

# Nanoflagellates from coastal waters of southern Brazil (32°S)

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## Abstract

A taxonomic survey of nanoflagellates in the extreme southern Brazilian coastal waters of the Patos Lagoon estuary and adjacent coastal shelf was undertaken using transmission electron microscopy. A total of 44 species was recorded including 42 nanoflagellates, one scale-bearing amoeba and two species *incertae sedis*. Scale-bearing haptophytes predominated (27 species), followed by prasinophytes (7), chrysophytes (6), pedinellids (2), *incertae sedis* (2) and one scale-bearing amoeba. Most of the species (42) are new records for Brazilian marine waters. Among the haptophytes, *Chrysochromulina* was the most species-rich genus, occurring mainly in high salinity waters, and *C. simplex* was the most frequent species. Scales of undescribed *Chrysochromulina* species were observed and documented. Other haptophytes present were *Prymnesium parvum* and *Phaeocystis scrobiculata*. Five scale-bearing prasinophytes of the genus *Pyramimonas* were observed including the uncommon species *P. longicauda*, in addition to *Micromonas pusilla* and *Nephroselmis pyriformis*. Among the chrysophytes, the scale-bearing *Paraphysomonas* species (4) and the loricate *Dinobryon belgica* were recorded. The pedinellids (*Apedinella radians* and *Parapedinella reticulata*), the amoeba *Rabdiophrys* sp. and the *incertae sedis* (*Meringosphaera mediterranea*, *Thaumatomastix* sp.) are all scale-bearing protists. The 44 taxa (mostly scale-bearing flagellate species) identified and the finding of further undescribed *Chrysochromulina* species indicate that only a fraction of the species present in the region are known, and that an effort must be made to increase general knowledge of biodiversity in these southern waters.

**Keywords:** Chrysophyceae; Haptophyta; pedinellids; Prasinophyceae; taxonomy.

## Introduction

Nanoflagellates (<20 µm) are a group of abundant and phylogenetically diverse, fast-growing autotrophic and heterotrophic organisms that commonly dominate microbial communities (Raven 2000). Autotrophic/mixotrophic nanoflagellates constitute the main component of oce-

anic phytoplankton biomass and are sometimes responsible for most of the primary production. Mixotrophic/heterotrophic species feed on bacteria, channeling production to higher trophic levels, and are responsible for nutrient remineralization (Azam et al. 1983, Laybourn-Parry and Parry 2000).

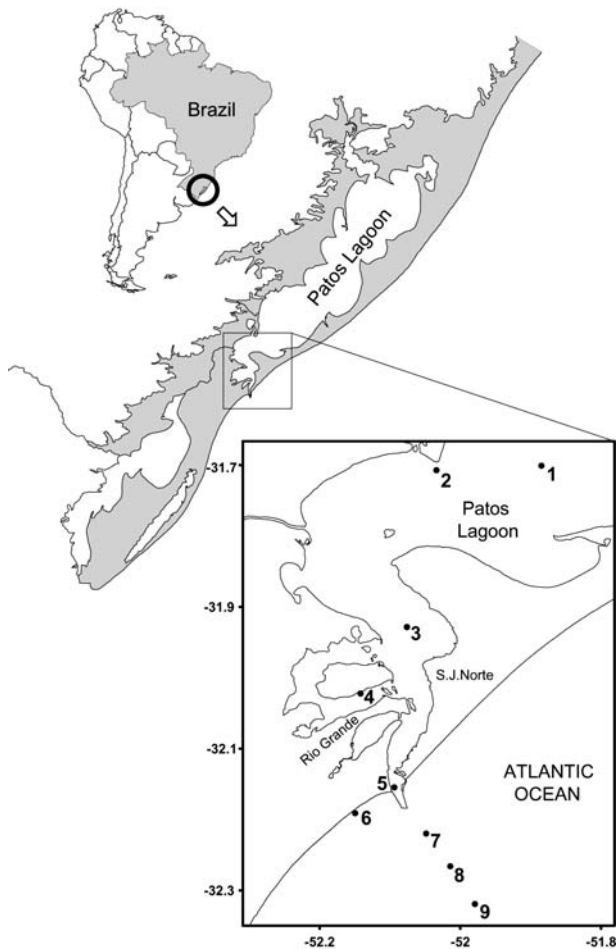
Nanoflagellate species identification is hampered by their small size and often requires electron microscopy. Through routine use of the electron microscope, knowledge of biodiversity has increased tremendously with the recognition of new taxonomic entities. Interest in small forms has increased, especially since the recognition that several species are toxic, and toxic blooms may cause deaths and economic losses in many parts of the world (Moestrup 1994, LeRoi and Hallegraeff 2004).

Nanoflagellates are poorly known in the southwestern Atlantic Ocean, partly due to the need to work with electron microscopy (Patterson and Lee 2000, Bergesch and Odebrecht 2001). Knowledge of the nanoflagellates from the Southern Hemisphere increased through the work of LeRoi and Hallegraeff (2004, 2006) in Australian waters, who demonstrated the apparent cosmopolitanism of many scale-bearing nanoflagellate species. A taxonomic and ecological study of oceanic and shelf phytoplankton between latitudes 18°S and 29°S conducted by Ribeiro (1996) with scanning and transmission electronic microscopy showed a predominance of coccolithophorids (73 species), followed by chrysophytes (9 species) and prasinophytes (5 species). Southwards (32° to 34°S), nanoflagellates were found to be dominant in oceanic areas under the influence of tropical waters, while sharing dominance with diatoms and dinoflagellates over the coastal shelf (Odebrecht and Garcia 1998). In the Patos Lagoon estuary, nanoflagellates are abundant (Odebrecht and Abreu 1998); however, the species composition is poorly known. Only a small number of species, including cryptophytes (9), chlorophytes (8), euglenophytes (3) and raphidophytes (3) have been identified in southern Brazilian marine waters (Odebrecht and Abreu 1995, Torgan 1997, Persich et al. 1998). The aim of the present study is to provide information on the taxonomic composition of scale-bearing nanoflagellates from the Patos Lagoon estuary and adjacent coastal waters in southern Brazil, and to contribute to the knowledge on their geographical distribution.

## Materials and methods

### Study area

The Patos Lagoon in southern Brazil (Figure 1) is the world's largest choked lagoon with a surface of 10,227 km<sup>2</sup> (Asmus 1998). Choked lagoons are characterized by high surface area and a narrow mouth connecting with coastal waters. The estuary proper in the south comprises 10% of the lagoon's area and has highly



**Figure 1** Location of the Patos Lagoon in southern Brazil with the positions of the sampling stations (1–9).

variable hydrographic characteristics due to short-term, seasonal and long-term fluctuations. Under *El Niño* events in the Pacific Ocean, freshwater discharge exceeds mean values in southern Brazil, and the reverse occurs during *La Niña* phenomena (Berlato and Fontana 2003). The second key factor is wind forcing with SE/SW winds pushing seawater into the estuary during low fluvial discharge, while NE winds favor fluvial discharge and cause salinity decrease in the lower lagoon. Periods of strong freshwater discharge with seawater intrusion induced by southerly winds cause vertical stratification and the formation of a saline wedge (Garcia 1998). Thermohaline conditions of the adjacent continental shelf are influenced by the freshwater outflow from the Patos Lagoon and La Plata River, and by tropical and subantarctic waters during summer and winter, respectively. Tropical waters transported by the Brazilian Current are the main outer shelf and slope waters, while on shelf, diluted waters of subtropical and subantarctic origin prevail in winter and summer, respectively (Ciotti et al. 1995, Piola et al. 2000, Soares and Möller Jr. 2001).

### Sampling

Water samples were collected with 5 l Niskin bottles monthly (a) from surface waters and near the bottom at four stations (1, 2, 3 and 5) between February 1999 and January 2000, (b) from surface waters at three stations

(4, 5 and 6) between October 1998 and May 2000, and (c) at 0, 10, 20, 30, 50, 75 and 100 m along a cross-shelf transect to the estuary at three stations (7, 8 and 9). Eight additional samples were collected in March 1999 at station 5 close to the mouth of the estuary, and in December 1999 and March 2000 on the continental shelf (Figure 1). Temperature and salinity were measured with a mercury thermometer and a thermo salinometer (YSY 33 or CTD), respectively (Table 1).

### Fixation and preparation of samples

Water samples were fixed with a mixture of Lugol's and glutaraldehyde solution (final concentration 1% Lugol's and 0.25% glutaraldehyde) in dark 1 l flasks (Jensen 1998a) and filtered through a 20  $\mu\text{m}$  membrane to remove larger particles that might interfere with observations of nanoflagellates. Samples were left settling for two days or more before removal of the supernatant, and they were subsequently transferred to 60 ml brown flasks and stored in the dark at 4°C. Whole mount preparations for scales studies followed Moestrup and Thomsen (1980). Grids were shadowcast with chromium at an angle of 20–30° using an Edwards E12E vacuum evaporator. One to three grids per sample were examined in a JEM (JEOL, Tokyo, Japan) 100SX transmission electron microscope (TEM). The size of scales and specimens was measured manually on TEM micrographs.

