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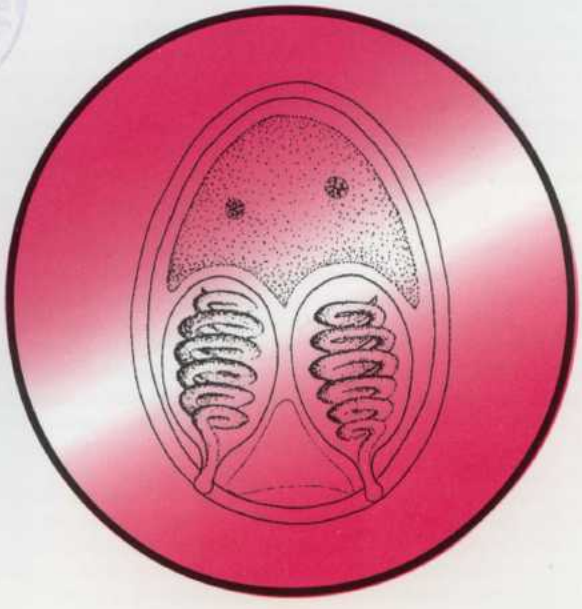

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Flagellates from Stromatolites and Surrounding Sediments in Shark Bay, Western Australia

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Summary. Hamelin Pool in Western Australia is home to distinctive shallow-water marine benthic communities dominated by stromatolites-structures formed by bacterial assemblages. We have sought to establish if the principal consumers of bacteria, the flagellates, form a distinctive community. We present observations of 87 species of flagellates, of which three are new (*Notosolenus hamelini, Ploeotia laminae, Ancyromonas sinistra*), and 15 (12 of which are dinoflagellates) have not been previously reported in Australia. The communities have been compared with other communities from marine sediments using cluster analysis. The community of flagellates in Hamelin Pool was found not to be significantly different from communities from other marine benthic habitats, and abundances (about 1 x 10^5 cells/cm^3) are also comparable to those in other sediments. We find no evidence that the formation of stromatolites is made possible by a reduced predation pressure from heterotrophic flagellates.

Key words: *Acanthoeca, Amastigomonas, Amphidiniella, Amphidinium, Ancyromonas, Ancyromonas sinistra* sp. n., *Anisonema,* biogeography*, Bodo, Bordnamonas, Caecitellus, Carpediemonas, Cercomonas, Chasmostoma, Clautriavia, Colpodella, Cryptaulax, Diaphanoeca, Dinema,* dinoflagellates*, Dolium, Entomosigma,* euglenids*,* flagellates*, Glissandra, Goniomonas, Gymnodinium, Gyrodinium, Hemistasia, Heteronema, Jakoba, Jenningsia, Karlodinium, Katodinium, Malawimonas, Massisteria, Menoidium, Metopion, Metromonas, Notosolenus, Notosolenus hamelini* sp. n., *Pendulomonas, Petalomonas, Ploeotia, Ploeotia laminae* sp. n., *Prorocentrum,* protists*, Rhynchobodo, Rhynchomonas, Rhynchopus, Salpingoeca,* stromatolites.

INTRODUCTION

Stromatolites are lithified masses formed mainly by cyanobacterial assemblages, and occur in intertidal and subtidal regions worldwide. Those of the Shark Bay region in Western Australia are particularly well known (Schopf 1968, 1983; Playford and Cockbain 1976; Skyring and Bauld 1990; Awramik 1992). This is partly because they are extensive and diverse, and partly because of their geographical association with early fossil stromatolites.

Stromatolite communities are examples of microbial dominated ecosystems - in this case the predominant organisms are cyanobacteria. The other microbial components of stromatolite ecosytems have been rarely studied. Some studies conducted on eukaryotic microalgae on stromatolites have focused on the diatoms (e.g. John 1990, 1991, 1993; Hein *et al*. 1993). To date there has been no study of the heterotrophic flagellates associated with stromatolites.

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One explanation for why stromatolites develop is that the high salinities inhibit predators (e.g. Playford and Cockbain 1976, Macintyre *et al*. 1996, Steneck *et al*. 1998). Hamelin Pool is hypersaline (55-70 ‰) (Bauld 1984), suggesting that potential predators may be excluded by the extreme characteristics of the habitat (Playford and Cockbain 1976). These models tend to focus on invertebrate grazers (Hoffman 1976). In many ecosystems, including the open ocean and marine and freshwater sediments, protozoa, especially flagellates, are the dominant consumers of bacteria (Fenchel 1986). Protozoa can survive in a very wide variety of environmental conditions, and individual species may have a relatively high tolerance to factors such as salinity (Noland and Gojdics 1967, Patterson *et al*. 1989). Protozoa may therefore play a significant role in determining the composition and abundance of stromatolite communities.

This study was undertaken to establish if the composition of the communities of flagellates present on the algal mat of the stromatolites and the surrounding sediments in Hamelin Pool is distinctive in terms of composition or abundance.

MATERIALS AND METHODS

This study was carried out at Hamelin Pool, Western Australia (114° E; 26° 50'S) from 19th Aug. 1999 to 27th Aug. 1999. During this period, water temperature and salinity were about 67 ‰ and 17 °C respectively. Samples were processed as described elsewhere (Larsen and Patterson 1990, Lee and Patterson 2000). Samples were collected daily from the top centimetre layer of the bacterial mats that form the lateral surfaces of stromatolites and which may cover the sediment between stromatolites. Samples were also taken from adjacent sediments without mats. The samples were placed in plastic trays in 1 cm deep layers. The sediments were covered with lens tissue and No. 1 22 x 22 mm coverslips were placed on the lens tissue. After 24 h the coverslips were removed and flagellates were observed using a Zeiss Axiophot microscope and Leica DMR equipped with photographic facilities (Patterson 1982). The flagellates were also recorded on U-MATIC video tapes and records were also made with video prints. Specimens were also drawn. Some samples were maintained at room temperature for 8 days to get anoxic material. We restrict our surveys to light-microscopical observations of living cells; as our objective is to obtain the most extensive list of species possible and to use techniques consistent with previous surveys so that we may explore the factors which determine the composition of the communities of flagellates.

Nomenclature follows the International Code of Zoological Nomenclature (International Commission of Zoological Nomenclature 1999).

To estimate the abundance of heterotrophic bacteria and flagellates, these organisms were preserved in a microtubule stabilising fixative (Weerakoon *et al*. 1999), extracted using the decant/fix method from sediments (Lee and Patterson 2002) and refrigerated until quantified using epifluorescence microscopy (Leica DMR). Two to five ml of the supernatant obtained from the extraction was stained with 5 µg/ml of DAPI (final concentration), and then filtered through a 0.22 µm black polycarbonate Nucleopore filter (Lee and Patterson 2002).

To measure the species richness on the stromatolites and the surrounding sediments, we catalogued flagellates on 3 coverslips from a 1 m x 1 m area of each of the algal mat and sediment, and repeated this for samples collected on three consecutive days. For each coverslip, we identified and counted every cell present across a grid of 1 mm x 1 mm, so that sampling effort was standardised. We assumed that the process by which cells or species from the underlying sediment became attached to the coverslip was basically random. The data were analysed by assuming an asymptotic relationship between the cumulative species number and the cumulative cell number present in the sampling area. The cumulative species number (S) were transformed into log $[1-S/S_{\infty}]$, where S_{∞} was the assumed asymptote, to linearise the equation, and a linear regression was performed. The value of S_{∞} that gave the best regression coefficient was used. The data per coverslip were compared by a t test.

Analysis of similarities among species assemblages was conducted using cluster analysis, a hierarchical classification technique based on the Bray-Curtis Similarity Coefficient, calculated on presence / absence-transformed data employing group average sorting, using PRIMER version 4.0 beta (Clarke 1993, Lee and Patterson 1998, Patterson and Lee 2000). The community from Shark Bay was compared with the communities from other sites (Lee and Patterson 1998, Patterson and Lee 2000).

OBSERVATIONS

A species list encountered during this study is presented in Table 1. In the descriptions 'n =' refers to the number of cells described.

Choanoflagellida Kent, 1880

***Acanthoeca spectabilis* Ellis, 1930 (Figs 1a, 2b)**

Description: The cell body is elongate with a pointed posterior end that fits into the main chamber of the lorica, the cell is about 6 µm long and has one flagellum. The pseudopodial collar is shorter than the cell, and the lorica is conical and tapers posteriorly with a stalk. One cell was observed from sediment.

Remarks: This common choanoflagellate has been found from marine sites in Antarctica, subtropical Australia, Canada, Denmark, England, Gulf of Finland, France, Norway and USA (Ellis 1930; Norris 1965; Boucaud-Camou 1967; Leadbeater 1972a, b, 1979; Thomsen 1973, 1992; Leadbeater and Morton 1974; Hara and Takahashi 1984; Marchant 1985; Vørs 1992a; Smith and Hobson 1994; Tong 1997a, c; Tong *et al*. 1998). The previously reported cell length ranges from 3.5 to 9 µm

(Tong 1997a, c; Tong *et al*. 1998). The genus contains only one other species, *A. brevipoda* Ellis, 1930. *Acanthoeca spectabilis* can be distinguished from *A. brevipoda* by its tapering lorica and by the greater length of the stalk.

***Diaphanoeca multiannulata* Buck, 1981 (Figs 1b, 2a)**

Description: The cell outline is oval, about 6.5 µm long, with a collar and one flagellum of about the cell length. The cell is suspended in a lorica about 34 µm long and 11 µm wide and which opens anteriorly and is closed posteriorly. The lorica has about six transverse costae and several longitudinal costae. Its posterior end is connected to a stalk which attaches to the substrate. One cell was observed from sediment.

Remarks: This species has been reported from the Weddell Sea, Antarctica (Buck 1981; Takahashi 1981; Buck and Garrison 1983, 1988; Marchant 1985; Thomsen *et al*. 1990, 1997) and from West Greenland (Hansen *et al*. 1989), and the previously reported overall lorica lengths range from 33-87 µm. Although our cell is in the lower range of the lorica length, we assign it to *D. multiannulata* because it has six transverse costae. *Diaphanoeca multiannulata* has 3-6 transverse costae (Thomsen *et al*. 1990), whereas other *Diaphanoeca* species usually have 2-3 transverse costae. It can be distinguished from *D. glacialis* Thomsen, Garrison et Kosman, 1997; which Thomsen treats as a subspecies of *D. multiannulata* (*D. multiannulata* subsp. *glacialis*), because *Diaphanoeca glacialis* has only three distinctive transverse costae (Thomsen *et al*. 1997).

***Salpingoeca camelopardula* Norris, 1965 (Figs 1c, 2c)**

Description: Cells are about 12-16 µm long (n = 3) in a lorica that has a long (about 8-10 µm), narrow neck. The collar emerges at opening of the neck with one short flagellum. The cells attach to the substrate by the posterior end of the lorica. The cells were observed from sediment samples.

Remarks: *Salpingoeca camelopardula* Norris, 1965 has been found in marine sites in Belize (central America), Gulf of Elat (Israel) and USA, and previously reported cell lengths range from 7-20 µm (Norris 1965, Thomsen 1978, Vørs 1993). The length of the neck of this species may vary from 3.5 to 15 µm (Thomsen 1978, Vørs 1993). This species is similar to *S. amphoridium* James-Clark, 1867; but can be distinguished by the length of neck relative to the length of the body. *Salpingoeca camelopardula* is distinguished from other *Salpingoeca* species by the long and slender neck. We regard *S. camelopardula* as indistinguishable from *Salpingoeca carteri* Kent, 1880; but *S carteri* has not been used subsequent to its initial observation, so we apply Article 23.9 of the International Code for Zoological Nomenclature to promote nomenclatural stability.

***Salpingoeca ringens* Kent, 1880 (Figs 1d, 2d)**

Description: The one cell observed (from a mat sample) was about 6 µm long and sat within a lorica. The lorica was ovoid with a broad, flaring neck and a pointed posterior end which attaches to the substrate via a short pedicel. The single flagellum was about twice as long as the cell and was thin at its tip. The cell filled out the posterior part of the lorica.

Remarks: Generally, our observations are in agreement with those of Vørs (1993), but the cell described here had a shorter pedicel. This species has been found in marine sites in Belize (central America) and the USA (Kent 1880-2, Vørs 1993). *Salpingoeca eurystoma* Stokes, 1886 is a junior synonym of *S. ringens* (Vørs, 1993). *Salpingoeca ringens* is similar to *S. convallaria* Stein 1878 in having a short pedicel, but is distinguished by the flaring neck and because the length of lorica is 1.5-2 times shorter than that of *S. convallaria*.

Cryptomonadida Senn, 1900

***Goniomonas amphinema* Larsen et Patterson, 1990 (Figs 1e, 2e)**

Description: Cells are 5-7 µm long (n = 36), anteriorly truncate and posteriorly rounded, dorso-ventrally flattened, with several delicate longitudinal stripes on both sides and with a line of extrusomes near the anterior end. The two flagella are unequal in length and insert obliquely in an anterior depression near one margin of the cell. The short flagellum is directed forwards. The long flagellum is slightly longer than the cell and usually trails. This species was found from both sediment and mat samples.

Remarks: This species has been observed at marine sites worldwide and the reported size range is 4-8 µm (Lee and Patterson 1998, 2000). The longitudinal ridges may be overlooked and extrusomes may or may not be present. *Goniomonas amphinema* is distinguished from other *Goniomonas* by having unequal flagella, the longer of which trails behind the cell.

Table 1. Species encountered during the survey of free living flagellates associated with stromatolites and associated microbial mats (M) and adjacent sediments (S) in Shark Bay

	Habitat
Choanoflagellida Kent, 1880	
Acanthoeca spectabilis Ellis, 1930	S
Diaphanoeca multiannulata Buck, 1981	S
Salpingoeca camelopardula Norris, 1965	S
Salpingoeca ringens Kent, 1880	M
Cryptomonadida Senn, 1900	
Goniomonas amphinema Larsen et Patterson, 1990	M, S
Goniomonas pacifica Larsen et Patterson, 1990	M, S
Kinetoplastida Honigberg, 1963	
Bodo caudatus (Dujardin, 1841) Stein, 1878	M
Bodo cygnus Patterson et Simpson, 1996	M, S
Bodo designis Skuja, 1948	M, S
Bodo platyrhynchus Larsen et Patterson, 1990	M, S
Bodo saliens Larsen et Patterson, 1990	M, S
Bodo saltans Ehrenberg, 1832	S
Hemistasia phaeocysticola (Scherffel, 1900) Elbrächter, Schnepf et Balzer, 1996	M, S
Rhynchobodo simius Patterson et Simpson, 1996	M, S
Rhynchomonas nasuta Klebs, 1893	M, S
Euglenida Bütschli, 1884	
Anisonema cfr *acinus* Dujardin, 1841	S
Anisonema trepidum Larsen, 1987	S
Chasmostoma nieuportense Massart, 1920	S
Dolium sedentarium Larsen et Patterson, 1990	S
Heteronema exaratum Larsen et Patterson, 1990	S
Heteronema ovale Kahl, 1928	S
Jenningsia fusiforme (Larsen, 1987) Lee, Blackmore et Patterson, 1999	M, S
Notosolenus apocamptus Stokes, 1884	M, S
Notosolenus canellatus Skuja, 1948	S
Notosolenus hamelini sp. n.	S
Notosolenus ostium Larsen et Patterson, 1990	S
Notosolenus scutulum Larsen et Patterson, 1990	S
Notosolenus similis Skuja, 1939	M, S
Notosolenus urceolatus Larsen et Patterson, 1990	S
Petalomonas abscissa (Dujardin, 1841) Stein, 1859	S
Petalomonas minor Larsen et Patterson, 1990	M, S
Petalomonas minuta Hollande, 1942	M, S
Petalomonas poosilla Larsen et Patterson, 1990	M, S
Ploeotia azurina Patterson et Simpson, 1996	S
Ploeotia corrugata Larsen et Patterson, 1990	M, S
Ploeotia costata (Triemer, 1986) Farmer et Triemer, 1988	S
Ploeotia laminae sp. n.	S
Ploeotia pseudanisonema Larsen et Patterson, 1990	M, S
Ploeotia punctata Larsen et Patterson, 1990	S
Ploeotia robusta Larsen et Patterson, 1990	M, S
Ploeotia vitrea Dujardin, 1841	S
Euglenozoa *incertae sedis*	
Bordnamonas tropicana Larsen et Patterson, 1990	M, S
Rhynchopus amitus Skuja, 1948	M, S
Dinoflagellida Bütschli, 1885	
Amphidiniella sedentaria Horiguchi, 1995	M
Amphidinium carterae Hulburt, 1957	M, S
Amphidinium elegans Grell et Wohlfarth-Bottermann, 1957	S
Amphidinium cfr *incoloratum* Campbell, 1973	S
Amphidinium mammillatum Conrad et Kufferath, 1954	S
Amphidinium operculatum Claparède et Lachmann, 1859	S
Amphidinium psittacus Larsen, 1985	S

Table 1 (contd)

Amphidinium salinum Ruinen, 1938	S
Amphidinium scissum Kofoid et Swezy, 1921	S
Amphidinium semilunatum Herdman, 1924	S
Gyrodinium dominans Hulburt, 1957	M
Gyrodinium estuariale Hulburt, 1957	S
Gyrodinium cfr *mundulum* Campbell, 1973	S
Gyrodinium oblongum Larsen et Patterson, 1990	S
Gyrodinium pavillardi Biecheler, 1952	S
Katodinium asymmetricum (Massart, 1920) Loeblich, 1965	S
Katodinium dorsalisulcum Hulburt, McLaughlin et Zahl, 1960	S
Katodinium fungiforme (Anissimowa, 1926) Loeblich, 1965	M, S
Prorocentrum cfr *concavum* Fukuyo, 1981	S
Prorocentrum mexicanum Osorio Tafall, 1942	M
Alveolates	
Colpodella unguis Patterson et Simpson, 1996	M, S
Stephanopogonidae Corliss, 1961	
Stephanopogon apogon Borror, 1965	M
Stramenopiles Patterson, 1989	
Actinomonas mirabilis Kent, 1880/*Pteridomonas danica* Patterson et Fenchel, 1985	S
Bicosoeca gracilipes James-Clark, 1867	M, S
Cafeteria roenbergensis Fenchel et Patterson, 1988	M, S
Ciliophrys infusionum Cienkowski, 1876	M, S
Pendulomonas adriperis Tong, 1997	M, S
Pseudobodo tremulans Griessmann, 1913	M
Stramenopiles *incertae sedis*	
Developayella elegans Tong, 1995	M, S
Hamatores	
Caecitellus parvulus (Griessmann, 1913) Patterson, Nygaard, Steinberg et Turley, 1993	M, S
Apusomonadidae Karpov et Mylnikov, 1989	
Amastigomonas debruynei De Saedeleer, 1931	M, S
Amastigomonas mutabilis (Griessmann, 1913) Molina et Nerad, 1991	M, S
Cercomonadida Vickerman, 1983	
Cercomonas longicauda Dujardin, 1841	M
Cercomonas sp.	M, S
Massisteria marina Larsen et Patterson, 1990	M, S
Excavates Patterson et Simpson, 1999	
Carpediemonas bialata (Ruinen, 1938) Lee et Patterson, 2000	S
Carpediemonas membranifera Ekebom, Patterson et Vørs, 1996	M, S
Protista *incertae sedis*	
Ancyromonas melba Patterson et Simpson, 1996	M
Ancyromonas sigmoides Kent, 1880	M, S
Ancyromonas sinistra sp. n.	M, S
Clautriavia cavus Lee et Patterson, 2000	S
Glissandra innuerende Patterson et Simpson, 1996	M, S
Metopion fluens Larsen et Patterson, 1990	M, S
Metromonas grandis Larsen et Patterson, 1990	M, S
Metromonas simplex (Griessmann, 1913) Larsen et Patterson, 1990	S

***Goniomonas pacifica* Larsen et Patterson, 1990 (Figs 1f, 2f)**

Description: Cells are 4-8 μm long (n = 18), anteriorly truncated and posteriorly rounded, with several longitudinal ridges on both sides of the cell. A row of extrusomes is present near the anterior end of the cell. Two equal flagella, somewhat shorter than the cell emerge from a small anterior depression. The flagella beat anteriorly and laterally in opposed directions during swimming. Cells were observed in both sediment and mat samples.

Remarks: *Goniomonas pacifica* has been found at marine sites worldwide, with cell lengths of 3-15 μm (Lee and Patterson 1998, 2000). This species is distinguished from *G. amphinema* in having two equally long anterio-laterally directed flagella. The distinction between *G. pacifica* and *G. truncata* is unclear (Lee and Patterson 2000), and we follow past practice (e.g.

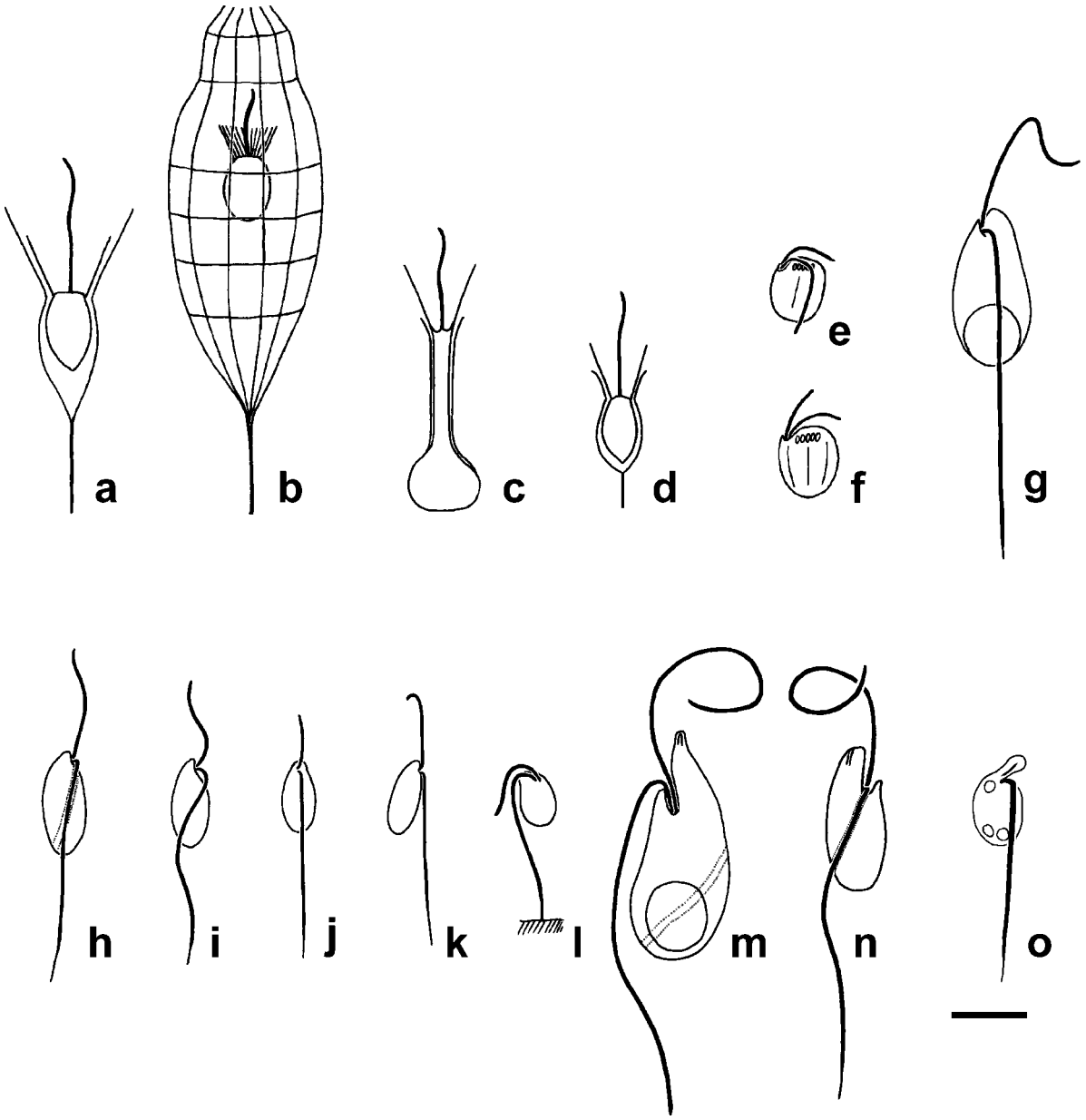

Fig. 1. (**a**) *Acanthoeca spectabilis*, (**b**) *Diaphanoeca mutiannulata*, (**c**) *Salpingoeca camelopardula*, (**d**) *Salpingoeca ringens*, (**e**) *Goniomonas amphinema*, (**f**) *Goniomonas pacifica*, (**g**) *Bodo caudatus*, (**h**) *Bodo cygnus*, (**i**) *Bodo designis*, (**j**) *Bodo platyrhynchus*, (**k**) *Bodo saliens*, (**l**) *Bodo saltans*, (**m**) *Hemistasia phaeocysticola*, (**n**) *Rhynchobodo simius*, (**o**) *Rhynchomonas nasuta*. Scale bar - 5 μm

Patterson and Simpson 1996, Lee and Patterson 2000) of referring to small cells from marine habitats as *G. pacifica*.

Kinetoplastida Honigberg, 1963

***Bodo caudatus* (Dujardin, 1841) Stein, 1878 (Figs 1g, 2l, m)**

Description: Cells are 11-16 μm long (n = 15) and are compressed and plastic, but usually ovate in profile. Two flagella emerge from a subapical pocket. The anterior flagellum is slightly longer than the cell, and beats with a paddling motion ahead of the cell during movement. The acronematic posterior flagellum is 2-2.5 times the cell length and appears to attach to the cell body or to lie in a depression when the cell is not moving. There is a prominent apical mouth. Cells consumed *Bodo designis*. The cells move by skidding or swimming. This species was observed in mat samples.

Remarks: This species has usually been reported from freshwater sites (e.g. Dujardin 1841, Stein 1878, Aléxéieff 1910, Hänel 1979). Previous marine records were made from hypersaline habitats in Canada (Wailes 1928/1929, 1939), India, Indonesia, Portugal and south and west Australia (Post *et al*. 1983, Ruinen 1938), but not from habitats of normal salinity. Cells have previously been reported with lengths of 10-22 µm. For synonymies see Hänel (1979). *Bodo caudatus* is distinguished from other *Bodo* species by the compressed cell body and the orientation and beat pattern of the anterior flagellum, and prominence of the mouth.

***Bodo cygnus* Patterson et Simpson, 1996 (Fig. 1h)**

Description: Cells are 8-11 µm long (n = 27) and elliptical, with a subapical pocket from which two unequal flagella emerge. The anterior flagellum is about the cell length and wraps around the anterior end of the cell when the cell is feeding. The acronematic posterior flagellum is about 2.5 times the length of the cell. Cells have a spiral groove that is easy to overlook, but distinctive. It extends from the subapical indentation to the posterior end of the cell. Cells move by rotating swimming movements. Cells were observed in both sediment and mat samples.

Remarks: This species has been described from marine sites in subtropical Australia and the previously reported sizes are 8-13 µm (Patterson and Simpson 1996, Lee and Patterson 2000). Our observations are in accord with previous accounts although the groove was distinctive. *Bodo cygnus* is distinguished from *B. designis* by its spiral groove, but the groove can be hard to see in swimming cells. Previously reported large *Bodo designis* may be referable to *B. cygnus* because the spiral groove may have been overlooked.

***Bodo designis* Skuja, 1948 (Figs 1i, 2g)**

Description: Cells are 6-10 µm long (n = 94) and flexible, usually with an elliptical outline. Two unequal flagella emerge from a subapical pocket. The anterior flagellum is about the length of the cell or slightly shorter and curves back over the rostrum, which contains a mouth. The anterior flagellum wraps around the anterior part of the cell and the mouth is pressed against food particles when the cell is feeding. The acronematic posterior flagellum is 2-3.5 times the cell length. Cells rotate when swimming. Cells were observed in both sediment and mat samples.

Remarks: This species has previously been reported with lengths of 4-15 µm from marine sites world-wide (Lee and Patterson 1998, 2000). *Bodo designis* is similar to *B. cygnus*, but lacks a spiral groove.

***Bodo platyrhynchus* Larsen et Patterson, 1990 (Figs 1j, 2h)**

Description: Cells are 4 to 11 µm long (n = 14), have an ovoid outline, are dorso-ventrally flattened, and are slightly flexible. The anterior margin of the cell is flattened. Two flagella insert subapically. The anterior flagellum is shorter than the cell and beats anteriorly. The trailing posterior flagellum is 2-4 times the length of the cell, is acronematic, and partly sticks to the cell. Cells usually glide slowly with a waggling movement or may swim with a slow rotating movement. Cells were observed in both sediment and mat samples.

Remarks: This species is rarely encountered, it was described from marine sites in subtropical Australia, Brazil and Hawaii with lengths from 3.5 to 7.5 µm (Larsen and Patterson 1990, Lee and Patterson 2000). Our observations here extend the range of sizes. The assignment of this taxon to *Bodo*, or to the kinetoplastids, needs to be verified (Lee and Patterson 2000).

***Bodo saliens* Larsen et Patterson, 1990 (Figs 1k, 2i)**

Description: Cells are 6-11 µm long (n = 32), usually elongate and elliptical, and somewhat flexible. Two flagella emerge from a shallow, subapical pocket. The anterior flagellum is as long as the cell and is held forwards with a single anterior curve held perpendicular to the substrate. The acronematic posterior flagellum is 2-3 times the cell length and typically directed straight behind the cell. Cells swim in rapid darts in straight lines. Cells were observed in both sediment and mat samples.

Remarks: This species has been reported from marine sediments worldwide, with sizes from 4 to 15 µm (Vørs 1992b; Lee and Patterson 1998, 2000). Generally, our observations agree with previous reports. This species is distinguished from other small heterotrophic flagellates by its rapid darting movement.

***Bodo saltans* Ehrenberg, 1832 (Figs 1l, 2j)**

Description: Cells are 5-6 µm long (n = 5) and somewhat elliptical, two flagella insert subapically. The anterior flagellum is slightly shorter than the cell and is usually held in a curve. The non-acronematic posterior flagellum is about 3 times the cell length. Cells often

attach to the substrate by the tip of the posterior flagellum. When so attached, the flagellum often bends quickly so that the cell jerks violently. This species was observed in sediment samples.

Remarks: This species is not usually reported from normal marine habitats. It is common in freshwater sites (e.g. Hawthorn and Ellis-Evans 1984, Vørs 1992a), and has been reported from brackish or hypersaline sites in Antarctica, subtropical Australia, North Atlantic, Gulf of Finland, Denmark, Belize and in the Bering Sea (Moiseev 1987; Vørs 1992a, b, 1993; Patterson *et al*. 1993; Patterson and Simpson 1996; Tong *et al*. 1997). Patterson and Simpson recorded this species in hypersaline habitats from the Shark Bay region, including saturated brine. *Bodo saltans* can be easily distinguished from other flagellates by its jerking behaviour.

***Hemistasia phaeocysticola* (Scherffel, 1900) Elbrächter, Schnepf et Balzer, 1996 (Figs 1m, 2k)**

Description: Cells are elongate ovate, somewhat metabolic and 16-25 μm long when extended (n = 3). The anterior quarter of the cell forms a rostrum containing a long, straight, tubular ingestion apparatus which opens immediately below an apical papilla. Two thickened flagella about 1.5 times the length of the cell insert in parallel in a deep antero-laterally directed pocket located at the base of the rostrum. A deep spiral groove originates from the flagellar pocket and continues to near the posterior end of the cell. The groove may have a 'doubled' appearance. During swimming the anterior flagellum beats ahead of the cell or to the side, the posterior flagellum trails and the cells rotate. A large, posterior food vacuole can be present. The round nucleus contains condensed chromatin and is located medially. This species was observed in both sediment and mat samples.

Remarks: The genus is monotypic, with *H. klebsi* Griessmann, 1913; *Entomosigma peridinioides* Schiller, 1925 and *Cryptaulax marina* sensu Throndsen, 1969 being junior synonyms of *H. pheocysticola* (Elbrächter *et al*. 1996; Bernard *et al.* 2000). This species has been reported from marine sites in subtropical Australia, Germany and in the Wadden Sea, with cell lengths of 12-25 μm (Scherffel 1900, Griessmann 1913, Elbrächter *et al*. 1996) under the names given above. Our observations are similar to previous reports and we confirm that the spiral groove is a discrete feature, not an artefact of flagellar beating as suggested by Elbrächter *et al*. (Elbrächter *et al*. 1996). The doubled appearance of the groove was noted by Griessmann (1913). *Hemistasia* has been shown to be a kinetoplastid euglenozoon (Elbrächter *et al*. 1996). The combination of an apical papillum and spiral groove distinguishes *Hemistasia* from similar taxa (Bernard *et al*. 2000).

***Rhynchobodo simius* Patterson et Simpson, 1996 (Fig. 1n)**

Description: Cells are 10-16 μm long (n = 41) and fusiform with a conspicuous rostrum containing a tubular ingestion apparatus that opens apically. Two thickened flagella insert in well-developed pocket at the base of rostrum. The anterior flagellum is about 2 times body length and, the posterior one is about 3 times body length. A spiral groove runs from the flagellar pocket to near the posterior end of cell. A fine groove also runs from the flagellar pocket to the tip of the rostrum. Cells were not seen to glide, skid, deform or squirm. There may be a large posterior food vacuole, containing eukaryotic prey. Cells were observed in both sediment and mat samples.

