		
C. interrupta Grunow			x										x													
C. lauriensis Frenguelli & Orlando																		O					x			
C. litigiosa V. Heurck						O												x		x						
C. schuettii var. *litigiosa* (V. Heurck) M. Peragallo										x									x							
C. magnifica Janisch										x																
C. marginata Kützing		x?																								
(*C. maronensis* Schmidt)													x													
C. melchiori Frenguelli & Orlando																		O				x	x			
C. molesta Kützing													x													
C. molesta* var. *amygdalina (Brébisson ex V. Heurck) Grunow													x													
C. molesta* var. *crucifera Grunow													x													
C. notata Petit		O																								
C. orbicularis Frenguelli & Orlando																		O				x		x		
**C. pediculus* Ehrenberg																										
C. pellucida Hantzsch		x		x								x														
C. pellucida* var. *minor Grunow													x													
C. pinnata Gregory ex Greville			x							x			x	x				x		x			x			
**C. placentula* Ehrenberg													x													
**C. placentula* var. *lineata* (Ehrenberg) V. Heurck													x					x								
C. lineata Ehrenberg		x								x																
C. problematica VanLandingham																										
C. grunowii A. Schmidt																		x								
C. pseudomarginata Gregory			x										x		x											
C. harioti Petit			O																							
C. major Gregory		x																								
C. pseudomarginata* var. *intermedia Grunow		x																								
C. quarnerensis (Grunow) A. Schmidt													x													

C. reticulata var. ***deceptionis*** Frenguelli & Orlando																		O								
****C. St. Pauli*** Heiden													O													
C. schleinitzii Janisch													x													
****C. schmidtii*** Heiden													O													
C. schuettii V. Heurck							O				x	x							x	x	x		x			
C. schuettii var. ***minor*** V. Heurck							O				x	x						x	x							
C. japonica Pantocsek var. *antarctica* V. Heurck							O																x			
****C. scutellum*** Ehrenberg	x	x	x								x				x			x								
****C. adjuncta*** A. Schmidt								x																		
C. adriatica Kützing		x																								
C. baldjickiana (Grunow) Grunow													x													
C. mediterranea Kützing.		x																								
****C. scutellum*** var. *ampliata* Grunow								x					x													x
C. scutellum var. *genuina* Cleve									x																	
****C. scutellum*** var. *ornata* Grunow			x										x													x
C. scutellum var. ***japonica*** (A. Schmidt.) Skvortzow.																										
C. japonica A. Schmidt													x													
***** (*C. scutellum* var. *kerguelense* Grunow)								x																		
C. scutellum var. *mediterranea* Rabenhorst			x																							
****C. scutellum*** var. ***parva*** (Grunow) Cleve													x													
C. scutellum var. *schmidtii* Frenguelli																		x								
*****C. *scutellum* var. *stauroneiformis* W. Smith.			x	x									x													
****C. stauroneiformis*** (W. Smith) Okuno																										x
C. paniformis Brun												x														
C. similis Karsten					o?																					
C. vanheurckii Cleve													x													
C. surirelloides Grunow				x?																						
C. wienckensis Petit						O						x														
Cocconeis sp													x													

** *C. sancti-pauli* Heiden has been transferred to the genus *Achnanthes* see text

Table III: References of the works corresponding to the numbers quoted in Table IV.

N°	Area	Reference
	Fresh water	
1	Kerguelen, cap Horn	(Ehrenberg 1854)
2	Kerguelen	(Bourrelly and Manguin 1954)
3		(Thomas 1965 *in* Prescott 1979)
4	Antarctic, Kerguelen	(Hirano 1965 *in* Prescott 1979)
5	Kerguelen -soil-	(Larson 1974 *in* Prescott 1979)
6	Kerguelen	(Le Cohu and Maillard 1983)
7	Deception Island, Antarctica	(Zhu 1989)

Table IV: References of *Cocconeis* in fresh water Antarctic and Subantarctic areas:
Species are listed following VanLandingham (1968, 1979).
In bold italics: valid name in VanLandingham (1968)
In brackets: species not referred to in VanLandingham (1968)
In italics: species quoted as uncertain or badly defined in VanLandingham (1968)
In italics and indented: species quoted as synonyms in VanLandingham (1968).
+ = present; O = quoted as new by the author; * = Kerguelen Islands
?= annotation of the author

	1	2	3	4	5	6	7
******C. borealis* Ehrenberg	?+						
******C. costata* Gregory				+			+
******C. costata* var. ***kerguelensis*** (Petit) Cleve							+
C. distans var *minima* H. Peragallo				+			
C. fasciolata (Ehrenberg) Brown							
**C. costata* var. *pacifica* (Grunow) Cleve				+			+
******C. feuerbornii* Hustedt						+	
****C. kerguelenensis*** Manguin		o		+	+		
C. litigiosa V. Heurck				+			
******C. pediculus* Ehrenberg				+		+	
******C. placentula* Ehrenberg			+	+	+	+	
******C. placentula* var. ***lineata*** (Ehrenberg) V. Heurck				+	+		
C. lineata Ehrenberg	+						
******C. scutellum* Ehrenberg		+		+		+	
**C. scutellum* var. *ampliata* Grunow						+	
******C. striata* Ehrenberg		+					
******(C. therezieni* Le Cohu & Maillard*)*						o	
C. wienckensis Petit			+				

** *C. kerguelenensis* Manguin has been transferred to the genus *Achnanthes*, see text.

CYMBELLA TRIANGULUM (EHRENB.) CLEVE (BACILLARIOPHYCEAE) , UN TAXON NOUVEAU POUR LA FLORE EUROPÉENNE - CARACTÉRISTIQUES MORPHOLOGIQUES ET ÉCOLOGIQUES.

Jean BERTRAND[1] et Michel COSTE[2]

(1) 42, rue Malvoisine - 45800 Saint Jean de Braye
(2) CEMAGREF , 50 avenue de Verdun 33610 Cestas

RÉSUMÉ. - *Cymbella triangulum* (Ehrenb.) Cleve a été récolté dans le canal latéral à la Loire près d'Orléans. Cette espèce d'origine américaine, décrite par Ehrenberg au siècle dernier, n'a pas été recensée à ce jour en Europe. Le polymorphisme valvaire est examiné en microscopie photonique et électronique à balayage. L'absence de données autécologiques détaillées conduit à aborder l'examen des exigences de l'espèce à l'aide des formes dominantes associées. Les causes de sa dissémination pourraient être attribuées à l'introduction récente de la tortue de Floride.

ABSTRACT. - *Cymbella triangulum* was collected for the first time in a canal running along the river Loire near by Orleans in the centre of France. This species, described by Ehrenberg in 1845, is known until now, only from North American countries. The most recent statement of its distribution is due to Patrick & Reimer 1975. Morphological aspects and polymorphism are examined under light and scanning microscopes. Few ecological data are available about this species and its main requirements are inferred from associated taxa. Distribution could be assigned to the recent invasion of French running waters by the Floridean turtle.

Mots-clés. - Bacillariophyceae, *Cymbella triangulum*, distribution, systématique, morphologie, ultrastructure, Europe.

INTRODUCTION

Cymbella triangulum appartient au cortège des espèces récemment apparues dans les eaux françaises dont l'introduction volontaire ou non est liée à des activités d'aquariophiles. Sa dissémination comme ses possibilités de prolifération dépendent des conditions environnementales et la température apparaît comme le principal facteur limitant en raison de ses origines subtropicales. L'examen des caractéristiques du milieu comme celui des modifications morphologiques contribuent à une meilleure connaissance de cette espèce rarement évoquée dans la littérature scientifique dont il sera intéressant de suivre la progression.