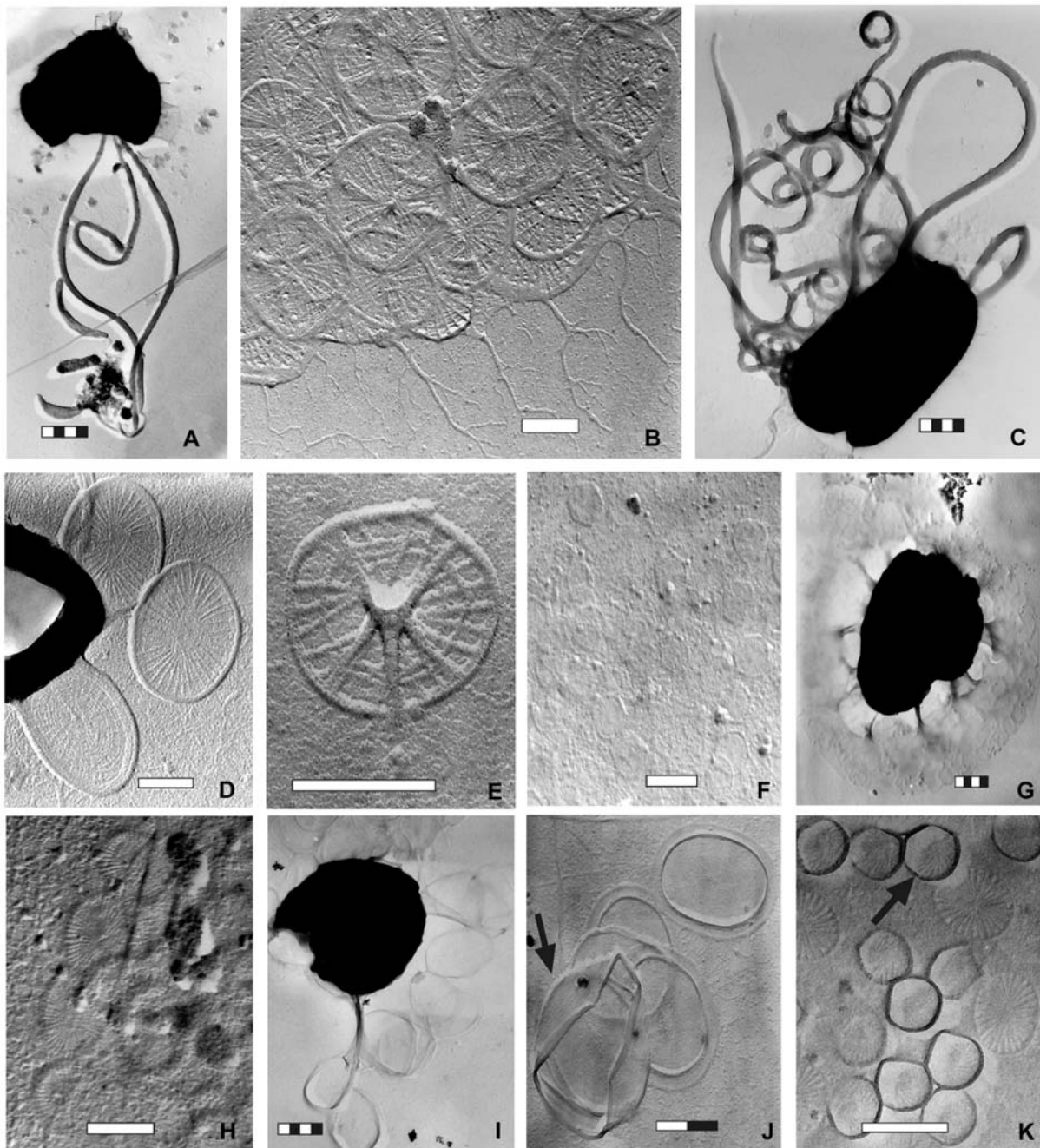
### Results

From the total of 44 nanoflagellate taxa, 40 were identified to species level. Scale-bearing protists (42) predominated in the southern Brazilian coastal waters of the Patos Lagoon and haptophytes represented the most species-rich group with 32 species. Other groups were prasinophytes (7), chrysophytes (5), pedinellids (2), two *incertae sedis* and one scale-bearing amoeba (Table 1). Among species with mineralized scales, we found *Wigwamma arctica*. Other haptophytes present were *Prymnesium parvum*, *Phaeocystis scrobiculata* and several described (21) and unknown species of *Chrysochromulina*. Five scale-bearing species of the prasinophycean genus *Pyramimonas* were observed, including the uncommon *P. longicauda*, in addition to *Micromonas pusilla* and *Nephroselmis pyriformis*. Among chrysophytes, scale-bearing *Paraphysomonas* (4) species and the loricate *Dinobryon belgica* were recorded. The pedinellids (*Apedinella radians* and *Parapedinella reticulata*), the amoeba *Rabdiophrys* sp., the *incertae sedis* species *Meringosphaera mediterranea* and *Thaumatomastix* sp. are all scale-bearing protists. A short description of each species is given below, including the observed characteristics, cell dimensions, lengths of flagella, and size of scales and lorica. As fixed material was used for the measurements in the TEM, changes in the observed cell size and shape are expected, mainly because of shrinking. The size and shape of the flagella, scales and lorica should not have been altered during preparation.

**Table 1** Nanoplankton protists recorded in the Patos Lagoon and on the southern Brazil coast, between 1998 and 2000, with station number (Figure 1), water depth, salinity and temperature.

Taxon	Salinity (psu)	Temperature (°C)	Station (depth) (m)	Date
<b>Haptophytes</b>				
<i>Chrysochromulina adriatica</i>	23; 30	24–28; 24	4 (0 m); 5 (0 m)	Jan. 1999, 2000
<i>C. ahrengotii</i>	36	28	5 (0 m)	Jan. 2000
<i>C. apheles</i>	36	18	9 (100 m)	Feb. 2000
<i>C. bergensis</i>	36	24; 24	8 (20 m); 10 (50 m)	Feb. 2000
<i>C. brachycylindra</i>	32	22	4 (0 m)	Dec. 1998
<i>C. cymbium</i>	35	19	9 (50 m)	Dec. 1999
<i>C. elegans</i>	36	24	9 (50 m)	Feb. 2000
<i>C. ehippium</i>	14	23	3 (0 m)	Dec. 1999
<i>C. cf. ericina</i>	32		4 (0 m)	Jan. 1999
<i>C. cf. hirta</i>	33	23	5 (8 m)	Apr. 1999
<i>C. kappa</i>	36; 30	23	7 (5 m)	Feb., Oct. 2000
<i>C. latilepis</i>	36	24	9 (20 m)	Feb. 2000
<i>C. megacylindra</i>	23	28	5 (0 m)	Jan. 2000
<i>C. minor</i>	31	23	6 (0 m)	Jan. 1999
<i>C. cf. parkeae</i>	35	24	9 (50 m)	Feb. 2000
<i>C. pringsheimii</i>	14–32	23	3 (0 m); 9 (10 m; 30 m)	Dec. 1999 Oct. 2000
<i>C. pyramidosa</i>	30	24	5 (0 m)	Jan. 1999
<i>C. cf. rotalis</i>	31	23	6 (0 m)	Jan. 1999
<i>C. scutellum</i>	27	19	5 (0 m)	Nov. 1999
<i>C. simplex</i>	27	20	6 (0 m)	Nov. 1999
<i>C. spinifera</i>	32	23	4 (0 m)	Jan. 1999
<i>C. sp. 2</i>	36; 32	24; 17	8 (20 m); 10 (10 m; 20 m)	Feb., Oct. 2000
<i>C. sp. 13</i>	36	24	9 (20 m)	Feb. 2000
<i>C. sp. 32</i>	30–31	24	5 (0 m), 6 (0 m)	Jan. 1999
<i>C. sp. 34</i>	36	24	9 (20 m))	Feb. 2000
<i>C. sp. 35</i>	36	24	8 (0 m)	Dec. 1999
<i>Corymbellus aureus</i>	8–35; 8	18–24	8 (75 m); 9 (0 m; 20 m)	Feb. 2000
<i>Imantonia</i> sp.	32	13	7 (20 m)	Oct. 2000
<i>Phaeocystis scrobiculata</i>	30	14	7 (5 m)	Oct. 2000
<i>Phaeocystis</i> sp.	27; 36	19	5 (0 m); 10 (75 m)	Nov. 1999, Feb. 2000
<i>Prymnesium parvum</i> f. <i>patelliferum</i>	30	19	5 (0 m)	May 1999
<i>Wigwamma arctica</i>	36	24	8 (0 m)	Feb. 2000
<b>Chrysophytes</b>				
<i>Dinobryon belgica</i>	35	17	9 (30 m)	Oct. 2000
<i>Paraphysomonas butcheri</i>	6; 30	28	3 (0 m); 6 (0 m)	Mar. 1999, Oct. 1998
<i>P. foraminifera</i>	15	23	4 (0 m)	Apr. 1999
<i>P. imperforata</i>	15; 2	20; 23	3 (0 m); 4 (0 m)	Aug., Apr. 1999
<i>P. vestita</i>	2	14	3 (0 m)	Aug. 1999
<b>Pedinellids</b>				
<i>Apedinella radians</i>	33	23	5 (15 m)	Apr. 1999
<i>Parapedinella reticulata</i>	4; 14	19; 16	3 (0 m); 5 (0 m)	Sept., Dec. 1999
<b>Prasinophytes</b>				
<i>Micromonas pusilla</i>	36	24	9 (10 m)	Feb. 2000
<i>Nephroselmis pyriformis</i>	30	19	6 (0 m)	Oct. 1998
<i>Pyramimonas disomata</i>	33; 15	24; 23	4 (0 m); 5 (0 m)	Mar., Apr. 1999
<i>P. grossii</i>	23–33	20–28	4 (0 m); 5 (0 m)	Jan. 2000, Jan. 2000 Apr., Nov. 1999
<i>P. longicauda</i>	30; 29	18; 15	5 (0 m); 6 (0 m)	Oct. 1998, Jun. 1999
<i>P. obovata</i>	32	14	8 (10 m)	Oct. 2000
<i>P. orientalis</i>	27	20	5 (0 m)	Nov. 1999
<b>Amoebae</b>				
<i>Rabdiphrys</i> sp.	2	19	3 (0 m)	Sept. 1999
<b>Incertae sedis</b>				
<i>Meringosphaera mediterranea</i>	33; 32	25	4 (0 m); 8 (20 m)	Mar. 1999, Oct. 2000
<i>Thaumatomastix</i> sp.	32	23	4 (0 m)	Jan. 1999





**Figure 2** Transmission electron micrographs of cells and scales of *Chrysochromulina* from the Patos Lagoon, southern Brazil. (A) *C. adriatica*, complete cell, (B) scales. (C) *C. ahrengotii*, whole cell with a long coiled haptonema, (D) plate scales, (E) spine scale. (F) *C. apheles*, scale details. (G) *C. bergensis*, cell with many scales spread around, (H) scale details. (I) *C. brachycylindra*, cell with detached scales, (J) plate and cylinder scales (arrow). (K) *C. cymbium*, plate scales and cup scales (arrow). Scale bars: 0.25  $\mu\text{m}$  per segment.