Remarks: *Rhynchobodo* has had an uncertain history. Bernard *et al*. (2000) synonymised *Cryptaulax* with *Rhynchobodo*. The species of *Cryptaulax* have been reviewed by (Ekelund and Patterson 1997) and the actions of Bernard *et al*. (2000) transfer seven species of *Cryptaulax* to *Rhynchobodo*. *Rhynchobodo* now contains 11 species of kinetoplastid flagellates with an apical mouth (but no papilla), a stiff anterior part of the body and, in some cases, a spiral groove. Many synonyms probably still exist. Most *Rhynchobodo* are reportedly more or less metabolic whereas *R. simius* is inflexible. We distinguish *R. simius* from *R. armata* Brugerolle 1985 and *R. agilis* Lackey, 1940 because these taxa lack a spiral groove. *Rhynchobodo simius* can be distinguished from *R. taeniata* (Skuja, 1956) Vørs, 1992 by its slightly smaller size (*R. taeniata*, 13-20 μm) and because *R. taeniata* has a more conspicuous spiral groove. This species has been previously reported from hypersaline sites in the Shark Bay area, with lengths of 9-16 μm (Patterson and Simpson 1996).

***Rhynchomonas nasuta* (Stokes, 1888) Klebs, 1893 (Fig. 1o)**

Description: Cells are 4-7 μm long (n = 123), are flattened, flexible, and have a bulbous motile snout. The snout, which contains a mouth, beats slowly. The anterior flagellum lies alongside the snout and is hard to see, and the trailing flagellum is 2-2.5 times the cell length and

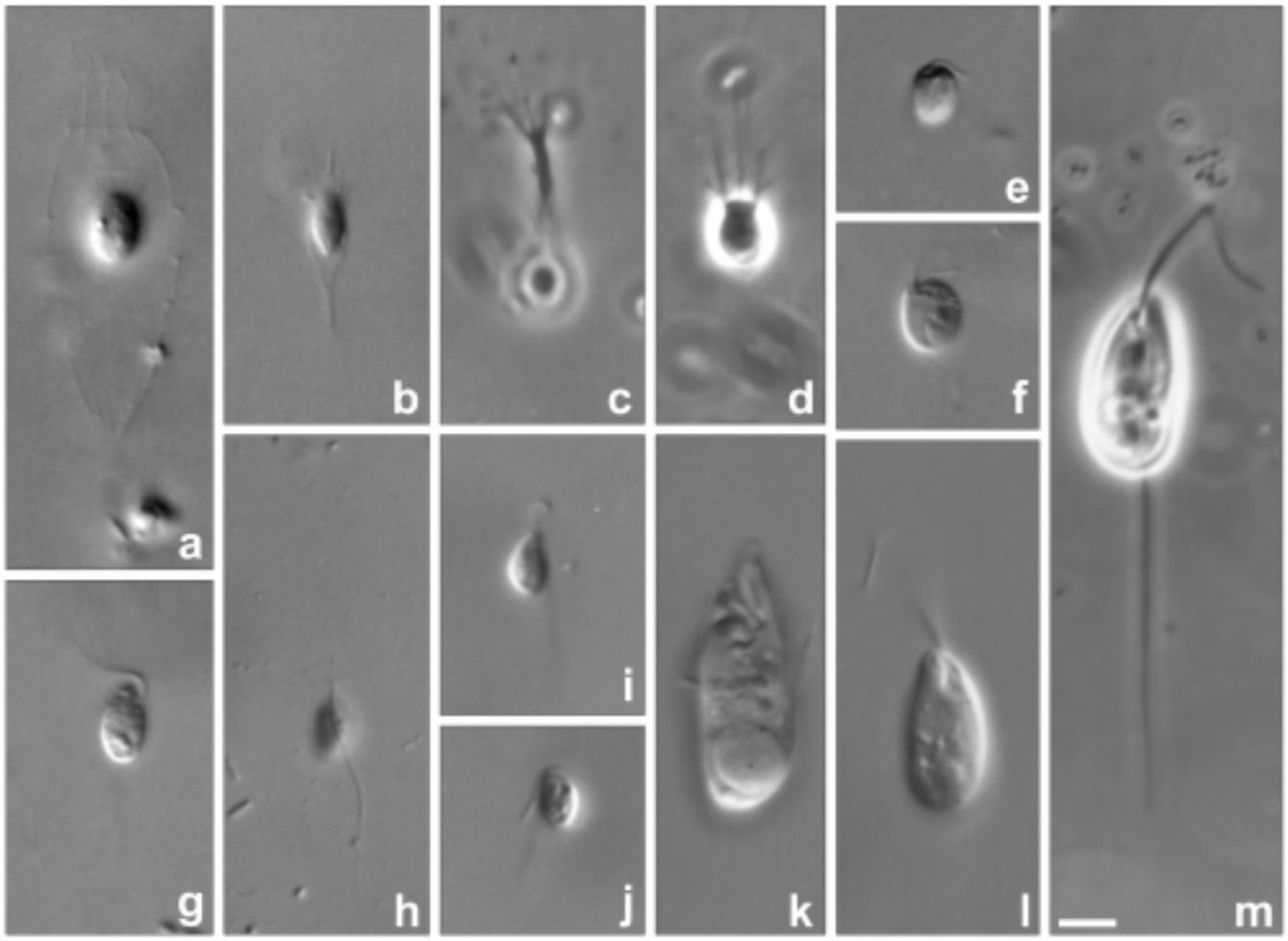

Fig. 2. (**a**) *Diaphanoeca mutiannulata*, (**b**) *Acanthoeca spectabilis*, (**c**) *Salpingoeca camelopardula*, (**d**) *Salpingoeca ringens*, (**e**) *Goniomonas amphinema*, (**f**) *Goniomonas pacifica*, (**g**) *Bodo designis*, (**h**) *Bodo platyrhynchus*, (**i**) *Bodo saliens*, (**j**) *Bodo saltans*, (**k**) *Hemistasia phaeocysticola*, (**l-m**) *Bodo caudatus*. All micrographs here and following are DIC images of living cells except where indicated. Figs 2 (**d**) and (**m**) are phase contrast images. Scale bar - 5 μm

is acronematic. Cells move by gliding. Cells were found in both sediment and mat samples.

Remarks: This species has been reported from diverse sites worldwide (Lee and Patterson 1998, 2000), including hypersaline habitats in Australia (Post *et al*. 1983, Patterson and Simpson 1996). The bulbous, flexible snout is distinctive (Larsen and Patterson 1990).

Euglenida Bütschli, 1884

***Anisonema* cfr *acinus* Dujardin, 1841 (Figs 3a, 4a, b)**

Description: The cells are 23-48 μm long and 12-27 μm wide (n = 40), are eliptical to ovate, and flattened. The cell surface has widely spaced longitudinal pellicular striations. Two flagella emerge subapically. The anterior flagellum is 1-1.5 times the cell length. The recurrent flagellum is thicker and 2-3.5 times the cell length, lying in a ventral groove. In cells otherwise indistinguishable, an ingestion organelle was either not seen, or was weakly developed, or was well developed. When visible, it consists of two rods, and extends about half way to the posterior end. The flagellar pocket is situated in the left side of the cell. The nucleus lies on the right side or posteriorly. The cells are often filled with granules of various sizes and may contain ingested pennate diatoms. The cells move by gliding, but occassionally jerk backwards. This species was observed in sediment samples.

Remarks: *Anisonema acinus* has been reported from marine sites worldwide, with cell lengths from 21 to 37 μm (Lee and Patterson 2000). We tentatively assign the individuals observed here with a well-developed ingestion organelle to *Anisonema. Anisonema*

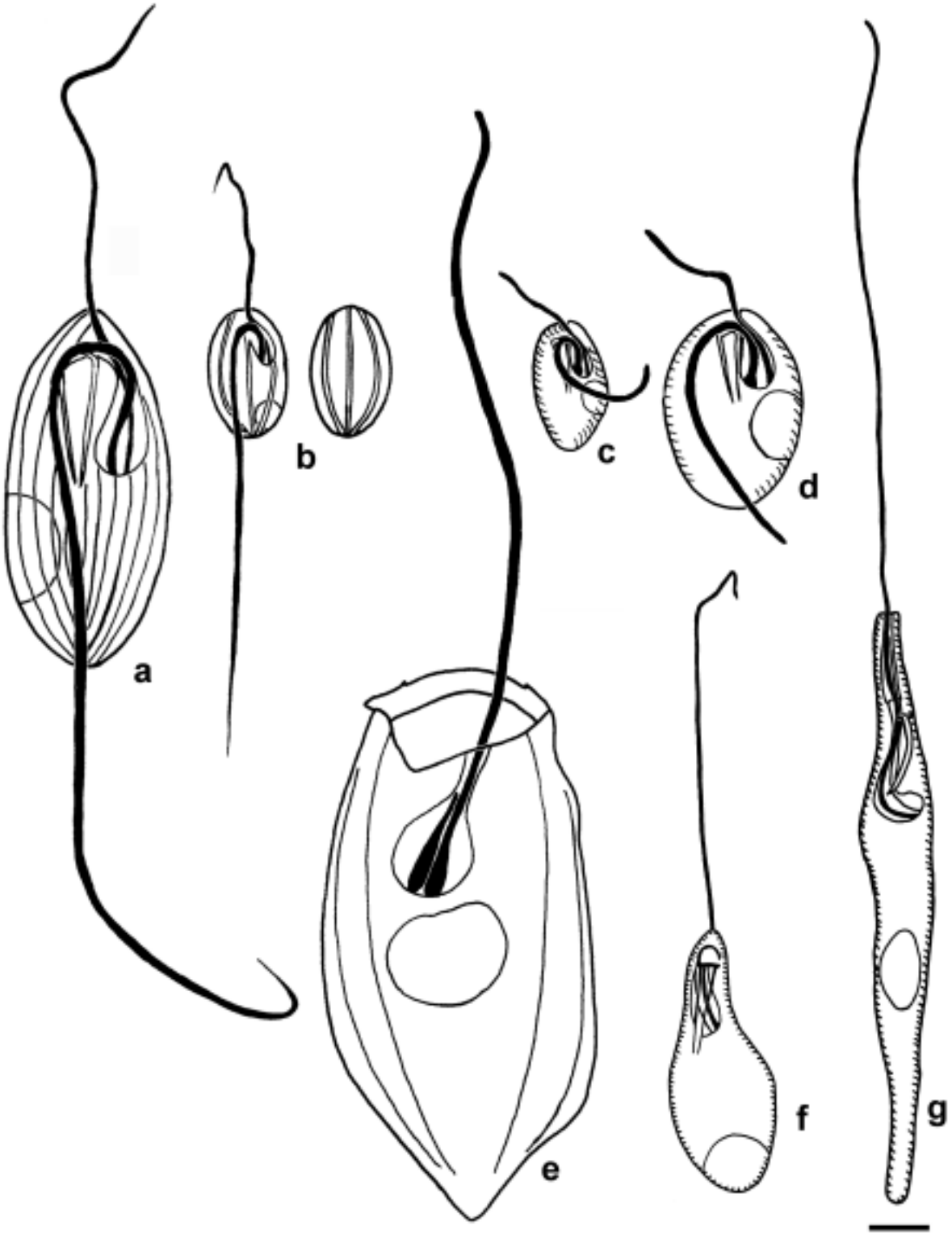

Fig. 3. (**a**) *Anisonema* cfr *acinus*, (**b**) *Anisonema trepidum*, (**c**) *Heteronema exaratum,* (**d**) *Heteronema ovale*, (**e**) *Dolium sedentarium*, (**f**) *Jenningsia fusiforme*, (**g**) *Chasmostoma nieuportense.* Scale bar: (a - f) - 10 µm; g- 15 µm

acinus was described by Dujardin (1841) as the type species of the genus. Dujardin did not refer to the presence of an ingestion apparatus. Some later authors asserted that *A. acinus* has a well-developed ingestion apparatus (Bütschli 1878; Stein 1878; Kent 1880-2; Klebs 1883, 1893; Aléxéieff 1913; Hollande 1942), although the identities of the taxa studied are often unclear. Hollande (1942) noted that the ingestion apparatus is difficult to observe, but becomes very distinct after staining with haematoxylin. Other authors denied or did not refer to the presence of this structure (Lemmermann 1910, 1913; Playfair 1921; Walton 1915; Skuja 1939; Huber-Pestalozzi 1955; Larsen 1987; Larsen and Patterson 1990; Lee and Patterson 2000). This raises the question of whether *A. acinus* is a single species in which the ingestion apparatus is variably visible, or whether *A. acinus* houses two discrete taxa: one with an ingestion apparatus and one without.

Some cells without visible ingestion apparatuses contain large pieces of food (e.g. diatoms) suggesting that a feeding apparatus is present even when not visible by light-microscopy. Electron microscopy of *Anisonema costatum* Christen, 1962 (Mignot, 1966) and of an unnamed species of *Anisonema* (Triemer, 1985) establish the presence of an ingestion apparatus in at least some *Anisonema*. It is possible that all species assigned to *Anisonema* have such organelles, although in only some species is the organelle visible by light microscopy. Structures interpreted as ingestion organelles have been reported in photosynthetic euglenids (Willey and Wibel 1985) and in some heterotrophs not previously regarded as having ingestion organelles, such as *Petalomonas* and *Notosolenus* (Mignot 1966, Cann et Pennick 1986, Larsen and Patterson 1990, Triemer and Farmer 1991, Lee and Patterson 2000). Ultrastructural work on strains without a mouth visible by light microscopy is now needed to determine if a revision of the genus is required. The interpretation that the type species of *Anisonema* has an ingestion apparatus will require a revision of this genus, of *Ploeotia* and perhaps of *Dinema*. Such a revision is beyond the scope of this paper, and we therefore make our identification tentative.

Skuja (1939) described *Anisonema prosgeobium,* which resembles *A. acinus,* but lacks pellicular striations. Lee and Patterson (2000) regarded *A. prosgeobium* as a junior synonym of *A. acinus*. *Anisonema acinus* resembles *A. glaciale* Larsen et Patterson, 1990, but *A. acinus* lacks the 'arresting' behaviour and the nucleus is in a different position. *Anisonema acinus* is distinguished from *A. costatum* Christen, 1962 because the pellicle in *A. costatum* has well-developed spiral ribs. This species is similar to *Dinema validum* Larsen et Patterson, 1990 and *Dinema platysomum* (Skuja, 1939) Lee et Patterson, 2000 in general outline, but *A. acinus* is not flexible and lacks a thickened pellicle. The cells observed here resemble *Ploeotia robusta* Larsen et Patterson, 1990 in cell shape and size, but can be distinguished because *P. robusta* has fewer, more deeply indented grooves.

***Anisonema trepidum* Larsen, 1987 (Figs 3b, 4c, d)**

Description: The cells are 10-18 μm long, 4.5-8 μm wide (n = 9), oblong in profile, and flattened. There are three fine longitudinal ventral grooves and 3-4 fine, longitudinal, dorsal grooves, which are widely spaced and extend the length of the cell. Two flagella emerge subapically. The anterior flagellum is 0.7-1.5 times the cell length, the recurrent flagellum is thick and 3-4 times the length of the cell. The flagellar pocket is pear-shaped and located in the left side of the cell. The nucleus lies near the posterior end of the cell. The cell moves by rapid gliding, regularly retracting by swift jerks to change direction. This species was observed from sediment samples.

Remarks: This species has been found in marine sites worldwide, with a size range of 9-20 μm (Lee and Patterson 1998, 2000). Our observations are broadly in agreement with previous observers. Larsen and Patterson (1990) distinguished *A. trepidum* from other small species in the genus, except for *A. glaciale* Larsen et Patterson, 1990, because its movement is interrupted by sudden, prolonged stops. However, we did not observe this behaviour. *Anisonema trepidum* can be distinguished from *A. solenatum* Stokes, 1887 by having a long recurrent flagellum and lacking a deep dorsal groove. This species also can be distinguished from *A. obliquum* Roskin, 1931 by cell outline shape and because the latter has a smooth pellicle. It is similar to *Ploeotia pseudanisonema* Larsen et Patterson, 1990 in general appearance, but is less rounded and lacks a visible ingestion apparatus.

***Chasmostoma nieuportense* Massart, 1920 (Figs 3g, 5c-e)**

Description: Cells are elongate but highly metabolic, measuring 80-100 μm long when fully extended (n = 4).

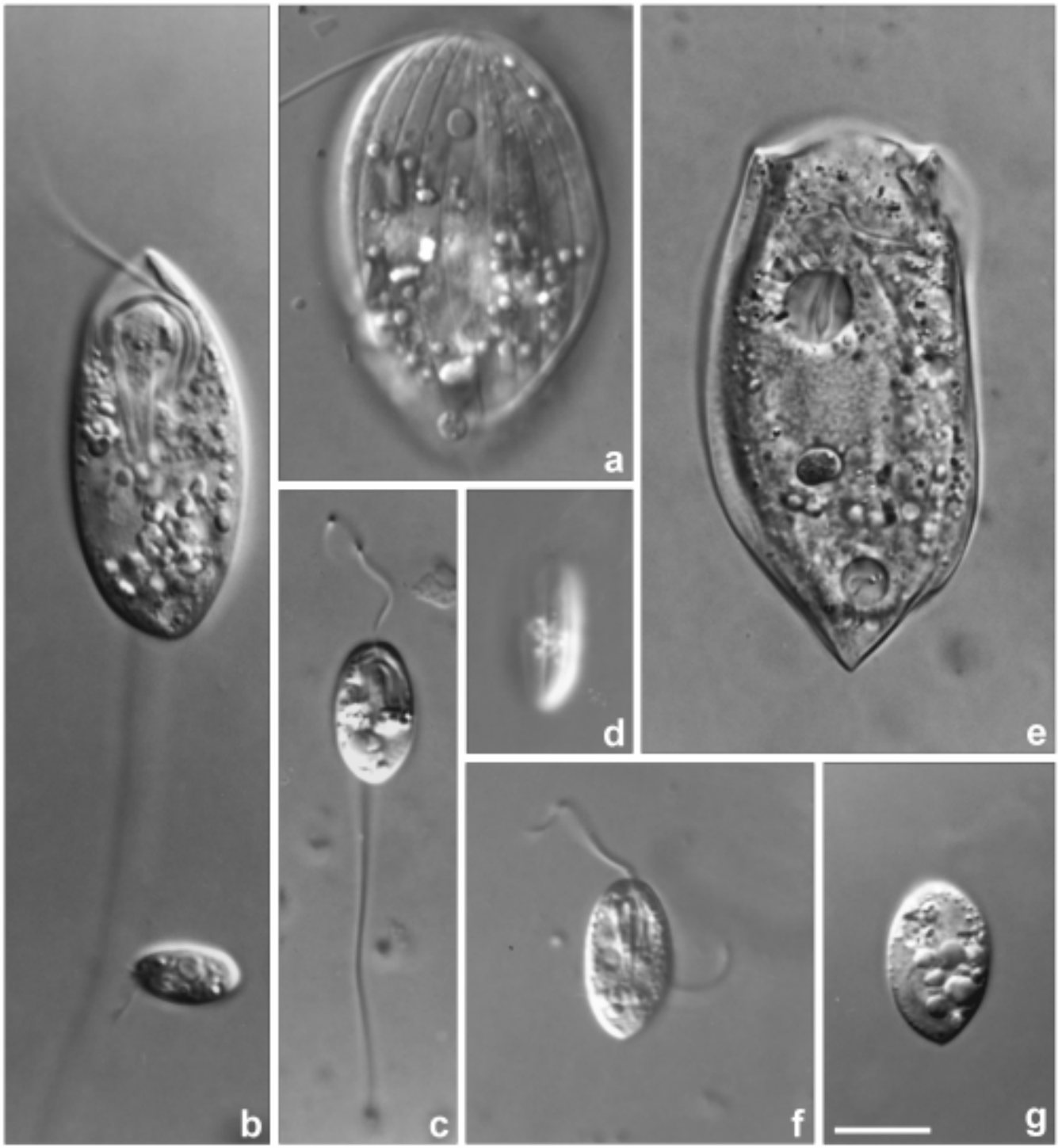

Fig. 4. (**a, b**) *Anisonema* cfr *acinus*, (**a**) dorsal view showing the pellicular striations, (**b**) general appearance, (**c, d**) *Anisonema trepidum*, (**c**) general appearance, (**d**) dorsal view showing grooves, (**e**) *Dolium sedentarium* showing general appearance, (**f, g**) *Heteronema exaratum*, (**f**) general appearance, (**g**) showing posterior pointed end. All micrographs are DIC images. Scale bar - 10 μm

The elongate flagellar pocket lies one third of the way down of the cell. It is connected to a large cavity that makes up most of the anterior part of the cell and opens at the anterior apex of the cell. Two flagella are present, but only one emerges from the flagellar cavity. This flagellum is slightly longer than the cell body. It can be withdrawn and coiled inside the cavity (i.e. can be swallowed), re-emerging slowly after a few moments. The ingestion apparatus is curved and lies beside the flagellar pocket. It is composed of two rods each with a swollen anterior end. The nucleus is situated two thirds of the way down the cell. The cell surface has pellicular striations following an **S** - helix; they are widely spaced over the cavity. The striations have an irregular appearance as if underlain by small extrusomes. The cytoplasm is filled with granules of various sizes. Movement is by gliding with the emergent flagellum extended anteriorly. This species was observed in sediment samples.

Remarks: *Chasmostoma nieuportense* was described from marine sites in Belgium (Massart 1920). The original account is poor. The species was next reported from a prawn farm in Queensland (Lee *et al*. 1999), and has also been seen in a salt marsh in Massachusetts (http://www.mbl.edu/microscope) and in Botany Bay (Lee unpublished observation). The present observations confirm the presence of a second flagellum in the flagellar pocket, and the possible presence of small extrusomes. The observations are in good agreement with those of Lee *et al*. (1999).

Dolium sedentarium **Larsen et Patterson, 1990 (Figs 3e, 4e)**

Description: The single cell observed was vase-shaped, 63 μm long, 31 μm wide, slightly flattened with a sharply pointed posterior by which it attached to the substrate. The anterior end has a wide opening with a raised margin. Six prominent longitudinal ridges lay roughly equally spaced around the cell. The flagellar pocket was spherical and was situated anteriorly in the right side of the cell. Two flagella were present, both with swollen bases. Only one flagellum was emergent and was about the length of the cell body. The nucleus was rounded and was located medially. The cell contained a few food vacuoles and many globular granules of various sizes. The cell was sessile. This species was observed in a sediment sample.

Remarks: This species has been reported from marine sites from tropical and subtropical Australia, Brazil and Fiji, with cell lengths 40-70 μm (Lee and Patterson 1998). Generally, our observations agree with previous accounts. *Dolium sedentarium* is distinguished from most other heterotrophic euglenids by its sedentary habit.

Heteronema exaratum **Larsen et Patterson, 1990 (Figs 3c, 4f, g)**

Description: Cells are ovate in outline, 12-19 μm long, 8-10 μm wide (n = 5), flattened, and somewhat metabolic. The posterior end of the cell is usually rounded but two cells had pointed posterior ends during metaboly. The pellicular striations follow a distinct **S** - helix, with the dorsal striations more developed than the ventral striations. Globular granules lie along the striations. Two flagella emerge subapically. The anterior flagellum is about the cell length and is directed to the right side while swimming. The posterior flagellum has a knob near its base, is slightly thicker and longer than the anterior flagellum, and is directed to the left and posteriorly. The ingestion apparatus is well developed, with two rods extending about half way to the posterior end. The flagellar pocket and nucleus are situated in the left side of the cell. The cells move by rapid skidding and consume diatoms. This species was observed in sediment samples.

Remarks: This species has been reported from marine sites in tropical and subtropical Australia and Fiji, with a range of cell length of 8-20 μm (Lee and Patterson 1998, 2000). *Heteronema larseni* Lee et Patterson, 2000 can be distinguished from *H. exaratum* because it is larger (35-45 μm), and has a pointed posterior end, less vigorous metaboly, a slower skidding movement, better developed pellicular striations and a more conspicuous ingestion apparatus. *Heteronema exaratum* can be distinguished from *H. ovale* Kahl, 1928 by different pellicular striations on the dorsal and ventral sides, a less conspicuous ingestion device, less vigorous metaboly, and by having a slightly pointed posterior end during metaboly.

Heteronema ovale **Kahl, 1928 (Figs 3d, 5a, b)**

Description: The cells are ovate, 13-25 μm long, 10-19 μm wide (n = 12), flattened, and highly metabolic. The pellicular striations follow an **S** - helix. Small globular bodies may be irregularly distributed along the striations. Two flagella emerge subapically. The anterior flagellum is as long as the cell or slightly longer, beats actively on the right hand side of the cell. The posterior flagellum is slightly thicker and longer than the anterior one and it has a distinct knob at the flagellar base. The ingestion device is well developed, with two rods extending more than half way to the posterior end. The flagellar pocket and

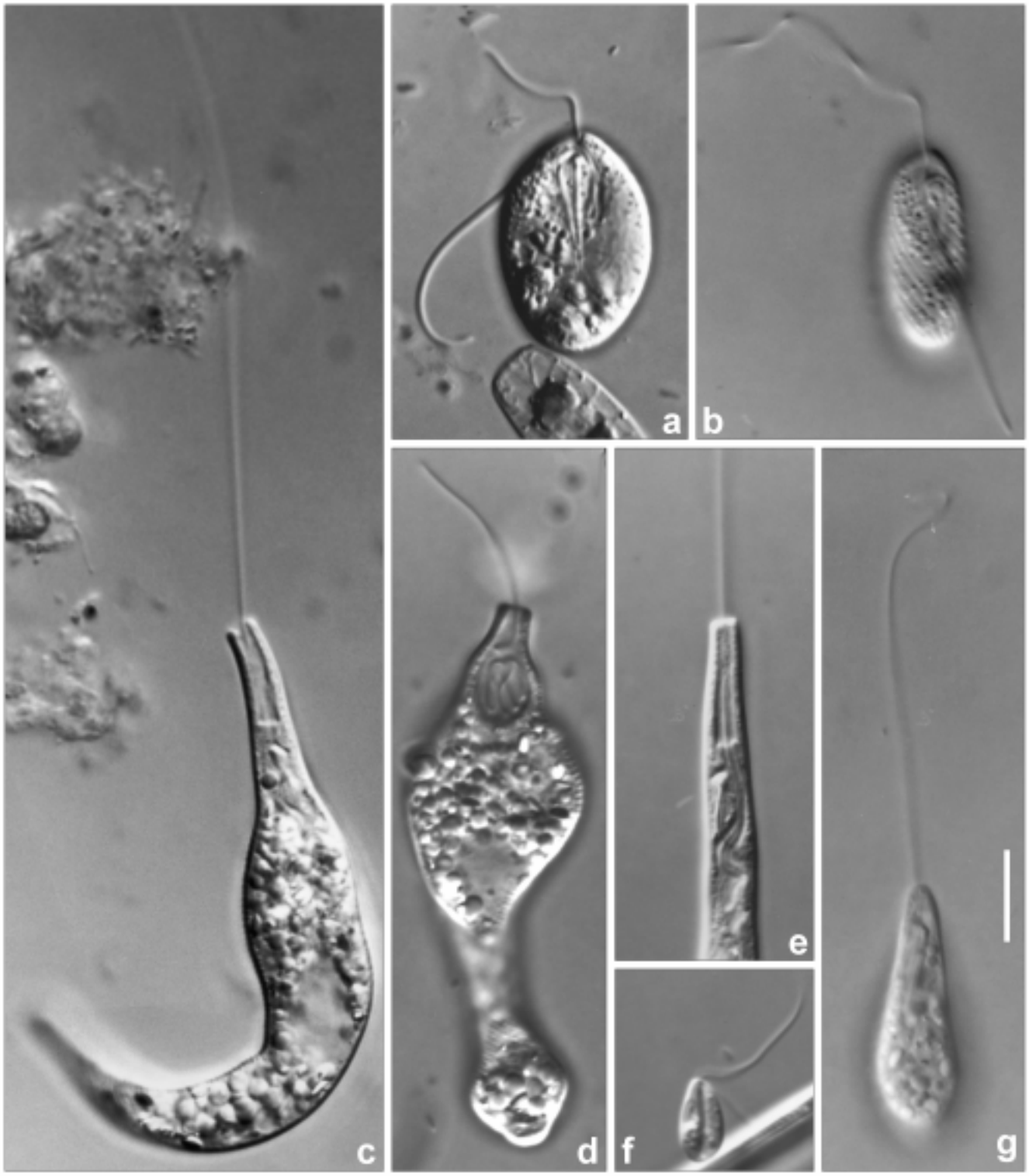

Fig. 5. (**a, b**) *Heteronema ovale*, (**a**) general appearance, (**b**) ventral view showing pellicular striations of elongated cell, (**c-e**) *Chasmostoma nieuportense*, (**c**) general appearance, (**d**) showing coiled anterior flagellum, (**e**) ventral view showing ingestion organelle and reservoir contain two flagella, (**f**) *Notosolenus apocamptus* showing a dorsal groove, (**g**) *Jenningsia fusiforme* showing general appearance. All micrographs are DIC images. Scale bar - 10 μm

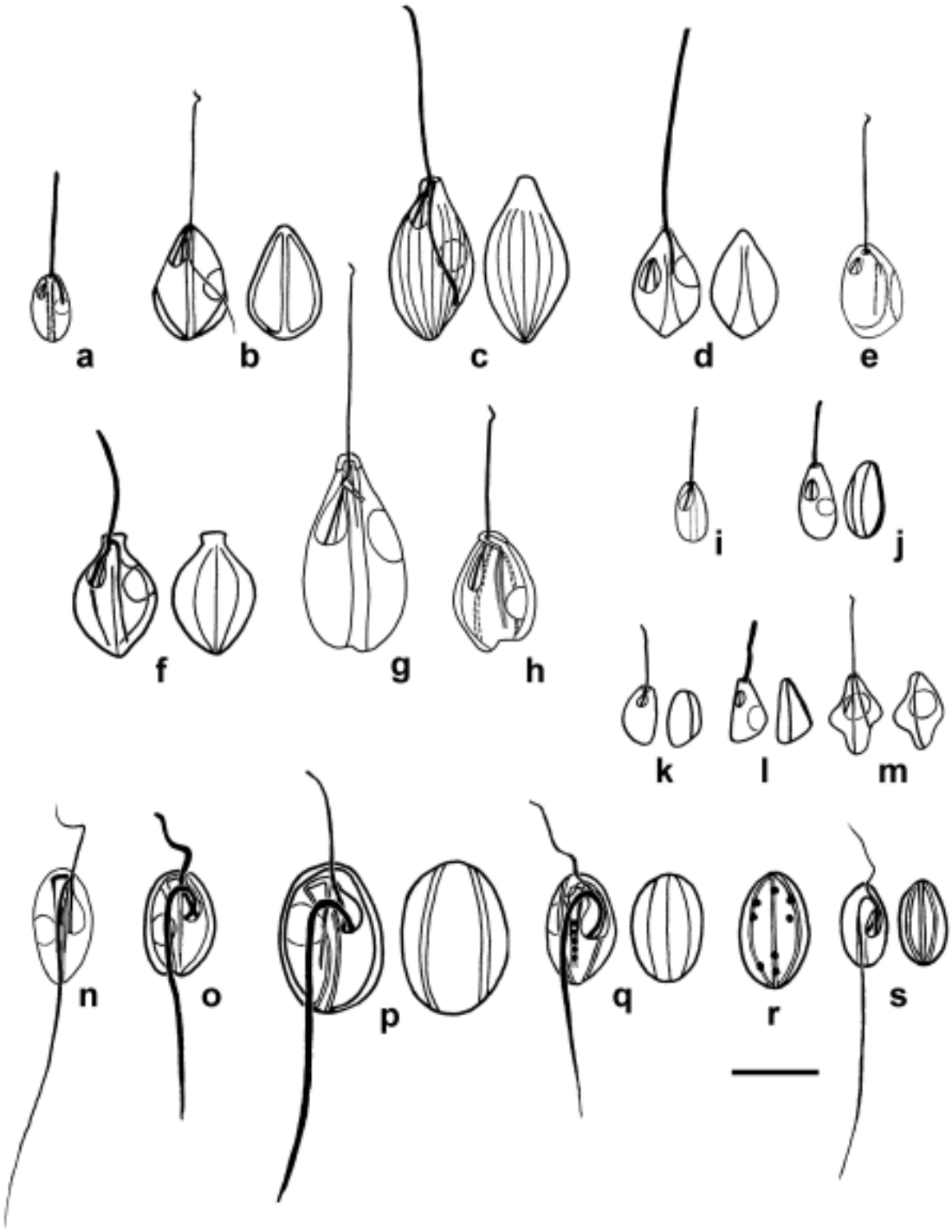

Fig. 6. (**a**) *Notosolenus apocamptus*, (**b**) *Notosolenus canellatus*, (**c**) *Notosolenus hamelini* sp. n., (**d**) *Notosolenus scutulum*, (**e**) *Notosolenus similis*, (**f**) *Notosolenus urceolatus*, (**g**) *Notosolenus ostium*, (**h**) *Petalomonas abscissa*, (**i**) *Petalomonas minuta*, (**j**) *Petalomonas poosilla*, (**k-m**) *Petalomonas minor,* (**n**) *Ploeotia azurina*, (**o**) *Ploeotia corrugata*, (**p**) *Ploeotia costata*, (**q, r**) *Ploeotia punctata*, (**s**) *Ploeotia* cfr *punctata.* Scale bar - 10 μm

nucleus are located on the left-hand side of the cell. The cells move by skidding, and also by squirming. This species was observed in sediment samples.

Remarks: Our observations agree with the original observation (Kahl 1928) from the Baltic Sea. Lee and Patterson (2000) observed this species from marine sites in Australia, with cell lengths from 15-30 µm. As pointed out by Lee and Patterson, the organism described by Larsen (1987) and the large-sized cells described by Larsen and Patterson (1990) and Ekebom *et al*. (1996) as *H. ovale* are not the same species as that described by Kahl and was given the name *H. larseni* Lee et Patterson, 2000.

***Jenningsia fusiforme* (Larsen, 1987) Lee, Blackmore et Patterson, 1999 (Figs 3f, 5g)**

Description: The cells are elongate, 22-37 µm long (n = 80), anteriorly narrowed, and posteriorly rounded, but actively metabolic. The fine pellicular striations follow an **S -** helix. Two flagella are present in the elongate flagellar pocket/reservior, which is situated in the anterior third of the left hand side of the cell. Only one flagellum is emergent, and is 1.2-1.8 times the cell length. The ingestion organelle includes two tapering rods that lie alongside the flagellar pocket. A conspicuous cytoskeletal arc arises from the right rod and curves towards the anterior part of the cell. The nucleus is located posteriorly. The cells move by gliding interrupted by squirming. The cells were found in sediments and mat samples.