MATÉRIEL ET MÉTHODES

L'échantillonnage a été réalisé par simple grattage des substrats immergés et expression de macrophytes aquatiques. Les diatomées ont été nettoyées à l'eau oxygénée concentrée (130 vol.) avant d'être montées dans une résine réfringente, le Naphrax (IR=1,74).

Les observations ont été effectuées en microscopie photonique sur Olympus BH2 pour le matériel vivant et sur Leitz DMRB pour le matériel nettoyé.

Les illustrations en microscopie électronique à balayage ont été réalisées à Pura (Suisse) par W. Güttinger sur ISI Super IIIA à 30 KV après filtration sur filtres nuclépores et métallisation à l'or fin sur appareil Balzers.

DESCRIPTION

Taxonomie

Cymbella triangulum (Ehr.) Cleve 1894 p.168, Boyer 1916 p.63 f.18/24 et Boyer 1927 p.284, Hustedt 1931 in Schmidt *et al.* 1874 pl.377/5-6, Patrick & Reimer 1975 p.45 pl. 7 fig. 7-10.

Synonymes :

Gloeonema triangulum Ehrenberg 1845 p.77: Ehrenberg 1854 35A/7 fig.10
Encyonema triangulum (Ehrenberg 1845) Kützing 1849, p. 62
Cymbella rotundata Chase 1886
Cymbella rhomboidea Boyer 1916 p.63 pl.18/11 selon Hustedt (1955)

Patrick & Reimer contestent la synonymie avec *C. gibba* Bailey, arguant du fait que les observations n'ont pas porté sur le matériel d'origine.

Cymbella triangulum var. gracilis Hustedt 1931 in Schmidt *et al.*, 1874 t..374/5, lac d'eau douce du Mexique ex Simonsen 1987 p.131 pl.216/3

La variété *gracilis* validée tardivement par Simonsen en 1987 correspond aux plus grands individus observés dans le canal de la Loire et n'a pas été distinguée de l'espèce au cours de nos investigations.

Parmi les synonymes possibles il faudrait ajouter *Cymbella mutica* Torka dont les dimensions et la morphologie coincident parfaitement à ceux de nos specimens in Sieminska (1964, p.439 fig. 788) mais il ne nous a pas été possible de nous procurer le matériel de référence.

Morphométrie

L'espèce présente les principaux caractères du genre *Encyonema* réhabilité par Round, Crawford & Mann (1990, p.490), plutôt considéré jusqu'alors comme un sous-genre dans les flores modernes (Krammer, 1982). A une forte dissymétrie dorsi-ventrale s'ajoute habituellement un mode de vie colonial en tubes muqueux qui n'a pas été observé chez cette espèce probablement solitaire.

a) Diatomée vivante (Fig. 1 à 6)

En vue valvaire, les chloroplastes de dimensions variables, remplissent largement la cellule et laissent libre les extrémités avec une forte indentation le long du canal raphéen ainsi que sur la partie ventrale médiane (fig. 1 flèches). La présence d'un pont sombre au-dessus de la partie ventrale est un caractère constant y compris sur les cellules initiales (fig. 2). En vue connective les chloroplastes sont formés de deux lames plaquées sur les faces valvaires avec un retour vers le centre près de la face ventrale et forment ainsi le pont décrit plus haut. Le noyau apparaît en position dorsale alors que des inclusions lipidiques de formes sphériques sont fréquentes avec un pyrénoide plus ou moins visible.

b) Frustule (fig. 7 à 14)

Dans les nombreuses récoltes effectuées nous avons observé un éventail complet des divers stades de croissance. Les longueurs s'étendent de 25 à 75 µm (90 µm sur une cellule initiale). Ces dimensions sont légèrement plus importantes que celles relevées par Patrick & Reimer (1975) qui signalent des valves de 30 à 70 µm de long. Les populations récoltées en février ont des longueurs moyennes (30-35 µm) sensiblement inférieures à celles de juin (40-45 µm) pour des valeurs extrêmes très proches.(fig. 15a). La largeur des cellules est variable et s'étend de 12.5 µm à 20.5 µm en accord avec les valeurs mesurées par Patrick & Reimer.

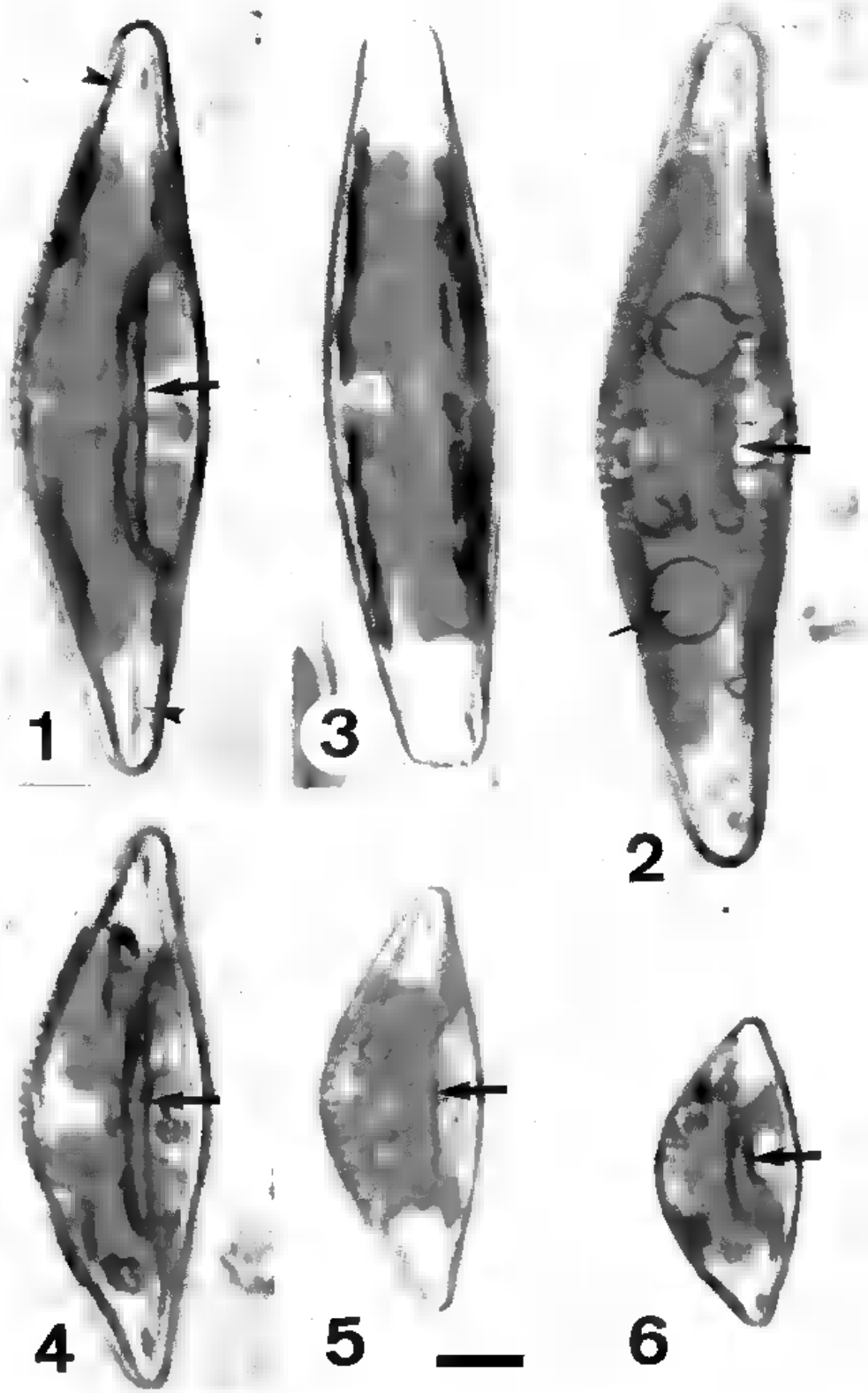
Le contour valvaire fortement dissymétrique, avec un côté dorsal toujours fortement convexe, présente une partie ventrale à convexité variable parfois aplatie ou même légèrement concave chez certains individus de petite taille. Les valves de longueur inférieure à 40 µm présentent des extrémités pointues ou émoussées; au-delà, les cellules sont toutes apiculées.