## Haptophyta

***Chrysochromulina adriatica* Leadbeater (Figures 2A, B)** Cells sub-spherical (3–4  $\times$  3  $\mu\text{m}$ ), two equal-length flagella (8–9  $\mu\text{m}$ ). Only oval plate scales were observed, probably of two types, both with scattered ridges in a concentric pattern. One of the scale types with a thickened rim (0.7–1.1  $\mu\text{m}$ ), the other lacking the rim or with a narrow inflexed rim on one face (0.7–0.8  $\times$  0.5  $\mu\text{m}$ ). Scale size range similar to Jensen (1998b), but larger than dimensions (0.6  $\times$  0.5  $\mu\text{m}$ ) reported by Leadbeater and Manton (1971).

***Chrysochromulina ahrengotii* Jensen et Moestrup (Figures 2C–E)** Cells saddle-shaped in outline (4  $\times$  3  $\mu\text{m}$ ), with equal-length flagella (12  $\mu\text{m}$ ) and a long coiling haptonema. Cell covered by two types of plate scales. One type is oval (0.6  $\times$  0.3–0.4  $\mu\text{m}$ ) with a system of concentric lines on the distal face, which possesses an inflexed rim. The proximal face has a pattern of ribs extending from the periphery, but without reaching the centre. Spine scales are more rounded (0.3–0.4  $\mu\text{m}$ ), with a spine supported by four struts extending to the thickened rim. The spineless side has radiating lines extending from the rim to the scale centre. The spine-

bearing upper side has a system of concentric ridges, and the radial fibrils on the proximal were visible through the scales. The spine is 0.3  $\mu\text{m}$  long.

***Chrysochromulina apheles* Moestrup et Thomsen (Figure 2F)** Cells spherical (2–3  $\mu\text{m}$ ). Periplast covered by two types of scales of the same size (0.2  $\mu\text{m}$ ). One type of scale with a thickened raised rim, giving an angulated appearance to the scale.

***Chrysochromulina bergenensis* Leadbeater (Figures 2G, H)** Cells oval (3–4  $\mu\text{m}$ ) with two types of scales: (1) numerous scales of a smaller oval type (0.4 $\times$ 0.3  $\mu\text{m}$ ), with inflexed rims and radial ridges on the proximal face, but patternless on the distal face; (2) larger and more delicate scales (1  $\mu\text{m}$  in diameter), apparently rimless but with a ridged proximal face.

***Chrysochromulina brachycylindra* Hällfors et Thomsen (Figures 2I, J)** Only two cells observed, both sub-spherical in shape (3  $\mu\text{m}$  in diameter), with slightly unequal flagella and a partially coiled haptonema. Two types of scales were present; one consisting of an oval plate scale (1.1–1.2 $\times$ 0.9–1.0  $\mu\text{m}$ ) concentrically striated on the distal face and with radial ridges on the proximal face. The second type is cylindrical with an oval basal plate (1.3 $\times$ 1.0  $\mu\text{m}$ ) and the cylinder (0.4  $\mu\text{m}$ ) with concentric striation on the distal face.

***Chrysochromulina cymbium* Leadbeater et Manton (Figure 2K)** Only incomplete cells observed, with two types of scales present: plate and cup scales. Plate scales (0.3 $\times$ 0.2  $\mu\text{m}$ ) with 28 ribs radiating from the centre, cup scales (0.2  $\mu\text{m}$ ) with slightly curved ribs radiating from the centre.

***Chrysochromulina elegans* Estep, Davis, Hargraves et Sieburth (Figure 3A)** Only one incomplete cell observed with three types of scales. Plate scales (0.8  $\mu\text{m}$ ) with radial ribs on one face and an inflexed rim on the other. Two types of spine scales are of the same size (0.8  $\mu\text{m}$ ) with an inflexed rim. One type of spine scale has a short spine (0.3  $\mu\text{m}$ ) and radial ribs visible on both faces. The morphological details of the long spines (4.5–5.0  $\mu\text{m}$ ) base plates were not observed (Estep et al. 1984).

***Chrysochromulina ehippium* Parke et Manton (Figures 3B, C)** Cells saddle-shaped (3  $\mu\text{m}$  in diameter) with two types of scales. Plate scales (0.5–0.6  $\mu\text{m}$ ) with radial ribs on both faces and an inflexed rim. Spine scales (0.3–0.4  $\mu\text{m}$ ) have radial ribs on the proximal face and a thickened rim on the distal face. The spine length equals the cell diameter or is a little longer (Jensen 1998b).

***Chrysochromulina cf. ericina* Parke et Manton (Figure 3D)** Cells ovoid to oblong (2 $\times$ 4  $\mu\text{m}$ ). One type of scale is without a spine (1.0 $\times$ 0.9  $\mu\text{m}$ ), while the other has a long spine, however, no details were observed in either scale type.

***Chrysochromulina cf. hirta* Manton (Figure 3E)** The species has three different types of scales, but only spine scales were observed, scattered around the protoplast. The basal plate (1.7 $\times$ 2.0  $\mu\text{m}$ ) is concave and the spine was long (3.8  $\mu\text{m}$ ). The proximal face possesses radial ribs and a narrow inflexed rim.

***Chrysochromulina kappa* Parke et Manton (Figures 3F, G)** Cells sub-spherical (2 $\times$ 3  $\mu\text{m}$ ), with equal-length flagella (10  $\mu\text{m}$ ) and a coiled haptonema. Two types of plate scales were observed, the larger (0.6–0.7 $\times$ 0.5  $\mu\text{m}$ ) with radial ribs and a thickened rim. The smaller type (0.4 $\times$ 0.3  $\mu\text{m}$ ) with radial ribs on one face and disordered microfibrils on the other. The original description refers to a scale type (0.3–0.4 to 0.5–0.8  $\mu\text{m}$ ) that in some cases bears a spine; however in our material, spine scales were not observed (Eikrem et al. 1999). *Chrysochromulina kappa* may be confused with *C. minor*, differing in the morphology of the plate scales. In *C. kappa* the distal scale face has disordered microfibrils, while *C. minor* has radial ribs.

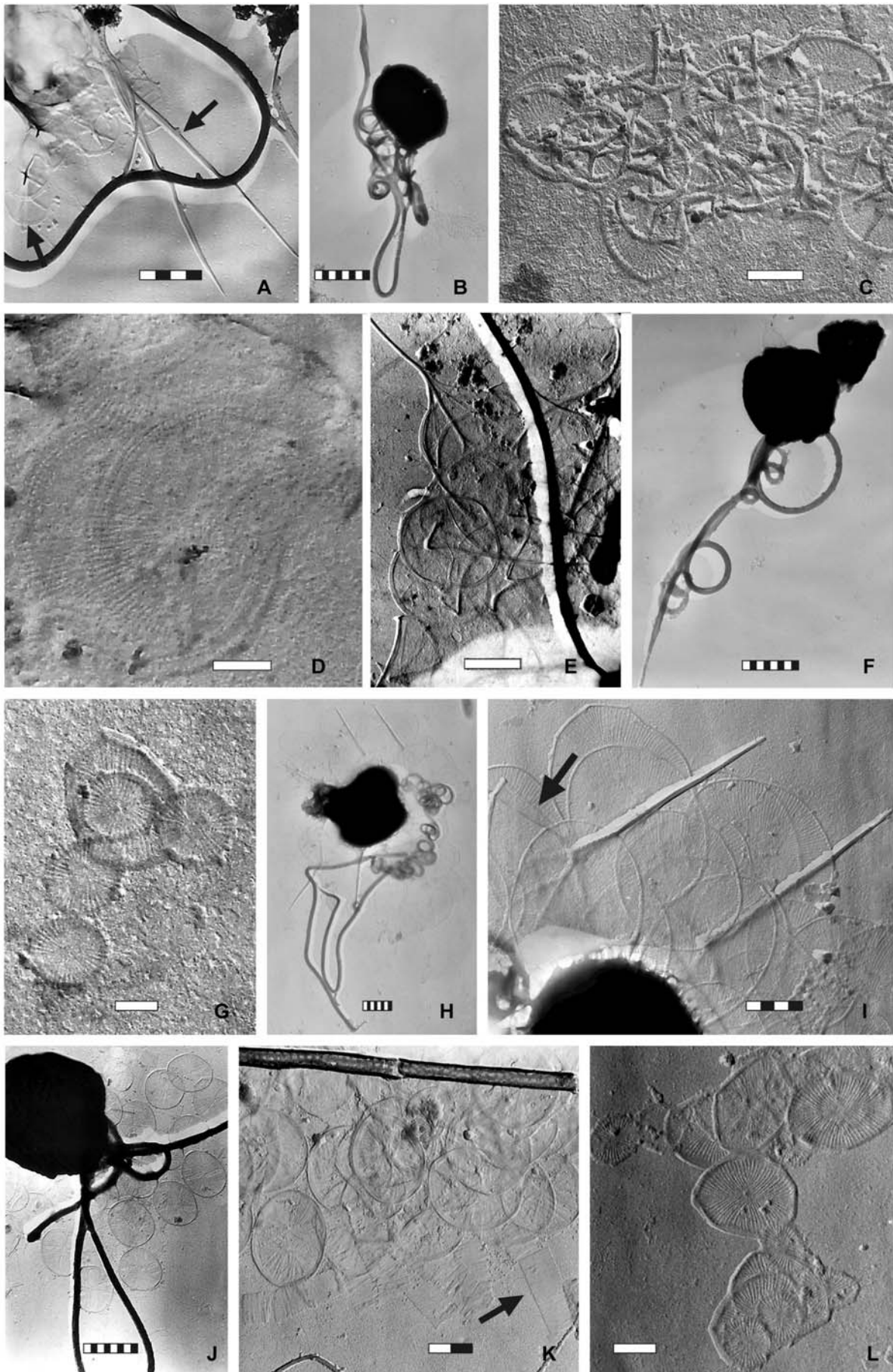
***Chrysochromulina latilepis* Manton (Figures 3H, I)** Cells ovoid to spheroidal (5 $\times$ 6  $\mu\text{m}$ ), with two types of scales. Plate scales very thin (2.0–2.4 $\times$ 1.6–1.9  $\mu\text{m}$ ), with radial ridges arranged in quadrants on the proximal face, on the distal face the pattern seems to be of radial ribs, at least in the marginal area. Spine scales a little larger (2.0 $\times$ 2.6  $\mu\text{m}$ ) but equally thin and with the same pattern as the plate scales. The slender spine sustained by four equally spaced thin struts extending to the edge. The small internal scales of the periplast were not observed.