Remarks: This species has been reported from marine sites worldwide with cell lengths ranging from 20 to 45 µm (Lee and Patterson 1998, 2000). Our observations are in agreement with previous accounts, as reinterpreted by Lee *et al*. (1999). *Jenningsia fusiforme* resembles *J. macrostoma* (Ekebom, Patterson et Vørs, 1996) Lee, Blackmore et Patterson, 1999 but the latter is larger (64-87 µm), has a strong ingestion organelle, and has well developed pellicular striations. This species is similar to *J. diatomophaga* Schaeffer, 1918; but the latter is about 180 µm long. Species of *Jenningsia* are distinguished from *Peranema* by the absence of an emergent recurrent flagellum (Lee *et al*. 1999).

***Notosolenus apocamptus* Stokes, 1884 (Figs 5f, 6a)**

Description: Cells are 5-12 µm long, 3-6 µm wide (n = 10), flattened, rounded posteriorly and narrowed towards the anterior. The dorsal surface has a deep longitudinal median groove extending the length of the cell. Two flagella emerge subapically. The anterior flagellum is 1.3-2.8 times the cell length. The posterior flagellum is 0.3-0.9 times the cell length. The flagellar pocket is located on the right side of the cell and the nucleus to the left side. Cells move by gliding. This species was observed in sediment and mat samples.

Remarks: Previously reported cells from marine sites in subtropical and tropical Australia, Brazil and Fiji range in size of 7-18 µm (Lee and Patterson 1998, 2000). Our observations agree with previous accounts. *Notosolenus apocamptus* resembles *N. similis* Skuja, 1939; but can be distinguished by the medial position of the dorsal groove. This taxon is similar to *Notosolenus orbicularis* Stokes, 1884 in general outline, but the latter has a very wide dorsal groove extending almost to the margins of the cell. This species is distinguished from *Petalomonas minuta* Hollande, 1942 by the presence of the recurrent flagellum.

***Notosolenus canellatus* Skuja, 1948 (Figs 6b, 7a-c)**

Description: Cells are 12-15 µm long, 6-10 µm wide (n = 11), pear-shaped, dorsally convex and ventrally flattened. The anterior end is broadly pointed and sometimes has a small collar. The dorsal surface has three longitudinal grooves, one median groove and two lateral grooves (giving the posterior end a flanged appearance). The ventral surface has a shallow longitudinal median groove. Two flagella emerge subapically from the cell collar. The anterior flagellum is 0.9-1.4 times the cell length. The posterior flagellum is 0.8-1.2 times the cell length. The cells are often filled with globular granules of various sizes. The flagellar pocket is located on the right hand side of the cell and the nucleus is on the left side. Cells move by gliding. This species was observed in sediment samples.

Remarks: This species was previously reported from marine sites in tropical Australia and the Danish Wadden Sea, with lengths of 12-15 µm (Lee and Patterson 1998). Our observations are in accord with these accounts. This species resembles *N. pyriforme* Lee et Patterson, 2000 and *N. lashue* Lee et Patterson, 2000 in general appearance, but these species have only one dorsal groove.

***Notosolenus hamelini* sp. n. (Figs 6c, 7d-f)**

Diagnosis: *Notosolenus* with 8 ventral ridges and 5 dorsal ridges.

Description: The cell shape is ovate, 18-20 µm long, 10-11 µm wide, and slightly flattened. Cells are anteriorly

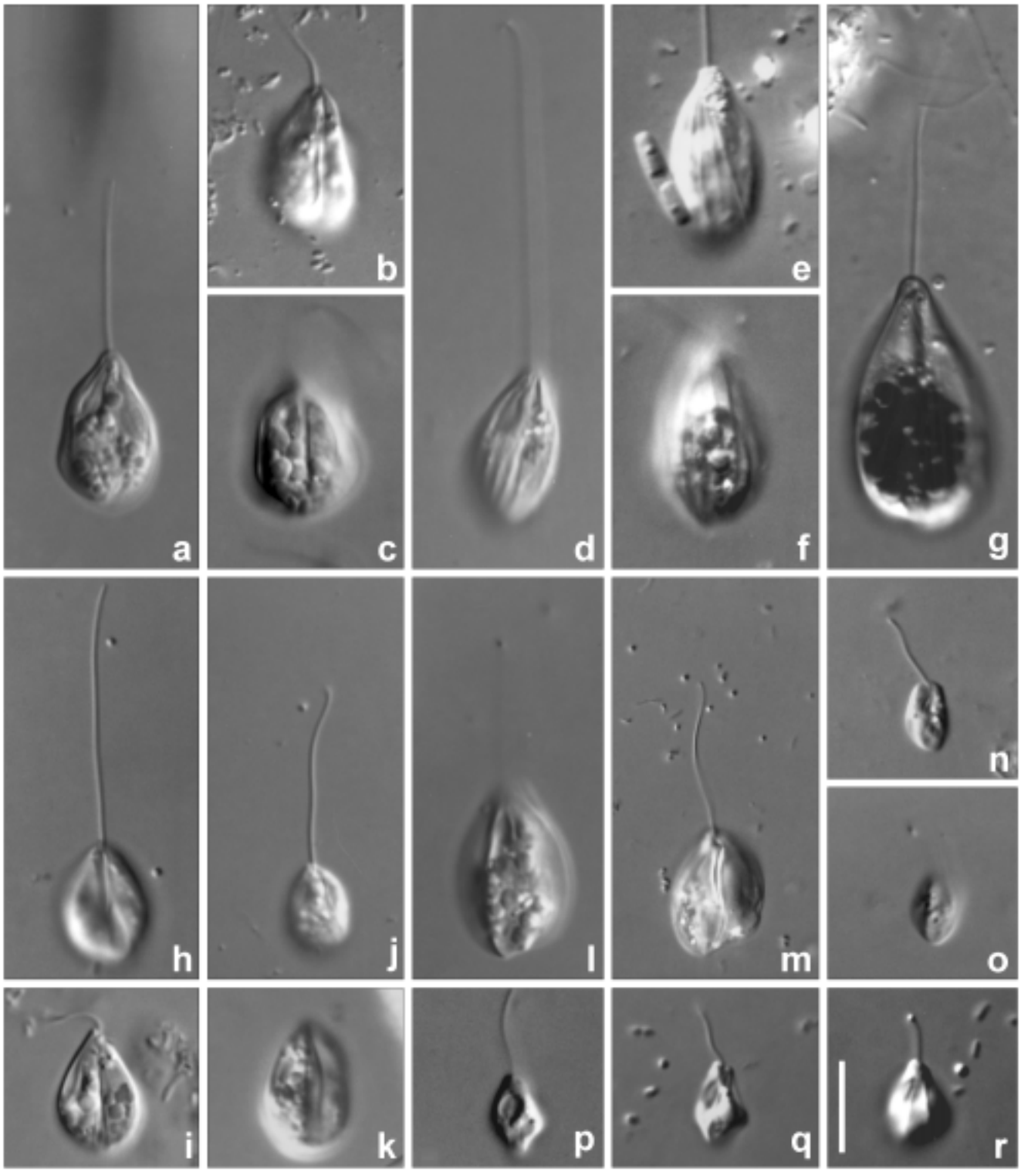

Fig. 7. (**a-c**) *Notosolenus canellatus*, (**a**) general appearance, (**b**) ventral view showing a shallow longitudinal groove, (**c**) dorsal view showing three grooves, (**d-f**) *Notosolenus hamelini* sp. n., (**d**) general appearance, (**e**) ventral view showing ridges, (**f**) dorsal view showing ridges, (**g**) *Notosolenus ostium* showing general appearance, (**h, i**) *Notosolenus scutulum*, (**h**) general appearance, (**i**) dorsal view showing a groove, (**j-k**) *Notosolenus similis*, (**j**) general appearance, (**k**) dorsal side, (**l, m**) *Petalomonas abscissa*, (**l**) dorsal view showing two ridges, (**m**) general appearance showing a ridged groove, (**n-r**) *Petalomonas minor*, (**n, o**) first form, (**n**) general appearance, (**o**) dorsal view showing a keel, (**p, r**) second form, (**p**) general appearance, (**q**) ventral view showing lateral depression, (**r**) ventral view showing two ridges. All micrographs are DIC images. Scale bar - 10 μm

narrowed with a small neck surrounding the flagellar canal, and posteriorly obtuse or slightly pointed. Cells have 13 moderately conspicuous longitudinal ridges: eight ventrally and five dorsally. Two flagella emerge subapically. The anterior flagellum is 1.3-2 times the cell length. The posterior flagellum is 0.65-0.8 times the cell length. The flagellar pocket is located on the right hand side of cell and the nucleus is on the left side. The cells move by gliding with both flagella in contact with the substrate. Two cells observed in sediment samples. This species has since been observed in Port Botany (Al-Qassab unpublished observation).

Remarks: This species is assigned to *Notosolenus* because it is rigid, flattened, has 2 emergent flagella, glides with the anterior flagellum adpressed to the substrate and has no visible ingestion organelle. *Notosolenus hamelini* is similar to *N. urceolatus* Larsen et Patterson, 1990 in general appearance, but *N. urceolatus* has 3-4 dorsal ridges and three ventral ridges. This species resembles *N. hemicircularis* Lee et Patterson, 2000 (which has a length of 9-10 µm) in general outline, but the latter species can be distinguished because it is smaller, has a semicircular collar around the short anterior neck, and has five dorsal and three ventral ridges. It resembles *N. canellatus* Skuja, 1948 in general shape, but *N. canellatus* can be distinguished because it has three dorsal grooves and a shallow median groove. *Notosolenus hamelini* is similar to *N. pyriforme* Lee et Patterson, 2000 in general appearance, but the latter species can be distinguished because it has a groove and a ridge on ventral side and has a dorsal ridge. This species resembles *N. rhombicus* Larsen, 1987 (which has a length of 8-12 µm), but the latter species can be distinguished because it is smaller, has a tiny horse-shoe-shaped collar and has a smooth pellicle. It resembles *N. chelonides* Skuja, 1939 (which has a length of 27-35 µm) and *N. intermedius* Christen, 1962 (which has a length of 37-39 µm) in general outline, but the two latter species can be distinguished because they are larger and they have several keels. *Notosolenus hamelini* is similar to *Petalomonas cantuscygni* Cann et Pennick, 1986 in general appearance, but the latter species can be distinguished because it has no second flagellum, a ventral groove and six dorsal keels.

Although it may be unusual to describe a species on the basis of two cells, in this case it was considered reasonable due to the distinctiveness of the cells and because of their similarity to each other.

***Notosolenus ostium* Larsen et Patterson, 1990 (Figs 6g, 7g)**

Description: The single cell observed was elongately ovate, 23 µm long, 12 µm wide, flattened with a fine apical collar and a small indentation in the posterior end. The ventral surface had a wide, shallow longitudinal groove. There was a deep but narrow median dorsal groove. Two flagella emerged subapically from a collar. The anterior flagellum was about 1.5 times the cell length; the posterior flagellum was about 0.4 times the cell length. The flagellar pocket was situated in the right side of the cell and the nucleus was in the left side. The cell had a fine, obliquely-oriented ingestion organelle situated under the collar. The cell moved by gliding. This species was observed in a sediment sample.

Remarks: This species has been reported from marine sites worldwide, with a size range of 23-56 µm (Lee and Patterson 1998, 2000). The combination of relatively large size and single conspicuous dorsal groove is distinctive, but this species resembles *Petalomonas mediocanellata* Stein, 1878 in cell length and having grooves. *Petalomonas mediocanellata* has a short second flagellum in the ventral groove by Schroeckh *et al.* (unpublished observations) and Al-Qassab (unpublished observation), but differs from *N. ostium* in its smaller size, shape, and in not having a well-developed dorsal groove.

***Notosolenus scutulum* Larsen et Patterson, 1990 (Figs 6d, 7h, i)**

Description: Cells are 9-14 µm long, 7-8 µm wide (n = 5), rhomboid in outline, flattened, and widest below the middle. The anterior end is narrowed and the posterior end is rounded or slightly narrowed. There is a narrow, deep, median, longitudinal dorsal groove, which become wider towards the posterior. There is a similar, but shallower ventral groove. The two flagella emerge subapically. The anterior flagellum is about 2 times the cell length. The recurrent flagellum is 0.4-0.5 times the cell length. The reservoir lies in the right side of the cell and the nucleus is in the left side. The cells move by gliding. This species was observed in sediment samples.

Remarks: This species has been found in marine sites in Australia and Brazil, with cell lengths of 9-15 µm (Larsen and Patterson 1990, Lee and Patterson 1998, 2000). This taxon resembles *Notosolenus lashue* Lee et Patterson, 2000 and *N. pyriforme* Lee et Patterson, 2000 in the number and appearance of the grooves.

However, in *N. lashue* both ventral and dorsal grooves lie close to the cell margins, while *Notosolenus pyriforme* has lateral hyaline flanges, a conspicuous collar, and a fine ventral ridge.

***Notosolenus similis* Skuja, 1939 (Figs 6e, 7j, k)**

Description: Cells are 7-12 µm long, 5-8 µm wide (n = 9), oblong to ovate, flattened, narrowed anteriorly to form a blunt point, and rounded posteriorly. The curved longitudinal groove runs along the left dorsal margin of the cell. The ventral surface may have a median shallow longitudinal groove. The two flagella emerge subapically. The anterior flagellum is 1-2 times the cell length and the posterior flagellum is 0.4-0.6 times the cell length. The flagellar pocket is situated in the right side of the cell and the nucleus is in the left side. Usually the cell is filled with granules of various sizes. The cells move by gliding. This species was observed in sediment and mat samples.

Remarks: This species was originally reported from freshwater sites (Skuja 1939) and has been found in marine sites in subtropical and tropical Australia and Brazil, with lengths of 10-22 µm (Lee and Patterson 1998, 2000). Our observations extend this size range slightly. *Notosolenus similis* is similar to *N. stenoschismus* Skuja, 1939 in general appearance, but *N. stenoschismus* has longitudinal ridges that are difficult to observe and a deep ventral groove. This species resembles *N. apocamptus* Stokes, 1884, but the dorsal groove of *N. apocamptus* is narrowed and located in the middle of the cell, not to the left side. *Notosolenus lashue* Lee et Patterson, 2000 can be distinguished from *N. similis* in having a deep ventral groove, a median ventral ridge and a straight dorsal groove. *Notosolenus similis* resembles *Petalomonas involuta* Skuja, 1939 in having a dorsal groove, but *N. similis* has a recurrent flagellum, *P. involuta* does not.

***Notosolenus urceolatus* Larsen et Patterson, 1990 (Fig. 6f)**

Description: Cells are 14-16 µm long, 7-8 µm wide (n = 2), rhomboid to ovate, and flattened. The canal opening is surrounded by a small horse-shoe-shaped collar opening obliquely towards the left side of the cell. There are three dorsal ridges, three ventral ridges and a ventral groove. The two flagella emerge subapically. The anterior flagellum is 1.5-2 times the cell length and the posterior flagellum is 0.7-1 times the cell length. The flagellar pocket located on the right side of the cell and the nucleus on the left side. The cells move by smooth gliding. This species was observed in sediment samples.

Remarks: This taxon has been reported from marine sites in subtropical and tropical Australia and Brazil, with a size range of 11-22 µm (Lee and Patterson 1998, 2000). Lee and Patterson observed two different shapes of this species; urceolate cells which are broader and have a slightly more developed neck than ovate cells. The organisms observed here agree with the urceolate form. Larsen (1987) erected *Notosolenus esulcis*, which resembles *N. urceolatus,* but can be distinguished because the pellicle has fine distant longitudinal striations only on the dorsal surface. We are of the view that there is considerable similarity between these two species. *Notosolenus chelonides*, Skuja 1939 (which has a length of 27-35 µm) can be distinguished from *N. urceolatus* because it is larger in size and has several dorsal keels. *Notosolenus urceolatus* resembles *N. hemicircularis* Lee et Patterson, 2000 in general outline, but the latter species can be distinguished because it has a semicircular hyaline collar around the anterior end of the cell.

***Petalomonas abscissa* (Dujardin, 1841) Stein, 1859 (Figs 6h, 7l, m)**

Description: Cells are 9.5-19 µm long, 7.5-14 µm wide (n = 10), flattened, slightly narrow anteriorly with the flagellar opening surrounded by a fine collar, broadly rounded or with an oblique concave margin posteriorly, and with lateral hyaline flanges. There are two strongly developed longitudinal dorsal ridges. A narrow median groove with margins strengthened by ridges runs obliquely on the ventral surface from the flagellar opening, then sharply bends to run longitudinally. One flagellum emerges subapically and is 1-1.5 times the length of the cell. The reservoir is situated the right side of the cell and the nucleus is in the left side. Some cells are filled with granules of various sizes. The cells move by smooth gliding. This species was observed in sediment samples.

Remarks: This species was described from marine sites worldwide, with the range of the lengths of 10-25 µm (Lee and Patterson 1998, 2000). Our observations agree with those of previous observers. *Petalomonas abscissa* resembles *P. raiula* Larsen et Patterson, 1990; *P. spinifera* (Lackey, 1962) Lee et Patterson, 2000 and *P. mira* var. *bicarinata* Skuja, 1939 in possessing a double ventral keel, but *P. abscissa* can be distinguished from all because these species have more strongly developed protrusions. This taxon resembles

P. ventritracta Skuja, 1939 in having a ventral double keel, but the latter species lacks dorsal keels.

***Petalomonas minor* Larsen et Patterson, 1990 (Figs 6k-m, 7n-r, 8a, b)**

Description: Cells are 6-13 µm long, rhomboid to triangular, flattened but with a longitudinal dorsal keel. The nucleus is located on the left side of the cell. The single emergent flagellum is about cell length. The cells move by gliding. We have observed three forms, but we do not feel confident that they can be consistently distinguished as to be treated as separate species.

The first form (Figs 6k, 7n, o) is ovate-rhomboid, 5.5-8 µm long, 3-5 µm wide (n = 6). The dorsal side is concave and the keel lies to the right of the midline of the cell. The flagellar pocket is located in the right side of the cell. This species was observed in sediment and mat samples.

The second form (Figs 6m, 7p-r) is rhomboid, is larger than the others at 10-12 µm long and 5-6 µm wide (n = 5), and is slightly flattened. The anterior and posterior ends are narrowed. The ventral surface has two fine ridges; the dorsal keel is less pronounced than in other forms. Sometimes the left lateral surface of the cell is depressed. The flagellar pocket is large and medially located. This species was observed in sediment samples.

The third form (Figs 6l, 8a, b) has a more triangular shape, is 6-7 µm long and 3-5 µm wide (n = 4), and is slightly flattened. The right half of the cell is slightly thicker and longer than the left half. The posterior end of the cell is truncated or pointed. The flagellar pocket is located in the right hand side of the cell. The dorsal keel lies to the right of the midline. The left margin of the cell is slightly thickened. This species was observed in sediments and mat samples.

Remarks: This species has been reported from marine sites in tropical and subtropical Australia and Fiji, with lengths from 6 to 11 µm (Lee and Patterson 1998, 2000). We treat the different forms we observed as infraspecific variants because we suspect that shape of the cells and the visibility of grooves and ridges may depend on the environmental factors such as the osmotic pressure of the water and the amount and nature of the ingested food. *Petalomonas minor* is similar to *P. poosilla* Larsen et Patterson, 1990 in general appearance and size (*P. poosilla* is 5-12 µm long), but is distinguished by the cell shape and by having a prominent dorsal ridge. This species resembles *P. minuta* Hollande, 1942 in general outline, but the latter species has a dorsal groove. It is similar to *P. iugosus* Lee et Patterson, 2000 in general shape, but the latter species has three dorsal ridges.

***Petalomonas minuta* Hollande, 1942 (Figs 6i, 8c, d)**

Description: Cells are 5-10 µm long, 3-6 µm wide (n = 8), ovate in outline and flattened. There is a deep, median, longitudinal dorsal groove. Some cells have one or two ventral fine ridges. One flagellum emerges subapically and is 0.8-1.8 times the cell length. The cells move by gliding. This species was observed in sediment and mat samples.

Remarks: Our observations are broadly in agreement with those of Patterson and Simpson (1996). *Petalomonas minuta* has been reported in marine sites worldwide, with the range of lengths of 6-12 µm (Lee and Patterson 1998, 2000). This species resembles *Notosolenus apocamptus* Stokes, 1884 in general outline and size, but can be distinguished because it lacks a posterior flagellum. It is similar to *P. ventritracta* Skuja, 1939 (which has a length of 24-30 µm) and *P. acuminata* Hollande, 1942 (which has a length of 18-27 µm) in general appearance, but the latter two species can be distinguished by their larger size and the possession of a ventral groove. We are unable to distinguish *Petalomonas dilatata* Hollande, 1942 (length about 15 µm) and *P. regularis* Christen, 1958 (length 17-22 µm) from *P. acuminata* Hollande, 1942 because all have a similar shape, size and ventral groove. We therefore regard these species as being the same. *Petalomonas minuta* resembles *P. poosilla* Larsen et Patterson, 1990 and *P. minor* Larsen et Patterson, 1990; but the latter two species have no dorsal groove.

***Petalomonas poosilla* Larsen et Patterson, 1990 (Figs 6j, 8e-g)**

Description: Cells are 5-9.5 µm long, 3-5 µm wide (n = 17), ovate in outline and flattened. The dorsal surface may or may not have up to three fine ridges. The ventral surface may or may not have up to two distinct ridges. The flagellum is 0.7-1.6 times the cell length and emerges subapically. The flagellar pocket lies on the right side of the cell and the nucleus in the left side. The cells glide. This species was observed in sediment and mat samples.

Remarks: This species has been widely reported from marine sites worldwide, with lengths of 4-12 µm

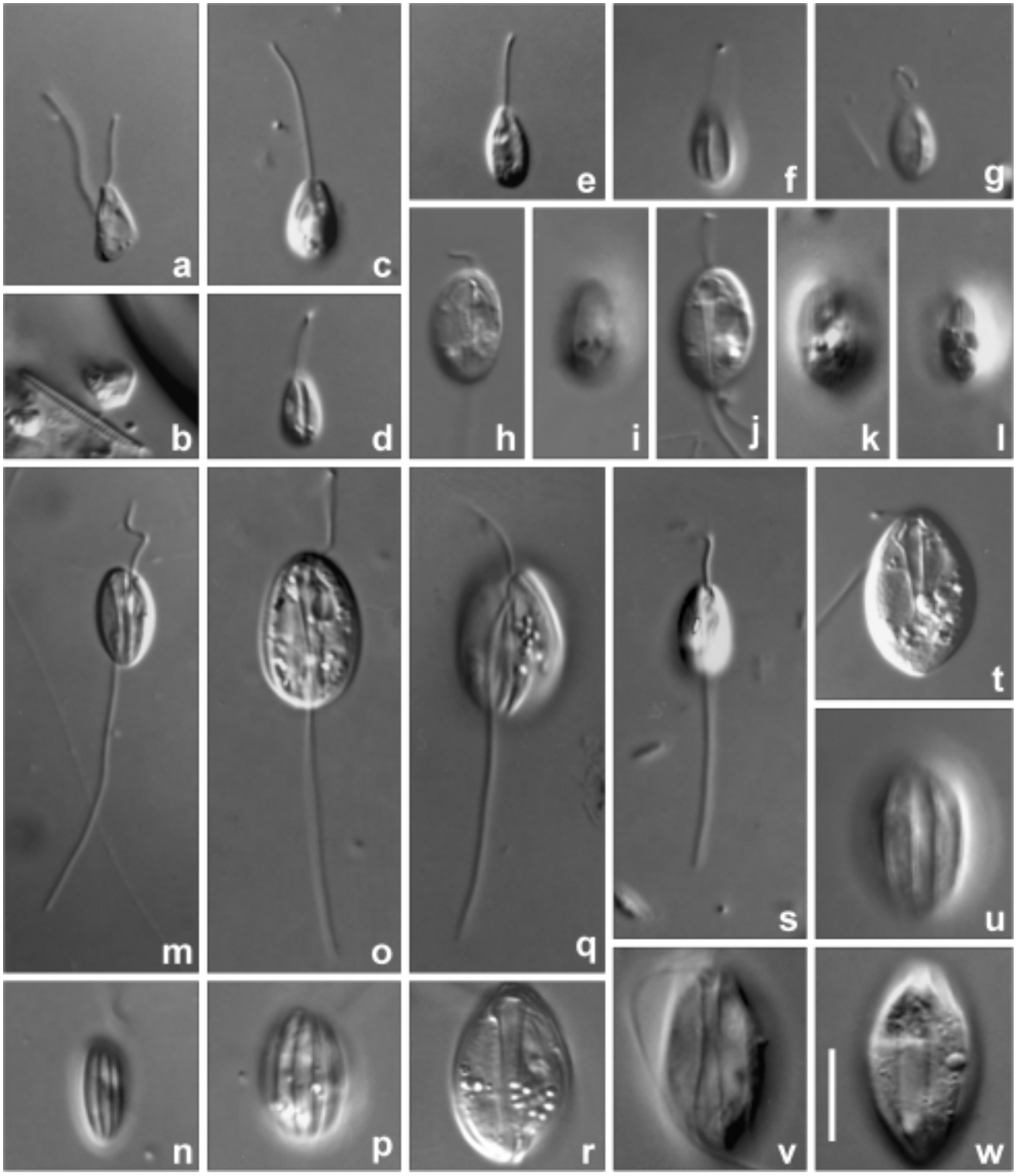

Fig. 8. (**a, b**) *Petalomonas minor*, third form, (**a**) general appearance, (**b**) transverse section, (**c, d**) *Petalomonas minuta,* (**c**) general appearance, (**d**) dorsal view showing a groove, (**e-g**) *Petalomonas poosilla*, (**e**) general appearance, (**f**) dorsal view showing three ridges, (**g**) dorsal view showing one ridge, (**h-k**) *Ploeotia punctata*, (**l, s**) *Ploeotia* cfr *punctata*, (**l**) dorsal view showing grooves, (**s**) general appearance, (**m, n**) *Ploeotia azurina*, (**m**) general appearance, (**n**) dorsal view showing ridges, (**o, p**) *Ploeotia corrugata*, (**o**) general appearance, (**p**) dorsal view showing ridges, (**q, r**) *Ploeotia costata*, (**q**) general appearance, (**r**) ventral view, (**t-w**) *Ploeotia vitrea*, (**t**) general appearance, (**u**) dorsal view showing ridged grooves of same cell, (**v**) ventral view of different cell, (**w**) dorsal view showing ridged grooves. All micrographs are DIC images. Scale bar - 10 μm

(Lee and Patterson 1998, 2000). Lee and Patterson (2000) described two forms of *P. poosilla*: small cells (5-9 μm) and large cells (10-12 μm) differing in shape, size and visibility of ridges. Our observations are in close agreement with the small form. The presence or visibility of ridges in cells might depend on environmental conditions such as osmotic pressure or amount of food. *Petalomonas poosilla* resembles *P. minor* Larsen et Patterson, 1990 in general appearance, but can be distinguished by the ovate-shape and because *P. minor* has a prominent dorsal ridge. This species is similar to *P. acuminata* Hollande, 1942 (length 18-25 μm) in general appearance, but is smaller and lacks a ventral groove.

***Ploeotia azurina* Patterson et Simpson, 1996 (Figs 6n, 8m, n)**

Description: Cells are 10-17 μm long, 5-10 μm wide (n = 7), elongate, slightly obovate and slightly flattened. The dorsal side is convex with seven prominent (corrugate) longitudinal ridges. Usually only six ridges can be observed in dorsal view. Two ridges form the margin. The ventral side is slightly flattened with one or several ridges. Two flagella emerge subapically. The anterior flagellum is the cell length and the posterior flagellum is 2-3.5 times the cell length. Small individuals (about 10 μm) were flatter and had longer posterior flagella than larger cells (13-17 μm), and had only a single visible ventral ridge. The ingestion device is well developed, has two rods, and extends slightly obliquely along the cell length. The flagellar pocket is located on the left side of the cell and the nucleus on the right side. The cells move by gliding. This species was observed in sediment samples.

Remarks: This species has only been reported in Australia (Patterson and Simpson 1996) with cell length ranges of 10-16 μm. Patterson and Simpson (1996) suggested that the cell described under the name of *P. punctata* by Ekebom *et al*. (1996) and the cells described under the name of *P. punctata* may be *P. azurina*. Patterson and Simpson (1996) observed the small and large forms in this species, and intermediates. Our observations agree well with theirs and encompass both forms. This species resembles *P. corrugata* Larsen et Patterson, 1990 in general appearance, but *P. corrugata* is broader, with a flattened ventral side, a shorter recurrent flagellum and a posterior indentation. *Ploeotia azurina* is similar to *P. decipiens* Larsen et Patterson, 1990, but *P. decipiens* is broader with thinner and widely separated dorsal ridges.

***Ploeotia corrugata* Larsen et Patterson, 1990 (Figs 6o, 8o, p)**

Description: Cells are 7-16 μm long, 5.5-11 μm wide (n = 9), elliptical in outline but often with a small posterior indentation, ventrally flattened and dorsally convex. The right half of the cell is slightly thicker than the left. There are seven longitudinal dorsal ridges, two of them forming marginal rims. A fine ventral longitudinal ridge lies to the left of centre. Two flagella emerge subapically. The anterior flagellum is about the cell length and the recurrent flagellum is slightly thicker and about 2 times the cell length. The ingestion device is well developed with two rods extending obliquely along the cell length. The flagellar pocket lies on the left side of the cell and the nucleus is on the right side. The cells move by gliding. This species was observed in sediment and mat samples.

Remarks: This species has been reported in marine sites worldwide, with lengths of 7-20 μm (see Lee and Patterson 1998, 2000). Our observations are in good agreement with previous observers. *Ploeotia corrugata* resembles *P. azurina* Patterson et Simpson, 1996, but can be distinguished (Remarks of *P. azurina* above). This species resembles *Ploeotia decipiens* Larsen et Patterson, 1990; but *P. decipiens* has dorsal ridges, which are not corrugated and it lacks a posterior indentation.

***Ploeotia costata* (Triemer, 1986) Farmer et Triemer, 1988 (Figs 6p, 8q, r)**

Description: Cells are 16-19 μm long, 10-11.5 μm wide (n = 5), oval or ovate, ventrally flattened and dorsally slightly convex. The cells have five longitudinal (slightly twisted) grooves: two dorsal, two lateral and one ventral. These grooves are strengthened along their edges. The two flagella emerge subapically. The anterior flagellum is shorter than the cell length and the posterior flagellum is 2-2.5 times the cell length. The ingestion apparatus has two well-developed rods. The right rod has a small protrusion. The flagellar pocket lies in the left side of the cell and the nucleus is in the right side. The cells move by gliding. This species was observed in sediment samples.

Remarks: This species has been reported from marine sites in subtropical and tropical Australia, Brazil, Hawaii and USA, with lengths of 10-25 μm (Lee and Patterson 1998, 2000). Our observations correspond well with those of previous observers. *Ploeotia costata* closely resembles *Ploeotia oblonga* Larsen et Patterson, 1990; the latter can be distinguished only by its oblong cell shape. Further work is required to establish whether

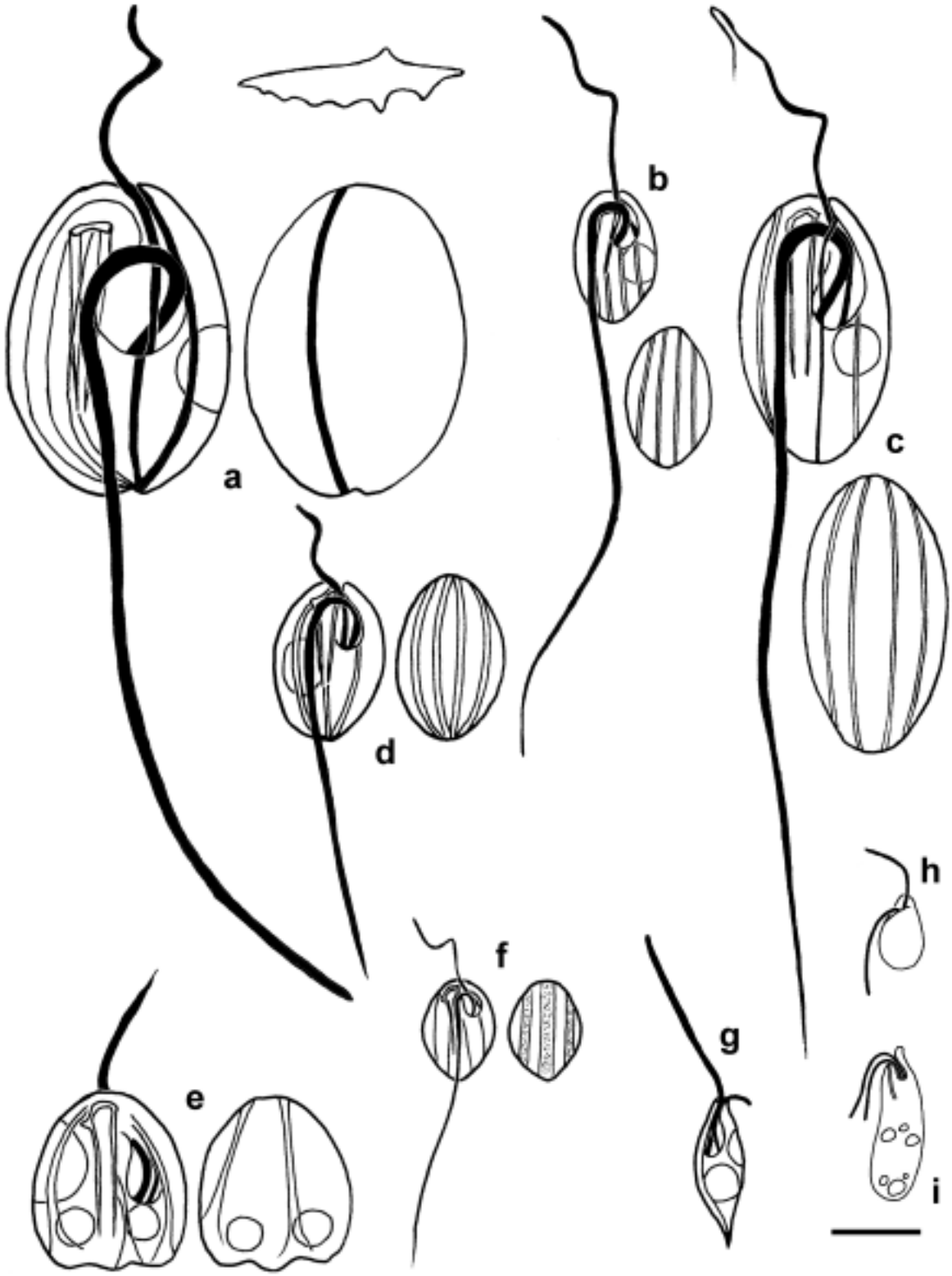

Fig. 9. (**a**) *Ploeotia laminae* sp. n. , general appearance, (**b**) *Ploeotia pseudanisonema* (**c**) *Ploeotia robusta*, (**d**) *Ploeotia vitrea*, (**e**) *Ploeotia* sp.1, (**f**) *Ploeotia* sp.2, (**g**) *Sphenomonas* sp., (**h**) *Bordnamonas tropicana*, (**i**) *Rhynchopus amitus*. Scale bar - 10 μm

these forms can be consistently distinguished. This species is similar to *P. vitrea* Dujardin, 1841 but the latter has ten separate ridge/groove structures.