Les stries toujours bien nettes sont parallèles et perpendiculaires au raphé pour les petites formes. Elles deviennent légèrement convergentes vers le centre pour les grandes diatomées, mais ce caractère n'est pas constant. Le nombre de stries est variable sur le même individu : 9 à 11 stries dorsales pour 10 à 12 ventrales dans la partie médiane. Près des apex, ce nombre peut atteindre 11 à 18 en 10 µm. Ces valeurs sont en accord avec celles fournies par Patrick & Reimer (1975). La présence, côté ventral, d'une à deux stries médianes plus courtes peut être considérée comme un bon caractère discriminant (fig. 10 à 14).

La ponctuation bien nette, espacée au centre avec 10 à 15 points en 10 µm, est très variable puisqu'elle atteint 15 à 18 points en 10 µm au niveau des extrémités. Elle est plus dense chez les petits individus.

L'area longitudinale est étroite et varie peu 2 à 2.5 µm. L'aire centrale est pratiquement inexistante sur la partie dorsale, elle est très faible sur la partie ventrale.

Le raphé presque rectiligne, légèrement incurvé vers la partie ventrale est de type 3 (Krammer & Lange Bertalot, 1986, p. 691, fig.124:12). Il est situé à peu près au 2/3 de la largeur centrale de la valve (rapport Vent./dors = 0.665 = +0.07 indépendant de la longueur du frustule, corr. = 0.363). Les fissures terminales du raphé s'incurvent par un crochet plat vers la partie ventrale. Inversement les terminaisons médianes sont incurvées vers le côté dorsal.



Les principaux critères de distinction par rapport aux autres espèces du genre peuvent être résumés comme suit (Tableau I):

Contour valvaire fortement dissymétrique à convexité dorsale marquée

Nombre de stries en 10µm (10-11 en moyenne)

Présence d'une à deux stries médianes plus courtes coté ventral.

Area longitudinale étroite (2 à 3 µm).

Terminaisons polaires du raphé incurvées côté ventral, inversement au centre.

Indentation des chloroplastes en forme de pont sur la partie ventrale.

			Mesures effectuées au niveau de l'area centrale (sur 52 valves)					
			Nombre de stries		Nombre	distance du raphé au bord :		
	Longueur	Largeur	dorsales	ventrales	de points	ventral (V)	dorsal (D)	V/D
maxi	74,0	20,6	11	12	15	9,64	11,63	0,88
mini	25,0	12,6	7	7	8	4,98	7,62	0,45
moyenne	44,7	16,8	8,6	10,0	12,3	6,7	10,1	0,67
écart-type	11,15	1,83	1,38	0,83	1,83	1,06	1,01	0,09
Coef. de variation	0,25	0,11	0,16	0,08	0,15	0,16	0,10	0,13

Ultrastructure

Au M.E.B. les intervalles des rangées de points se révèlent être des côtes fortement dessinées avec un renforcement marqué sur la partie dorsale intérieure (fig. 16 flèche). La structure des points est plus complexe. Ils apparaissent bien nets et circulaires au centre à l'intérieur (fig. 17) alors qu'ils sont séparés par des barres près des apex (fig. 18 flèche). D'autre part, à l'extérieur du frustule, ils sont représentés par des fentes parallèles à l'axe apical (fig. 19) qui se transforment parfois en croix ou en T (fig. 20-21 flèches). Le raphé est constitué à l'extérieur comme à l'intérieur par une ligne fine, légèrement sinueuse et se termine sur les apex par un crochet plat (fig. 21) qui est parfois visible ■ microscopie photonique. En vue interne, le raphé se termine sur les apex dans l'hélictoglosse qui forme une protubérance bien individualisée située à 3 ou 4 µm de l'extrémité. La fissure du raphé en forme de crochet correspond à la partie hyaline (fig. 18 flèche) située entre l'hélictoglosse et l'extrémité du frustule. Dans la partie centrale du raphé, les deux pores bien dessinés sur l'extérieur de la valve (fig. 20) se transforment en deux crochets tournés vers la partie dorsale à l'intérieur. Cette disposition est connue chez les *Cymbella* (Krammer & Lange Bertalot, 1986).

Ecologie

Distribution

Tous les individus décrits dans l'Atlas de Schmidt proviennent d'Amérique du Nord (Pensacola Floride pour l'espèce et Mexique pour la variété *gracilis*). Patrick &

Fig. 1 à 6: *Cymbella triangulum* , matériel vivant. Fig. 1: Vue valvaire d'un spécimen de 80 µm - On remarque : les extrémités lancéolées (tête de flèche) - Le pont ventral formé par les chloroplastes (flèche). Fig. 2: Vue valvaire d'une cellule initiale de 90 µm - On distingue le pont déjà formé (flèche forte) et l'accumulation de globules lipidiques (flèches fines). Fig. 3: Vue connective - Les chloroplastes sont plaqués sur les valves. Fig. 4-5-6: *Cymbella triangulum* à divers stades de croissance - On notera la présence constante du pont formé par les chloroplastes ainsi que le remplissage plus ou moins régulier. Échelle 10 µm