***Chrysochromulina megacylindra* Leadbeater (Figures 3J, K)** Cells conical (3  $\mu\text{m}$  in diameter), with two types of scales. Plate scales oval, with radial ridges on both faces (1.1–1.2 $\times$ 0.9–1.0  $\mu\text{m}$ ) and a cruciform central pattern. Concentric striations are seen on the margin of the distal face. Outermost scales cylindrical and narrower than described in the type material (1.1–1.3 $\times$ 0.3–0.4  $\mu\text{m}$ ) (Leadbeater 1972).

***Chrysochromulina minor* Parke et Manton (Figure 3I)** A few scales of two types were found: large scales with a thickened rim (0.7–0.8  $\mu\text{m}$ ) and small scales with an inflexed rim. The original description mentions one scale type only, but in the present study scales of intermediate size were not found. *Chrysochromulina minor* is easily confused with *C. kappa*, but the former lacks spine scales. The two species also differ in the presence of radial ribs on the distal face of *C. minor* scales, compared to disordered microfibrils in *C. kappa*.

***Chrysochromulina cf. parkeae* Green et Leadbeater (Figures 4A, B)** Cells elongate (3 $\times$ 6  $\mu\text{m}$ ), flagella 18  $\mu\text{m}$  long, haptonema shorter. The cell of this species is known to possess spine scales but only plate scales were found in this study. Narrow elliptical plate scales (1.5–1.6 $\times$ 1.0  $\mu\text{m}$ ) have an irregular fibrillar pattern on the distal face and an enlarged rim. The proximal face has radial ridges.







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**Figure 3** Transmission electron micrographs of cells and scales of *Chrysochromulina* from the Patos Lagoon, southern Brazil.

(A) *Chrysochromulina elegans*, spine scales with long (right arrow) and short spine (left arrow). (B) *C. ephippium*, whole cell, (C) plate and spine scale details. (D) *C. cf. ericina*, scale details. (E) *C. cf. hirta*, scale details. (F) *C. kappa*, whole cell (G) plate scale details. (H) *C. latilepis*, whole cell, (I) plate and spine scales, the arrow showing the basal part of the spine scale. (J) *C. megacylindra*, whole cell with scattered scales, (K) plate and cylinder scales (arrow). (L) *C. minor*, plate scales of different size. Scale bars: 0.25  $\mu\text{m}$  per segment.

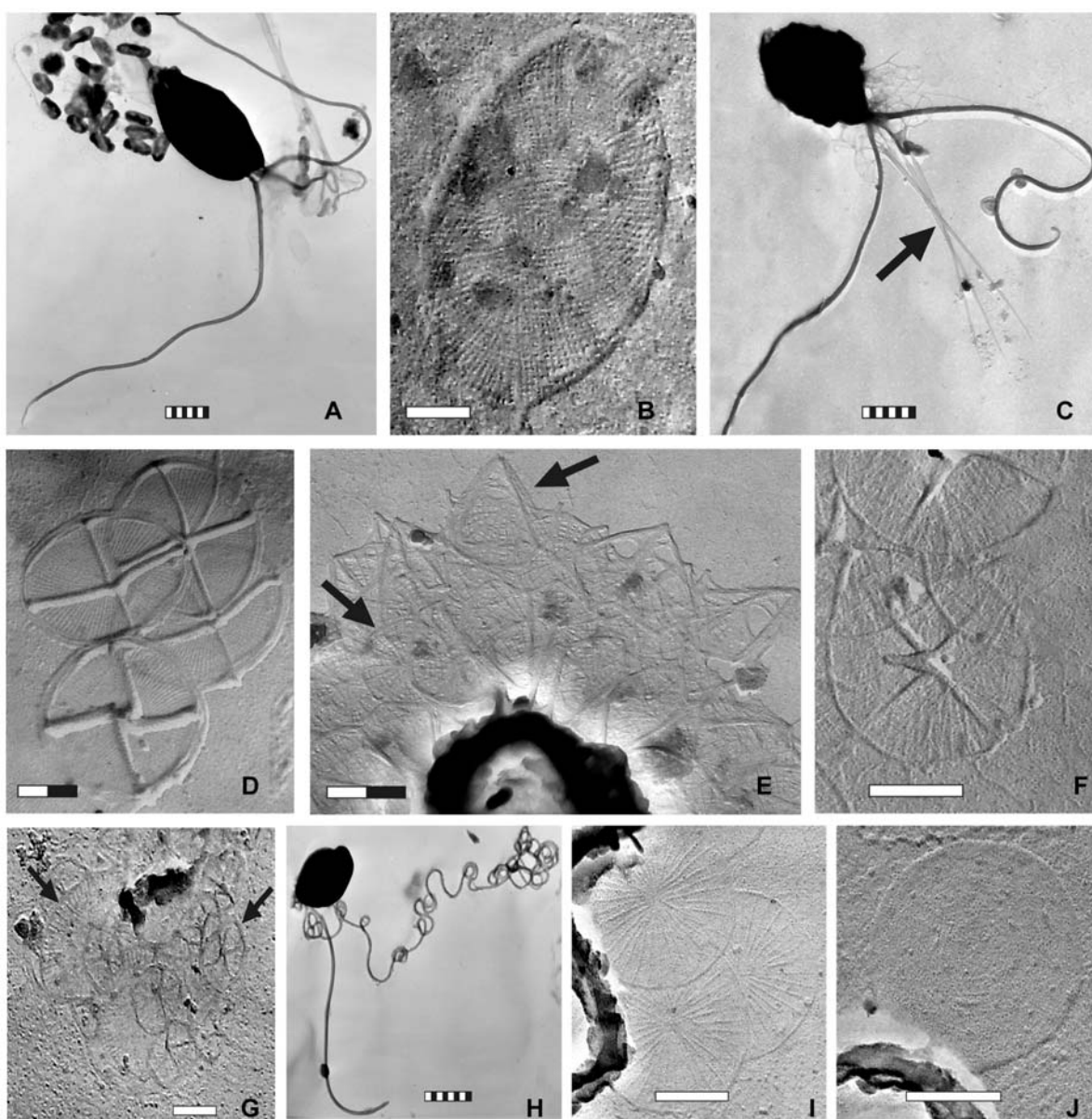
***Chrysochromulina pringsheimii* Parke et Manton (Figures 4C, D)** Cells elongate, from cylindrical to ovoid ( $3 \times 4 \mu\text{m}$ ). Of the four types of scales typical of the species, only three were observed in this study. The plate

scales ( $1.0 \times 1.1 \mu\text{m}$ ) with radial ridges resemble the short spine scales. These possess radial ridges on both faces and a thickened rim ( $1.3 \times 1.1 \mu\text{m}$ ). The short spine is sustained through four struts. The second spine scale type is long ( $8 \mu\text{m}$ ) and located on the posterior part of the cell.

***Chrysochromulina pyramidosa* Thomsen (Figure 4E)**

Cells sub-spherical ( $3 \mu\text{m}$  in diameter). Two types of scales observed: plate scales are circular ( $0.6\text{--}0.7 \mu\text{m}$ ) with radial ridges and rings, the marginal ring thicker than the others. The second scale type with a flat base ( $0.7 \times 1.0 \mu\text{m}$ ) from which four thick, ramifying structures extend forming a pyramidal structure.

***Chrysochromulina cf. rotalis* Eikrem et Thronsen (Figure 4F)** A few spine scales were found in the sam-



**Figure 4** Transmission electron micrographs of cells and scales of *Chrysochromulina* from the Patos Lagoon, southern Brazil. (A) *Chrysochromulina cf. parkeae*, whole cell, (B) scale details. (C) *C. pringsheimii*, whole cell with spine scales (arrow), (D) short spine scales. (E) *C. pyramidosa*, scale details. (F) *C. cf. rotalis*, spine scale details. (G) *C. scutellum*, plate scales (left arrow) and spine scales (right arrow). (H) *C. simplex*, whole cell and scales, (I) proximal face, (J) distal face. Scale bars: 0.25  $\mu\text{m}$  per segment.

ple; these are circular (0.6  $\mu\text{m}$  in diameter) with a pattern of concentric fibrils on the distal face and a radiate pattern on the other. The spine scales are similar and in the same size range as those of Eikrem and Throndsen (1999).