***Ploeotia laminae* sp. n. (Figs 9a, 10a-c)**

Diagnosis: *Ploeotia*, 22-40 μm long, flattened, ventral side with six ridges, dorsal side with one prominent ridge.

Description: Cells are 22-40 μm long, 15-20 μm wide (n = 9), eliptical in outline and flattened. The cell has lateral hyaline flanges and a small indentation of the posterior ventral margin. The ventral surface is slightly convex and has six ridges, two of which are more prominent. The dorsal surface has a prominent ridge situated in the left side of the cell. Two flagella, which are unequal in length, emerge subapically. The anterior flagellum is about the cell length. The recurrent flagellum is thicker and hooked at its base, and is about 3 times the cell length. The ingestion organelle is well developed with two strong rods extending the length of the cell. The reservoir and the nucleus are situated in the left hand side of the cell. The cells are often filled with granules of various sizes. The cells move by gliding. This species was found in sediment samples.

Remarks: We assigned this species to *Ploeotia* because it is rigid, it has an ingestion organelle that is not protrusible, the two flagella are of unequal length and the reservoir is on the left side. The assignment of this species to *Ploeotia* is tentative pending a review of the status of the genus *Anisonema* (see comments to *Anisonema* cfr *acinus* above). *Ploeotia laminae* can be distinguished from *Ploeotia adhaerens* Larsen et Patterson, 1990; because the latter has blunt protrusions at the anterior end, has four prominent ridges on both dorsal and ventral sides, has no hyaline margin and has an unusual behaviour in which it clamps to the substrate. *Ploeotia laminae* can be distinguished from *P. decipiens* Larsen et Patterson, 1990; *P. corrugata* Larsen et Patterson, 1990 and *P. azurina* Patterson et Simpson, 1996 because all have a greater number of prominent dorsal ridges. *Ploeotia punctata* Larsen et Patterson, 1990 can be distinguished because it has three dorsal ridges and two fine ventral ridges. *Ploeotia laminae* can be distinguished from *P. heracleum* Larsen et Patterson, 1990 because the latter species is smaller and has four dorsal ridges and four ventral grooves. *Ploeotia laminae* is similar to *P. robusta* Larsen et Patterson, 1990 in general appearance but the latter species has delicate grooves rather than ridges. We are therefore unable to identify this species as any other species in the genus *Ploeotia*, and assign it to a new species. Similar organisms currently referred to *Anisonema* can be distinguished from *P. laminae* because they have delicate grooves/ridges arranged more-or-less evenly around the cell and lack hyaline margins.

***Ploeotia pseudanisonema* Larsen et Patterson, 1990 (Figs 9b, 10f-i)**

Description: Cells are oblong, 12-20 μm long, 6-12 μm wide, (n = 6) and flattened. The cells have dorsally 3-4 longitudinal grooves and ventrally 4-5 longitudinal grooves extending the entire length of the cell. Two flagella of unequal length emerge subapically. The anterior flagellum is 1-1.8 times the cell length, and the recurrent flagellum is thicker and lies in a ventral groove, and is 4-5 times the cell length. The ingestion organelle has two well-developed rods running along the length of the cell. The reservoir is situated in the left side of the cell and the nucleus is in the right side. The cells move rapidly by gliding, interrupted by swift jerks-like *Anisonema*. This species was found in both sediment and mat samples.

Remarks: This species has been reported from marine sites with a cell length of 10-20 μm (Lee and Patterson 1998, 2000). The cells observed here are slightly different in shape from the original description (Larsen and Patterson 1990), having a more oblong shape. Generally, our observations are consistent with those of Patterson and Simpson (1996). The cells observed here and by Patterson and Simpson (1996) are similar to *P. robusta sensu* Patterson et Simpson, 1996 (which is 28-32 μm long), but can be distinguished by their smaller size. The cells are similar to *P. longifilum* Larsen et Patterson, 1990 in general appearance, size and the possesion of a long recurrent flagellum, but *P. longifilum* can be recognised by its greater width and by one ventral groove.

Ploeotia pseudanisonema sensu Larsen et Patterson, 1990 resembles *P. punctata* Larsen et Patterson, 1990 in general outline, but it can be distinguished by its greater number of grooves, and the greater thickness of the anterior part of the recurrent flagellum. This species can be confused with *Anisonema trepidum* Larsen et Patterson, 1990, but can be distinguished by the presence of an ingestion organelle. It can be distinguished from *Anisonema obliquum* (Roskin 1931) by the presence of an ingestion organelle. As discussed above under *Anisonema* cfr *acinus*, the presence/absence of a mouth in *Anisonema* may need to be re-evaluated.

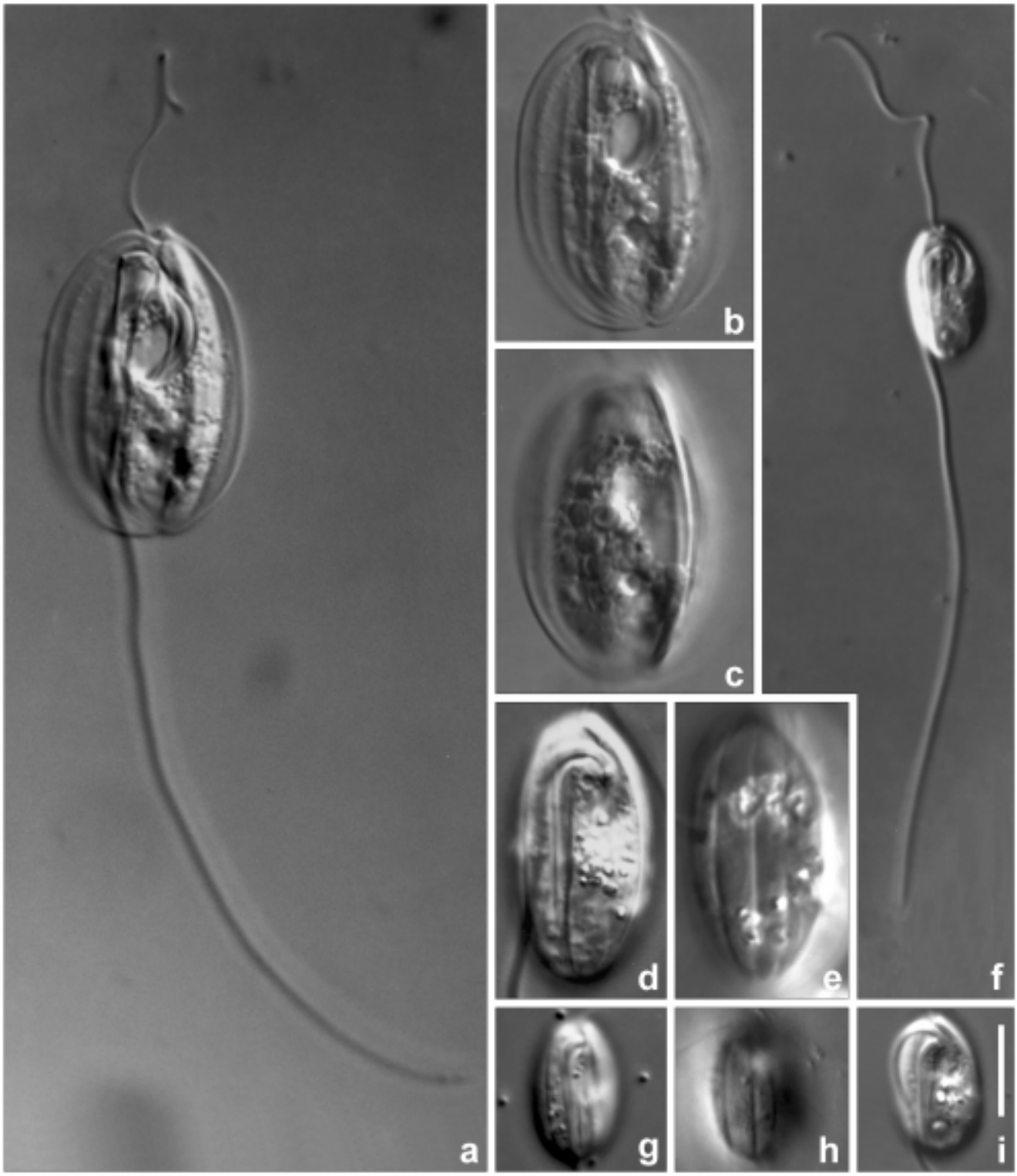

Fig. 10. (**a-c**) *Ploeotia laminae* sp. n. (**a**) general appearance, (**b**) ventral view, (**c**) dorsal view showing a ridge, (**d, e**) *Ploeotia robusta*, (**d**) ventral view of large form, (**e**) dorsal view showing grooves, (**f-i**) *Ploeotia pseudanisonema*, (**f**) general appearance, (**g**) ventral view, (**h**) dorsal view, (**i**) ventral view showing a discontinuity. All micrographs are DIC images. Scale bar - 10 μm

***Ploeotia punctata* Larsen et Patterson, 1990 (Figs 6q-s, 8h-l, s)**

Description: The cells are elliptical to ovate, 12-13 µm long, 8 µm wide (n = 2) and slightly flattened. The anterior end is more rounded than the posterior end. The dorsal surface may have three longitudinal ridges (Fig. 8i) or three longitudinal ridged grooves (Fig. 8k). The ventral surface has two longitudinal ridges. Small refractile bodies lie along these ridges. Two flagella of unequal length emerge subapically. The anterior flagellum is the same as the cell length and the recurrent flagellum is 1.6-2.5 times the cell length. The ingestion organelle is wide anteriorly and has two rods, which run along the entire length of the cell. The reservoir lies to the left and extends to the middle of the cell. The nucleus is situated on the right side of the cell. The cells move by gliding and may jerk backwards, like *Anisonema*. This species was found in sediment samples.

Remarks: This species has been reported in tropical and subtropical Australia and Hawaii with cell lengths of 7-16 µm (Larsen and Patterson 1990, Ekebom *et al*. 1996, Lee and Patterson 1998). The cell with three dorsal longitudinal ridges is consistent with the original account of Larsen and Patterson (1990). Ekebom *et al.* (1996) described *P. punctata* with 3 dorsal longitudinal grooves and one to several delicate ventral grooves. We believe that these authors may have regarded the dorsal ridges as grooves. We believe that these two forms are different, but as we do not believe they can be reliably and consistently distinguished, we prefer to treat them as probably assignable to the same species. We also observed three cells (Figs 8l, s) which are consistent with *P.* efr *punctata* described by Patterson and Simpson (1996).

Ploeotia punctata resembles *P. pseudanisonema* Larsen et Patterson, 1990 in general shape, but the latter species is distinguished because it has a very long recurrent flagellum, more grooves and no punctae. This species resembles *P. heracleum* Larsen et Patterson, 1990 in general appearance, but *P. heracleum* has four grooves and four ridges, and lacks punctae.

***Ploeotia robusta* Larsen et Patterson, 1990 (Figs 9c, 10d, e)**

Description: Cells are obovate, 19-30 µm long, 7-15 µm wide and flattened, (n = 4) and have a single ventral discontinuity arising from the ventral opening of the reservoir at the anterior end. The ventral surface has 3-4 ridges and the dorsal surface has 4-5 ridges. Two flagella, unequal in length, emerge from under a lip-like flange. The anterior flagellum is 1.2-1.6 times the cell length, and the recurrent flagellum is thicker and is 3.5-5 times the cell length. The ingestion organelle with two well-developed rods extends the length of the cell. The reservoir and the nucleus are located on the left side of the cell. The cells are filled with food vacuoles and refractile granules of various sizes. The cells move by gliding and capable of *Anisonema* like reversals. This species was found in sediment samples.

Remarks: This species has been observed from marine sites in Western Australia, Hawaii and UK (Lee and Patterson 1998). The range of the lengths was previously reported to be 28-40 µm (Larsen and Patterson 1990, Patterson and Simpson 1996). We assigned the cells observed here to *Ploeotia robusta*, although there are some differences such as cell outline and the number of grooves from the original observation of *P. robusta* by Larsen and Patterson (Larsen and Patterson 1990). *Ploeotia robusta* sensu Larsen and Patterson 1990 has an oblique and acute posterior end, the right hand side of the cell is thinner than the left side and the cell has only one ventral groove. We believe that other 2-3 ventral grooves may have been overlooked. Our observations are in good agreement with *Ploeotia robusta* sensu Patterson and Simpson 1996. *Ploeotia robusta* is similar to *Anisonema acinus* Dujardin, 1841 in cell shape, size and the pattern of movement, but can be distinguished because the grooving patterns are different. This species resembles *P. pseudoanisonema* Larsen et Patterson, 1990 (which has a length of 10-20 µm) in general outline, but can be distinguished because it is larger. The reversal behaviour in the genera *Ploeotia* and *Anisonema* suggests that they are similar (see discussion under *Anisonema* cfr *acinus*).

***Ploeotia vitrea* Dujardin, 1841 (Figs 8t-w, 9d)**

Description: Cells are ovate to broadly ellipsoid, 13-24 µm long, 7-12 µm wide (n = 11) and not flattened. The cells have 10 longitudinal double raised ridges, four dorsally, two laterally and four ventrally. The two flagella emerge subapically. The anterior flagellum is about the length of the cell. The posterior flagellum is thicker and about 2 times the cell length. The ingestion apparatus has two well-developed rods, extends the length of the cell and has a small lateral protrusion at the top of the right hand rod. The flagellar pocket is located on the left hand

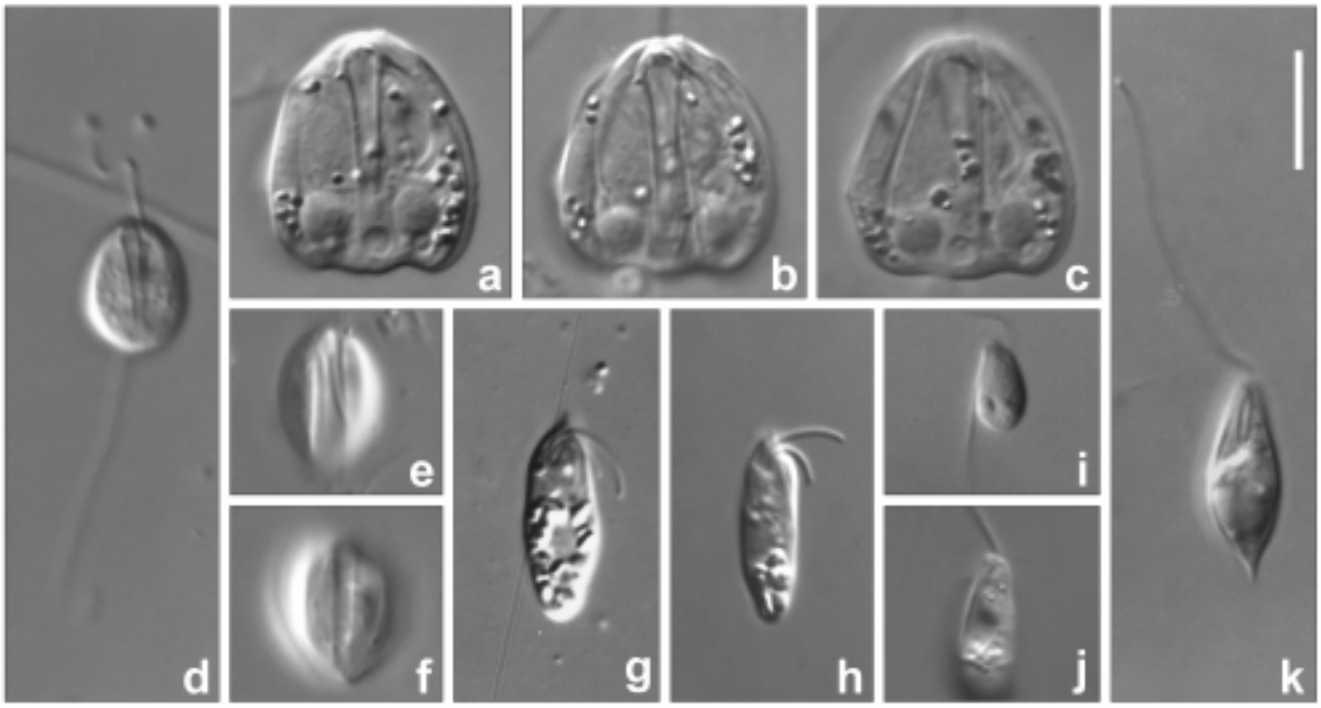

Fig. 11. (**a-c**) *Ploeotia* sp.1, (**a**) general appearance, (**b**) ventral view, (**c**) dorsal view showing grooves, (**d-f**) *Ploeotia* sp.2, (**d**) general appearance, (**e**) ventral view showing a wide groove, (**f**) dorsal view, (**g, h**) *Rhychophus amitus*, (**i**) *Bordnamonas tropicana*, (**j, k**) *Sphenomonas* sp., (**k**) general appearance. All micrographs are DIC images. Scale bar - 10 µm

side of the cell and the nucleus is on the right side. The cells move by gliding with the posterior flagellum. This species was observed in sediment samples.

Remarks: This species has been found from marine sites worldwide, with a cell length of 16-25 µm (Lee and Patterson 1998, 2000). The presence of 10 more or less equally spaced, very prominent, doubled ridges is distinctive

Euglenozoa *incertae sedis*

***Bordnamonas tropicana* Larsen et Patterson, 1990 (Figs 9h, 11i)**

Description: Cells are 5-11 µm long (n = 64), flattened, anteriorly narrow and posteriorly broad, and with an apical mouth. Two slightly thickened, non-acronematic flagella insert subapically in the right ventral side of the cell and are about 1.5 times the cell length. The anterior flagellum is held in a sigmoid arc and beats stiffly. The posterior flagellum is directed towards the rear of the cell. Cells move by gliding or rapid skidding movements close to the substrate. This species was often observed in both sediment and mat samples.

Remarks: This species has been reported from marine sites worldwide, with cell lengths of 4-20 µm (Lee and Patterson 1998, 2000). Simpson (1997) presents arguments for inclusion in Euglenozoa. *Bordnamonas tropicana* may be confused with *Bodo curvifilus* Griessmann, 1913 and *Bodo caudatus* (Dujardin, 1841) Stein, 1878 in that the anterior flagellum is held anteriorly in a curve, but is distinguished by its pattern of swimming in arcs and its non-acronematic posterior flagellum.

***Rhynchopus amitus* Skuja, 1948 (Figs 9i, 11g, h)**

Description: Cells are 11-18 µm long (n = 12), sac shaped and metabolic. There is a fine apical papilla with a slightly thickened margin. A conspicuous flagellar pocket, about 2 µm deep opens immediately below the papilla and is directed anterio-laterally. Most cells have very short flagella that do not emerge from the pocket, but a few cells had two thickened flagella, 0.5-1 times the length of the cell. These flagella curve posteriorly and are usually inactive; movement is by gliding. The posterior half of the cell often contains large food

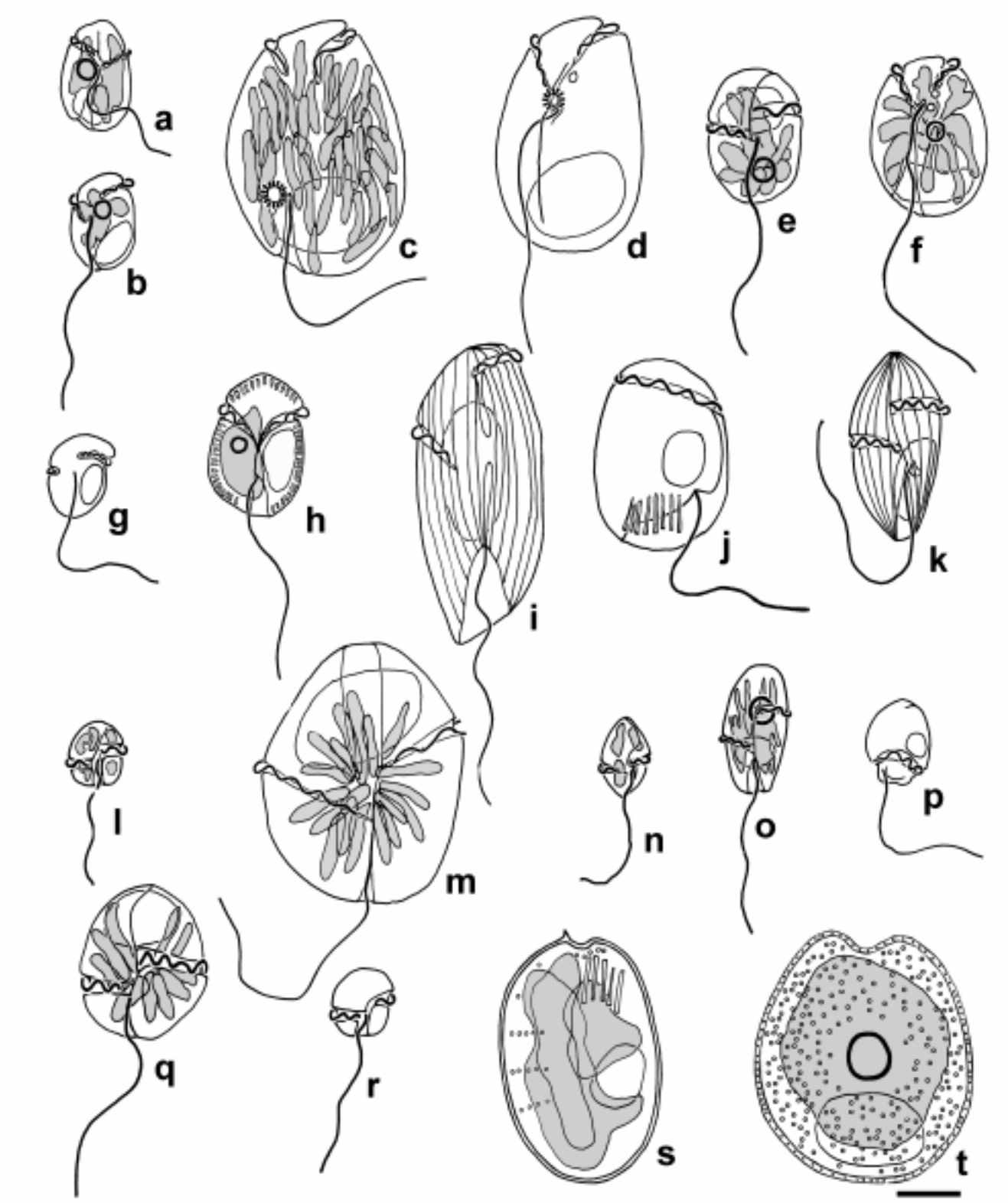

Fig. 12. (**a**) *Amphidiniella sedentaria*, (**b**) *Amphidinium carterae*, (**c**) *Amphidinium elegans*, (**d**) *Amphidinium* cfr *incoloratum*, (**e**) *Amphidinium mammillatum*, (**f**) *Amphidinium* cfr *operculatum*, (**g**) *Amphidinium psittacus*, (**h**) *Amphidinium salinum*, (**i**) *Amphidinium scissum*, (**j**) *Amphidinium semilunatum*, (**k**) *Gyrodinium dominans*, (**l**) *Gyrodinium* cfr *estuariale*, (**m**) *Gyrodinium pavillardi*, (**n**) *Gyrodinium mundulum*, (**o**) *Gyrodinium oblongum*, (**p**) *Katodinium asymmetricum*, (**q**) *Katodinium dorsalisulcum*, (**r**) *Katodinium fungiforme*, (**s**) *Prorocentrum mexicanum*, (**t**) *Prorocentrum* cfr *concavum*. Scale bar - 10 µm

vacuoles. Occasionally observed in both sediment and mat samples.

Remarks: This species was described from freshwater (Skuja 1948). Schnepf (1994) described *Rhynchopus conscinodiscivorus* from a marine habitat in Northern Europe, distinguishing it from *R. amitus* solely by the absence of a contractile vacuole. We believe that this feature, in isolation, is inappropriate to distinguish flagellate taxa (Larsen and Patterson 1990) and regard *R. coscinodiscivorus* as a junior synonym of *R. amitus*. Our observations generally agree with those of both Skuja (1948) and Schnepf (1994), although we observed slightly smaller cells than previous accounts (20-25 µm). We did not observe the feeding apparatus reported by Schnepf (1994), but this may be difficult to discern. *Rhynchopus* may be distinguished from all flagellates except *Diplonema* and *Hemistasia* by the combination of a deep flagellar pocket and fine apical papillum (Bernard *et al.* 2000). *Hemistasia* cells always have long, thickened flagella and a spiral groove. Trophic cells of *Diplonema* have emergent flagella of 'normal' thickness. *Rhynchopus amitus* has a 'dispersal phase' with emergent flagella thickened by paraxonemal rods (Simpson 1997). We believe that the cells we observed with emergent flagella are partially converted dispersal phases of *Rhynchopus,* rather than trophic cells of *Diplonema*, because the flagella are thicker and longer than reported for *Diplonema*.

Entz (1883) described a flexible organism with two short flagella as *Menoidium astasia*. *Menoidium* Perty, 1852 is a taxon of euglenids with a single emergent flagellum and rigid body (Huber-Pestolozzi 1955). The organism observed by Entz (1883) is probably referable to *Diplonema.* Ruinen (1938) also described a flexible organism with a deep flagella pocket under the name *Menoidium astasia*. Ruinen's account includes cells with no flagella, and cells with two long flagella, and is not referable to *Diplonema*, instead being very similar to our observations and previous accounts of *Rhynchopus amitus*. Ruinen's observations are therefore a previous record of this organism from a hypersaline environment (60-180 ‰) in Australia (Marion Bay, South Australia).

Alveolates - Dinoflagellida, Bütschli 1885

***Amphidiniella sedentaria* Horiguchi 1995. (Figs 12a, 13a-c)**

Description: Cells are ellipsoid, dorso-ventrally flattened, 17-19 µm long, 11-12 µm wide, with a dorso-ventral depth of 6-7 µm (n = 2). The species is armoured, with delicate thecal plates. The epicone has an obvious ridge leading from the sulcus to the anterior end. An apical pore is present. The cingulum is an ascending spiral, starting about one third of the way down the cell, extending forward in a curve to with about one tenth cell length away from the apex, then descending on the ventral side and displaced about one cingular width. The sulcus has a prominent right ridge, initially narrow but broadening as it reaches the posterior end. The longitudinal flagellum arises at the anterior end of the sulcus. The nucleus is round, 5 µm diameter, in the posterior of the cell. The plastid appears to be single and lobed, and is yellow-brown. The pyrenoid is central, and 3 µm diameter. Cells were found in mat samples.

Remarks: This monotypic genus (Horiguchi 1995) is the only known small armoured species with a cingulum in the same position as in *Amphidinium* and otherwise like *Amphidinium*. Its plate pattern is distinctive and, along with size and presence of plastids, distinguishes it from the larger, heterotrophic *Amphidiniopsis* Woloszynska, 1928. The plate pattern of the organisms observed here was not established but, in all other aspects recorded, our observations agree well with the original observation. This species has been reported in marine sediment from Japan and South Africa (Horiguchi 1995).

***Amphidinium carterae* Hulburt, 1957 (Figs 12b, 13d, e)**

Description: Cells are ovoid from the ventral side, dorso-ventrally flattened, 10-17 µm long, 7-11 µm wide, with a dorso-ventral breadth of about 6 µm (n = 15). The epicone is crescent-shaped from the ventral side, clearly deflected towards the left, arising from the hypocone to the right of the mid-ventral line. The cingulum is asymmetrical on the ventral side, its proximal end is about half way down the hypocone and the distal end about a third of the way down. The sulcus arises slightly below the left end of the girdle about midway down the hypocone, continuing to the posterior of the cell. No apical groove was observed. The longitudinal flagellum is 20-30 µm long. The nucleus is round and in the posterior part of the hypocone. The plastid is greenish-yellow and appears to be single with multiple lobes, arising from the obvious central pyrenoid (about 3 µm diameter). This species was found in mat and sediment samples.

Remarks: This commonly reported species is in need of further study to establish the relationship between it and *Amphidinium operculatum* Claparède et Lachmann, 1859 (see *Amphidinium operculatum*; Maranda and Shimizu 1996). In this study, the two

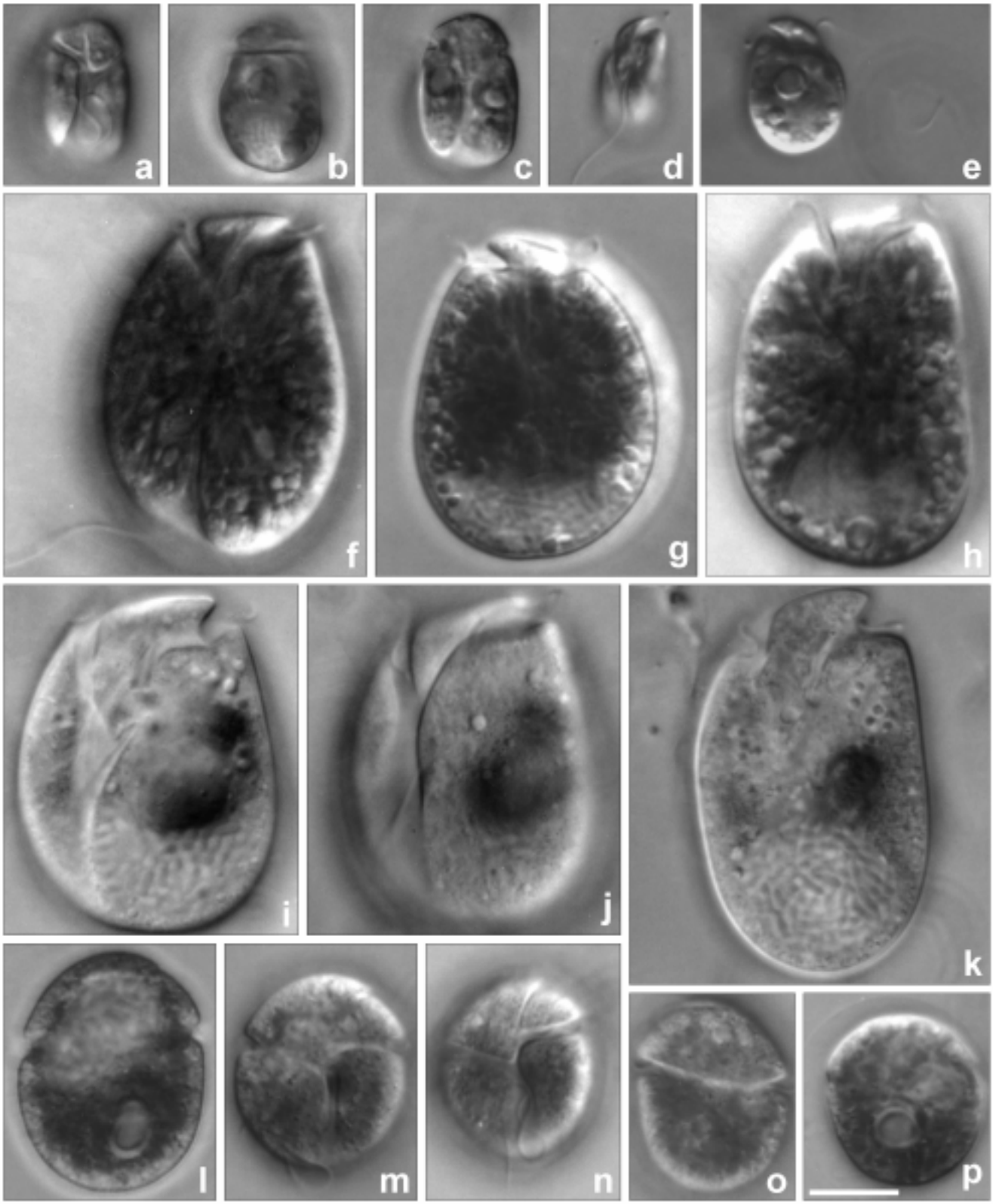

Fig. 13. (**a-c**) *Amphidiniella sedentaria*, (**a**) ventral view, (**b**) dorsal view (**c**) showing plastids, (**d, e**) *Amphidinium carterae,* (**d**) ventral view, (**e**) dorsal view, (**f-h**) *Amphidinium elegans,* (**f**) ventral view, (**g**) dorsal view, (**h**) ventral view, with higher pusule position, (**i-k**) *Amphidinium* cfr *incoloratum,* (**i**) ventral view, (**j**) ventral view showing 'flap' (**k**) ventral view showing pusules, (**l-p**) *Amphidinium mammillatum*, (**l**) ventral view showing nucleus, (**m**) showing flagellar insertion, (**n**) showing apical groove, (**o**) dorsal view showing apical groove, (**p**) non-motile cell. All micrographs are DIC images. Scale bar - 10 μm

species were separated by a discontinuity in size range. *Amphidinium carterae* has been reported in marine sediments and from the water column from England (Dodge 1982), USA (Hulburt 1957), Japan (Ono *et al*. 1999), Portugal (Sampayo 1985), the Society Islands of French Polynesia, New Caledonia, and the Ryukyu Islands (Fukuyo 1981), Belize (Faust 1995), Egypt (Ismael *et al*. 1998) and tropical Australia (Larsen and Patterson 1990).