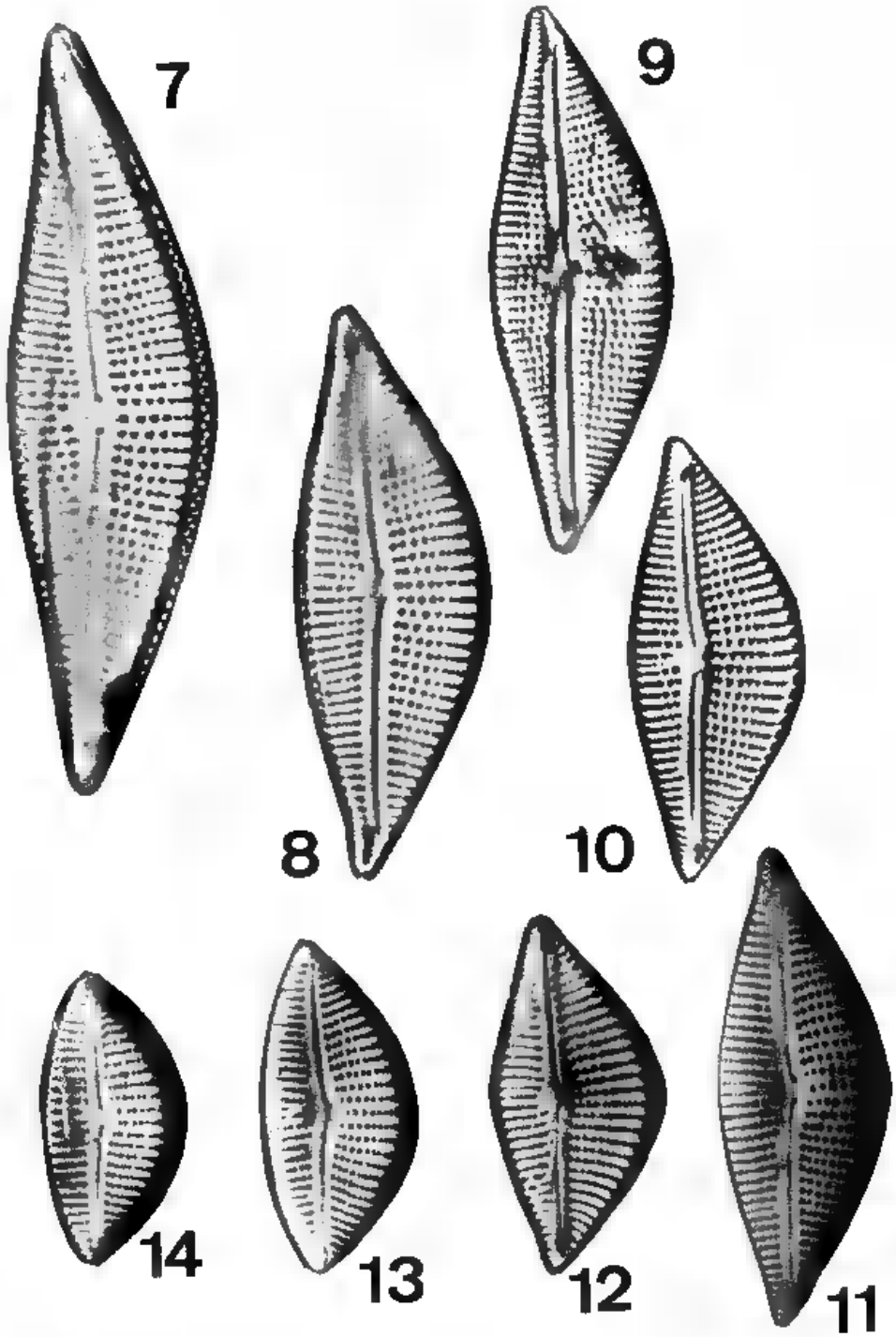
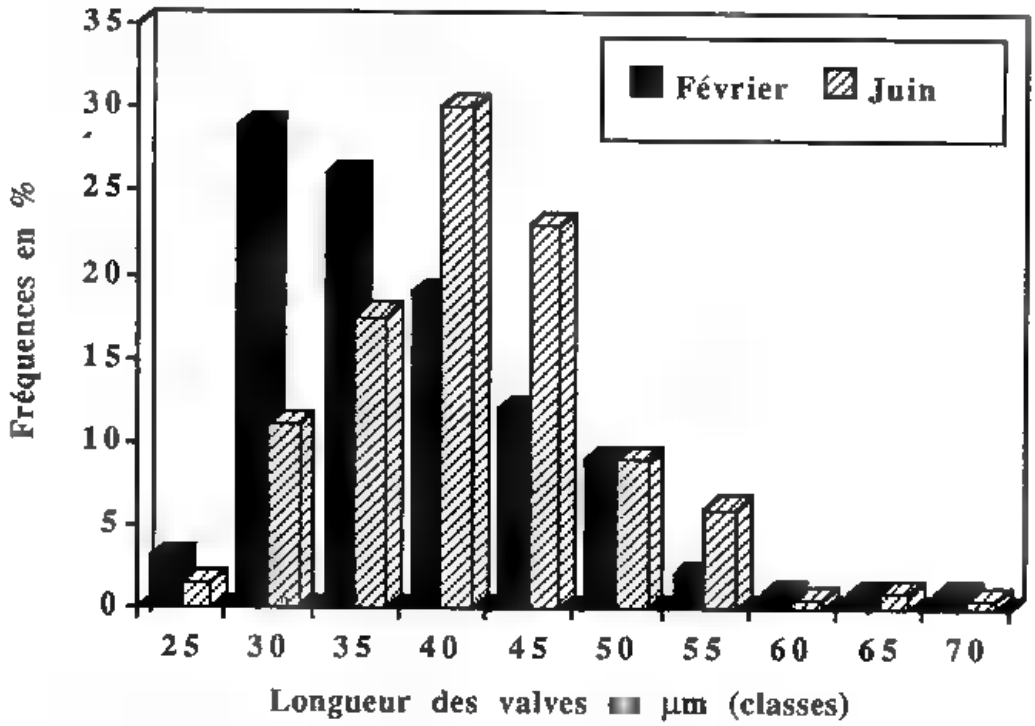


Fig. 7 à 14: *Cymbella triangulum* (microscopie photonique) - Évolution de la forme dorsi-ventrale au cours de la régression dimensionnelle - Le raccourcissement d'une ou deux stries ventrales centrales est une constante de détermination. Échelle 10 µm



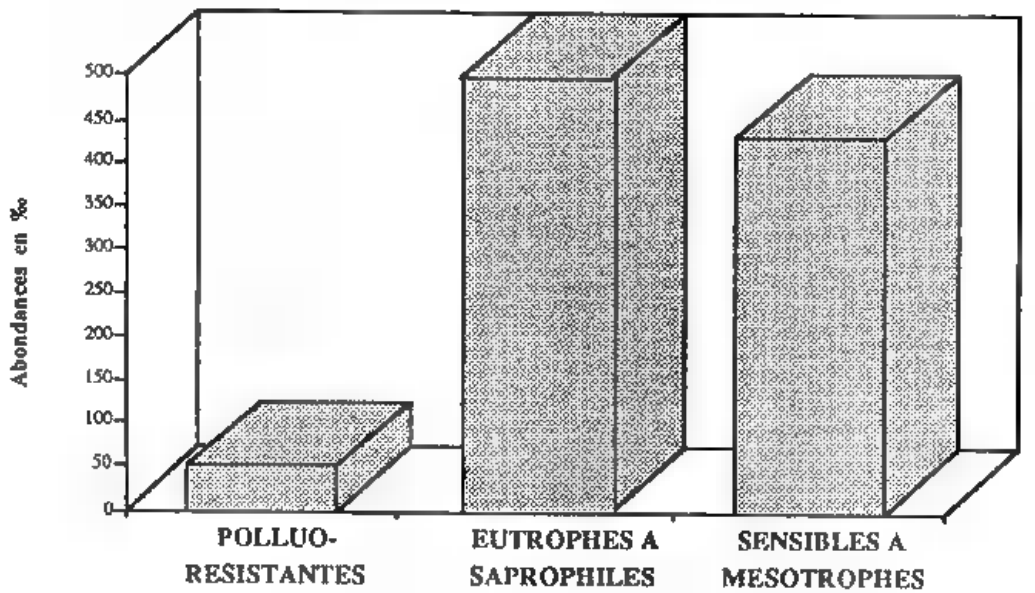
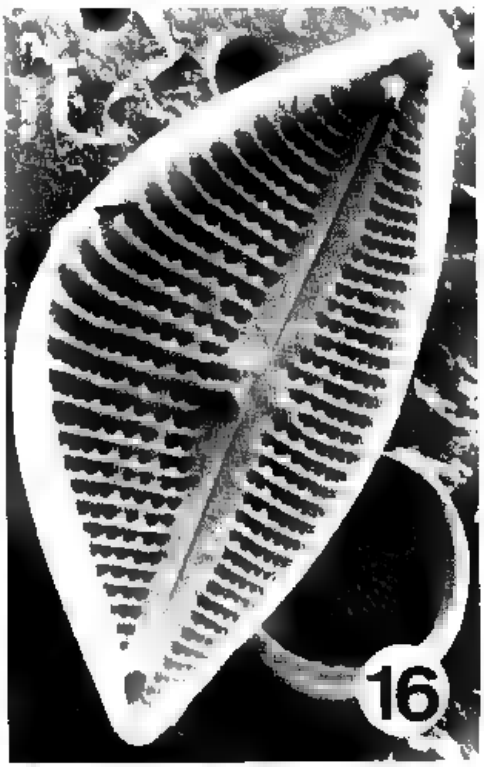
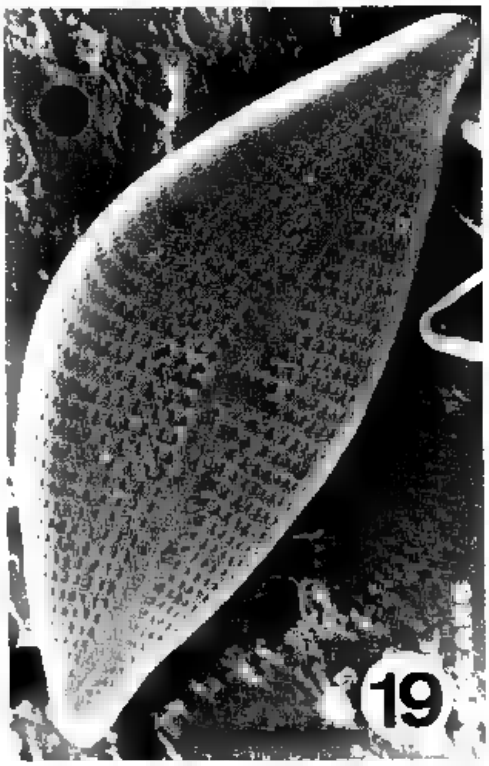
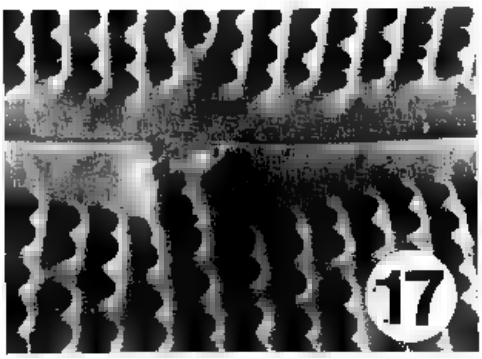