***Chrysochromulina scutellum* Eikrem et Moestrup (Figure 4G)** Cells saddle-shaped (3  $\mu\text{m}$ ). Plate scales (0.8 $\times$ 0.5  $\mu\text{m}$ ) and two types of spine scales, one with a raised rim and a spine length that is shorter or equals the base plate radius (0.30–0.35  $\mu\text{m}$ ), while the other type without a raised rim, was not observed.

***Chrysochromulina simplex* Estep, Davis, Hargraves et Sieburth emend. Birkhead et Pienaar (Figures 4H–J)** Cells saddle-shaped (3 $\times$ 4  $\mu\text{m}$ ) with a very long haptonema. With plate scales only, slightly oval (0.5–0.7  $\mu\text{m}$ ) and showing little size variation. The proximal face of the scale has a radial pattern and the distal face a pattern of concentric fibrils. This species was the most abundant in our samples.

***Chrysochromulina spinifera* (Fournier) Pienaar et Norris (Figures 5A–C)** Cells slightly elongate (3.5 $\times$ 3.8  $\mu\text{m}$ ), flagella 4–5  $\mu\text{m}$  long, with two types of scales. Plate scales oval (0.6–1.1 $\times$ 0.5–0.7  $\mu\text{m}$ ) with ridges extending from the longitudinal and transverse scale axis. The distal face with unordered fibrils and a thickened rim. The other scale is tubular (11.3 $\times$ 0.2  $\mu\text{m}$ ) with four small spines on the top. The basal part of this scale was not observed.

***Corymbellus aureus* Green (Figures 5D, E)** Cells cordate (5  $\mu\text{m}$  in diameter) with two sub-equal flagella (12.5 and 14.5  $\mu\text{m}$  in length) and a short haptonema (2  $\mu\text{m}$ ). Body scales are large and oval (0.4  $\mu\text{m}$ ) with a raised rim and a pattern of radiating ridges, and a short four-strutted spine.

***Imantonia* sp. (Figure 5F)** Cells spherical (3  $\mu\text{m}$  in diameter) to cordate (2  $\mu\text{m}$  length). One type of scale (0.5  $\mu\text{m}$ ) similar in size to *I. rotunda* Reynolds but not well defined, as observed by Jensen (1998b). Scales with rim thickened as in *I. rotunda* were not observed.

***Phaeocystis* sp. (Figure 5G)** Cells lacked visible scales; however, filamentous structures were arranged in pentagonal patterns. Such pentagonal structures are known in other *Phaeocystis* species, and identification at the species level requires information about colony shape (Moestrup 1979, Throndsen 1997, Marchant et al. 2005). This unidentified *Phaeocystis* species was occasionally very common.

***Phaeocystis scrobiculata* Moestrup (Figures 5H, I)** Cells covered with organic scales of two different sizes. The small scales are circular-oval (0.4  $\mu\text{m}$ ) while the large scales are oval (0.7 $\times$ 0.5  $\mu\text{m}$ ). Both scales have radiating ribs on one face, while the opposite face is without any pattern. The small scales with a thickened rim, the large scales with an erect rim. The smaller scales observed were larger than those described by Moestrup (1979).

Cells produce filamentous structures arranged as a figure of nine, four pairs and one single.

***Prymnesium parvum* f. *patelliferum* (Green, Hibberd et Pienaar) A. Larsen (Figures 5J, K)** Cells elongate (12  $\mu\text{m}$  long), with an obliquely truncated anterior end giving rise to the flagella and the haptonema. Flagella equal or sub-equal (10–14  $\mu\text{m}$ ); haptonema short (4–6  $\mu\text{m}$ ). Two green yellowish chloroplasts visible in light microscopy on opposite sides of the cell. Plate scales of one type observed, with radial ridges on both faces and a protuberance in the central area of the distal face.

***Wigwamma arctica* Manton, Sutherland et Oates (Figure 5L)** A few circular scales of one type were observed (= coccoliths, 0.5–0.6  $\mu\text{m}$ ). The base plate possesses a thickened narrow rim; a pyramidal calcified superstructure is composed of four converging rods attached to the rim.

## Heterokontophyta

### Chrysophyceae

***Dinobryon belgica* Meunier (Figure 5M)** Cells located in a short and wide lorica (8 $\times$ 27  $\mu\text{m}$ ). The original diagnosis of this species did not provide any information about cell size (Meunier 1910) and our cells measurements are a little larger than those reported by Throndsen (1997).

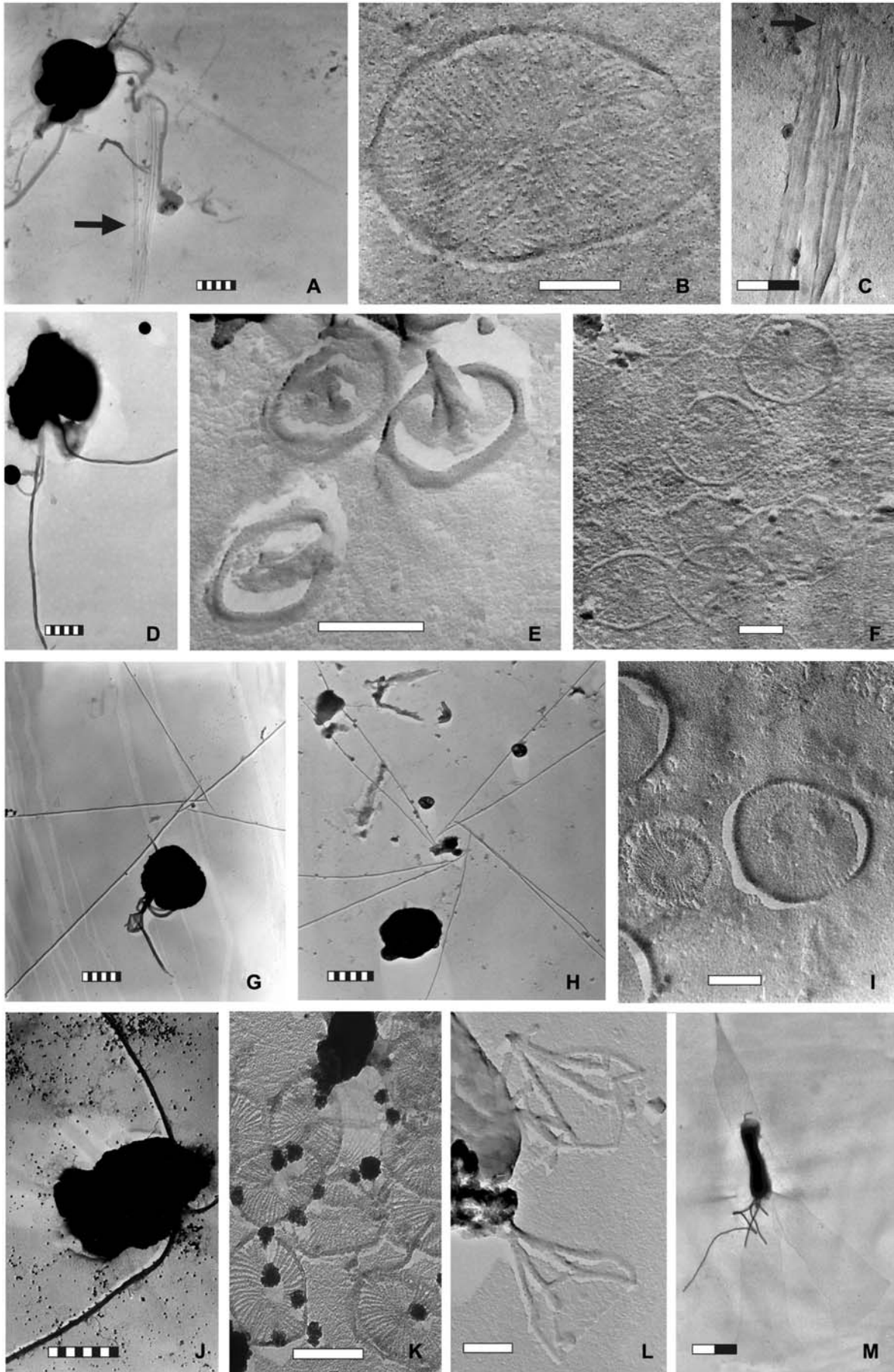
***Paraphysomonas butcheri* Pennick et Clarke (Figure 6A)** Cells ovoid (2–3  $\mu\text{m}$  in diameter), with two unequal flagella (2.5 and 8.5  $\mu\text{m}$  long). Two types of elliptical scales with a reticulated pattern formed by openings arranged in concentric rings, the outermost ring occasionally larger. The number of openings and scale sizes vary for a single cell. The second scale type formed has an elaborated crown. Cell dimensions from this study agree with those reported by Preisig and Hibberd (1982a).

***Paraphysomonas foraminifera* Lucas (Figure 6B)** Cells rounded to ovoid (2.5  $\mu\text{m}$  in diameter) with two unequal flagella (12.5 and 3  $\mu\text{m}$  long). Periplast scales are of one type formed by a circular basal plate (0.7  $\mu\text{m}$  in diameter) with perforations forming rings and a central spine (1.0  $\mu\text{m}$  long).