***Amphidinium elegans* Grell et Wohlfarth-Bottermann, 1957 (Figs 12c, 13f-h)**

Description: Cells are ellipsoidal to ovoid from the ventral side, dorso-ventrally flattened, 33-40 µm long, and 21-28 µm wide (n=7). The epicone is 7-10 µm wide, anteriorly flat and deflected to the left. The right anterior corner forms almost a 90° angle; the left corner is extended into a 30-45° angle. The cingulum is relatively deep. The proximal end is about 0.25 of the way down the cell, extending anteriorly then continuing almost horizontally around the cell, with its distal end slightly higher. The hypocone is rounded and slightly asymmetrical, with the right side more curved than left. The sulcus is relatively deep, beginning to the right of mid-ventral line, either 0.6-0.7 of the way down cell, or about 0.4 of the way down cell. Two pusules are present: one is obvious, about 2 µm diameter, to the right of origin of the sulcus, the second is less obvious, also 2 µm diameter, and just below and to the left of the start of the cingulum. The plastids are in long thin strands, and yellow-brown. The nucleus is crescent-shaped or ellipsoidal, 8-10 µm by 12-18 µm, in the posterior part of the hypocone, and contains very fine chromosomes. Red-orange eyespots are present in some cells, just above the nucleus. Colourless globules are also present. Cells were observed in sediment samples.

Remarks: This species was originally described as a marine organism growing on macroalgae from the USA (Grell and Wohlfarth-Bottermann 1957). In *Amphidinium,* only *A. operculatum* Claparède et Lachmann, 1859 (= *A. klebsi* Kofoid et Swezy, 1921) and *A. herdmani* Kofoid et Swezy, 1921 are of a similar size and shape and possess plastids. When *Amphidinium elegans* was described, only *A. operculatum* (as *A. klebsi*) was compared, and distinguished from *A. elegans* by its anteriorly rounder epicone with a less constricted base (Grell and Wohlfarth-Bottermann 1957). *Amphidinium elegans* was described with a longitudinal flagellum inserted 0.6-0.7 of the way down the cell, an orange eyespot just above the nucleus, and an average size of 39 µm by 22 µm. In this study, *Amphidinium operculatum* could be distinguished from *A. elegans* by its more anterior flagellar insertion, more distinct sulcus, absence of an eyespot, and smaller size. We distinguish *Amphidinium elegans* from *A. herdmani* because *A. herdmani* has a wide, symmetrical, rectangular epicone protruding above the hypocone on the dorsal side, and *A. elegans* has an asymmetrical epicone clearly deflected to the left. *Amphidinium herdmani* has a large rounded hypocone with no distinct indentation at the posterior end. We treat a cell described from tropical Australia and referred to as *A. herdmani* (Larsen and Patterson, 1990) as identical to the species described here. This cell was not identified as *A. elegans* because it did not possess an 'eyespot'. In this study, the possession of red granules, possibly eyespots, was a variable character, and is not an appropriate distinguishing feature.

***Amphidinium* cfr *incoloratum* Campbell, 1973 (Figs 12d, 13i-k)**

Description: Cells are broadly ellipsoidal to ovoid from the ventral side, with a relatively straight left side and a convex right side and are slightly obliquely dorso-ventrally flattened. The lengths range from 35- 42 µm, the width is 22-30 µm (n = 6). The epicone is 5-7 µm at its widest, and deflected to the left. The cingulum and sulcus are asymmetrical, located one third of the cell width from the right side. The cingulum is proximally shallow and becomes relatively deep and wide (about 2 µm), with the distal end about 2 cingulum-widths lower than the proximal. The sulcus begins below and to left of the distal end of the cingulum, and is pronounced, forming a flap, with the left side much more distinct than the right. No apical groove was observed. The longitudinal flagellum arises in a pocket to the left and just below the start of the sulcus. Two pusules are present, one to the right of the anterior end of the sulcus, and another just below the origin of the cingulum. The nucleus is round, 10-15 µm diameter, and in the posterior part of the hypocone. Plastids are not present. The cytoplasm is filled with large, colourless lipid globules and occasional food particles. This species was found in sediment samples.

Remarks: *Amphidinium incoloratum* Campbell, 1973 was first described as similar to *A. operculatum* (as *A. klebsi* Kofoid et Swezy, 1921) in size and the shape of the epicone, but is distinguished by its lack of plastids, more globular hypocone, and shorter longitudinal flagellum. We refer these cells to *A. incoloratum* tentatively.

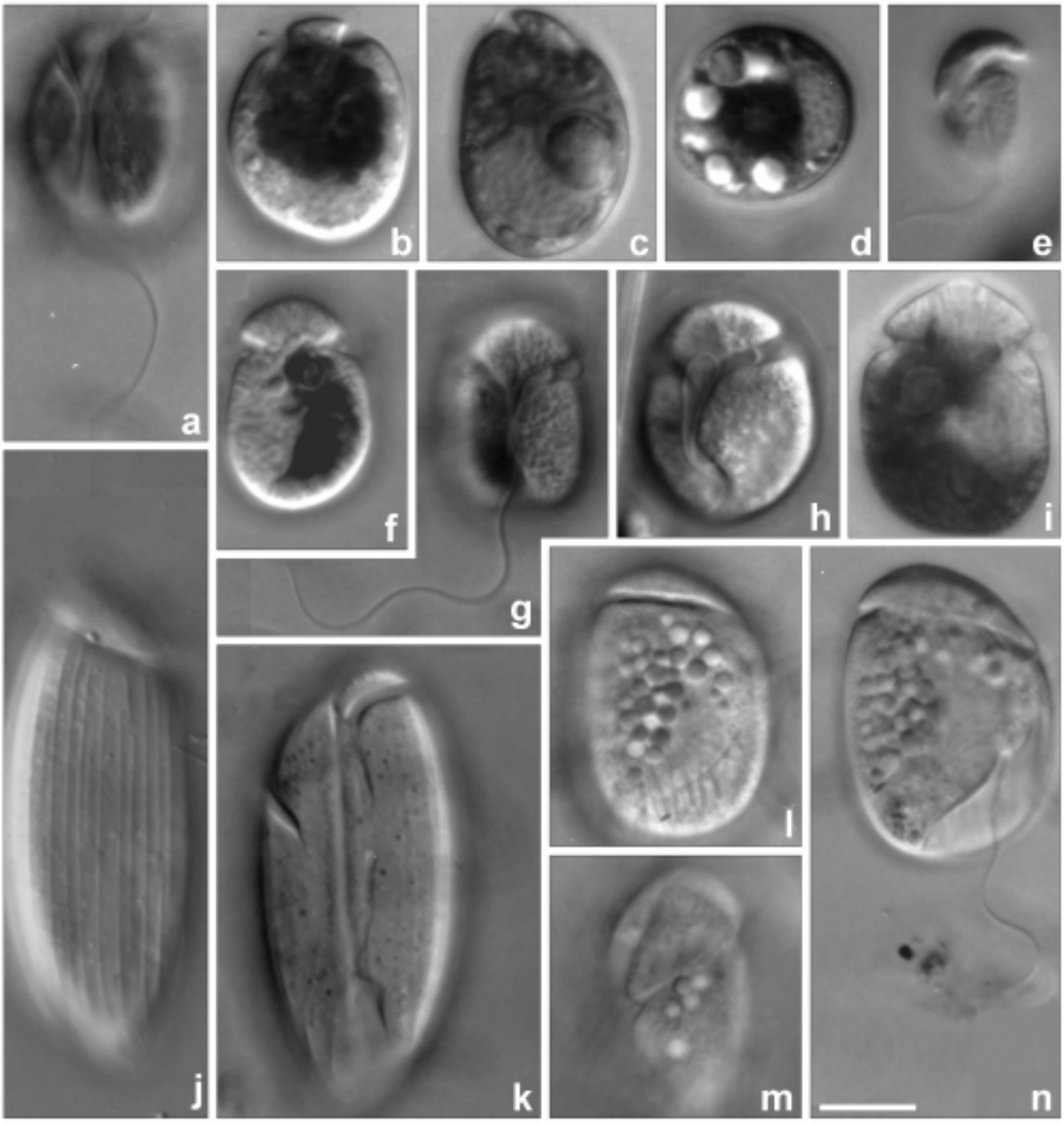

Fig. 14. (**a-d**) *Amphidinium operculatum*, (**a**) ventral view, (**b**) showing plastids and pusules, (**c**) dorsal view, (**d**) non-motile cell, (**e**) *Amphidinium psittacus,* ventral view, (**f-i**) *Amphidinium salinum*, (**f**) ventral view showing plastid, (**g**) ventral view, (**h**) non-motile cell, (**i**) showing ejectosomes, (**j, k**) *Amphidinium scissum,* (**j**) dorsal view, (**k**) ventral view showing flagellar pockets, (**l-n**) *Amphidinium semilunatum,* (**l**) right lateral view, (**m**) showing apical groove, (**n**) showing flagellar insertion. All micrographs are DIC images. Scale bar - 10 μm

In comparison with the original records, the cells reported here are larger, 35-42 µm compared to 21-24 µm (Campbell 1973) and 20-30 µm (Hansen and Larsen 1992), have a higher position of longitudinal flagellar insertion, a more pronounced sulcal 'flap' and two pusules. This species has been reported rarely, and it is difficult to judge how variable the size and sulcus may be. The species reported as *Amphidinium incoloratum* by Larsen and Patterson (1990) differs from the original account because it is round, has an excavated sulcus, a very narrow, straight epicone that barely curves to the left, and is shorter (about 15 µm). This species has been observed in Port Botany, eastern Australia (Murray unpublished observation). We believe that this is not referable to *A. incoloratum*. *Amphidinium incoloratum* has been reported previously from estuarine water, in the USA (Campbell 1973) and the marine water column in Denmark (Hansen and Larsen 1992).

Amphidinium mammillatum **Conrad et Kufferath, 1954 (Figs 12e, 13l-p)**

Description: Cells are rounded to ellipsoidal from the ventral side, slightly dorso-ventrally flattened, 16-24 µm long, and 11-18 µm wide (n = 18). The width of this species is variable. The epicone is a little over a third of the cell length. The cingulum is relatively wide (about 2 µm). The proximal end is just to the left of the mid-ventral line, ascending slightly initially, then descending as it passes across the dorsal side towards its distal end, 1 cingulum-width lower than the proximal end. The sulcus curves towards the left, becomes less well defined posteriorly and does not reach the antapex. The left side of the sulcus is much more distinct than the right. The apical groove begins to the left of the mid-ventral line at the junction of the cingulum and sulcus, follows a slightly curved path and reaches the anterior end to the left of the apex, then curves back towards the apex on the dorsal side. The longitudinal flagellum arises in a pocket just below the start of the cingulum, to the left of the mid ventral line, and opens into the sulcus about half way down the cell. The nucleus is round to ellipsoidal, in the epicone, and about 10 µm diameter. The plastid is yellow-green, single, in diffuse strands, and mainly in the hypocone, with a large (4-5 µm) pyrenoid. Red granules are occasionally present in the hypocone. Non-motile cells with hyaline membranes were occasionally found. Cells were observed in sediment samples.

Remarks: *Amphidinium mammillatum* Conrad et Kufferath, 1954 was originally described from brackish waters in Belgium. The present observations agree with the original observation in size, shape, the description of the plastids and the path of the cingulum, but it differs in that *A. mammillatum* was not described with a pyrenoid or an apical groove that continues to the anterior end. We believe these features might have been overlooked. This species is similar in shape and size to *Gymnodinium limitatum* Skuja, 1956, but differs in that *G. limitatum* appears to have a larger epicone than *A. mammillatum.* Other similar species are *Amphidinium inflatum* Kofoid, 1931 which is larger (47 µm), and *Gymnodinium cassiei* Norris, 1961; which is slightly smaller (15-17 µm), and despite a more detailed observation than that of *A. mammillatum*, was not reported to possess a pyrenoid or an apical groove.

Amphidinium operculatum **Claparède et Lachmann, 1859 (Figs 12f, 14a-d)**

Description: Cells are ellipsoidal from the ventral side, dorso-ventrally flattened, 20-30 µm long, 15-20 µm wide (n = 7). The epicone is about 6 µm at its widest, and deflected to the left. The distal end of the cingulum is about 1 cingulum-width lower than the proximal. The sulcus is to the right of the mid-ventral line and initially deep and wide. It begins at the distal end of the cingulum and becomes less distinct posteriorly. No apical groove was observed. Two pusules are present, one below the origin of the cingulum, the other near the origin of the sulcus. The longitudinal flagellum arises in a pocket just to the left of and below the origin of the sulcus. The nucleus is in the posterior part of the hypocone, about 10 µm diameter. The plastids are yellow-brown, in strands radiating from the central pyrenoid or more globular around the pyrenoid. Non-motile cells are round and surrounded by a transparent layer. Cells were found in sediment samples.

Remarks: This species, the type of the genus, is commonly reported but has been the subject of considerable uncertainty (Maranda and Shimizu 1996). Possible synonyms include: *Amphidinium carterae* Hulburt, 1957; *A. hoefleri* Schiller et Diskus, 1955; *A. herdmani* Kofoid et Swezy, 1921; *A. klebsi* (Klebs, 1884) Kofoid et Swezy, 1921; *A. steini* (Lemmerman, 1910) Kofoid et Swezy, 1921; *A. rhynchocephalum* Anissimowa, 1926; *A. massarti* Biecheler, 1952; and *A. wislouchi* Hulburt, 1957. The view of Dodge (1982) is followed, and all of the above species are considered to be synonymous with *Amphidinium operculatum* except for *A. carterae,* which is distinguished by size and the plastid arrange-

ment, and *A. herdmani*, which is presumably distinguished by the shape of the epicone (although this was not discussed by Dodge). In this study, *A. operculatum* was distinguished from *A. carterae* primarily by a discontinuity in the size ranges of the two species (Hulburt 1957), rather than by the arrangement of the plastid (Taylor 1971), as the latter feature may be unreliable (Fukuyo 1981). *Amphidinium operculatum* and its synonyms have been reported frequently from marine sediment and the water column from all over the world (Dodge 1982).

***Amphidinium psittacus* Larsen, 1985 (Figs 12g, 14e)**

Description: This species is ellipsoidal from the ventral side and slightly dorso-ventrally flattened, about 14 μm long, 7 μm wide. The epicone is asymmetrical, narrower on the left side, and bending to the left. The apex is rounded. The cingulum forms a descending spiral, beginning one third of the way down the cell, then descending across the dorsal side, with the distal end about half way down the cell. The sulcus originates 2-3 μm below the origin of the cingulum. No apical groove was observed. The longitudinal flagellum arises at the anterior end of the sulcus. The nucleus is in the anterior left side of the cell, 4-5 μm diameter. Plastids are absent. One cell was found in sediment samples.

Remarks: This species was first described from marine sediments in Denmark (Larsen 1985b). This observation agrees well with the original observation. This species has also been reported from marine sediment from tropical Australia (Larsen 1985b, Larsen and Patterson 1990).

***Amphidinium salinum* Ruinen, 1938 (Figs 12h, 14f-i)**

Description: Cells are broadly ellipsoidal from the ventral side, with a truncate hypocone and a rounded to slightly conical epicone; slightly dorso-ventrally flattened, 18-25 μm long, 13-19 μm wide (n = 10). The epicone narrows to a 'stem' where it meets the hypocone on the mid-ventral line. The cingulum is relatively wide, about 2 μm, and ascends slightly initially, then descends towards its distal end. The cingulum is not displaced in the vertical direction, but the ends do not meet due to the stem of the epicone. The sulcus is initially narrow, curving to the left and widening at the posterior end. No apical groove was observed. The longitudinal flagellum arises where the sulcus widens, just to the left of the mid-ventral line, 35-45 μm long. The nucleus is round to ellipsoidal, located in the hypocone, and about 8 μm diameter. The plastid is yellow-brown with one or two obvious pyrenoids (2-4 μm diameter), does not fill the cytoplasm, and its size and position is variable. Extrusomes (1-4 μm) are present around the periphery of the cell. The cell surface is rugose. Non-motile cells may be enclosed in a hyaline sheath. Cells were found in sediment samples.

Remarks: This species was first described from marine sediments from temperate Australia (Ruinen 1938). It has since been recorded once, in high salinity sites in Belgium (Conrad and Kufferath 1954). The original report does not mention pyrenoids, records larger cells (35-40 μm, compared to 18-25 μm) without a rugose surface. The shapes of the epicone and the plastid are unusual and distinctive. The variable size and position of the plastid suggests the possibility of kleptoplasty. Even allowing for this species being mixotrophic, there are very few similar species. *Gymnodinium verruculosum* Campbell, 1973 is similar in shape, the rugose surface and single plastid. It differs in the vertical displacement of its cingulum, rather than the stem shaped posterior end of the epicone in *A. salinum*, and is slightly smaller (15-20 μm). *Amphidinium lacunarum* Skuja, 1964, also has a stem-shaped epicone but does not have a rugose surface, has its nucleus located in the epicone not hypocone, and has blue-green rather than yellow-brown plastids with pyrenoids.

***Amphidinium scissum* Kofoid et Swezy, 1921 (Figs 12i, 14j, k)**

Description: Cells are elongate ellipsoids from the ventral side, are dorso-ventrally flattened, 43-60 μm long, 20-22 μm wide, about 12 μm deep dorso-ventrally (n = 9). The epicone is asymmetrical, and slopes down towards the right. The cingulum is relatively deep and forms a descending spiral. The distal end narrows 4-5 μm to the right of the mid-ventral line. The cingulum is displaced about by 0.2-0.3 of the cell length. The transverse flagellum arises in a pocket that begins 1/4 of the way down the cell to the left of the mid-ventral line and leads to the cingulum. The sulcus is narrow and shallow, reaching to the posterior end, where it opens into an obvious wedge-shaped indentation. An apical groove is present, reaching from the sulcus to the apex, which it encircles. The longitudinal flagellum arises in a pocket to the left of the mid-ventral line, about one third of the way

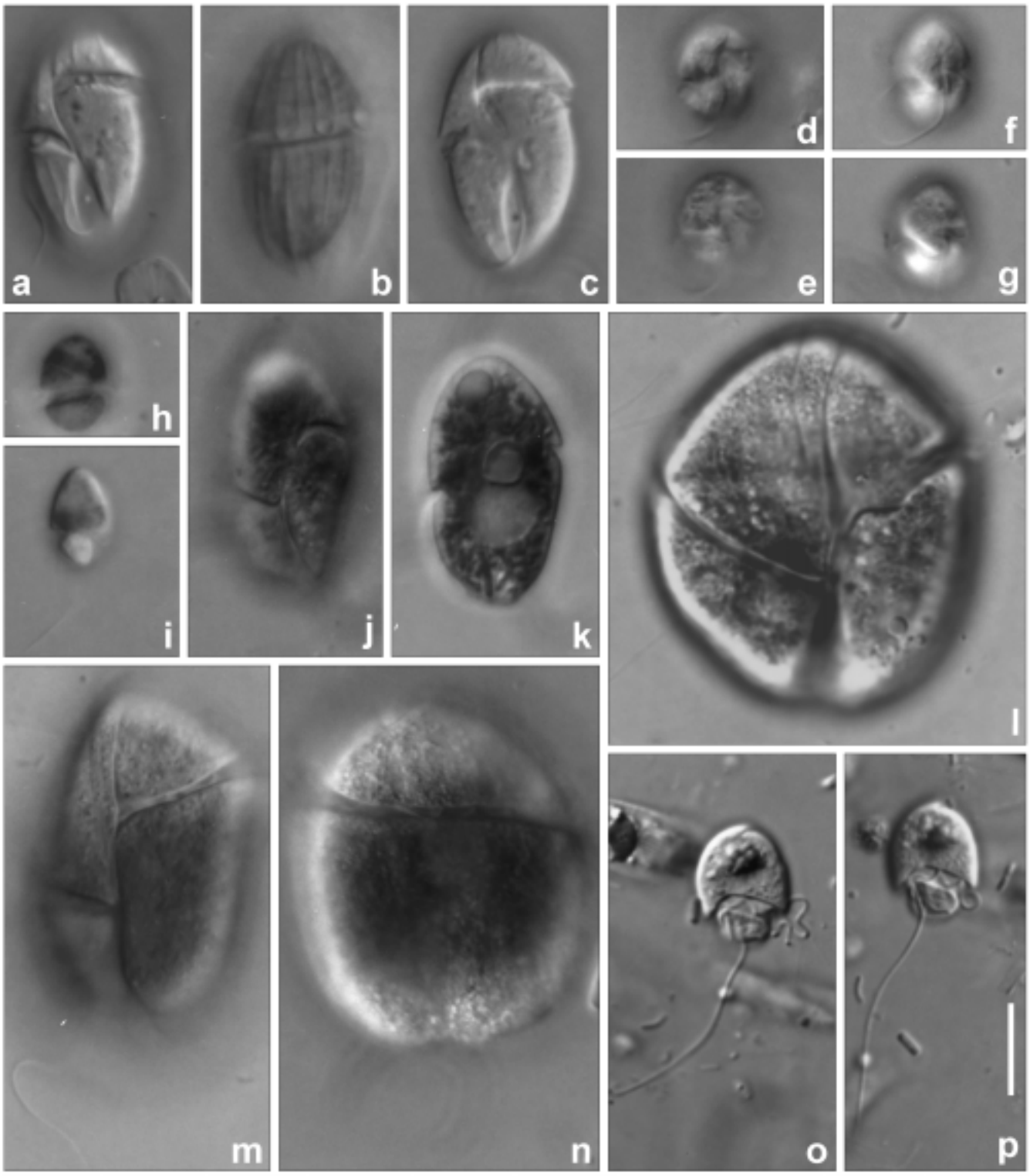

Fig. 15. (**a-c**) *Gyrodinium dominans*, (**a**) ventral view, (**b**) dorsal view, (**c**) ventral view showing flagellar pocket, (**d, e**) *Gyrodinium* cfr *estuariale,* (**d**) ventral view showing apical groove, (e) ventral view, (**f-i**) *Gyrodinium mundulum*, (**f**) ventral view showing apical groove and stigma, (g) showing cingulum, (**h**) dorsal view, (**i**) lateral view, (**j, k**) *Gyrodinium oblongum,* (**j**) ventral view, (**k**) showing pyrenoid and nucleus, (**l-n**) *Gyrodinium pavillardi,* (**l**) ventral view showing apical groove, (m) ventral/lateral view, (**n**) dorsal view, (**o, p**) *Katodinium asymmetricum*, (**o**) ventral view showing apical groove, (**p**) showing sulcus. All micrographs are DIC images. Scale bar - 10 μm

down the cell and then leads to the widened sulcus. The nucleus is ellipsoidal, (10 µm by 20 µm), in the central-right side of the hypocone. Plastids are not present. Ingested diatoms and other food particles may be present. About 18 fine longitudinal surface striations are present across the cell surface. Cells were observed in sediment samples.

Remarks: This species was originally described from marine sediments in the USA (Kofoid and Swezy 1921). The present observation complies with the original observation in size, shape of the cingulum and epicone, and the presence of surface striations. It differs in the position of the sulcus, which was originally reported to run from the cingulum to the antapex, and the observation of the flagellar pocket, differences we do not regard as significant. A species appearing identical to this one has been recorded in the German Wadden Sea, but was not named (Hoppenrath and Okolodkov 2000).

Amphidinium semilunatum **Herdman, 1924 (Figs 12j, 14l-n)**

Description: Cells are ellipsoidal to oblong in right lateral view, laterally compressed, about 30 µm long, 21 µm wide, and 6 µm deep (n = 1). The cingulum has its origin towards the dorsal side of the cell, then tilts downwards, with the distal end about 1 cingular width higher than the proximal end. The sulcus is initially narrow, then widens towards the posterior of the cell and becomes relatively deep (about 12 µm). An apical groove is present, arising from the junction of the cingulum and sulcus. A row of large (5-6 µm) extrusomes is present in the posterior end of the cell. The longitudinal flagellum arises in the sulcus. The nucleus is 7-8 µm diameter, in the middle of the hypocone. Plastids are absent. A number of food particles and oil globules may be present. This species was found in sediment samples.

Remarks: *Amphidinium semilunatum* Herdman, 1924 was first described from marine sediments in England. The cell observed here is very similar to the original observation (Herdman 1924), but differs in the additional detail of the apical groove, the smaller size (32 µm, compared to 50 µm) and the presence of extrusomes. As the possession of extrusomes may be a variable character, this species is referred to *Amphidinium semilunatum.* This species has been reported from marine sediment, in Denmark (Larsen 1985b), England (Dodge 1982, Herdman 1924), Canada (Baillie 1971), and tropical Australia (Larsen and Patterson 1990).

Gyrodinium dominans **Hulburt, 1957 (Figs12k, 15a-c)**

Description: Cells are fusiform when observed from the ventral side, and circular or slightly flattened in cross-section, 23-26 µm long, 13-15 µm wide (n = 4). The hypocone is moderately longer than epicone. Both have striations, about eight across the ventral faces. The cingulum is deep, with a slight overhang. The distal end is 2-3 cingulum-widths lower than proximal, displaced about one third of the cell length. The sulcus widens as it reaches the antapical end. The longitudinal flagellum arises at the anterior end of the sulcus, in a 2 µm pocket just to the left of the junction of the cingulum and sulcus. An apical groove extends from the cingulum to the apex. The nucleus is in the epicone and is ellipsoidal, 6-8 µm diameter. Plastids are absent. Cells were observed in mat samples.

Remarks: Our observations agree well with the original observation. The pocket of the longitudinal flagellum was not previously recorded. This species is similar to *Gyrodinium grossestriatum* Campbell, 1973; but differs in the lesser torsion of the body and the greater number of striations. *Gyrodinium pingue* (Schütt, 1895) Kofoid et Swezy, 1921 differs in the sulcal overhang and the shape of the sulcus, and *Gymnodinium hulburtii* Campbell, 1973 differs in having less cingular displacement. This species has been reported from the marine water column in Australia (Larsen 1996), Japan (Takayama 1985, Fukuyo *et al*. 1990), Denmark (Hansen and Larsen 1992) and the USA (Campbell 1973, Hulburt 1957).

Gyrodinium **cfr** ***estuariale*** **Hulburt, 1957 (Figs 12l, 15d, e)**

Description: Cells are round from ventral side, slightly dorso-ventrally flattened, 10-12 µm long, 9-10 µm wide (n = 3). The cingulum is wide, about 2 µm, with the proximal end, about 1 cingulum-width lower than the distal end. The sulcus is relatively wide, 1.5-2 µm. An apical groove is present, curving to the left towards the apex from the junction of the cingulum and sulcus. The longitudinal flagellum arises at the anterior end of the sulcus. An orange stigma-like body is present. The position of the nucleus was not observed. A number of small yellow-green plastids are distributed in the epicone and hypocone. Cells are generally very fast swimming, and were observed in sediment samples.

Remarks: This species was first described from the estuarine water column in the U.S.A (Hulburt 1957). The present report agrees with the original observation,

except that only the initial part of the apical groove was originally described. A number of other round, plastid possessing species in the same size range have been described: *Gymnodinium albulum* Lindemann, 1924; *Gymnodinium biciliatum* Ohno, 1911 and *Gymnodinium profundum* Schiller, 1933; all of which differ in having no cingular displacement, *Karlodinium micrum* (Leadbeater et Dodge, 1966) Larsen, 2000; *Gyrodinium galatheanum* Braarud, 1970; *Gyrodinium* sp. B (Throndsen, 1983); *Karlodinium veneficum* Ballantine, 1956 and *Karlodinium vitiligo* Ballantine, 1956; which differ in the lack of a stigma-like body. This may be a residuum of food, therefore this distinction may be artificial. Further study using electron microscopy is necessary to establish the identity of this species with confidence. *Gymnodinium punctatum* Pouchet, 1887; also appears similar in dimensions and possesses plastids, but is insufficiently described to establish synonymy. *Gyrodinium estuariale* has been reported from other locations in the USA (Campbell 1973, Gardiner *et al.* 1989) and from Norway (Throndsen 1969).

***Gyrodinium* cfr *mundulum* Campbell, 1973 (Figs 12n, 15f-i)**

Description: Cells are ellipsoidal from the ventral side, slightly dorso-ventrally flattened, 10-12 µm long, 7-8 µm wide (n = 17). The epicone is conical ventrally and wedge-shaped from the side, the hypocone is slightly shorter than the epicone ventrally and half as wide laterally. The cingulum is 1-1.5 µm wide, with the distal end 1-2 cingulum-widths lower than the proximal end and overhanging slightly. The cingulum narrows significantly at its proximal end. The sulcus is shallow and barely visible. An apical groove begins at the intersection of the cingulum and sulcus, curves initially to the left and then back to the right towards the apex. The longitudinal flagellum arises at the anterior end of the sulcus. An orange stigma is present in hypocone, just to the left of the origin of the sulcus. The nucleus lies in the hypocone. Several yellow-green plastids are present in the epicone and hypocone. Cells are generally very fast swimming, and were observed in sediment samples.

Remarks: This species was first described from the estuarine water column in the USA (Campbell 1973). Cells recorded here are very similar to the original observation in the slight sulcal overhang, the presence of a stigma, plastids and the apical groove. Cells from Hamelin Pool differ in not being dorso-ventrally flattened, having a rounder hypocone and the slightly larger sizes (12-22 µm, compared to 10-12 µm). Because of these differences, cells observed by us are tentatively referred to *G. mundulum*. Other small, similarly shaped species of *Gymnodinium* or *Gyrodinium* with plastids are *Gyrodinium zeta* Larsen, 1996 and *Gymnodinium amphityphlum* Larsen, 1994, which both differ in the absence of cingular overhang and in the positions of the apical grooves, *Gymnodinium pumilum* Larsen, 1994 and *Gymnodinium aurantium* Campbell, 1973; which have no cingular displacement; *Gymnodinium natalense* Horiguchi et Pienaar, 1994 and *Gyrodinium carteretensis* Campbell, 1973; which lack an apical groove and have no cingular overhang, *Gyrodinium complanatum* Campbell, 1973 and *Gyrodinium* sp. ined. 4 (Hansen et Larsen, 1992) which have no apical groove. *Gymnodinium* sp B, described by Throndsen (1983), is indistinguishable from the present species. This species has also been reported from the water column in Japan (Throndsen 1983).

***Gyrodinium oblongum* Larsen et Patterson 1990 (Figs 12o, 15j, k)**

Description: Cells are oblong from the ventral side, and dorso-ventrally flattened posteriorly; 17-25 µm long, 8-9 µm wide (n = 16). The epicone and hypocone are about equal in size. The cingulum is displaced 0.25-0.3 of cell length. The sulcus is initially narrow but widens at the posterior end. No apical groove was observed. The nucleus is in the hypocone, and about 5 µm diameter. The plastid is single, yellow-brown, and forms thin longitudinal strands towards the anterior and posterior ends. An obvious pyrenoid is present, 3-4 µm diameter, in the centre of the cell. Cells were observed in sediment samples.

Remarks: This species was described from marine sediments in tropical Australia (Larsen and Patterson 1990), and as yet, has only been recorded in Australia. This observation agrees well with the original observation in all aspects except for its greater length (17-25 µm, compared to 12-17 µm). *Gyrodinium oblongum* is distinguished from other species in this genus by its obvious large single pyrenoid.