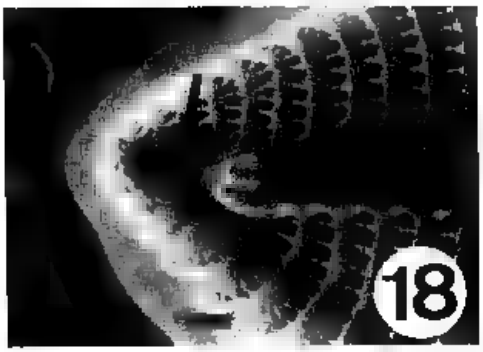
Fig. 15a. Distribution de *Cymbella triangulum* par classes de tailles (longueur) en Février et Juin 93
Fig. 15b. Distribution des classes de sensibilités des diatomées associées à *C. triangulum* en juin.

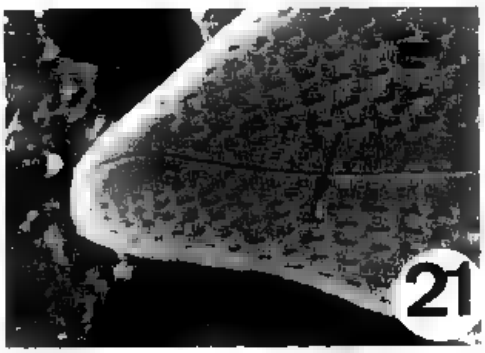
Reimer (1975) dressent une liste de stations de récoltes pour les États -Unis avec comme localité type l'aval des chutes du Niagara. *Cymbella triangulum* apparaît disséminé dans la plupart des États du centre et du sud aussi bien à l'est qu'à l'Ouest.. Aucune mention de cette espèce n'est faite dans les flores d'Europe où elle semble ne pas avoir été recensée à ce jour, du moins à notre connaissance.











La présence de *Cymbella triangulum* dans le centre de la France est nouvelle. Nous en avons, au moins la certitude pour la zone d'Orléans où les lieux de découverte étaient suivis systématiquement depuis 7 ans à raison de 20 à 25 prélèvements par an. Les récoltes ont toujours été exemptes de cette espèce. Le lieu de développement s'étend sur 6 kilomètres d'un canal de navigation utilisé depuis 50 ans uniquement pour la pêche. Le courant est faible à inexistant en période de basses eaux. Les premières récoltes ont été faites sur une dalle horizontale inondée d'un déversoir du canal. *Cymbella triangulum* était rare dans une population très dense de divers *Nitzschia* contenus dans des débris organiques, feuilles mortes et menus branchages. Par la suite, les recherches sur l'origine du peuplement nous ont conduit à prélever dans le canal; nous avons alors découvert *Cymbella triangulum* d'une manière constante sur les macrophytes immergés (*Ranunculus aquatilis, Potamogeton spp.*) mais également sur les feuilles de *Phragmites* immergées. La composition diatomique était alors différente. La localisation très restreinte de cette espèce nouvelle semble indiquer une colonisation accidentelle probablement importée d'aquarium possédant des espèces végétales ou animales originaires de Floride (USA) ou du Mexique. Nous pensons plus précisément aux tortues et en particulier à la tortue à tempes rouges dite "de Floride" *Chrysemys (Pseudemys)scripta elegans* qui colonise d'une manière permanente le canal où nous avons rencontré *Cymbella triangulum*. Si notre hypothèse se révèle exacte, nous devons nous attendre à rencontrer cette diatomée dans tous les lieux où la tortue de Floride, importée en grande quantité, a fait souche et forme des colonies sauvages. Elles ont été repérées dans les Pyrénées, l'Hérault, l'Allier, le Bordelais et en Ile de France. Selon Le Cohu (comm. verb.), la récolte récente de *Cymbella triangulum* dans le canal du Midi à Toulouse où la totrtue de Floride est également recensée, pourrait conforter cette hypothèse.

Caractéristiques écologiques

Cymbella triangulum semble être une espèce libre. Comme Ehrenberg, (1845, p. 77) qui notait déjà "Tubulos continuos non vidi", nous n'avons jamais observé dans les récoltes françaises de diatomées attachées ou possédant un reste de tube muqueux servant de fixation. Les déplacements sont relativement lents ; la vitesse varie de 5.75 à 8.35 µm/sec.-1 pour une moyenne de 6.45 µm/sec.-1.

Tous les mouvements propres aux *Cymbella* libres (Bertrand 1992) ont été observés et font l'objet d'un film vidéo de 15 minutes portant sur les séquences caractéristiques. Le déplacement apical sur la face valvaire s'exécute dans les plans horizontaux sur la lame, sous la lamelle couvre objet et également dans le plan vertical. On peut observer les mouvements transapicaux de la face valvaire vers la face

Fig. 16 à 21: *Cymbella triangulum*, vu en microscope électronique à balayage. Fig. 16: Vue intérieure d'un frustule - On remarque le renforcement des nervures dorsales (tête de flèche). Fig. 17: Vue intérieure centrale du raphé - On notera les crochets du nodule central. Fig. 18: Vue intérieure de l'apex - On distingue les barres de séparation des pores (flèche) et la partie hyaline opposée à la fissure du raphé. Fig. 19: vue extérieure d'un frustule. Fig. 20: vue extérieure des nodules centraux du raphé - Les fentes parallèles des points se transforment en croix (flèche). Fig. 21: vue extérieure de l'apex et la fissure terminale du raphé - Une fente en T (flèche). Échelle : fig. 16-19, 10 µm - fig. 17-18-20-21 ; 1 µm

connective étroite et vice-versa. Le pivotement polaire horizontal à partir d'un apex ou médian sur 60 à 200° est couramment exécuté alors que le pivotement polaire vertical est plus rarement observé car il nécessite un grand espace entre les deux surfaces de verre. Nous pouvons également remarquer des pivotements coniques verticaux ou obliques sur la diatomée en appui sur de la matière organique dans l'espace aquatique avec des glissements simultanés le long du raphé.