**Figure 5** Transmission electron micrographs of cells and scales of *Chrysochromulina*, *Prymnesium*, *Corymbellus*, *Imantonia*, *Phaeocystis*, *Wigwamma* and *Dinobryon* from the Patos Lagoon, southern Brazil.

(A) *Chrysochromulina spinifera*, cell with long spine scales (arrow), (B) plate scale on distal face, (C) tip of spine (arrow). (D) *Corymbellus aureus*, cordiform cell, (E) spine scale. (F) *Imantonia* sp., scale details. (G) *Phaeocystis* sp., filamentous pentagonal structure. (H) *P. scrobiculata*, characteristic filamentous structure, (I) scales. (J) *Prymnesium parvum*, cell, (K) scales. (L) *Wigwamma arctica*, scales with pyramidal superstructure. (M) *Dinobryon belgica*, cell in lorica. Scale bars: 0.25  $\mu\text{m}$  per segment.





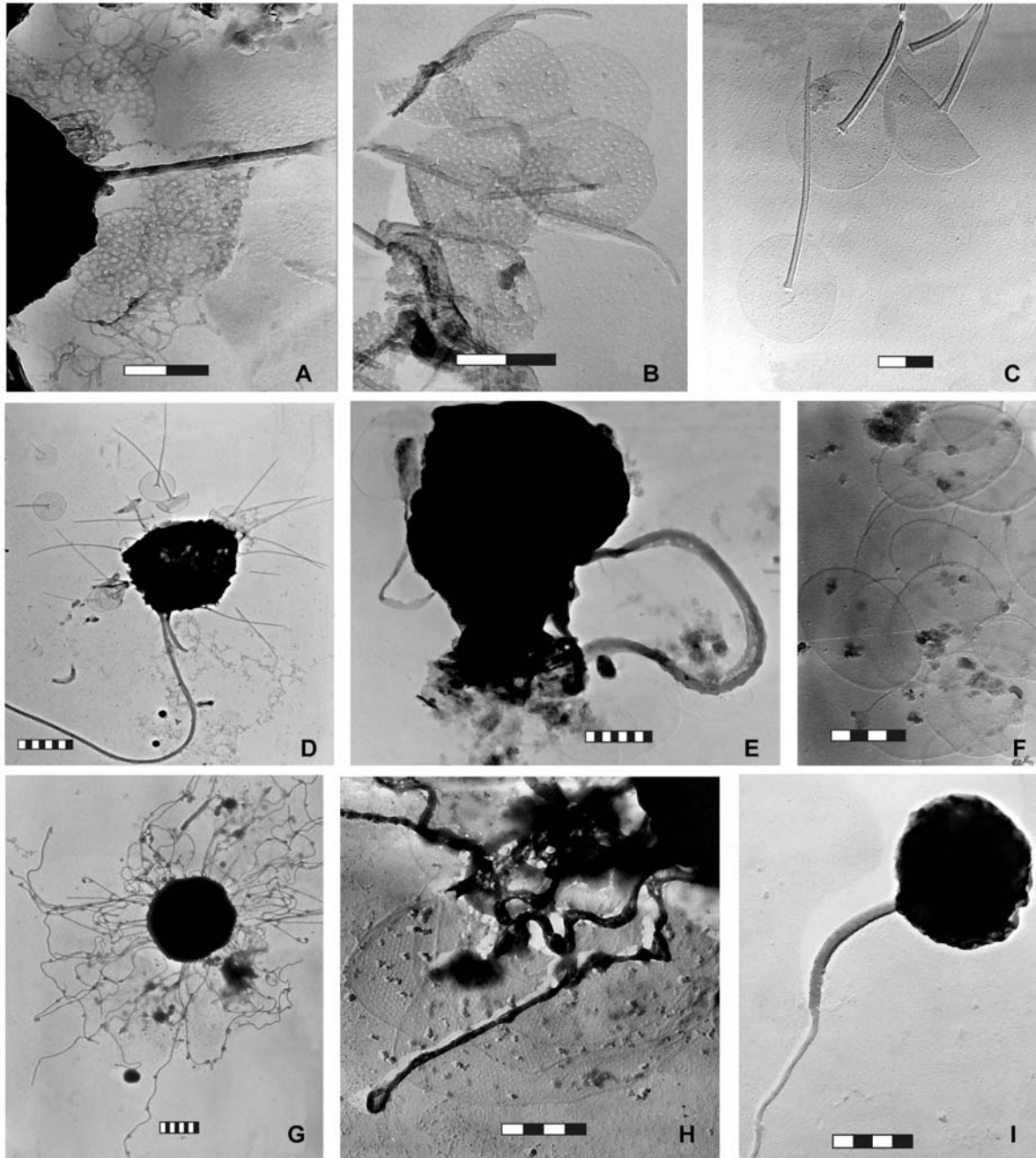
***Paraphysomonas imperforata* Lucas (Figure 6C)** Cells ovoid ( $2.2\ \mu\text{m}$  in diameter), with two unequal flagella ( $3$  and  $15\ \mu\text{m}$  long). Periplast formed by numerous scales with a circular base plate ( $1\ \mu\text{m}$ ) bearing a central spine ( $2\ \mu\text{m}$  long).

***Paraphysomonas vestita* (Stokes) de Saedeleer (Figure 6D)** Cells ovoid ( $4 \times 5\ \mu\text{m}$ ). Basal plates of scales are slightly curved ( $3.8\text{--}4.4\ \mu\text{m}$ ) with a spine and a thickened rim. Two smaller scales ( $1\ \mu\text{m}$ ) with shorter spines ( $1.1\text{--}2.5\ \mu\text{m}$ ) were also observed, and the scales

and spine dimensions are known to vary considerably (Preisig and Hibberd 1982b). Most previous findings are from freshwater (Preisig and Hibberd 1982b) although also recorded in the gulfs of Finland (Vørs 1992) and St. Lawrence, Canada (Bérard-Therriault et al. 1999).

#### Pedinellids

***Apedinella radians* (Lohmann) Campbell (Figures 6E, F)** Cells rounded ( $4\ \mu\text{m}$  in diameter), the periplast composed of plate scales of variable size ( $1.0\text{--}2.1 \times 0.8\text{--}1.5\ \mu\text{m}$ ) with a thickened rim and a pattern



**Figure 6** Transmission electron micrographs of cells and scales of *Paraphysomonas*, *Apedinella*, *Parapedinella* and *Micromonas* from the Patos Lagoon, southern Brazil.

(A) *Paraphysomonas butcheri*, scale details. (B) *P. foraminifera*, scale details. (C) *P. imperforata*, scale details. (D) *P. vestita*, cell with scattered scales. (E) *Apedinella radians*, cell, (F) scales. (G) *Parapedinella reticulata*, cell with axopods, (H) scales. (I) *Micromonas pusilla*, whole cell. Scale bars:  $0.25\ \mu\text{m}$  per segment.



of irregular fibrils. The spine scales observed by Thronsen (1997) were not observed in our specimens.

***Parapedinella reticulata* Pedersen et Thomsen (Figures 6G, H)** Cells spherical (4  $\mu\text{m}$  in diameter) covered by large oval scales (1.8–3.6 $\times$ 1.3–2.3  $\mu\text{m}$ ) with a pattern of loosely woven fibrils. The cell possesses numerous axopodia, sometimes with granular structures attached.

## Chlorophyta

### Prasinophyceae

***Micromonas pusilla* (Butcher) Manton et Parke (Figure 6I)** Small autotrophic flagellate more or less rounded (2  $\mu\text{m}$ ) with one laterally inserted flagellum (4  $\mu\text{m}$ ). The cell lacks scales and the identification is based on the cell size and flagellum shape. In our material, the flagellum was 4  $\mu\text{m}$  long, ca. 1.7  $\mu\text{m}$  in the proximal part and ca. 2.2  $\mu\text{m}$  in the thin distal part. This species is widely distributed (Moestrup 1979).

***Nephroselmis pyriformis* (Carter) Ettl (Figure 7A)** Cells pyriform (2 $\times$ 3  $\mu\text{m}$ ) with two unequal flagella (6.7 and 11.7  $\mu\text{m}$  long). When alive, the laterally inserted, short flagellum is held in front of the cell during swimming, while the long flagellum is directed backwards (Moestrup 1983).

***Pyramimonas disomata* Butcher (Figures 7B, C)** Cells obovoid to cuneiform (3 $\times$ 5–6  $\mu\text{m}$ ), with four apical flagella. Three types of scales were observed: square body scales (box scales), sometimes with a central spike, in addition to limuloid and crown scales. In a study of the genus *Pyramimonas* from southeastern Australia, McFadden et al. (1986) stated that the presence of a central spike in box scales is an inconstant feature even in single cells of *P. disomata*. Some other box scales in our samples had the distinctive pattern of concentric squares typical of *P. obovata* Carter (McFadden et al. 1986).

***Pyramimonas grossii* Parke (Figures 7D, E)** Cells obovoid to pyramidal (3.3 $\times$ 3.4  $\mu\text{m}$ ) with four broad anterior lobes. Four equal flagella, slightly longer than the cell. Only box scales were observed, they are flat and the rim seems to have one dentate side. The structure of the basal plate has a pattern of eight radial perforations forming a ring close to the center of the scale (Figure 7E), as reported by McFadden et al. (1986), Figure 8D.

***Pyramimonas longicauda* van Meel emend. Inouye, Hori et Chihara (Figures 7F, G)** Only two types of scales belonging to the intermediate and outermost layers on the cell were observed. Scales of the intermediate layer were square (1.2  $\mu\text{m}$ ), the four sides forming a frame; scales of the outermost layer formed by a square basal plate (0.8  $\mu\text{m}$ ) and a basket superstructure.