***Gyrodinium pavillardi* Biecheler 1952. (Figs 12m, 15 l-n)**

Description: Cells are round to rhomboidal from the ventral side, are dorso-ventrally flattened, 39-55 µm long, 30-35 µm wide (n = 7). The epicone is asymmetrical, with the apex of the cell to left of the mid-ventral line. The cingulum is narrow, about 1 µm, initially ascending on the ventral side, then descending across the dorsal

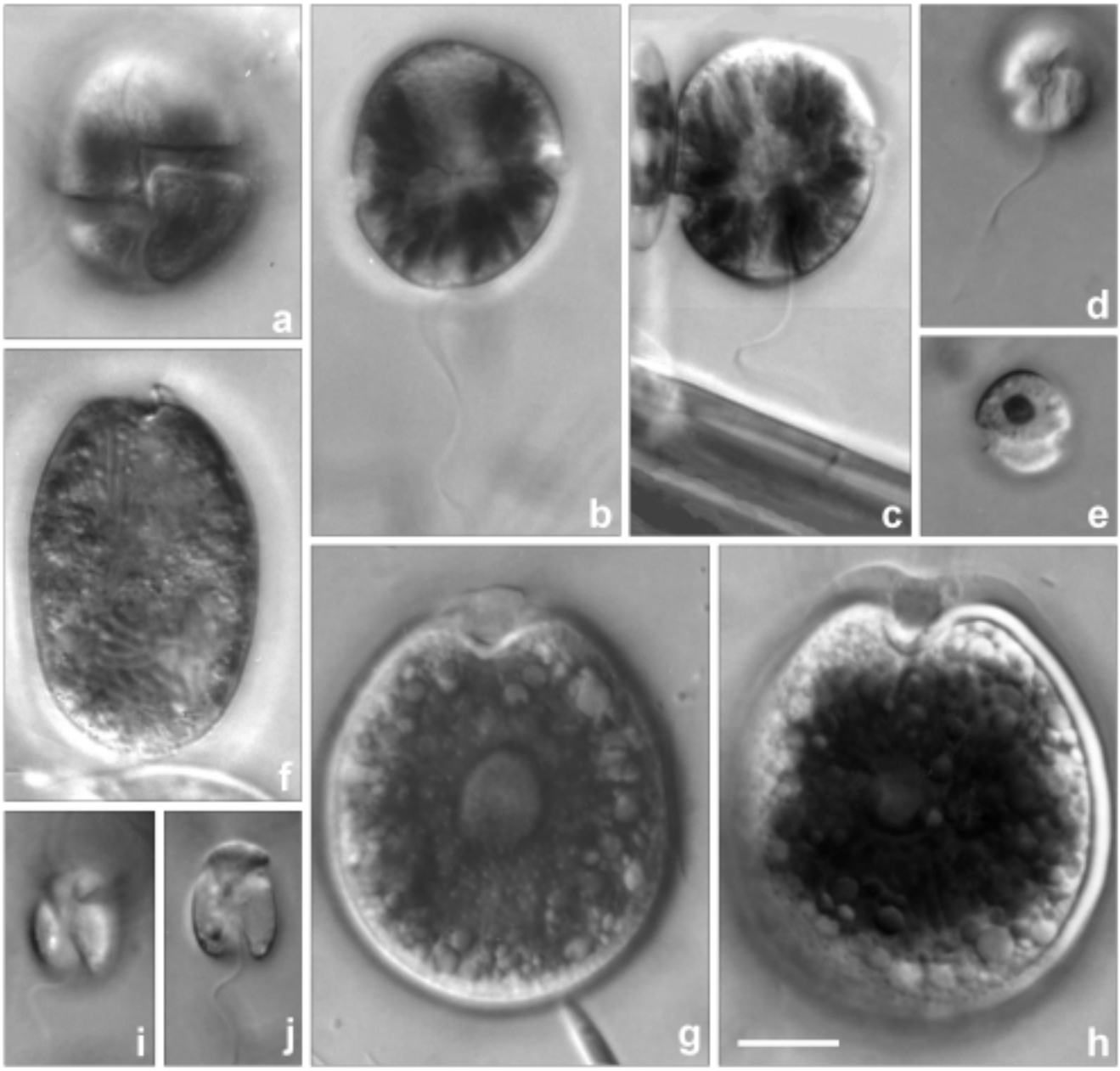

Fig. 16. (**a-c**) *Katodinium dorsalisulcum*, (**a**) ventral view, (**b**) showing nucleus, (**c**) showing plastids and pusules, (**d, e**) *Katodinium fungiforme,* (**d**) ventral view, (**e**) dorsal view, (**f**) *Prorocentrum mexicanum*, (**g, h**) *Prorocentrum* cfr *concavum*, (**g**) showing pyrenoid, (**h**) non-motile cell, (**i, j**) *Amphidinium* sp, (**i**) ventral view, (**j**) showing cingulum. All micrographs are DIC images. Scale bar - 10 µm

side until it meets the sulcus. The cingulum is displaced by 0.25-0.3 of the cell length. The sulcus is narrow initially, and widens at the posterior end. An apical groove ascends in a straight path from the sulcus to the apex, loops around the apex then descends in a straight path, ending to the right of the junction with the sulcus. Two small pusules are present, one below the start of the cingulum, the other at the junction of the sulcus and the right end of the cingulum. The longitudinal flagellum arises at the anterior end of the sulcus. The nucleus is round to oval, about 15 µm diameter, in the epicone. The plastids are yellow-brown, in sparse radiating strands. No pyrenoid was observed. An orange refractile globule, possibly an eyespot, is present in the posterior of the cell.

Round non-motile cells are occasionally formed. The cells were observed in sediment samples.

Remarks: *Gyrodinium pavillardi* was originally described from the marine water column in France (Biecheler 1952). Cells observed in Hamelin Pool are very similar to the original observation, in terms of plastids, shape, size, refractile globule, the position of the nucleus, and the apical groove looping around the apex and returning on the ventral side. The original account differs from the present cell in lacking mention of a posterior stigma-like inclusion, a difference that may be variable and we do not consider significant. Three other species of *Gyrodinium* are similar to *G. pavillardi* in size, shape, the path of the cingulum, anterior position of the nucleus, plastids and the lack of striations, but were not described as having apical grooves which loop around and return on the ventral side of the epicone: *Gyrodinium instriatum* Freudenthal et Lee, 1963; *G. prunus* (Wulff, 1916) Lebour, 1925; and *Gyrodinium* sp. (Sousa E. Silva, 1959). *Gyrodinium instriatum* was described from the marine water column in the U.S.A, without reference to *G. pavillardi*, and is very similar, but was not described as having an apical groove which returns on the ventral side (Freudenthal and Lee 1963). Specimens identified as *G. instriatum* from the Inland Sea of Japan (Takayama 1985) have an apical groove that loops around the apex and return on the epicone, and therefore probably represent *G. pavillardi.*

***Katodinium asymmetricum* (Massart, 1920) Loeblich, 1965 (Figs 12p, 15o-p)**

Description: The cells are ovoid from the ventral side, slightly obliquely dorso-ventrally flattened; about 12 µm long. The epicone is about 10 µm wide, the hypocone is about 5 µm wide (n = 1). The epicone is dome-shaped, about two third of the total cell length, and has a notch just to the left of the apex. The cingulum is very wide, initially descending, and slightly displaced. The sulcus is narrow, on the right side of the hypocone, and turning to the left as it descends towards the posterior end. The longitudinal flagellum arises at the anterior end of the sulcus. The nucleus is in the left half of the cell, and is about 4 µm diameter. Plastids are not present. Food particles are occasionally present. This species was observed in sediment samples.

Remarks: The species from Hamelin Pool is very similar to that described in the original observation (Massart 1920) in size, shape, the lack of plastids and the position of the sulcus, but differs in having the apical notch. Massart may have overlooked this notch (Carter 1937, Larsen 1985b, Larsen and Patterson 1990). Some later observations (Biecheler 1952, Conrad and Kufferath 1954, Hulburt 1957, Campbell 1973) differ slightly from the original observation in that the sulcus is placed centrally and not to the right of the cell. There may be some local variation in this. The only other small, colourless species with a similarly sized hypocone are: *K. austriacum* (Schiller, 1955) Loeblich, 1965, *K. crassifilum* (Schiller, 1954) Loeblich, 1965; and *K. mazuricum* Javornicky, 1965, which differ in the possesion of a stigma and the lack of an apical notch, *K. campylops* (Harris, 1940) Loeblich, 1965 differs in having a stigma and a sulcal extension onto the epicone. This species has been reported from marine sediments and the water column in Belgium (Massart 1920, Conrad and Kufferath 1954, Van Meel 1969), England (Carter 1937, Dodge 1982), France (Biecheler 1952), USA, (Hulburt 1957, Campbell 1973), Denmark (Larsen 1985b), and tropical Australia (Larsen and Patterson 1990).

***Katodinium dorsalisulcum* Hulburt, McLaughlin et Zahl, 1960 (Figs 12q, 16 a-c)**

Description: Cells are circular to ovoid from the ventral side, slightly dorso-ventrally flattened; 24 µm long, 20 µm wide (n = 3). The epicone is somewhat flat anteriorly, 0.6-0.7 of the cell length. The cingulum is wide and relatively deep, beginning about half way down the cell. It descends slightly across the ventral and dorsal sides, and its distal end is one cingulum-width lower than the proximal end. The sulcus is narrow, curving first to the right and then to the left. The longitudinal flagellum arises at the anterior end of the sulcus, in a pocket extending directly posteriorly. An apical groove arises at the junction of the cingulum and sulcus, and curves to the left where it notches the epicone. Plastids are numerous, yellow-brown, and may radiate from the centre. A pyrenoid is present in some cells. The nucleus is about 8 µm diameter, located in the hypocone. Cells were observed in sediment samples.

Remarks: This species was described from cultures made from 'cyst-like bodies' collected in marine sediment in Bimini in the Caribbean (Hulburt *et al*. 1960). To our knowledge, it has not been reported since. Cells observed in Hamelin Pool agree well with the original observation, differing only in the occasional pyrenoid, which was not reported in the original. *Katodinium dorsalisulcum* is similar to several species of *Gyrodinium* because of the cingular displacement, but was placed in the genus *Katodinium* due to its larger epicone in comparison to the size of the hypocone. The species

most similar to *K. dorsalisulcum* in size, shape, cingular displacement and the possession of plastids are: *Gymnodinium impudicum* (Fraga et Bravo, 1995) Hansen et Moestrup, 2000; differing in the shape of the hypocone and the formation of chains; *Gyrodinium resplendens* Hulburt, 1957; differing in the path of the apical groove and the less curved path of the sulcus; *Gymnodinium microreticulatum* Bolch et Hallegraeff, 1999; differing in the more ellipsoidal shape and the lesser degree of cingular displacement; and *Gymnodinium aureolum* (Hulburt, 1957) Hansen, 2001; differing in the less curved path of the sulcus.

***Katodinium fungiforme* (Anissimowa, 1926) Loeblich, 1965 (Figs 12r, 16 d, e)**

Description: Cells are round to slightly ellipsoidal from the ventral side, slightly dorso-ventrally flattened, 10-12 µm long, 9-10 µm wide (n = 12). The cingulum is 1.5-2 µm wide, with the distal end about 1 cingulum-width lower than the proximal. The sulcus is about 2 µm wide, and slightly sloped to the right. No apical groove was observed. The longitudinal flagellum is 27-30 µm, arising at the anterior end of the sulcus. Some cells contain many small colourless lipid globules or food particles. The nucleus is in the hypocone, and is about 5 µm diameter. Plastids are absent. Cells generally swim very fast. This species was found in mat and sediment samples.

Remarks: This species was first described from saline waters and sediments in Russia (Anissimowa 1926). The organisms reported here differ slightly from the original observation in that the path of the cingulum and sulcus were not originally described, and they have more equally sized epicones and hypocones. Later observers (Biecheler 1952, Spero 1982, Throndsen 1983) have described the cingular displacement and the central path of the sulcus consistent with the present observations. A number of small, round, colourless species of *Gymnodinium*, *Gyrodinium* and *Katodinium* have been described. *Gymnodinium galesianum* Campbell, 1973 was described without reference to the present species and appears to differ only in its more rounded shape and less displaced cingulum. We regard it as a synonym. *Gyrodinium metum* Hulburt, 1957; *Gymnodinium subroseum* Campbell, 1973; and *Gymnodinum amplinucleum* Campbell, 1973 have a more conical epicone, *Gyrodinium lebourae* Herdman, 1924 has a more displaced cingulum and a stigma, *Gymnodinium albulum* Lindemann, 1924 has an indistinct sulcus, *Gymnodinium roseostigma* Campbell, 1973 and *Katodinium hiemale* (Schiller, 1954) Loeblich, 1965 have a stigma. *Katodinium fungiforme* has been reported from saline waters in Russia (Anissimowa 1926), the Gulf of Riga (Skuja 1939), Japan (Throndsen 1983), U.S.A (Spero 1982), England (Harris 1940), and France (Biecheler 1952).

***Prorocentrum* cfr *concavum* Fukuyo, 1981 (Figs 12t, 16 g, h)**

Description: Cells are round to ovoid in valve view, widest below the middle, laterally flattened and apparently concave with lengths from 40 to 46 µm, width 34-38 µm (n = 15). The apical area is a small rounded triangular depression on the right valve and a slight indentation on the left, comprising several small plates. An apical spine was not present. Both valves are covered with numerous shallow depressions and pores of extrusomes, 1-3 µm apart, with fewer in the centre of the cell. The intercalary band is relatively wide, 1-2 µm, with many small depressions. The nucleus is in the posterior part of the valve. The plastid is yellow-brown and diffuse. A large (10 µm diameter) pyrenoid is present in the centre of the cell. Cells were occasionally seen covered by a colourless, mucoid cyst. This species was found in sediment samples.

Remarks: The species observed here is consistent with the original description (Fukuyo 1981) in terms of size and shape of the cell, the shape of the apical area, and the observation of pores and depressions. Two other species of *Prorocentrum* have similar features, *P. tropicalis* Faust, 1997; and *P. maculosum* Faust, 1993. *Prorocentrum concavum* is distinguished from *P. tropicalis* and *P. maculosum* partly by the arrangement, shape and size of the periflagellar plates, which are too small to be clearly seen under the light microscope and were not recorded in this study. Differences which are visible with the light microscope are that *P. maculosum* has fewer pores, while *P. tropicalis* does not have a pyrenoid. *Prorocentrum concavum* has been reported from marine sediment from French Polynesia, New Caledonia (Fukuyo 1981), Japan (Fukuyo 1981), Belize (Faust 1990), and Venezuela (Gamboa-Marquez *et al.* 1994).

***Prorocentrum mexicanum* Osorio Tafall, 1942 (Figs 12s, 16f)**

Description: Cells are asymmetrically ovoid in valve view, with the left side of the right valve slightly longer

at the apical end, and laterally compressed; 35-40 μm long, 22-27 μm wide (n = 7). The apical area is a small indentation, covered by many small pores. A small spine is present to the right of the apical area on the right valve. Both valves have many large pores, which lie in radially arranged rows. Large (3-5 μm) extrusomes are present in the anterior part of the valve. The nucleus is about 10 μm in diameter, in the posterior part of the valve. The plastid is yellow-brown, and diffuse. This species was found in mat samples.

Remarks: This species was originally described from the Gulf of Mexico (Osorio Tafall 1942). *Prorocentrum rhathymum* Loeblich, Sherley et Schmidt, 1979 was described without reference to Osorio Tafall and refers to the same species. This observation agrees well with the original observation, except that the pores were originally reported as spines. This species has also been reported from marine sediment and the water column in Japan (Fukuyo 1981, Okamoto 1992), New Caledonia (Fukuyo 1981), Venezuela (Gamboa-Marquez *et al*. 1994), Belize (Faust 1990), the Virgin Islands (Loeblich *et al*. 1979), the Sichang Islands, Gulf of Thailand (Piyakarnchana *et al*. 1986) and temperate Australia (Larsen and Moestrup 1989, Hallegraeff 1991).

Alveolates - Apicomplexa

***Colpodella unguis* Patterson et Simpson, 1996 (Fig. 17a)**

Description: Cells are 8-11 μm long (n = 4), bean-shaped in profile and somewhat flattened. Two flagella insert at right angles on one flat side about one third of the way down the cell. The anterior flagellum is nearly the length of the cell, inserts at the end of a deep, triangular curving depression. It is directed laterally and posteriorly. The posterior flagellum inserts at the top of a shallow longitudinal groove, is nearly 2 times the length of the cell, and is directed posteriorly. Some cells have large vacuoles containing eukaryotic prey material. Cells typically swim rapidly, usually in contact with the substrate. Cells erratically flip from one side to the other rather than rotating smoothly. This species was observed in sediment and mat samples.

Remarks: Our observations agree with the only previous records for this species (Patterson and Simpson 1996). Food vacuoles have not been previously observed. The observation of ingested eukaryotic material supports assignation of this organism to *Colpodella*, a taxon of apparently obligate predators of eukaryotes (Patterson and Simpson 1996, Simpson and Patterson 1996). However, the distinctive division cysts of *Colpodella* were not observed for *C. unguis* to date. Previously reported from hypersaline sites in Shark Bay (Patterson and Simpson 1996) and from Botany Bay, Australia (Lee unpublished observation).

Stephanopogonidae Corliss, 1961

***Stephanopogon apogon* Borror 1965 (Figs 17b, 18a)**

Description: One cell was observed, which was about 26 μm long and flattened, with a somewhat constricted anterior end bearing a broad apical mouth. Several rows of kineties run along the cell, cells can crawl or swim. This species was observed in a mat sample.

Remarks: This species has been found in tropical Australia and the USA (Lee and Patterson 1998) and also found in Botany Bay and Cape Tribulation, Australia (Lee unpublished observation). Previously reported cell lengths are 20-70 μm. Generally, our observation agrees with that of previous observers. *Stephanopogon* contains six nominal species: *S. apogon*, *S. colpoda* Entz, 1884; *S. mesnili* Lwoff, 1923; *S. minuta* Lei, Xu et Song, 1999; *S. mobilensis* Jones et Owen; 1974 and *S. paramesnili* Lei, Xu et Song, 1999. *Stephanopogon apogon* is distinguished from the others because it lacks anterior barbs.

Stramenopiles Patterson, 1989

***Actinomonas mirabilis* Kent, 1880 / *Pteridomonas danica* Patterson et Fenchel, 1985 (Fig. 17c)**

Description: The one cell observed was about 7 μm long, with one slightly thickened flagellum emerging from a small apical depression. The flagellum had an undulating beat and was about 1.5 times the cell length. The cell swam rapidly or attached to the substrate by a long posterior stalk (about 20 μm). Small particles were present on the cell surface. This species was observed in a sediment sample.

Remarks: *Pteridomonas danica* can be distinguished from *Actinomonas mirablis* by having flagellar transitional rings visible only at the ultrastructural level (Larsen 1985a, Larsen and Patterson 1990). At the light microscopical level, *A. mirabilis* and *P. danica* are hard to distinguish with confidence. *Actinomonas mirabilis* more frequently has posterior or lateral arms and more commonly has two anterior wreaths of arms (Lee and Patterson 2000). The arms were not seen here. Both taxa have been reported from marine sites worldwide

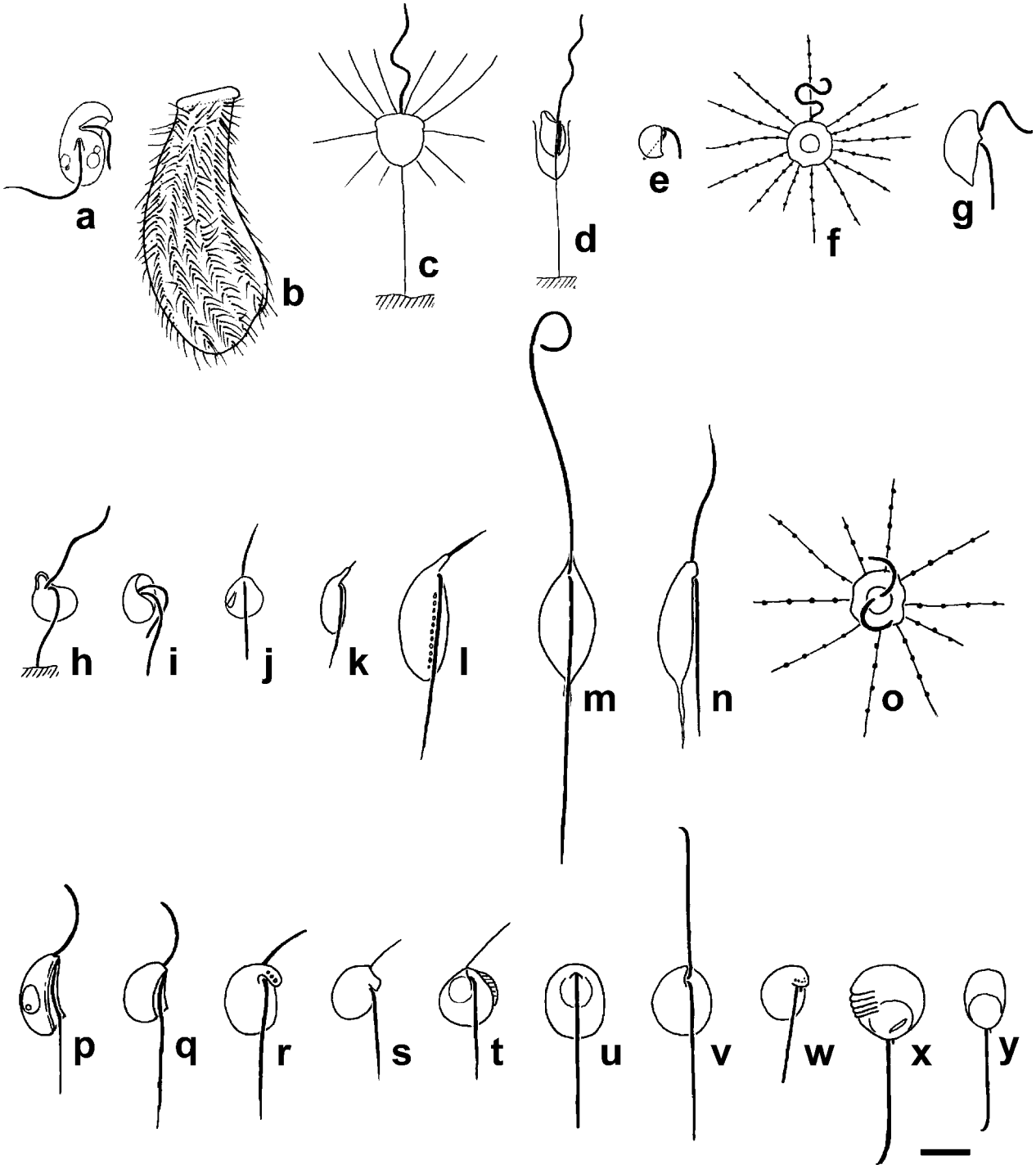

Fig. 17. (**a**) *Colpodella unguis*, (**b**) *Stephanopogon apogon,* (**c**) *Actinomonas mirabilis* / *Pteridomonas danica*, (**d**) *Biocosoeca gracilipes* (**e**) *Cafeteria roenbergensis*, (**f**) *Ciliophrys infusionum*, (**g**) *Pendulomonas adriperis*, (**h**) *Pseudobodo tremulans*, (**i**) *Developayella elegans*, (**j**) *Caecitellus parvulus*, (**k**) *Amastigomonas debruynei*, (**l**) *Amastigomonas mutabilis*, (**m**) *Cercomonas longicauda*, (**n**) *Cercomonas* sp., (**o**) *Massisteria marina*, (**p**) *Carpediemonas bialata*, (**q**) *C. membranifera*, (**r**) *Ancyromonas melba*, (**s**) *Ancyromonas sigmoides*, (**t**) *Ancyromonas sinistra*, (**u**) *Clautriavia cavus*, (**v**) *Glissandra innuerende*, (**w**) *Metopion fluens*, (**x**) *Metromonas grandis*, (**y**) *M. simplex*. Scale bar - 5 μm

(Lee and Patterson 1998, 2000), but not, to our knowledge, from markedly hypersaline habitats.

***Bicosoeca gracilipes* James-Clark, 1867 (Figs 17d, 18b-c)**

Description: Cells are oval, 3.5-5 μm long (n = 2) and lie in a cylindrical lorica about 9 μm long. Two flagella insert in a small fold at the anterior end of the cell. The anterior flagellum is about 15 μm long and beats with a sine-wave. The posterior flagellum is about 1.5 times the cell length, lies in a ventral groove and attaches to the lorica. The lorica attaches to the substrate by a pedicel that is about 15 μm long. Cells were observed in mat and sediment samples.

Remarks: This species has been reported from marine sites at Australia, England, Gulf of Elat and the USA (James-Clark 1867; Kent 1880-2; Wailes 1928/9, 1939; Thomsen 1978; Tong 1997b; Tong *et al*. 1998). The identities of species in *Bicosoeca* are not clear because nominal species are frequently distinguished by minor differences in cell or lorica size or shape (Tong 1997a). Generally, our observations are in agreement with those of Tong (Tong 1997b, Tong *et al*. 1998). *Bicosoeca gracilipes* is similar to *B. vacillans* Stôlc, 1888 in having a cylindrical lorica, but can be distinguished by the smaller size - *B. vacillans* has a lorica that is 17-25 μm long. It is distinguished from *B. conica* Lemmermann, 1914 by its cylindrical lorica. *Bicosoeca gracilipes* is distinguished from *B. epiphytica* Hilliard, 1971 by its smaller size of both the lorica and cell.

***Cafeteria roenbergensis* Fenchel et Patterson, 1988 (Fig. 17e)**

Description: Cells are 2.5-5 μm long (n = 65), D-shaped and laterally compressed, with a shallow groove on the left side. Two flagella emerge subapically and are slightly longer than the cell. Cells often attach to the substrate by the tip of the posterior flagellum, which is held in a curve. In attached cells the anterior flagellum is directed laterally. In swimming cells, the anterior flagellum is directed anteriorly and beats with a sine wave, while the posterior flagellum trails. Food particles are ingested near the posterior part of the ventral groove. Cells were observed in both sediment and mat samples.

Remarks: Our observations agree with the observations of previous observers. This species has been reported from marine sites worldwide with a cell length range of 2-10 μm (Lee and Patterson 1998, 2000). *Cafeteria roenbergensis* may not be clearly distinguished from *Acronema sippewissettensis* (Teal *et al.* 1998), which, at the light microscopical level, is held to differ from *Cafeteri*a because it has acronematic flagella.

***Ciliophrys infusionum* Cienkowski, 1876 (Figs 17f, 18d)**

Description: In the heliozoan stage, the cells are spherical, measure 4-6.5 μm across (n = 10) with a central nucleus and have one flagellum, which is held in a figure of eight and undulates slowly. Delicate pseudopodia extend radially from the body and bear extrusomes. Heliozoan cells feed on suspended bacteria that adhere to the pseudopodia and then are drawn to the cell body. Cells may change from this heliozoan stage to a swimming flagellate without pseudopodia. During swimming the flagellum beats rapidly and is directed anteriorly. Cells were observed from both mat and sediment samples.

Remarks: Generally, our observations are in agreement with observations by Larsen and Patterson (Larsen and Patterson 1990). This species has been found in marine sites worldwide (Lee and Patterson 1998, 2000). It is distinguished from the recently described *C. azurina* because the latter has peripheral nuclear material and tapering arms (Mikrjukov and Patterson 2001).

***Pendulomonas adriperis* Tong, 1997 (Figs 17g, 18f)**

Description: Cells are 5-11 μm long (n = 73), are ovoid or droplet-shaped (posteriorly pointed) with a rounded dorsal side, and are slightly flexible. Two non-acronematic flagella, each slightly longer than the cell, emerge subapically about one third of the way down the cell, apparently separated by a small protrusion. The anterior flagellum projects anteriorly and beats with an asymmetric pattern, and the posterior flagellum trails behind the body and may be held obliquely. Cells usually swim by slow rotating movements during which cells 'wag'. Cells may attach to the substrate by the tip of the posterior flagellum. Cells were observed in both habitats.

Remarks: This species has been reported from marine sites in subtropical Australia and England with a cell length of 4-9 μm (Lee and Patterson 1998, 2000). It can be distinguished from all bicosoecids and *Pseudobodo* by the orientation and beat pattern of the flagella during feeding. Moriya *et al*. (2000) described the very similar *Wobblia lunata* without any reference to *Pendulomonas*. *Wobblia* was later said to be distin-

guished from *Pendulomonas* because *Wobblia* has a small papillum between the flagella, and contains a double helix in the flagellar transition zone, rather than a single helix (Moriya personal communication). *Pendulomonas* also has a papillum, but it was described as a ridge in Tong (Tong 1997b). We believe this is only a difference in terminology. It is unclear whether *Pendulomonas* has a single or double helix as available electon micrographs are of limited quality (Fig. 19e in Tong 1997b). In all other respects *W. lunata* and *P. adriperis* appear indistinguishable (Lee and Tong personal communcations). *Wobblia lunata* is here regarded as a junior synonym of *P. adriperis*.

***Pseudobodo tremulans* Griessmann, 1913 (Figs 17h, 18i)**

Description: Cells are 4-5 μm long (n = 2) with a prominent anterior collar. Two flagella insert at the base of the collar. The anterior flagellum has a sine-wave beat pattern and is about 2.5 times the cell length. The posterior flagellum is about twice the cell length. During swimming the anterior flagellum is directed anteriorly and the posterior flagellum trails. Cells often attach to the substrate by the tip of the posterior flagellum. Cells were observed in mat samples.

Remarks: This species has been found in marine sites worldwide, with a cell length of 3-8 μm (Lee and Patterson 1998, 2000). It can be distinguished from *Cafeteria minuta* (Ruinen, 1938) Larsen et Patterson, 1990 by the presence of the collar. The cells described here have a slightly shorter anterior flagellum than in previous reports.

Stramenopiles *incertae sedis*

***Developayella elegans* Tong, 1995 (Fig. 17i)**

Description: Cells are about 5 μm long (n = 25) and ovoid, with two flagella emerging from a depression in the right anterior part of the ventral side of the cell. The anterior flagellum is about 1.3 times the cell length and the posterior flagellum is about 1.5 times the cell length. Cells attach to the substrate by the posterior flagellum. In attached cells the anterior flagellum is directed laterally, is held in a curve and beats slowly with a waving motion, while the posterior flagellum beats rapidly. The cells move by swimming. Cells were observed in both habitats.

Remarks: This species has been observed from marine sites worldwide, with a cell length of 3.5-10 μm (Lee and Patterson 1998, 2000). Generally, our observations agree with the observation of Tong (Tong 1995). The beat pattern of the flagella when the cell is attached is distinctive.

Hamatores

The hamatores is a new term to refer to a possibly paraphyletic group to include *Caecitellus* and the pseudodendromonads (O'Kelly and Nerad 1998, Karpov personal communication, O'Kelly personal communication). These two taxa lack flagellar hairs but share ultrastructural characteristics with the bicosoecids and so have a relationship to the stramenopiles. At this time, it is not clear whether the hamatores includes taxa that evolved prior to the stramenopiles, or if it is a subset of stramenopiles in which flagellar hairs have been secondarily lost.

***Caecitellus parvulus* (Griessmann, 1913) Patterson, Nygaard, Steinberg et Turley, 1993 (Figs 17j, 18e)**

Description: Cells are 3-6 μm long (n = 67) and somewhat triangular or rounded. A mouth protrudes on the right ventral side of the cell. Two flagella are about 2 times the cell length; the acronematic anterior flagellum inserts apically, and beats anteriorly and stiffly. The non-acronematic posterior flagellum emerges from the anterio-ventral face of the cell and trails under the body. Cells glide slowly with the anterior flagellum sweeping the substrate. Cells were observed in both sediment and mat samples.

Remarks: *Caecitellus parvulus* has been found in marine sites worldwide and the size range 2-7 μm has been reported (Lee and Patterson 1998, 2000). Cells reported by Ruinen (1938) as *Bodo parvulus* have both flagella directed anteriorly and appear to be incorrectly identified. Our observations are in accord with Patterson *et al*. (1993) and Lee and Patterson (Lee and Patterson 2000). Amongst gliding flagellates *Caecitellus parvulus* is distingiushed by its conspicuous ventral mouth and the orientation and beat pattern of the flagella.

Apusomonadidae Karpov et Mylnikov, 1989

***Amastigomonas debruynei* De Saedeleer, 1931 (Figs 17k, 18g)**

Description: Cells are 4-7 μm long (n = 19), dorsoventrally flattened, and plastic. The anterior flagellum is about 0.5 times the cell length and inserts apically but is directed somewhat laterally. A fine, flexible sleeve surrounds the flagellum for most of its length. The acronematic posterior flagellum, 1.3-1.7 times the length of the cell, inserts near the base of the sleeve. It is

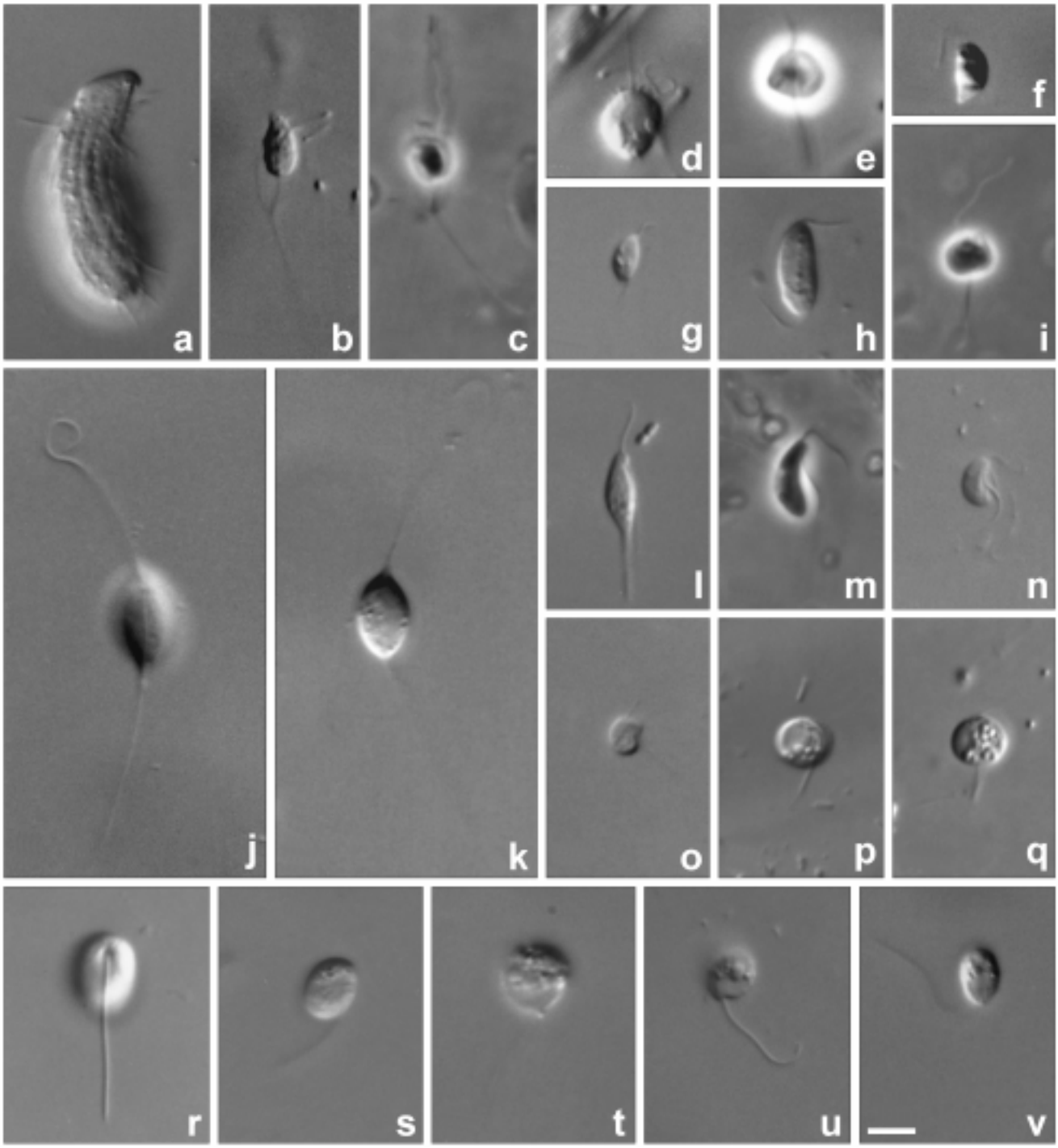

Fig. 18. (**a**) *Stephanopogon apogon,* (**b, c**) *Biocosoeca gracilipes,* (**d**) *Ciliophrys infusionum*, (**e**) *Caecitellus parvulus,* (**f**) *Pendulomonas adriperis*, (**g**) *Amastigomonas debruynei*, (**h**) *Amastigomonas mutabilis,* (**i**) *Pseudobodo tremulans*, (**j, k**) *Cercomonas longicauda*, (**l**) *Cercomonas* sp., (**m**) *Carpediemonas bialata*, (**n**) *C. membranifera*, (**o**) *Ancyromonas sigmoides*, (**p, q**) *Ancyromonas sinistra* sp. n., (**r**) *Clautriavia cavus*, (**s**) *Metopion fluens*, (**t, u**) *Metromonas grandis*, (**v**) *M. simplex*. All micrographs are DIC images except for micrographs (**e**) and (**i**), which are phase contrast images. Scale bar - 5 µm

directed posteriorly along the cell and is adherent. The cells move by gliding, with the anterior flagellum sweeping anterio-laterally. Cells were observed in both samples.