Cymbella triangulum parait relativement eurytherme aussi bien représenté dans les récoltes de février où la température n'excède pas 5°C que dans celles de juin où elle atteint 24°C. Le pH varie peu, de 6.5 en hiver à 7.5 en été. Les informations concernant l'écologie de l'espèce restent comme souvent, très fragmentaires. Pour Patrick & Reimer elle est fréquemment rencontrée dans des eaux des fleuves d'alcalinité moyenne et pourrait être considérée comme indifférente au pH à alcaliphile...

La composition des relevés effectués dans le canal apporte quelques informations complémentaires tirées des caractéristiques écologiques des dominantes.

L'utilisation des compilations bibliographiques proposées par Denys (1991) nous conduit a conclure que l'espèce affectionne les milieux neutres à alcalins et qu'elle est capable de supporter une charge en N et P non négligeable. La composition du peuplement diatomique de juin montre la juxtaposition de formes électives des milieux eutrophes, mésotrophes et oligotrophes ainsi que la présence d'espèces saprophiles à polluo-résistantes comme *Navicula subminuscula* ou *Nitzschia palea*, (Fig. 15b et tab. II).

La faible abondance de *Cymbella triangulum* et le nombre restreint de relevés ■ permettent pas de préciser plus avant les exigences écologiques de cette espèce dont la dissémination sera suivie avec intérêt.

DISCUSSION ET CONCLUSION

Cymbella triangulum vient s'ajouter aux espèces récemment répertoriées dans les eaux françaises et devrait contribuer modestement sans doute à la modification progressive de la microflore de nos eaux courantes. Sa persistance ou le succès de sa dissémination pourrait bien traduire des modifications à long terme qui échappent aux investigations de routine et que le programme international "Global Change" tente de recenser. Parmi les diatomées d'apparition récente dans les eaux françaises il faut signaler des formes planctoniques: *Skeletonema potamos* (Weber) Hasle, signalé pour la première fois dans la Seine par Belcher & Swale en 1978; *Hydrosera triquetra* Wallich recensé dans l'estuaire de la Gironde; des formes benthiques libres ou fixées comme *Gomphoneis herculeana* (Ehrenb.) Cleve (et formes affines) qui envahit les cours d'eau du Sud-Ouest et du Centre de la France (Coste *et al.*, 1991); plus récemment *Navicula jakovlevicii* Hustedt dans le Rhône et la Meuse, également cité par Reichardt (1992) en Suisse. La capacité d'adaptation des diatomées déversées dans nos caux tempérées est souvent importante et des espèces électives des milieux tropicaux paraissent s'acclimater avec succés à ces nouvelles conditions. C'est le cas de *Navicula confervacea* (Kütz.) Grunow observé dans la Seine et la Charente, d' *Hydrosera triquetra* Wallich déjà cité ou de taxons subcosmopolites comme *Stauroneis*

CANAL LOIRE JUIN 93 - Cymbella triangulum (Ehrenb.) Cleve	
Espèces dominantes en juin 1993	‰
Gomphonema parvulum Kutzing var. parvulum f. parvulum	220
Cymbella minuta Hilse ex Rabenhorst	101
Cyclostephanos dubius (Fricke) Round	82
Navicula tripunctata (O.F.M.) Bory	58
Melosira varians Agardh	55
Cocconeis placentula Ehrenb. var. placentula	42
Nitzschia fonticola Grunow	31
Stephanodiscus hantzschii Grunow in Cl. & Grun.	31
Navicula subminuscula Manguin	29
Navicula cryptotenella Lange-Bertalot	23
Navicula capitatoradiata Germain	22
Achnanthes minutissima Kutzing v.minutissima Kutzing	21
Aulacoseira granulata (Ehrenb.) Simonsen	21
Nitzschia paleacea Grunow in V.Heurck	21
Cocconeis placentula Ehrenb. var.euglypta(Ehrenb.)Grunow	14
Nitzschia palea (Kutzing) W.Smith	14
Stephanodiscus hantzschii fo.tenuis(Hustedt)Hakansson et Stoermer	13
Nitzschia amphibia Grunow f.amphibia	13
Cymbella triangulum (Ehrenb.) Cleve	10

Tableau II : Principales espèces dominantes dans le relevé de Juin 1993.

brasiliensis. (Zimmerman) Compère dans la Gartempe, *Navicula kotschyi* Grunow, dans la Drôme. Ces ensemencements, dont les conséquences peuvent être néfastes comme l'a montré l'exemple du *Caulerpa taxifolia* (Vahl) Agardh en Méditerranée, sont fréquemment imputés à des déversements accidentels ou provoqués d'organismes animaux ou végétaux utilisés en aquariophilie ou en aquaculture. Une certaine vigilance parait de rigueur, et la formation de diatomistes susceptibles d'apporter une contribution à la surveillance des milieux aquatiques devrait être encouragée.

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CAULERPA RACEMOSA (CHLOROPHYTA) ON THE GREEK COASTS

Panayotis PANAYOTIDIS[1] and Barbara MONTESANTO[2]

1. National Center of Marine Research (NCMR) 16604 Agios Kosmas Hellinikon, Athens, Greece.
2. Univ. of Athens, Ecology & Taxonomy Dep. 15784 Panepistimiopolis, Athens, Greece

The green alga *Caulerpa racemosa* (Forsskal) J. Agardh (Caulerpaceae, Bryopsidales) is a species with tropical and subtropical distribution (Taylor, 1960), rare in the Mediterranean (Boudouresque *et al.*, 1990). Suddenly a massive occurence is reported from the Mediterranean coasts of Egypt (Aleem, 1992) and one year later Alongi *et al.* (1993) reported *C. racemosa* in the southern Italian coasts. During the same period the expansion in the Mediterranean of the also tropical *Caulerpa taxifolia* (Vahl) C. Agardh is reported (Boudouresque *et al.*, 1992).

Samplings were carried out by SCUBA diving in Laganas Bay (Zakynthos Island, 37:40 N, 20:45 E) during summer 1993 and by draging in Pylos Bay (Western Greek coasts, 36:50 N, 21:40 E), during automn 1993. The *C. racemosa* specimens were found on *Posidonia oceanica* (L.) Delile beds at 25-35 m depth. Specimens were fixed in formalin 4% and examined in the NCMR laboratory of phytobenthos.

The examined specimens (Figure 1) are very similar with *C. racemosa v. macrophysa* (Kutzing) Taylor, described by Taylor, (1960). The cylindric stolons are 2-3 mm large, and the erect axes are 2 mm large and 3.0-10.0 cm long. The branchlets are attached to them at intervals of 3-5 mm. The branchlets are subconical 3-4 mm long, and 2-3 mm large.