***Pyramimonas obovata sensu Hallegraef 1983 (Figures 7H, I)*** Cells obovate with a posterior acute end (3.5 $\times$ 5.5  $\mu\text{m}$ ) and four equal flagella (8–9  $\mu\text{m}$  long). Only

square scales (0.3  $\mu\text{m}$  wide) were observed, with a thickening in the form of two squares, one inside the other (Hallegraef 1983).

***Pyramimonas orientalis* Butcher (Figures 7J, K)** Cells ovoid to pyramidal (2 $\times$ 3  $\mu\text{m}$ ) with four equally thick flagella. Two types of scales were observed, the body scales types 2 and 3 of Norris and Pienaar (1978). The crown scale is typical of *P. orientalis* but the basal plate of the square scale did not have the faintly striation typical of the distal side. Flagellar scales were not observed.

### Scale-bearing amoebae

***Rabdiophrys* sp. (Figure 7L)** Cells (4  $\mu\text{m}$  in diameter) covered by two types of scales, one type spineless the other with spine. Spineless scales oval with a faint concentric pattern and a marked central line in the larger axis. The other scale type has a hollow spine 4  $\mu\text{m}$  long with two teeth on its apex, and a basal patternless perforated plate (2.5  $\mu\text{m}$ ).

### *Incertae sedis*

***Meringosphaera mediterranea* Lohmann (Figure 7M)** The protoplast is spherical and covered by long wavy spines, radiating in all directions. The spine-bearing type has a small basal plate and the elliptical one has a narrow central thickening without a defined pattern. The systematic position of this species is uncertain (Adl et al. 2005).

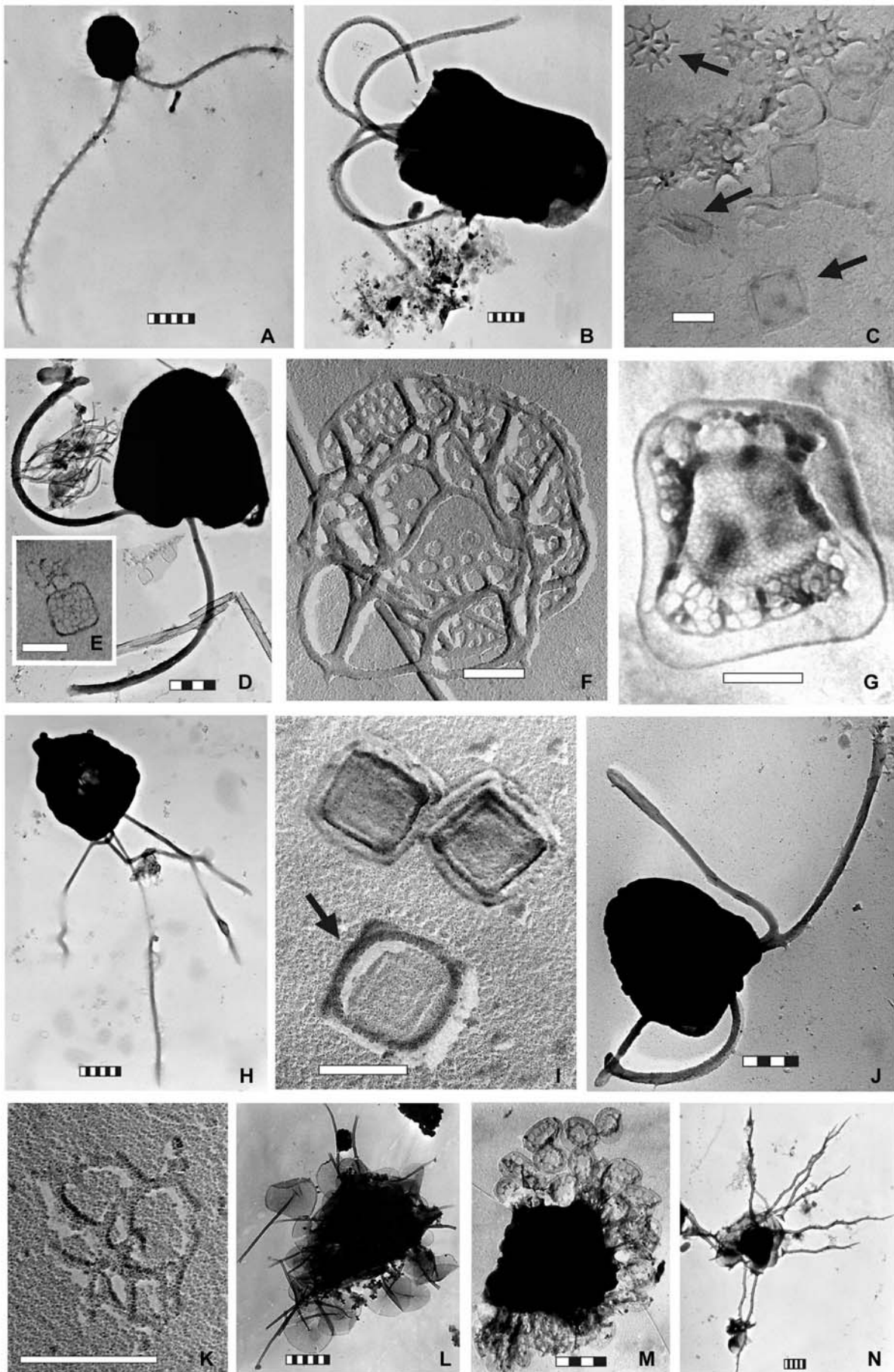
***Thaumatostix* sp. (Figure 7N)** Cells (2.3  $\mu\text{m}$  in diameter) with one type of silicified scales (Thomsen et al. 1993), which are elliptical in shape, perforated in the center, with a marginal thickening and a ring of round structures between the central perforation and the margin.

### Undescribed *Chrysochromulina* species

In addition to the 21 described species of *Chrysochromulina*, a certain number remained undescribed. Preliminary descriptions are given below with some morphological characteristics of the scales illustrated in Figure 8. All undescribed *Chrysochromulina* species are listed and numbered *sensu* Bergesch (2003).

***Chrysochromulina* sp. 2 (Figures 8A, B)** Only one type of scale was observed, with a large size variation (0.4–1.3  $\mu\text{m}$ ), a pattern of radial grooves on one face (60–90) and concentric rings on the other. A similar species was reported in Danish coastal waters by Jensen (1998b) as *Chrysochromulina* sp. 17, but this latter possessed two scale types (0.7 and 0.3  $\mu\text{m}$  in diameter), both with a pattern of radiating grooves on one face and concentric fibrils on the opposite face.

***Chrysochromulina* sp. 13 (Figures 8C, D)** Cells rounded with one type of scale (1.0–1.3  $\mu\text{m}$ ), showing radial ridges on one scale face and a concentric pattern on the other. The haptonema is probably slightly shorter than the flagella.



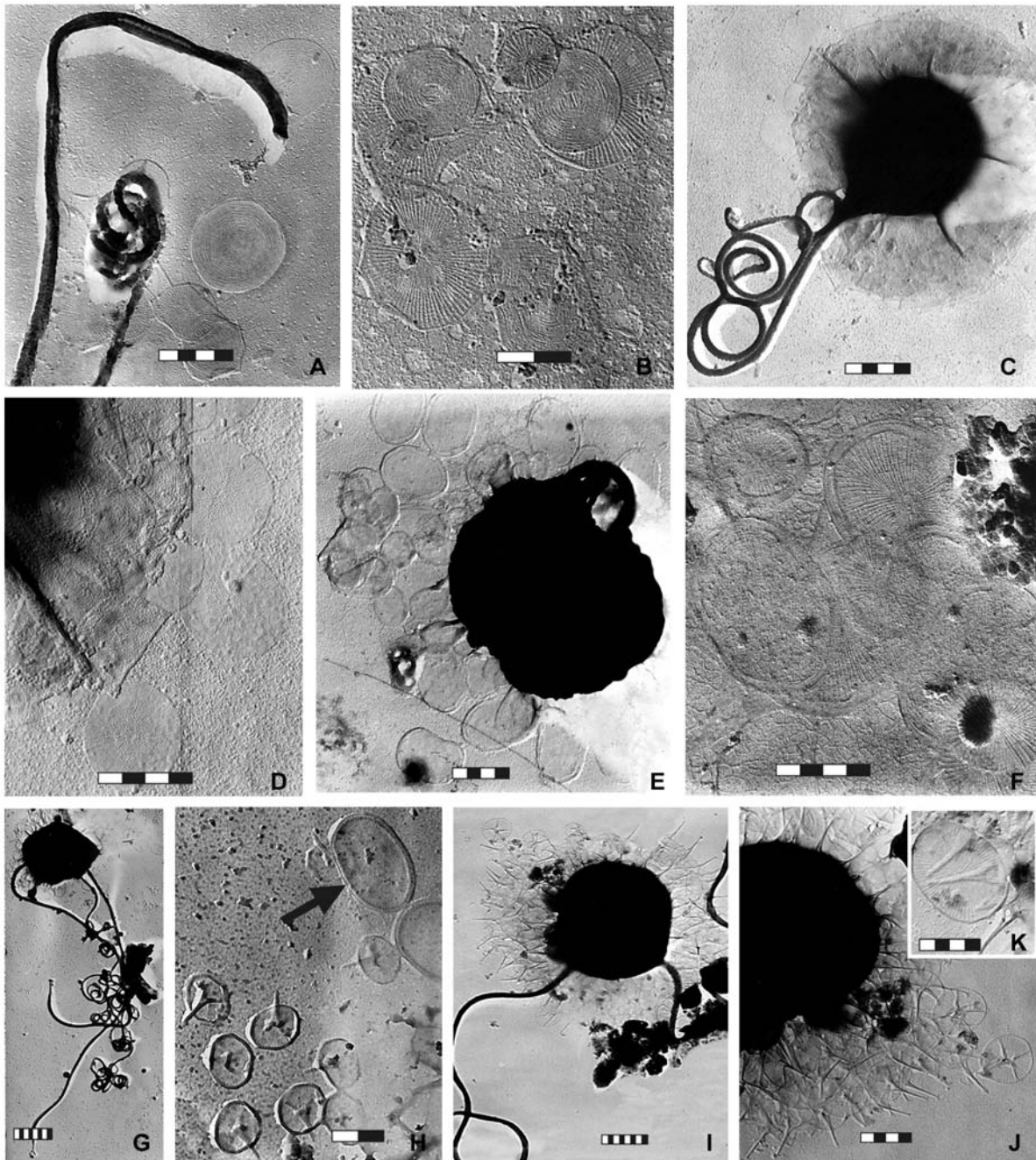


←  
**Figure 7** Transmission electron micrographs of cells and scales of *Nephroselmis*, *Pyramimonas*, *Rabdiophrys*, *Meringosphaera* and *Thaumatomastix*, from the Patos Lagoon, southern Brazil.