Remarks: This species has been recorded from marine sites worldwide, with cell lengths of 3-7.5 μm (Lee and Patterson 1998, 2000). The criteria for identifying species within *Amastigomonas* are given by Lee and Patterson (Lee and Patterson 2000).

***Amastigomonas mutabilis* (Griessmann, 1913) Molina et Nerad, 1991 (Figs 17l, 18h)**

Description: Cells are 12-14 μm long (n = 3), eliptical, dorso-ventrally flattened and plastic. Two flagella of equal thickness insert almost apically. The anterior flagellum is about three-quarters the length of the cell and is acronematic. A flexible sleeve surrounds its basal quarter. The posterior flagellum is more than 1.5 times the length of the cell, is acronematic and attaches loosely in a slight marginal, longitudinal groove. The groove margin includes a row of granules. Cells glide on the posterior flagellum with the anterior flagellum sweeping anteriorly. This species was observed in both mat and sediment samples

Remarks: This species has been reported from marine sites worldwide, ranging from 7 to 20 μm in size (Lee and Patterson 1998, 2000). *Amastigomonas bermudensis* Molina et Nerad, 1991 is similar in size and may be a junior synonym of *A. mutabilis* (Lee and Patterson 2000).

Cercomonadida Vickerman, 1983

***Cercomonas longicauda* Dujardin, 1841 (Figs 17m, 18j, k)**

Description: The single cell observed is about 10 μm long, is spindle shaped and flexible. Two flagella insert subapically into a small subapical depression. The anterior flagellum is non-acronematic and about 2.5 times the cell length, and its most basal part adheres to the anterior part of the body. The acronematic posterior flagellum is about 3 times the cell length, adheres to the length of the cell and has a slow sinusoidal beat during swimming. The cell moves by gliding or swimming. Strands of cytoplasm are drawn out behind the cell during movement. This species was observed in a mat sample.

Remarks: Our observations are generally in agreement with those of Dujardin (1841), Stein (1878) and Kent (1880-2). The previously reported cell lengths range from 9.4 to 48 μm and this suggests that more than one species may be included. *Cercomonas longicauda* is similar to *C. granulatus* Lee et Patterson, 2000 in general appearance, but can be distinguished by the lack of the row of granules. The anterior flagellum of *C. longicauda* is longer than that of *C. granulatus* and the posterior flagellum of *C. longicauda* appears to trail while that of *C. granulatus* beats slowly from side to side. *Cercomonas longicauda* is similar to *C. agilis* (Moroff, 1904) Mignot et Brugerolle, 1976 in cell length, but can be distinguished because of the narrow body of *C. agilis.* Species identities within *Cercomonas* are currently unclear and this genus is in urgent need of attention.

***Massisteria marina* Larsen et Patterson, 1990 (Fig. 17o)**

Description: Cells are 3-6 μm across (n = 92), and are dorso-ventrally flattened, with an irregular outline. Cells produce delicate pseudopodia with extrusomes that extend radially from the cell and normally adhere to the substrate. Two short, curved flagella arise from the dorsal side of the cell and are relatively inactive. Cells were observed in both habitats.

Remarks: This species has been reported from marine sites worldwide (Lee and Patterson 1998, 2000), with a size range of 2-9.5 μm (see Lee and Patterson 2000). The combination of extrusome-bearing pseudopodia and short, curved flagella are distinctive.

Excavates Patterson et Simpson, 1999

***Carpediemonas bialata* (Ruinen, 1938) Lee et Patterson, 2000 (Figs 17p, 18m)**

Description: Cells are 8-9 μm long (n = 2), and kidney shaped, with a longitudinal ventral groove. A fine membrane moves down along the groove. Two flagella emerge from the anterior end. The anterior flagellum bends backwards, is about the cell length and beats over the cell with a slow, sweeping motion. The posterior flagellum is acronematic and is about 1.5 times the cell length. It beats within the groove when the cell is not moving. Cells attach to the substrate by the tip of the posterior flagellum. Cells move by skidding or gliding with the anterior flagellum beating with a flicking motion. This species was observed in sediment samples.

Remarks: This species has been described from marine sites in subtropical Australia (Ruinen 1938, Lee and Patterson 2000) and previously reported cell lengths range from 6 to 14 μm. It was reported with *C. membranifera* under the name *Percolomonas membranifera* by Larsen and Patterson (1990). *Carpedimonas bialata* differs from *C. membranifera* in the cell length and shape. It is distinguished from

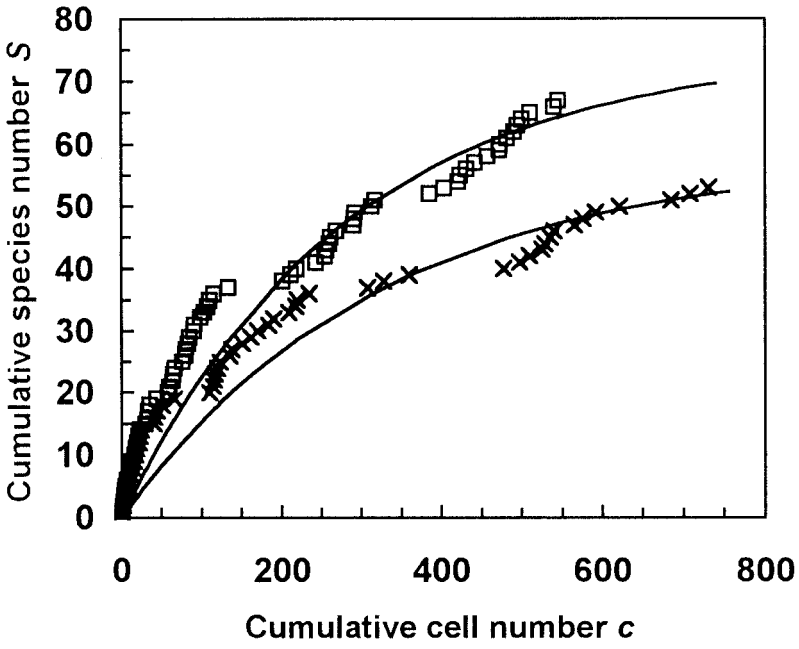

Fig. 19. Cumulative number of species observed as a function of cumulative number of cells observed; in samples of the stromatolite algal mat (crosses, regression equation $S_{mat}=58(1-0.0013^{c})$) and in samples of the surrounding sediment (open squares, regression equation $S_{sed}=75(1-0.0016^{c})$); regression curves do not saturate. Linear regression on transformed data coefficient results were $r^2 = 0.95$ for sediment and $r^2 = 0.93$ for algal mat

Jakoba libera (Ruinen, 1938) Patterson, 1990 by the length of the posterior flagellum, and from both *J. libera* and *J. incarcerata* Bernard, Simpson et Patterson, 2000 by the presence of a moving membrane and the tendency to attach to the substrate by the posterior flagellum, rather than the anterior flagellum. *Malawimonas jakobiformis* O'Kelly et Nerad, 1999 is generally smaller (4-8.5 μm) than *C. bialata,* has a groove that does not extend to the posterior end of the cell, has an anterior flagellum that crooks to the ventral side of the cell when at rest and has a non-acronematic posterior flagellum (O'Kelly and Nerad 1998). A 'moving membrane' has not been reported in *Malawimonas*.

***Carpediemonas membranifera* Ekebom, Patterson et Vørs, 1996 (Figs 17q, 18n)**

Description: Cells are 4-8 μm long (n = 46), obovate, with a longitudinal ventral groove that extends most of cell length. Cells are not rigid. Two flagella emerge from the anterior end of the cell. The anterior flagellum is about the cell length, curves dorsally and beats stiffly with a paddling motion. The acronematic posterior flagellum is 2.5-3 times the cell length and usually beats actively in the ventral groove. Cells usually move by skidding. Cells were observed in late observations of both types of sample.

Remarks: Our observations are in accordance with those of Lee and Patterson (2000). This species is most easily distinguished from *Carpediemonas bialata,* and from *Malawimonas* and *Jakoba* by the longer posterior flagellum. It thrives under low oxygen conditions (Simpson and Patterson 1999, Bernard *et al*. 2000). This species was previously reported from marine sites in Australia and Brazil (Lee and Patterson 1998, 2000).

Protista *incertae sedis*

***Ancyromonas melba* Patterson et Simpson, 1996 (Fig. 17r)**

Description: Cells are 6-8 μm long (n = 5), oval shaped, dorso-ventrally flattened, and have a ventral groove near an antero-lateral margin of the cell. The snout is set off by the ventral groove and has some granules along it. The thick, stiff anterior flagellum emerges from an anterior depression and is about the cell length. The acronematic posterior flagellum inserts subapically and ventrally, and is about 2 times the length

of the cell. Cells move by gliding with the posterior flagellum trailing behind the cell. This species was observed in mat samples.

Remarks: Our observations are in agreement with those of Patterson and Simpson (1996). This species has been reported from marine and hypersaline sites in subtropical Australia with cell lengths of 4-7 µm (Patterson and Simpson 1996, Tong *et al*. 1998 under the name of *A. magna*). This species can be distinguished from *A. sigmoides* Kent, 1880 and *A. sinistra* sp. n. because the anterior flagellum is as thick as the posterior flagellum. *Ancyromonas contorta* (Klebs, 1893) Lemmermann, 1913 has a contorted, rather than flat cell body, distiguishing it from *A. melba* and other members of *Ancyromonas.*

***Ancyromonas sigmoides* Kent, 1880 (Figs 17s, 18o)**

Description: Cells are 3-8 µm long (n = 80), oval in outline, and dorso-ventrally flattened, with a shallow ventral groove near an antero-lateral margin of the cell. A thin stiff anterior flagellum, which emerges from an anterior depression, can be easily overlooked, but it was present in one cell. The posterior flagellum is 1.5-2 times the length of the cell and is acronematic. Cells move by gliding with the posterior flagellum trailing. Cells were in both samples.

Remarks: This species has been reported from marine sites worldwide with previously reported cell lengths of 2-7.6 µm (Lee and Patterson 1998, 2000). Our observations are in agreement with those of previous authors. This species can be distinguished from *A. sinistra* sp. n. by the smoothness of the left anterior-lateral margin and the lesser degree of flattening. It can be distinguished from *A. melba* Patterson et Simpson, 1996 by the thinness/absence of the anterior flagellum. *Ancyromonas sigmoides* can be confused with *Metopion fluens* Larsen et Patterson, 1990, but is distinguished by the anteriorly directed flagellum; the second flagellum in *M. fluens*, when visible, is thicker and is directed posteriorly, and there is no apical depression.

***Ancyromonas sinistra*. sp. n. (Figs 17t, 18p-q)**

Diagnosis: *Ancyromonas* with rugose anterior-lateral margin and thin anterior flagellum.

Description: Cells are 5-6 µm long (n = 4), round, dorso-ventrally flattened with a rugose flange on the left anterior-lateral margin of the cell and with two flagella. The anterior flagellum inserts apically, is thin, is easy to overlook and is slightly longer than the cell. It is directed anterior-laterally and beats with a slow motion. The acronematic posterior flagellum emerges subapically from a ventral depression and is 2-2.5 times the cell length. Cells move by gliding. Cells were observed in both sediment and mat samples. This species was also observed in Botany Bay, Australia (Lee unpublished observation). The photographs are of cells from Botany Bay.

Remarks: Like the common and widespread species, *Ancyromonas sigmoides*, this species has two flagella emerging in different directions. The anterior flagellum in both species arises almost apically and is extremely thin. Both taxa have an anterio-lateral row of extrusomes. We assign this species to *Ancyromonas* because of these characters. *Ancyromonas sinistra* is distinguished from *A. melba* and *A. sigmoides* in that the margin containing the presumptive extrusomes is very thin and the presumptive extrusomes are more prominent. Also the anterior flagellum in *A. sinistra* is thin entirely. The cell body is flatter than in *A. sigmoides* and the posterior flagellum lies more directly under the cell. This species is distinguished from *A. contorta* (Klebs, 1893) Lemmermann, 1913 because it does not have a contorted body.

***Clautriavia cavus* Lee et Patterson, 2000 (Figs 17u, 18r)**

Description: Cells are 6-10 µm long (n = 2), oval, flattened and rigid. The single flagellum emerges from a ventral subapical depression, is directed posteriorly, and is about 2 times the cell length. The ventral face of the cell is slightly concave. The cell surface is warty. Food particles are seen in the posterior part of the cell. The cells move slowly by gliding with the posterior part slightly raised above the substrate. The nucleus is located subapically. This species was observed in sediment samples.

Remarks: This species was first described from subtropical Australia (Lee and Patterson 2000) with a cell length of 8-10 µm, and our observations agree with that observation. The genus *Clautriavia* contains three nominal species, *C. cavus* Lee et Patterson, 2000; *C. mobilis* Massart, 1900 and *C. parva* Schouteden, 1907. *Clautriavia cavus* can be distinguished from *C. mobilis* because it is half of the size and because its nucleus is located subapically, rather than posteriorly. *Clautriavia parva* may be the gliding stage of *Metromonas* (Patterson and Zölffel 1991). *Clautriavia*

Fig. 20. Dendrogram showing the (Bray-Curtis) similarity (%) between the communities from 38 surveys; taxonomic information based on species. Species data used for this analysis were from Lee and Patterson (1998), Lee (2001) and the present study. All sites are marine (inclusive of brackish and hypersaline) unless otherwise specified. *: Australian sites

can be distinguished from *Protaspis* because it has only one flagellum. It is distinguished from *Allantion* because *Clautriavia* does not have a rostral prominence at the anterior end of the cell and has a concave ventral face.

***Glissandra innuerende* Patterson et Simpson, 1996 (Fig. 17v)**

Description: Cells are 4.5-8 µm long (n = 25), ovoid, somewhat flattened, with a slight anterior depression with a raised right margin. Two flagella insert subapically and laterally into the groove. Both flagella are 2-2.5 times the cell length. The cell glides on both flagella with the anterior flagellum held directly anteriorly and the posterior flagellum trailing. This species was observed in both habitats.

Remarks: This species was found at marine sites in subtropical Australia with cell lengths of 5-8 µm (Patterson and Simpson 1996, Lee unpublished observation). The organism referred to as *G. innurende* by Lee and Patterson (2000) is different from *G. innurende sensu* Patterson et Simpson, 1996 because the cells were slightly flattened laterally and its flagella are thickened. *Glissandra innurende* Patterson et Simpson, 1996 is distinguished from other similar gliding flagellates (e.g. *Protaspis*) because of its tendency to glide on both anterior and posterior flagella simultaneously.

***Metopion fluens* Larsen et Patterson, 1990 (Figs 17w, 18s)**

Description: Cells are 4-8 µm long (n = 20), ovate and laterally compressed, with a small lobe anterior to the flagellar insertion. Small bodies are usually seen in the proximal anterior part of the cell. The two unequal flagella emerge in parallel from a ventral groove that is located in the left side of the cell at the base of the lobe. The long flagellum is 1.7-2 times the cell length. The short flagellum may be difficult to see. Cells contained small granules in the posterior part of the cell. The nucleus is located near the groove. Cells move by gliding. Cells were observed in both habitats.

Remarks: This species has been found in marine sites worldwide, with a cell length of 2-9 µm (Lee and Patterson 1998, 2000). Generally, our observations are in accordance with previous reports. *Metopion fluens* can be distinguished from *Ancyromonas sinistra* sp. n. by the broad anterior end and because the short flagellum of *M. fluens* is directed posteriorly, rather than anteriorly.

***Metromonas grandis* Larsen et Patterson, 1990 (Figs 17x, 18t-u)**

Description: Cells are 6.5-8 µm long (n = 32), roundish in profile and dorso-ventrally flattened. One side of the cell is ridged. Two flagella insert posteriorly in parallel. The major flagellum is 1.5-2 times the length of the cell. The minor flagellum is very short, is inactive, inserts to the right of the major flagellum, away from the ridged surface. The nucleus is located near the flagellar insertion. Cells attach to the substratum by the longer flagellum and move with a pendulum action. Cells may also glide on the longer flagellum. Cells were observed in both mat and sediment samples.

Remarks: *Metromonas grandis* has been reported from marine sites worldwide, with cell lengths of 5-14 µm (Lee and Patterson 1998, 2000). Generally, our observations are in agreement with the previous authors. This species may be distinguished from *M. simplex* by the typically larger size and the ridged margin.

***Metromonas simplex* (Griessmann, 1913) Larsen et Patterson, 1990 (Figs 17y, 18v)**

Description: Cells are about 6.5 µm long (n = 2), are abovate, dorso-ventrally flattened (especially posteriorly), and have a smooth surface. Two flagella of unequal length arise from the posterior end. The major flagellum is about 1.5 times the cell length. The minor flagellum is very short, inactive, inserts to the right of the major flagellum and may be difficult to see. Cells may attach to the substrate by the major flagellum and move with a pendulum action. Cells may also glide. Cells were observed in sediment samples.

Remarks: *Metromonas simplex* has been found in marine sites worldwide and has previously reported cell lengths from 3 to 9 µm (Lee and Patterson 1998, 2000). It can be distinguished from *M. grandis* because of the lack of the ridged margin and its smaller size.

Further species of the genera *Amphidinium* (Figs 16i, j), *Cercomonas* (Figs 17n, 18l), *Gyrodinium, Hemidinium, Ploeotia* (Figs 9e, f 11a-f)*, Sphenomonas* (Figs 9g, 11j, k), *Prorocentrum, Rhynchobodo* and *Scrippsiella* were found but could not be identified.

Cumulative species curves for samples from sediments and algal mats are given in Fig. 19. The curves indicated that further sampling would be necessary to find the total number of species present in the sampling area, because the regressed curves were not saturated (had not yet reached the asymptote). The sediment sampling area had significantly more species present than the algal mat sampling area (t statistic = 10.40, $df = 9$, $p<0.01$).

The abundance of heterotrophic flagellates on the stromatolite algal mat and in the surrounding sediments was found to be 1.23×10^5 and 1.19×10^5 cells/cm^3, respectively. Bacterial abundance (excluding cyanobacterial) was 2.21×10^8 and 2.87×10^8 cells/cm^3 respectively.

The results of the cluster analysis to assess similarity of communities (Lee and Patterson 1998) shows that the communities do not cluster with other studies from the same region, but rather with a group of communities from marine benthic habitats with standard salinities [Fig. 20 drawn from data of Lee and Patterson (1998), and of Lee (2001) which includes the present study].

DISCUSSION

The capacity of microbial communities of the hypersaline sediments of Hamelin Pool to form stromatolites led us to question whether the principle consumers of bacteria, the flagellates, formed an impoverished or an unusual community at this site. The results of the cluster analysis showed that, despite the hypersalinity and the remoteness of the site, the community was not distinctive. The communities of flagellates from Hamelin Pool

appear to be essentially indistinguishable from communities from normal salinity communities of the eastern seaboard of Australia (Fig. 20). They separate from the communities from much higher salinities and which, like other extreme communities, are species poor. This location of the benthic community in the dendrogram parallels the community of water column flagellates from a similar region, but contrasts with the previous study of flagellates from a similar location (Patterson and Simpson 1996). Prior to this investigation, the community of Hamelin Pool in Shark Bay clustered with other hypersaline habitats (Lee and Patterson 1998), but is now repositioned after more sampling. Patterson and Simpson reported only 17 species whereas this study increased the number to 77 species. We attribute the change in the clustering to under-reporting in the earlier study. The change in position may also reflect the differing taxonomic expertise and judgement of the workers who carried out the alpha taxonomy. Either way, this instability reflects the need to be vigilant in recognizing the continued vulnerability of our insights to extrinsic factors (Patterson and Lee 2000).

We can find no evidence in support of the argument that an impoverished or modified community of flagellates contributes to the formation of stromatolites. As noted above, the community is not distinctive. The cumulative species curves indicate that in the area where we sampled, the sediment is more species rich than the adjacent microbial mat. Further sampling on a wider spatial scale would be necessary to establish whether this is a consistent pattern in Hamelin Pool. The abundances of both flagellates and bacteria are within the range for marine sediments generally (e.g., Lee and Patterson submitted, references within).

We conclude that the flagellate community associated with the stromatolites in Hamelin Pool is not distinctive and this factor does not play a role in facilitating the development of stromatolites.

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ACTA
PROTOZOOLOGICA

New Contribution to the Morphology and Taxonomy of Four Marine Hypotrichous Ciliates from Qingdao, China (Protozoa: Ciliophora)

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Summary. The morphology and infraciliature of four marine hypotrichous ciliates, *Bakuella agamalievi* Borror & Wicklow, 1983, *Pseudokeronopsis flavicans* (Kahl, 1932) Borror & Wicklow, 1983, *Holosticha heterofoissneri* Hu & Song, 2001 and *Cyrtohymena marina* (Kahl, 1932), collected from the coastal water of Qingdao (Tsingtao), China are investigated and redescribed. *Pseudokeronopsis flavicans* (Kahl, 1932) is characterized by: yellow-coloured, flexible body, *in vivo* 200-300 x 40-55 µm that is slender ribbon-shape with a narrowed caudal portion; a bicorona comprising 5-9 pairs of frontal cirri; one buccal, 2 frontoterminal and 3-6 transverse cirri; midventral rows consisting of about 25-40 pairs of cirri which terminate caudally about 15 µm above the inconspicuous transverse cirri; 46-66 adoral membranelles; on average, 52 left and 60 right marginal cirri; 3-4 dorsal kineties; numerous macronuclear segments; colourless, sub-pellicular blood-cell-shape granules distributed over whole cell and bright yellow-brownish cortical granules grouped around cilia or cirri; one contractile vacuole lying in anterior 2/5 of body. The diagnosis for *Bakuella agamalievi* is as follows: 100-150 x 30-50 µm *in vivo* with elongated body shape; about 30 adoral membranelles; on average, 33 left and 43 right marginal cirri; 4-7 frontoterminal, 4 frontal, one buccal and 4-7 transverse cirri; 9-13 pairs of midventral cirri distributed mostly in frontal area; 3-6 ventral rows with 3-5 cirri each; consistently 3 dorsal kineties; numerous macronuclei; cortical granules grouped in short rows; one contractile vacuole in anterior 1/3 of body. *Holosticha heterofoissneri* is characterized by; slender, fusiform body, 110-150 x 30-60 µm *in vivo*; 14-21 macronuclear segments forming an elongate U-shape; single, post-equatorial contractile vacuole; small, sparsely distributed cortical granules; 50 adoral membranelles; one anteriorly positioned buccal cirrus; 4 frontal, 2 frontoterminal and 12-17 transverse cirri; midventral rows comprising about 13 pairs of cirri, which extend almost to caudal end; 5 dorsal kineties. The little-known *Cyrtohymena marina* is characterized by: body elongate oval to long elliptical shape, 100-150 x 30-50 µm *in vivo*; one pre-equatorially positioned contractile vacuole; 32-42 adoral membranelles; consistently 8 frontal, 5 ventral, 5 transverse and 2 to 3 caudal cirri; 17-21 left and 19-25 right marginal cirri; 2 to 3 caudal cirri; 9-11 dorsal kineties; 2 macro- and 2-5 micronuclei; 4 complete, and one fragment-like, dorsal kineties; cortical granules fine, inconspicuous, and very sparsely distributed.

Key words: *Bakuella agamalievi,* Ciliophora, *Cyrtohymena marina, Holosticha heterofoissneri,* Hypotrichida, marine ciliate, morphology and infraciliature, *Pseudokeronopsis flavicans*.

INTRODUCTION

Despite recent advances in ciliate taxonomy, species separation and identification among some groups within the hypotrichs remains problematic. This is especially true of marine hypotrichs, as compared with those inhabiting terrestrial and freshwater biotopes, because many marine forms remain insufficiently studied. Some recent studies have shown that the morphological descriptions of many species are confused or even highly contradictory (Kahl 1932, Borror 1972, Dragesco and Dragesco-Kernéis 1986, Foissner 1987, Carey 1991,

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Petz *et al.* 1995, Wilbert 1995, Song and Packroff 1997, Berger 1999, Song and Hu 1999).

The current work forms part of a faunistic study of ciliates in the coastal waters of the north China seas, which has been carried out for a period of over 10 years. In this paper, 4 previously known but poorly described species of hypotrichs, collected from offshore mariculture waters near Qingdao, China, are redescribed following examination using modern techniques.

MATERIALS AND METHODS

Sample sites. All samples were collected from mariculture waters near the coast of Qingdao (Tsingtao, 36°08'N; 120°43'E), China. *Bakuella agamalievi* and *Cyrtohymena marina* were isolated (6 May 1997) from a semi-closed pond used for mollusc culture (salinity 10-15 ‰). *Pseudokeronopsis flavicans* was isolated on two occasions (6 and 12 May 1997) from the same sampling site as above. Two populations of *Holosticha heterofoissneri* were collected on separate occasions (31 March 1995 and 28 October 1997) from an open scallop (*Chlamys farreri*) farming pond (salinity 31-32 ‰).

General methods. After collection and isolation, specimens were maintained in the laboratory for several weeks, either as pure or raw cultures in Petri dishes, with rice grains as food source for bacteria. Observations on living morphology were undertaken using Nomarski optics. Protargol staining (Wilbert 1975) was performed for revealing the infraciliature and nuclear apparatus. Counts and measurements were made at a magnification of x1250. Drawings were made with the help of a camera Lucida. Terminology is mainly according to Foissner (1982) and Hemberger (1985).

New type specimens. Since no type specimens of *Bakuella agamalievi, Cyrtohymena marina* or *Pseudokeronopsis flavicans* are known to exist, one neotype slide of protargol impregnated cells of each species has been deposited in the collection of the Natural History Museum, London, UK with the following registration numbers: *Bakuella agamalievi,* 2001:1z:z8:01; *Cyrtohymena marina,* 2001:1z:z8:02; *Pseudokeronopsis flavicans,* 2001:1z:z8:03. In addition one paraneotype of each has been deposited in the Laboratory of Protozoology, Ocean University of Qingdao, People's Republic China.

RESULTS

Bakuella agamalievi Borror & Wicklow, 1983 (Figs 1-8, 52-53; Table 1)

Syn. *Holosticha manca sensu* Agamaliev, 1972

Borror and Wicklow (1983) reclassified this species but did not give a clear definition; hence we supply here the species diagnosis based on the present studies.

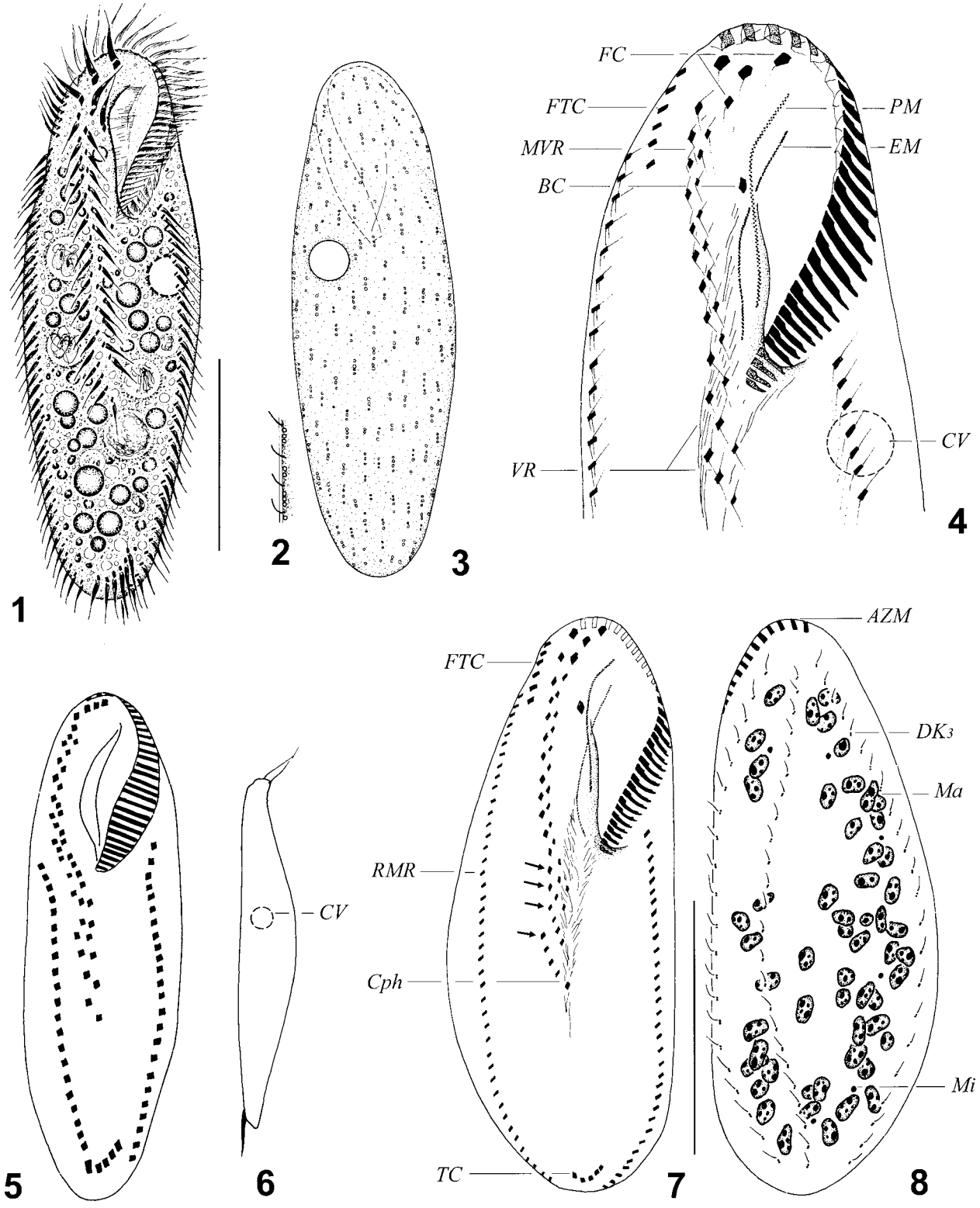
Improved diagnosis. Marine *Bakuella* with elongated body shape, 100-150 x 30-50 μm *in vivo*; about 30 adoral membranelles; on average, 33 left and 43 right marginal cirri; 4-7 frontoterminal, 4 frontal, one buccal and 4-7 transverse cirri; 9-13 pairs of midventral cirri distributed mostly in frontal area; 3-6 ventral rows with 3-5 cirri each; consistently 3 dorsal kineties; numerous macronuclei; cortical granules grouped in short rows; one contractile vacuole in anterior 1/3 of cell.

Morphological description. Body shape generally constant, length to width ratio about 3:1 with both ends well rounded; cell margins almost parallel with slight bulge in middle portion (Fig. 1). Buccal field about 1/3 to 2/5 body length (Fig. 1). Pellicle rigid, cortical granules colorless or slightly greenish when observed at low magnification, about 0.8 μm across, typically grouped together and sparsely arranged in short rows on both ventral and dorsal sides of cell (Figs 2, 3, 53). Cytoplasm colourless to grayish, usually containing numerous shiny globules (2-5 μm in diameter), which render the cell completely opaque. Food vacuoles large, usually several in number, and containing flagellate, small ciliates or bacteria. Contractile vacuole (CV) situated near left border about 1/3 to 2/5 of the way down the body (Figs 1, 3). Pulsation interval rather long (up to 5 min). About 50 ellipsoid macronuclear nodules, each *ca* 3-5 μm long and containing several large nucleoli; micronuclei globular, only recognized after protargol staining (Fig. 8).

Locomotion moderately fast, crawling on substrate, sometimes jerking back and forth.

Adoral zone of membranelles about 1/3 of cell length with distal end only slightly curved towards right border. Bases of membranelles *ca* 7-8 μm in length. Cilia of membranelles 15-20 μm long. Undulating membranes relatively long, slightly curved and noticeably crossed (Figs 4, 7).