The examined specimens were collected on seagrass beds where they formed small patches covering the free space between the *P. oceanica* shoots. The studied areas are oligotrophic (*P. oceanica* deeper limit at 40 m. depth). Ben Maiz (1984) and Aleem (1992) reported the presence of *C. racemosa* on shallow rocky coasts near urban areas. The examined specimens are different from those described by Ben Maiz (1984). Nevertheless, *C. racemosa* is a very polymorphic species and the genetic value of the varieties is doubtful. Calvert (1976) reports that assimilators of var. *macrophysa* in the 1350 lx culture are 1-3 cm tall and bear uncrowded imbricate ramuli, similar to the speciments of *C. racemosa* described by Ben Maiz (1984). The same material at 650 lx culture presented assimilators which showed a marked elongation up to 10 cm, the ramular placement became bilateral with opposite ramuli spaced at 5-7 mm, similar to our specimens. The var. *macrophysa* is new for the mediterranean but it is possible that the differences between our specimens and the earlier reports are due to the different light conditions of the sampling sites.

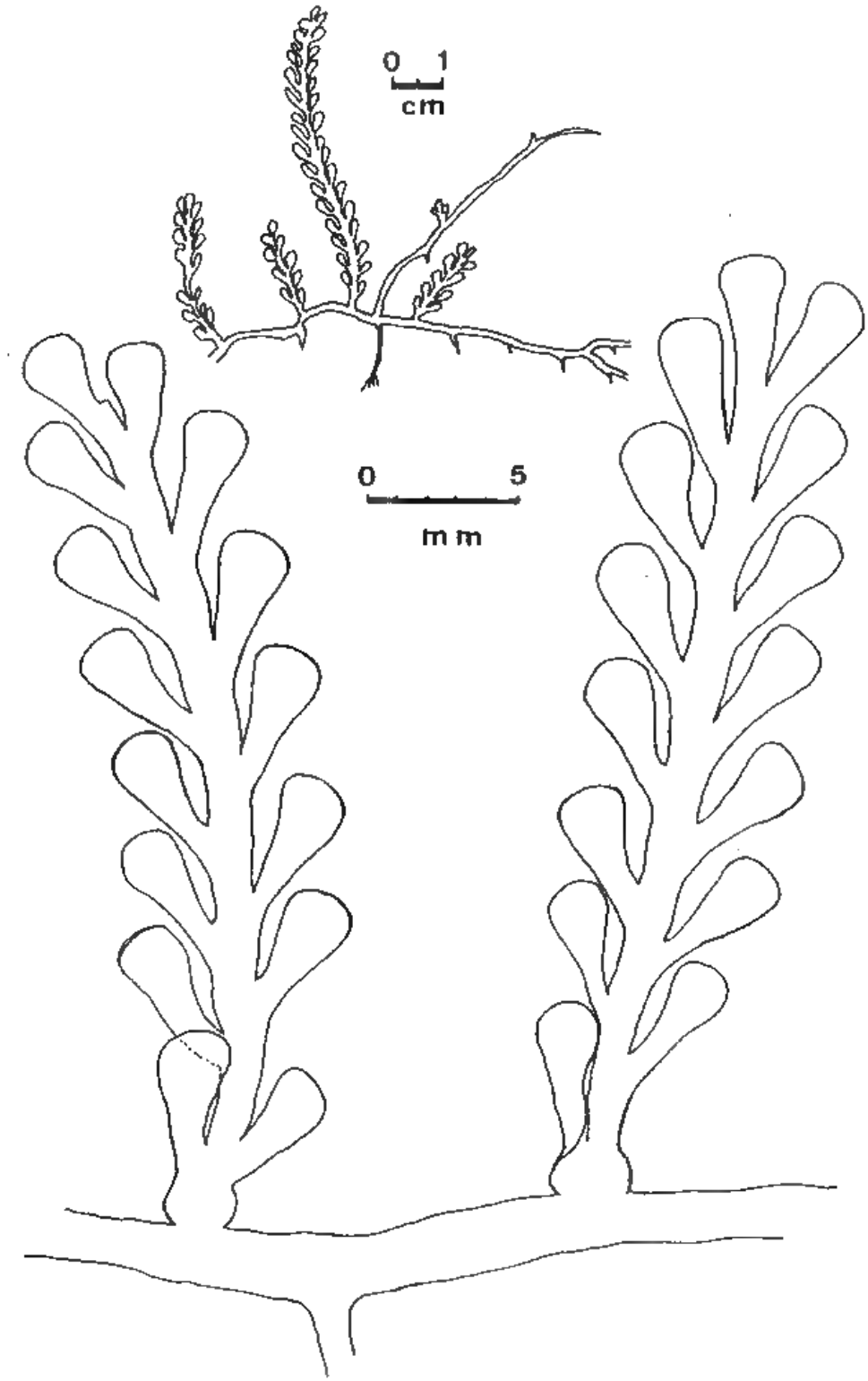

Figure 1. The examined specimens.

In the Mediterranean it is already known the opportunistic behaviour of some other subtropical species as *Halophila stipulacea* (Lessepsian migrator) and *Caulerpa prolifera*, which are very abundant in the free spaces between *P. oceanica* shoots, when there is a decline of the seagrass beds due to the pollution (Panayotidis, 1988). It is also known that the man introduced species *Caulerpa taxifolia* has an antagonistic behavior against *P. oceanica* (Villele & Verlaque, 1992). Thus, it is interesting to survey the distribution *C. racemosa* in the Greek coasts for the next years, in order to compare the ecological strategies of the tropical species (induced or not) in the Mediterranean under the global climat changes point of view.

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Source : MNHN, Paris

OUVRAGES REÇUS POUR ANALYSE

YARISH C., C.A. PENNIMAN & P. Van PATTEN (eds), 1990 - **Economically Important Marine Plants of the Atlantic. Their Biology and Cultivation,** Connecticut Sea Grant College Program, Marine Sciences Institute, University of Connecticut, Groton, viii + 158 p.

Cet ouvrage correspond à la publication des résultats du symposium du 2 octobre 1988: "Economically Important Marine Plants of the Atlantic: Their Biology and Cultivation" regroupant des scientifiques des Etats-Unis, du Canada et du Royaume Uni, des industriels et des représentants gouvernementaux. Certaines données, surtout économiques, sont par conséquent déjà anciennes, mais ce fascicule fait utilement le point sur un certain nombre de questions et consitute un apport bibliographique précieux. Il est subdivisé en neuf chapitres.

Le premier (J.P. van der Meer) est une présentation de l'état de nos connaissances sur la recherche génétique des algues et les impacts pratiques qui peuvent en résulter sur la production. La recherche en biologie des algues a apporté et continue d'apporter une contribution non négligeable à l'industrie mais les améliorations à cibler par la génétique moléculaire ne sont, selon lui, pas clairement définies.

Dans un deuxième chapitre, D.P. Cheney expose une synthèse de nos connaissances sur la technique de fusion des protoplastes (qui permet la réalisation d'hybrides somatiques, en particulier entre espèces sexuellement incompatibles mais à partir desquels on ne réussit pas toujours à régénérer une algue dont on a la certitude qu'il s'agit bien d'une vraie chimère "hybride").

Le chapitre trois (C.J. Dawes) est une revue de l'écophysiologie des iota-carraghénophytes, en particulier *Eucheuma isiforme*.

J.S. Craigie expose au chapitre 4 l'optimisation des techniques de culture de *Chondrus crispus* en bacs extérieurs, dans les provinces maritimes du Canada, notamment pour produire du lambda-carraghénane à partir des sporophytes.