(A) *Nephroselmis pyriformis*, whole cell. (B) *Pyramimonas disomata*, whole cell, (C) box, limuloid and crown scales (arrows). (D) *P. grossi*, whole cell, (E) detail of basal plate with a central ring of eight radial perforations. (F) *P. longicauda*, intermediate layer and scales, (G) external layer scales. (H) *P. obovata*, whole cell, (I) box scales with thickenings inside (arrow). (J) *P. orientalis*, whole cell, (K) crown scale. (L) *Rabdiophrys* sp., cell and scales. (M) *Meringosphaera mediterranea*, cell. (N) *Thaumatomastix* sp., cell with periplast. Scale bars: 0.25  $\mu\text{m}$  per segment.

***Chrysochromulina* sp. 32 (Figures 8E, F)** Two types of scales were observed: the larger (1.2 $\times$ 0.7  $\mu\text{m}$ ) with radial ridges on one face and disordered fibrils on the other and with a thickened rim on both faces; the small scales (0.6 $\times$ 0.3  $\mu\text{m}$ ) with radial ridges on both faces and an inflexed rim. These specimens correspond to *Chrysochromulina* sp. 18 reported by Jensen (1998b) from Danish coastal waters.

***Chrysochromulina* sp. 34 (Figures 8G, H)** Cells (3 $\times$ 4  $\mu\text{m}$ ) with equally long flagella (16  $\mu\text{m}$ ) and a very long haptonema. Two types of scales were observed,



**Figure 8** Transmission electron micrographs of cells and scales of undescrbed *Chrysochromulina* species collected in coastal waters.

(A–B) *Chrysochromulina* sp. 2. (C–D) *Chrysochromulina* sp. 13. (E–F) *Chrysochromulina* sp. 32. (G–H) *Chrysochromulina* sp. 34, two smaller scales and one large scale (arrow). (I–K) *Chrysochromulina* sp. 35. Scale bars: 0.25  $\mu\text{m}$  per segment.

one with radial ridges on one face and an inflexed rim on the other ( $0.9 \times 0.7 \mu\text{m}$ ). Spine scales are smaller ( $0.4 \times 0.3\text{--}0.4 \mu\text{m}$ ) with radial ridges on both faces, and an inflexed rim. Spines are  $0.3 \mu\text{m}$  long.

***Chrysochromulina* sp. 35 (Figures 8I–K)** Cells ( $4 \times 5 \mu\text{m}$ ), with two types of scales present. One scale type with a central spine and radial ribs and an inflexed rim on the distal face ( $0.8 \times 1.0 \mu\text{m}$ ). The spine with basal struts extends to the thickened rim. Each spine scale has a characteristic hole in the central base plate beneath the spine. The second scale type ( $1.3\text{--}1.6 \times 1.0\text{--}1.2 \mu\text{m}$ ) with radiating ribs on one face but probably patternless on the other (K). These specimens correspond to *Chrysochromulina* sp. 22 reported by Jensen (1998b) from Danish coastal waters.

## Discussion

The high species richness of haptophytes observed in our study indicates the importance of this group in the southwestern Atlantic Ocean, as in other coastal and oceanic environments of the world. Of the nearly 60 *Chrysochromulina* species so far described (Hu et al. 2005), 21 were recorded for the first time in the Patos Lagoon estuary and adjacent coastal waters of Brazil. These also represent new information regarding their geographical distribution in the southwestern Atlantic Ocean, indicating the cosmopolitan nature of several *Chrysochromulina* species (Moestrup and Thomsen 2004). In addition, there were many different types of scales present, some of them also reported for Scandinavian waters (Jensen 1998b). The high number of undescribed forms in several parts of the world attests to the incomplete general knowledge of this genus.

All identified species of *Chrysochromulina* have been reported previously from the Northern Hemisphere (Jensen 1998b, Bérard-Therriault et al. 1999) and several of them from Australasia (LeRoi and Hallegraeff 2004, 2006) and Antarctica (Marchant et al. 2005). In our study, *Chrysochromulina* species were frequent in estuarine and coastal high salinity waters, especially during summer. The most common species, *C. simplex*, is known for its wide temperature and salinity tolerance range (Moestrup 1979, Thomsen et al. 1994, Jensen 1998b).

There was little evidence of toxicity in any species of *Chrysochromulina* prior to the harmful bloom event of *C. polylepis* Manton et Parke in 1988 (Moestrup 1994). From the *Chrysochromulina* species recorded in this study only *C. kappa* was previously related to form harmful algal blooms (Moestrup and Thomsen 2004). Although the absence of overt toxicity is the usual case in *Chrysochromulina*, the noxious blooms of *C. breviturrita* Nicholls and *C. parva* Lackey in lakes, *C. birgeri* Hällfors et Niemi in brackish waters and *C. polylepis* and *C. leadbeateri* Estep, Davis, Hargraves et Sieburth in marine waters show the potential toxicity of members of this genus (Edvardsen and Paasche 1996). The other known toxic species observed in our study, *Prymnesium parvum* f. *patelliferum*, had not been observed previously in the southwestern Atlantic Ocean.

The haptophyte *Phaeocystis* was frequent in our samples although unlike *Chrysochromulina*, this genus contains few species; its taxonomy is in a transitional phase (Moestrup and Thomsen 2004). *Phaeocystis scrobiculata*, known from the Pacific and Atlantic Oceans only as a unicellular flagellate stage (Moestrup and Thomsen 2004), was previously recorded from the Brazilian southeastern coast and eastern continental shelf (Ribeiro 1996). Colony-forming *Phaeocystis* species previously reported in southwestern Atlantic waters were *P. pouchetii* (Hariat) Lagerheim in the mouth of the La Plata River, Argentina (Negri et al. 1988) and the Brazilian southeastern coast and eastern continental shelf (Ribeiro 1996, Mafra et al. 2006), and *P. globosa* Sherffel in coastal waters of southern Brazil (Proença and Tamanaha 2000, Mafra et al. 2006). Recent studies on the flagellate *P. pouchetii*-like morph, reported as frequent in Antarctic waters (with up to  $>10^6$  cells  $\text{l}^{-1}$ ; Marchant 1985), indicate it is very similar to *P. antarctica* Karsten, the only *Phaeocystis* species currently recognized in the area. No definitive characterization of its morphology has been published, and EM studies indicate similarity with descriptions of the *P. pouchetii* flagellar state, while life cycle and colony morphology resemble *P. globosa* (Marchant et al. 2005). Current knowledge restricts *P. pouchetii* to the Arctic and temperate waters of the Northern Hemisphere (Moestrup and Thomsen 2004) and *Phaeocystis*-like colonies in the southwestern Atlantic Ocean need to be investigated in more detail. The number of *Phaeocystis* species and their geographic distribution is still not known, and controlling factors are not completely understood due to the complexity of the life cycle (alternating between free living and colonial stages). Species delimitation is still being debated and may be resolved by examining the relationships between morphological and cytological aspects, phenotypic plasticity, and the analysis of genotypes (Baumann et al. 1994). Isolation of strains from the southwestern Atlantic Ocean is required to obtain detailed morphological (TEM) and genetic information.

The second most species group in the present study was the prasinophytes, with five described species of *Pyramimonas*, among them the uncommon *P. longicauda* (McFadden et al. 1986). Prasinophytes were found mostly in high salinity estuarine waters, although they were found in samples of a wide salinity range (1–33 psu). Only *P. grossii* has been reported previously from the Patos Lagoon (Torgan 1997). In addition, four *Pyramimonas* species could not be fully characterized, as complete cells with scales and flagella were not observed. The minute species *Micromonas pusilla* and the neritic species *Nephroselmis pyriformis* have a worldwide distribution (Moestrup 1979, 1983).

Colorless or heterotrophic chrysophytes such as *Parapedinella reticulata* and species of *Paraphysomonas* were among the few scale-bearing nanoflagellates observed predominantly in the innermost part of the estuary in low salinity water ( $<15$  psu) during autumn and winter, except for *P. butcheri*, which was also found in a coastal sample at a salinity of 30 psu. Some species of *Paraphysomonas* appear to have a considerable tolerance to salinity fluctuation and the genus also occur in freshwater. Previous



records of marine *Paraphysomonas* from the Southern Hemisphere have been restricted to Antarctica (Takahashi 1987, Marchant and Scott 2005).

The 44 taxa (mostly scale-bearing nanoflagellate species) identified in the present study increase the current knowledge of biodiversity in Brazilian marine waters. However, the finding of a high number of several undescribed *Chrysochromulina* species points to the fact that we actually know only a fraction, and that a significant effort has still to be made to understand their importance in coastal ecosystems.

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