Le chapitre 5, par C. Yarish *et al.*, comporte une réflexion et une synthèse intéressante sur la notion d'écotype et d'espèce chez les algues (en particulier brunes), ainsi qu'une étude de la taxinomie et de la biogéographie des espèces du genre *Laminaria* section *simplices*, dans l'Atlantique nord. Ces points sont suivis par une synthèse sur la détermination de la valeur systématique des caractères taxinomiques, la manière de mettre en évidence s'il sont déterminés génétiquement ou non et l'intérêt appliqué de cette détermination. Enfin, une méthodologie de sélection et le protocole utilisé à Long Island sont présentés.

J.M. Kain *et al.* (chapitre 6) s'interroge sur la possibilité de réussir, en Europe et avec des espèces indigènes de Laminariales, ce qu'ont réalisé les japonais avec *L. japonica*. Dans cette optique, les techniques de culture sur filins sont passées en revue; certaines paraissent économiquement utopiques, au moins pour l'instant, pour des algues destinées à l'alimentation et donc de relativement faible valeur ajoutée. L'intérêt des travaux réalisés par l'IFREMER dans ce domaine est reconnu et souligné.

J.D. Pringle & G.J. Sharp (chapitre 7) font une mise à jour des données concernant les cinq espèces récoltées à des fins commerciales dans l'est du Canada. L'importance, au plan économique et au plan de la qualité, du séchage, lorsque le transport le rend nécessaire, est soulignée. L'introduction des techniques norvégiennes de récolte mécanisée des *Ascophyllum* se traduit par une inquiétante surexploitation de cette matière première. Les auteurs constatent l'absence de politique de gestion des ressources naturelles végétales marines, même chez des pays leader dans l'exploitation de ces ressources, en dehors de la France et du Canada qui, dans ce domaine, sont aussi exigeants pour les ressources végétales que pour les ressources animales et fournissent un support financier. Les auteurs soulignent aussi le soutient scientifique fourni à l'aquaculture en Chine et aux USA. Un bilan des données nécessaires à connaître pour la bonne gestion des stocks est proposé.

Au chapitre 8, K.T. Bird rappelle que le marché mondial des algues marines benthiques est de l'ordre de 1 à 2 milliards de dollars. Il souligne aussi les aspects parfois fragmentaires des

marchés et les contraintes qu'imposent la concurrence internationale de pays où la main d'oeuvre est extrêmement bon marché. Pour cet auteur, la rentabilité de l'aquaculture des algues passe par une stratégie multiproduits privilégiant les substances à forte valeur ajoutée, les colloïdes très ciblés techniquement et ne pouvant être extraits des algues importées et les algues alimentaires vendables sur le marché local.

C.A. Penniman (chapitre 9) conclut par une discussion sur les perspectives d'avenir. Les problèmes sont clairement posés de la part de l'industrie (Marine colloids) et des représentants du gouvernement. Il faut noter que l'une des préoccupations majeures de l'industrie (outre, bien entendu, les propriétés physico-chimique des colloïdes) est d'avoir une meilleure connaissance systématique des espèces et une approche biochimique de la classification qui mette en relation cohérente la nature du polysaccharide pariétal et la position taxinomique. Rappelons que cette discussion date de 1988, ce n'est pas un hasard si la nouvelle classification des algues rouges qui se dessine est beaucoup plus cohérente avec les compositions pariétales.

B. de Reviers

FRIEDMANN E.I. (Ed.), 1993 - **Antarctic Microbiology,** 1 vol., 634 p. John Wiley & Sons, Inc., Price: 165 $.

Ce volume, composé avec la collaboration de A.B. THISTLE, montre bien l'importance des microorganismes dans l'Antarctique. Nous y trouvons l'étude du milieu marin: Microbial processes in the southern Ocean, par D.M. Karl; le phytoplancton par S.Z. El-Sayed et G.A. Fryxell; le protozooplancton avec des formes autotrophes (Cyanophycées, Diatomées, Dinoflagellés) et des hétérotrophes (Bactéries, Amibes, Ciliés, Foraminifères, Radiolaires, Héliozoaires) par D.L. Garrison et M.M. Gowing; A.C. Palmisano et D.L. Garrison étudient les microorganismes croissant sur les glaces de la mer polaires et montrent l'abondance des Diatomées et des Ciliés; D.C. White, G.A. Smith, J.B. Guckert et P.D. Nichols s'intéressent aux sédiments benthiques marins proches des côtes, sédiments riches en stérols; J.T. Staley et R.P. Herwig étudient la dégradation du matériel organique dans l'Antarctique (chitine, acide urique et kératine). La deuxième partie réunit les articles relatifs au milieu continental et d'eau douce: S.S. Abyzov signale les microorganismes de la glace (Bactérie Levures et Champignons); H.S. Vishniac étudie la microbiologie des sols antarctiques (Bactéries, Champignons, Protozoaires); J.A. Nienow et E.I. Friedmann analysent les communautés croissant sur les rochers (Lichens, Cyanophycées, Bactéries, Diatomées, Xanthophycées, Chlorophycées); P.A. Broady étudie la flore des sols réchauffés par les fumerolles volcaniques (Cyanophycées, Chlorophycées, Diatomées, Champignons, Bactéries); L. Kappen donne une étude sur l'écologie des Lichens antarctiques; G.M. Simmons Jr., J.R. Vestal et R.A. Wharton Jr. présentent l'écologie de la microfaune et de la microflore des lacs continentaux antarctiques et la formation des stromatolites; W.F. Vincent, C. Howard-Williams et P.A. Broady étudient les communautés microscopiques des eaux courantes de l'Antarctique durant la fonte des neiges. Dans la dernière partie H.G. Muchmore, E.N. Scott et A.J. Parkinson analysent les maladies infectieuses humaines dans l'Antarctique; C.P. McKay s'intéresse à l'exobiologie", c'est-à-dire à l'origine et à la distribution de la vie dans l'univers et compare le mode vivant de l'Antarctique à celui que l'on peut supposer exister dans la planète Mars.

Ce volume se termine par un article de S. Draggan qui propose une protection internationale de l'Antarctique dont 28 régions ont un intérêt scientifique spécial. Un index alphabétique termine ce volume. Nous souhaitons que ce bref aperçu montrera la valeur scientifique de cet ouvrage pluridisciplinaire.

P. Bourrelly

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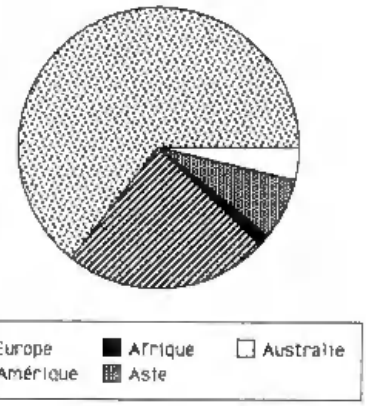

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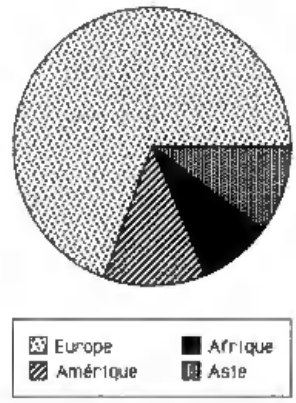

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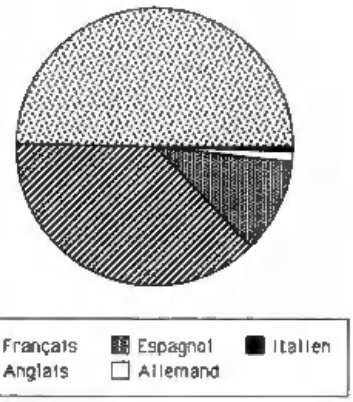

SOMMAIRE

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