Somatic ciliature typical of genus. Three slightly enlarged, and one smaller, frontal cirrus arranged in anteriormost portion of frontal area; cirri about 20 μm long. One buccal cirrus near anterior 1/3 of undulating membranes (Fig. 4). Mostly 6 frontoterminal cirri relatively fine, located at anterior terminus of right marginal row (Figs 4, 7). Midventral rows composed of closely spaced oblique pairs of cirri and extending to about the level of the cytostome (Fig. 7). Posterior to these midventral rows are (usually) 4-5 ventral rows (arrows in Fig. 7) each with 3 to 5 cirri, which are also obliquely



Figs 1-8. *Bakuella agamalievi* from life (1-3, 6), after silver nitrate (5) and protargol impregnation (4, 7, 8). **1** - ventral view of a typical specimen; **2** - optical section of cortex, to show the cortical granules; **3** - dorsal view from life; note the arrangement of the cortical granules; **4** - anterior portion of infraciliature, to show the buccal apparatus and the ciliature on ventral side; **5** - ventral side (redrawn from Agamaliev, 1972, called *Holosticha manca*); **6** - lateral view from life; **7, 8** - ventral and dorsal views of infraciliature; arrows in Fig. 7 mark the short ventral rows. AZM - adoral zone of membranelles, BC - buccal cirrus, Cph - cytopharynx, CV - contractile vacuole, DK3 - dorsal kinety No. 3, EM - endoral membrane, FC - frontal cirri, FTC - frontoterminal cirri, Ma - macronuclei, Mi - micronuclei, MVR - midventral rows, PM - paroral membrane, RMR - right marginal row, TC - transverse cirri, VR - ventral rows. Scale bars - 50 μm

Table 1. Morphometrical data of *Bakuella agamalievi* Borror & Wicklow, 1983 (upper line) and *Holosticha heterofoissneri* (lower line). All data are based on protargol impregnated specimens. Measurements in µm. Abbreviation: CV - coefficient of variation in %; Max - maximum, Min - minimum, n - number of cells measured; SD - standard deviation; SE - standard error of mean

Character	Min	Max	Mean	SD	SE	CV	n
Length of body	97	131	115.7	11.38	3.79	9.8	19
	90	133	116.5	18.02	6.37	15.5	8
Width of body	48	70	58.8	7.12	2.25	12.1	19
	30	56	44.9	10.65	4.03	23.8	7
Length of buccal field	37	52	43.8	5.62	1.69	12.8	19
	44	52	48.7	2.69	1.02	5.5	7
Number of membranelles	26	37	30.9	4.14	1.57	13.4	7
	41	49	44.6	3.25	1.08	7.3	9
Number of frontal cirri*	4	4	4	0	0	0	14
	4	4	4	0	0	0	12
Number of buccal cirri	1	1	1	0	0	0	14
	1	1	1	0	0	0	12
Number of frontoterminal cirri	4	7	5.6	0.88	0.29	15.9	9
	2	2	2	0	0	0	12
Number of transverse cirri	4	7	5.2	1.14	0.36	21.8	10
	12	17	14.3	1.49	0.50	10.4	8
Number of pairs of cirri in midventral rows	9	13	10.9	1.46	0.52	13.4	7
	12	15	13.4	1.27	0.48	9.5	7
Number of ventral rows**	3	6	4.0	1.16	0.37	28.9	10
	-	-	-	-	-	-	-
Number of cirri in left marginal row	30	38	32.9	3.67	1.39	11.2	7
	19	25	21.8	2.25	0.80	10.4	10
Number of cirri in right marginal row	40	47	43.4	3.21	1.43	7.4	7
	27	33	30.4	2.01	0.67	6.6	10
Number of macronuclei	47	60	-	-	-	-	4
	14	21	17.3	1.96	0.57	11.4	12
Length of macronuclei	5	7	-	-	-	-	-
	6	12	8.8	2.10	0.66	23.8	16
Width of macronuclei	3	5	-	-	-	-	-
	4	10	7.0	2.31	0.73	32.9	16
Number of dorsal kineties	3	3	3	0	0	0	14
	5	5	5	0	0	0	16

* Not including buccal cirrus
** Short rows behind the midventral rows

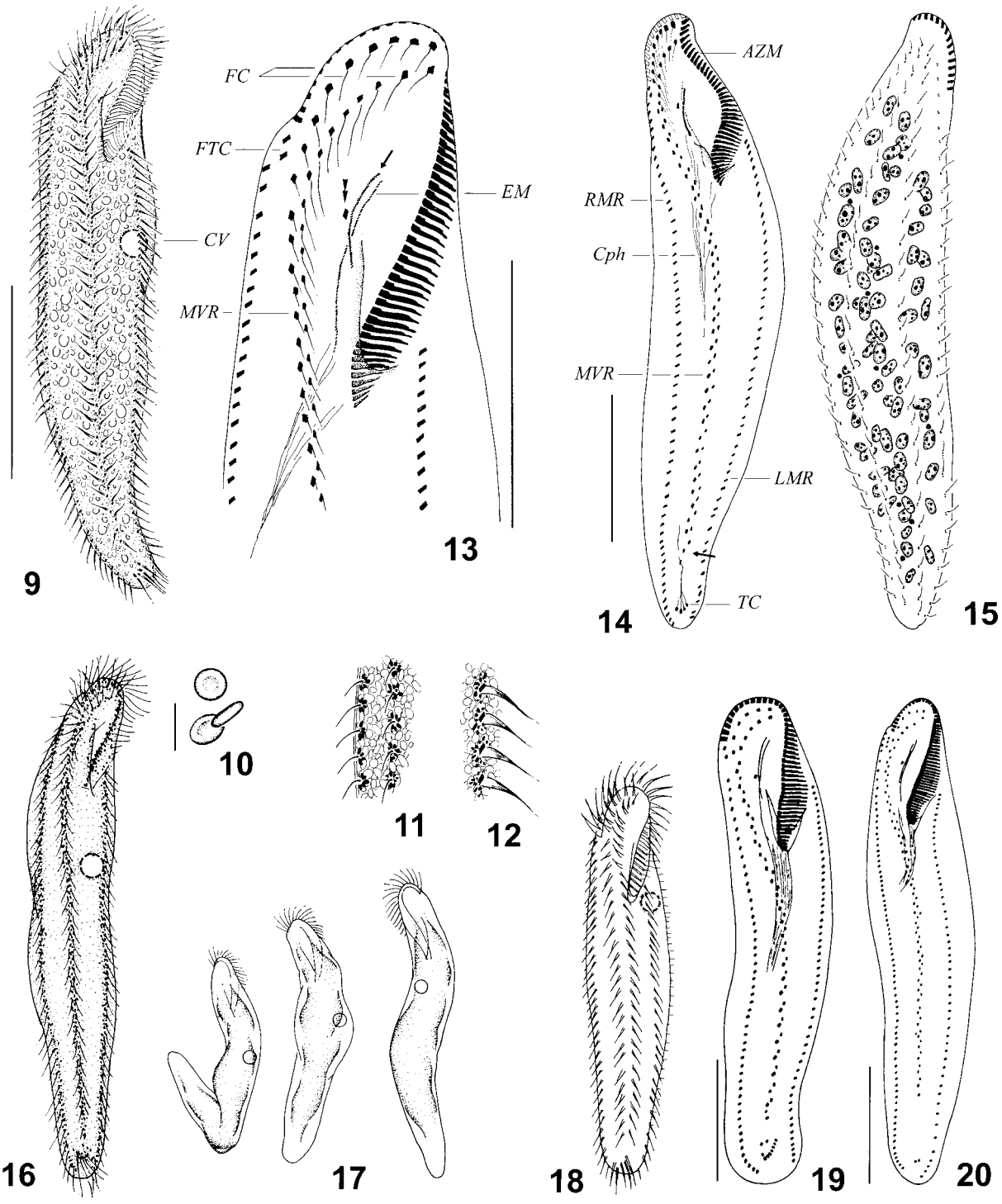
arranged and terminate at about posterior 1/3 of cell length. Almost always 5 fine transverse cirri, terminally positioned with relatively short cilia (*ca* 15 µm long), which extend only slightly beyond posterior margin of cell when observed *in vivo* (Fig. 1). Marginal rows separated posteriorly.

Dorsal cilia 2-3 µm long, consistently arranged in 3 dorsal kineties, which extend over entire length of cell; caudal cirri absent (Fig. 8).

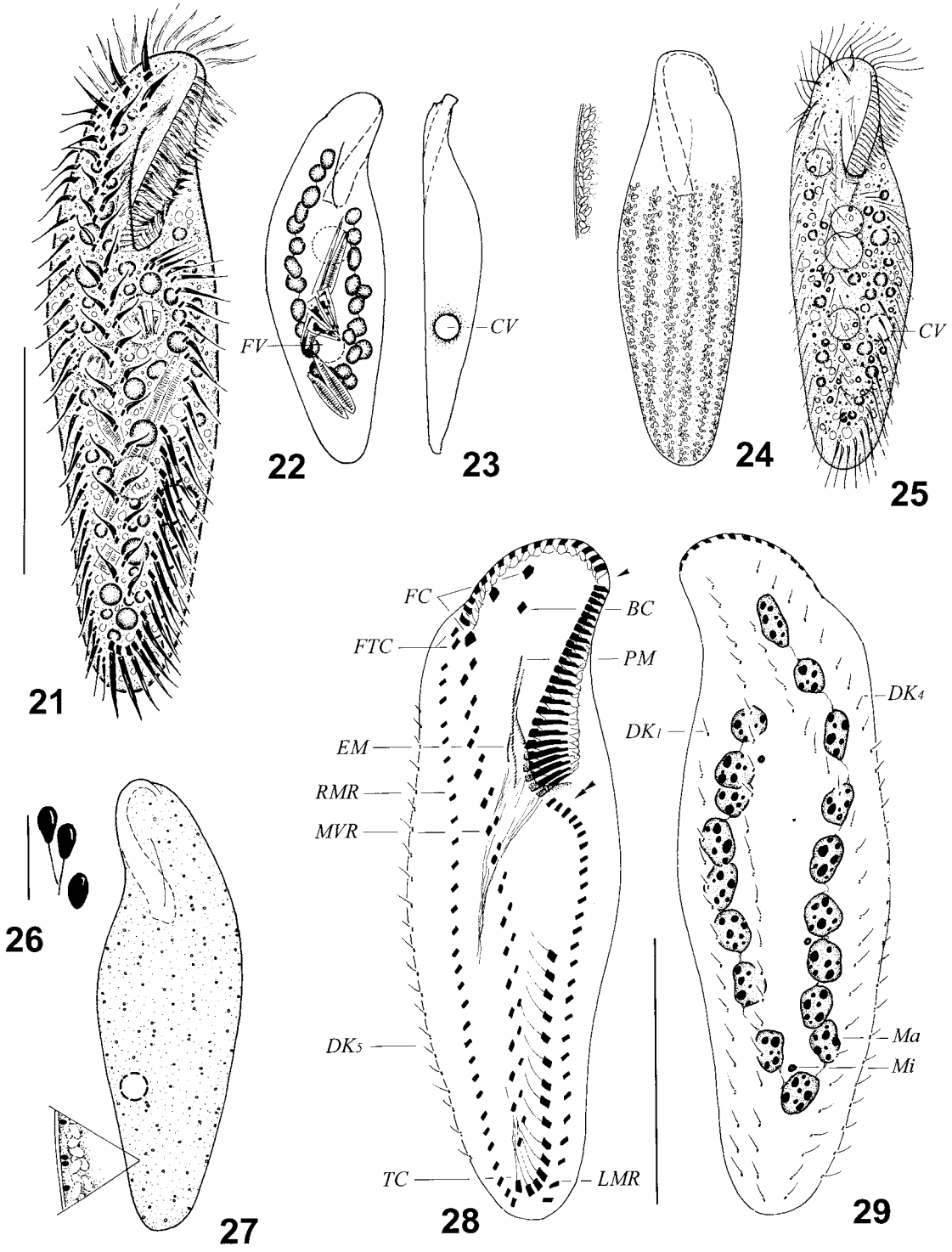
Comparison and discussion. This species was originally described by Agamaliev (1972) under the name *Holosticha manca*. We agree with Borror and Wicklow (1983) that it should belong to the genus *Bakuella* because it possesses several obliquely oriented ventral rows behind the midventral rows. The Qingdao population is considered conspecific with *B. amagalievi* because of its body shape and size, biotope, nuclear apparatus and the general pattern of its ciliature.

Compared with other congeners, this species can be recognized by having fewer and shorter ventral rows, fewer buccal cirri, a significantly smaller body size and a different biotope (Agamaliev and Alepterov 1976; Alekperov 1982, 1992; Mihailowitsch and Wilbert 1990; Song *et al.* 1992).

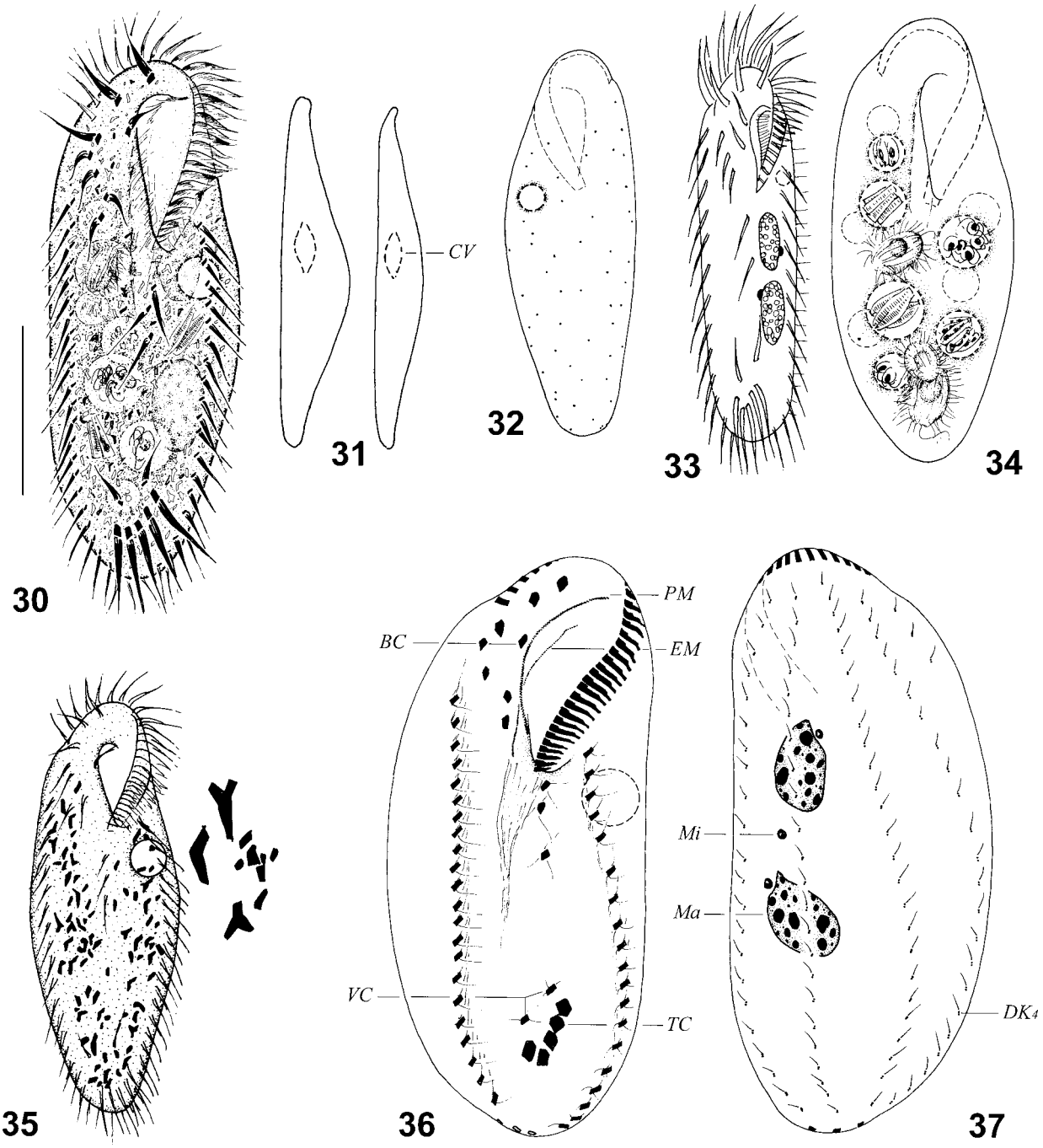
Morphologically, this species is also similar to *Holosticha manca* Kahl, 1932. According to the redescription by Song and Wilbert (1997), however, the latter is relatively smaller (42-106 *vs.* 97-131 µm after protargol impregnation), has fewer adoral membranelles



Figs 9-20. *Pseudokeronopsis flavicans* (9-18), *Pseudokeronopsis carnea* (19) and *Pseudokeronopsis flava* (20) from life (9-12, 16-18) and after protargol impregnation (13-15, 19, 20). **9** - ventral view of a normal individual; note the contractile vacuole positioned about 2/5 of the way down the cell; **10** - to show the colourless "cortical granules"; **11, 12** - to denote the distribution of the pigments; **13** - ventral view, to show the details of the infraciliature in the anterior portion; arrow indicates the short paroral membrane, while the double-arrowheads mark the buccal cirrus; **14, 15** - ventral and dorsal views of the infraciliature; arrow in Fig. 14 marks the position where the midventral rows terminate; **16** - ventral view of a slender form; note that the yellow granules (pigments?) are along the margins and the median of the cell; **17** - dorsal views, to show the appearance of some extended, bending or slightly contracted individuals; **18** - ventral view (redrawn after Kahl, 1932); **19, 20** - ventral view of infraciliature (after Wirnsberger *et al.* 1987); note that the midventral rows in Fig. 20 are significantly shortened and terminate well above the transverse cirri. AZM - adoral zone of membranelles, Cph - cytopharynx, CV - contractile vacuole, EM - endoral membrane, FC - frontal cirri, FTC - frontoterminal cirri, LMR - left marginal row, MVR - midventral rows, RMR - right marginal row, TC - transverse cirri. Scale bars: 9 -100 μm; 13-15, 19, 20 - 50 μm



Figs 21-29. *Holosticha heterofoissneri* from life (21-27) and after protargol impregnation (28, 29). **21** - ventral view of a typical individual; **22** - to show the arrangement of macronuclear segments and the food vacuoles; note the digested diatom; **23** - lateral view; note the posteriorly positioned contractile vacuole; **24** - dorsal view, to show the distribution of mitochondria; inset - optical section of cortex; **25** - ventral view of a thicker form; **26** - extrusomes (cortical granules); **27** - dorsal view, to show the arrangement of the cortical granules (note also the inset); **28, 29** - ventral and dorsal views of infraciliature; arrowhead marks the gap between the anterior and posterior portion of the adoral zone of membranelles; double-arrowheads indicate the close-set cirri in left marginal row. BC - buccal cirrus, CV - contractile vacuole, DK1, 4, 5 - dorsal kinety No. 1, 4, 5, EM - endoral membrane, FC - frontal cirri, FTC - frontoterminal cirri, FV - food vacuoles, LMR - left marginal row, Ma - macronuclei, Mi - micronuclei, MVR - midventral rows, PM - paroral membrane, RMR - right marginal row, TC - transverse cirri. Scale bars - 50 μm



Figs 30-37. *Cyrtophymena marina* from life (30-35) and after protargol impregnation (36, 37). **30** - ventral view of a typical individual; **31** - lateral view of two specimens, to show the difference in cell thickness; **32** - dorsal view, to show the sparsely distributed cortical granules; **33** - ventral view (redrawn after Kahl, 1932); **34** - ventral view of a well-fed cell; **35** - ventral view of a slender form, to show the distribution of crystals (see also the inset); **36, 37** - ventral and dorsal views of infraciliature. BC - buccal cirrus, CV - contractile vacuole, DK4 - dorsal kinety No. 4, EM - endoral membrane, Ma - macronuclei, Mi - micronuclei, MVR - midventral rows, PM - paroral membrane, TC - transverse cirri, VC - ventral cirri. Scale bar - 50 μm

(21-27 *vs.* 26-37) and frontoterminal cirri (2-4 *vs.* 4-7), and lacks ventral rows of cirri (*vs*. present in *B. amagalievi*).

Pseudokeronopsis flavicans (Kahl, 1932) Borror and Wicklow 1983 (Figs 9-20, 38-45; Table 2)

Syn. *Keronopsis flavicans* Kahl, 1932

To the best of the authors' knowledge, this species has never been reinvestigated using modern methods since it was described by Kahl (1932), who called it *Keronopsis flavicans*. Based on examinations of the

Table 2. Morphometric data for two populations of *Pseudokeronopsis flavicans*. All data are based on protargol impregnated specimens. Measurements in μm

Character	Min	Max	Mean	SD	SE	CV	n
Length of body	180	220	204.7	14.84	6.06	7.3	9
	193	238	209.1	21.16	7.99	8.1	14
Width of body	40	48	44.0	2.83	1.15	6.4	9
	56	65	60.1	4.67	1.77	6.2	14
Length of buccal field	60	67	64.7	4.50	1.84	7.0	9
	55	72	65.1	7.35	2.78	9.0	14
Number of membranelles	46	54	51.3	2.73	1.12	5.3	9
	50	66	56.1	5.49	2.08	9.8	7
Number of cirral pairs in bicorona*	5	7	-	-	-	-	5
	6	9	7.4	0.97	0.31	13.1	10
Number of buccal cirri	1	1	1	0	0	0	12
	1	2	1.1	0.33	0.10	30.9	9
Number of frontoterminal cirri	2	2	2	0	0	0	9
	2	2	2	0	0	0	11
Number of transverse cirri	3	5	4.1	0.52	0.15	12.6	10
	3	6	3.6	0.97	0.32	26.8	9
Number of pairs of cirri in midventral rows	25	32	28.3	2.35	0.78	8.3	7
	29	40	33.4	4.39	1.46	13.1	9
Number of cirri in left marginal row	40	51	-	-	-	-	5
	50	57	52.4	3.05	1.36	5.8	8
Number of cirri in right marginal row	44	57	-	-	-	-	5
	56	65	60.2	3.16	1.29	5.3	8
Number of macronuclei	-	-	-	-	-	-	-
	72	97	-	-	-	-	2
Number of dorsal kineties	4	5	4.1	0.32	0.10	7.7	10
	4	5	4.1	0.36	0.10	8.8	14

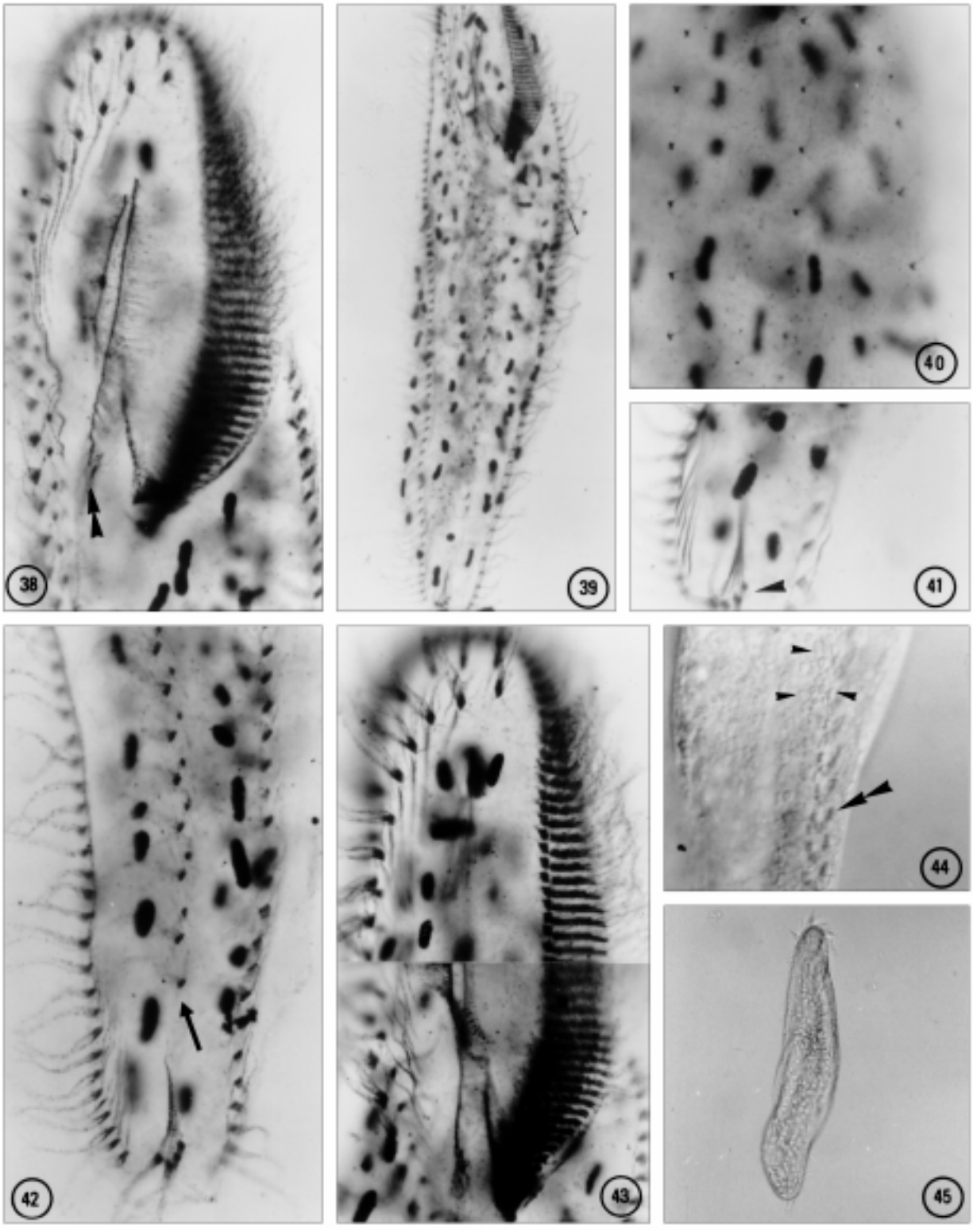
*Anteriormost frontal cirri which are positioned in two widely spaced rows forming the bicorona

Qingdao population, we here provide a detailed description and a new diagnosis.

Improved diagnosis. Marine *Pseudokeronopsis,* body slender, ribbon-shape with narrow caudal portion, 200-300 x 40-55 μm *in vivo* and yellow in colour. Ciliature comprising bicorona of 5-9 pairs of frontal cirri; one buccal, 2 frontoterminal and 3-6 transverse cirri; two midventral rows consisting of about 25-40 pairs of cirri which terminate caudally about 15 μm above the inconspicuous transverse cirri; 46-66 adoral membranelles; 40-57 (mean 52) left and 44-65 (mean 60) right marginal cirri; 4-5 dorsal kineties. Numerous macronuclear segments. One contractile vacuole anterior 2/5 of body. Bright yellow-brownish cortical granules grouped around cilia and cirri; colourless blood-cell-shaped granules lying just beneath pellicle and distributed throughout whole cell.

Morphological description. Cells flexible, slightly contractile and often somewhat distorted in middle portion, i.e. folded or twisted, when crawling or gliding on bottom of Petri dish (Fig. 17); body dorsoventrally flattened about 1:2. Body shape distinctly slender with posterior portion often distinctly narrowed; ratio of cell length to width about 5-6:1 (Figs 9, 16, 17); buccal field narrow and inconspicuous, about 1/4 - 1/5 of body length. Cell margins generally parallel but sometimes swollen in middle portion (Figs 9, 16). Dorsal side slightly uneven and irregularly bulged (Fig. 17).

Pellicle soft and thin. "Cortical granules" consisting of two types: type 1, which are spindle-shape, about 1-1.5 μm long, bright yellow-brownish and regularly grouped in dense rosette-pattern around both cirri (ventrally) and cilia (dorsally). These granules are thus distributed in belts or lines along cirral rows and dorsal kineties, rendering whole cell bright yellow (Figs 11, 12, 16). This species, however, also appears to contain some "dissolved pigments" because, in addition to these belts of pigment-like granules, other regions are also yellowish



Figs 38-45. Photomicrographs of *Pseudokeronopsis flavicans* from life (44, 45) and after protargol impregnation (38-41). **38** - ventral view of anterior portion, double-arrowheads mark the paroral membrane; **39** - infraciliature of ventral side; **40** - dorsal view, to show the cilia of dorsal kineties; **41** - ventral side of posterior end, to show the transverse cirri (arrowhead); **42** - ventral view of posterior portion; note that the mid-ventral rows extend to subcaudal region (arrow); **43** - to show the buccal apparatus; **44** - ventral view, to show the pigments (double-arrowhead) and "cortical granules" (arrowheads); **45** - a slightly contracted individual

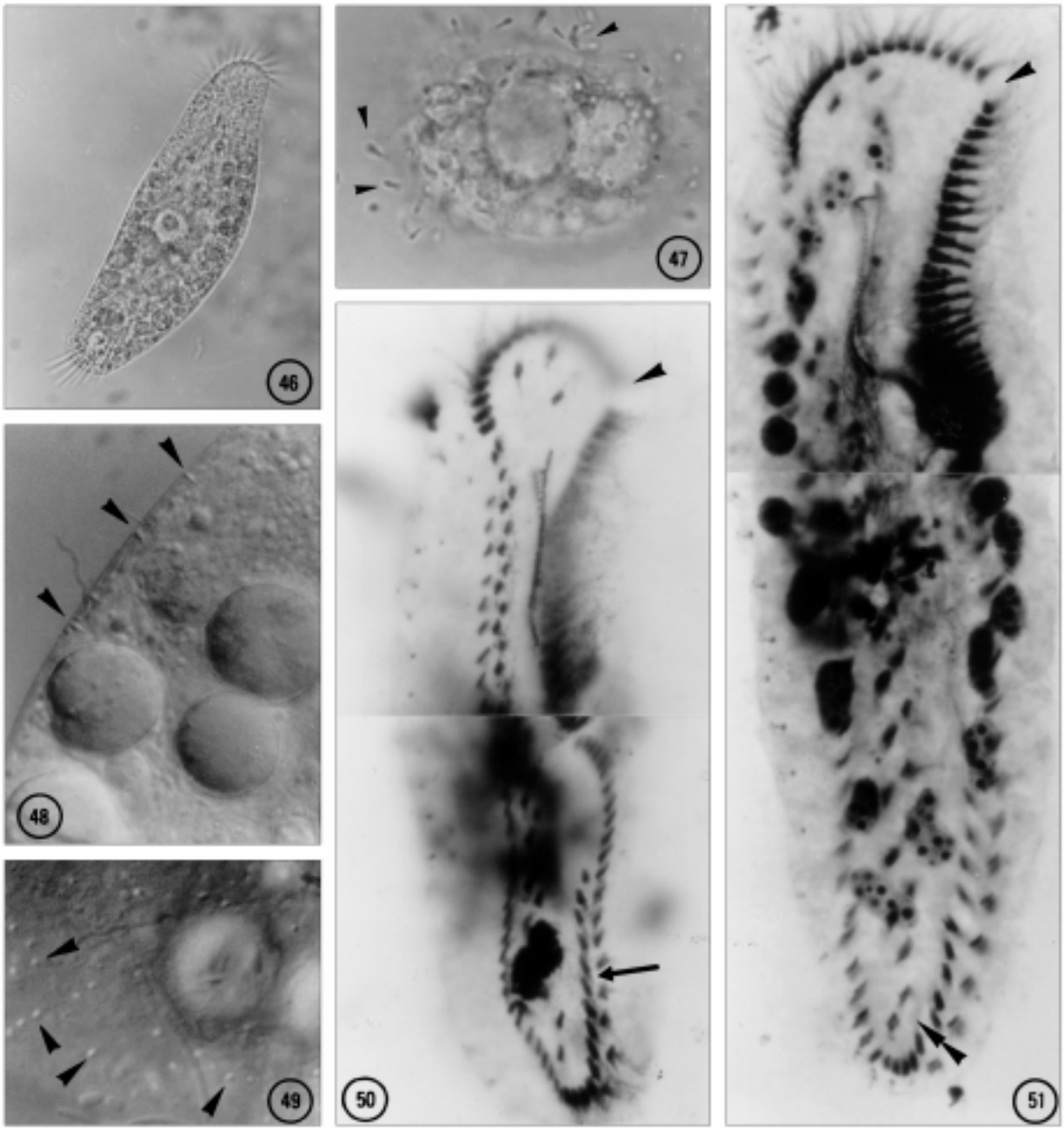
Table 3. Comparison of closely-related, yellow to orange marine *Pseudokeronopsis* species, which have been re-defined using modern methods. Measurements in μm

Characters	*P. flava*	*P. carnea*	*P. flavicans*	*P. flavicans*
Cell size *in vivo*	140-260 x 40-57	140-200 x 28-40	150-250 x ?	200-300 x 40-55
Body shape	belt-like with narrowed caudal region	belt-like, caudally less to not narrowed*	belt-like, caudal portion clearly narrowed	belt-like and slender with narrowed caudal region
Colour of cortical granules	yellow	orange-red	yellow	yellow-brownish
Colour of cell at low magnification	yellow	orange-red	yellow	bright yellow
Number of pairs of midventral cirri	28-38	22-44	?	25-40
Number of dorsal kineties	3-5	5-7	?	4-5
Number of membranelles	43-59	39-80	?	46-66
Number of cirral pairs in bicorona	*ca* 5	*ca* 5-7	?	*ca* 7 (5-9)
Number of frontoterminal cirri	3**	2**	?	2
Number of transverse cirri	1-3	5-9	ca 5	3-6
Position of contractile vacuole	?	?	anterior 1/3 of cell length	anterior 2/3 of cell length
Distance from the posterior end of midventral rows to the anteriormost transverse cirri	30 (22-42)	5-39	?	*ca* 15
Gap between posterior end of midventral rows and transverse cirri	always conspicuous	small to conspicuous	small and inconspicuous	small and inconspicuous
Geographical region	North Sea, Denmark	North Sea, German/ Denmark & Mediterranean, Yugoslavia	North Sea, Germany	Yellow Sea, China
References	Wirnsberger *et al.* 1987	Wirnsberger *et al.* 1987	Kahl, 1932	present paper

? data absent; * according to original descriptions; ** counted from the drawings

Table 4. Comparison of some closely-related *Holosticha* species with posteriorly-positioned contractile vacuoles, that have been re-defined following examination using modern methods. Measurements in μm

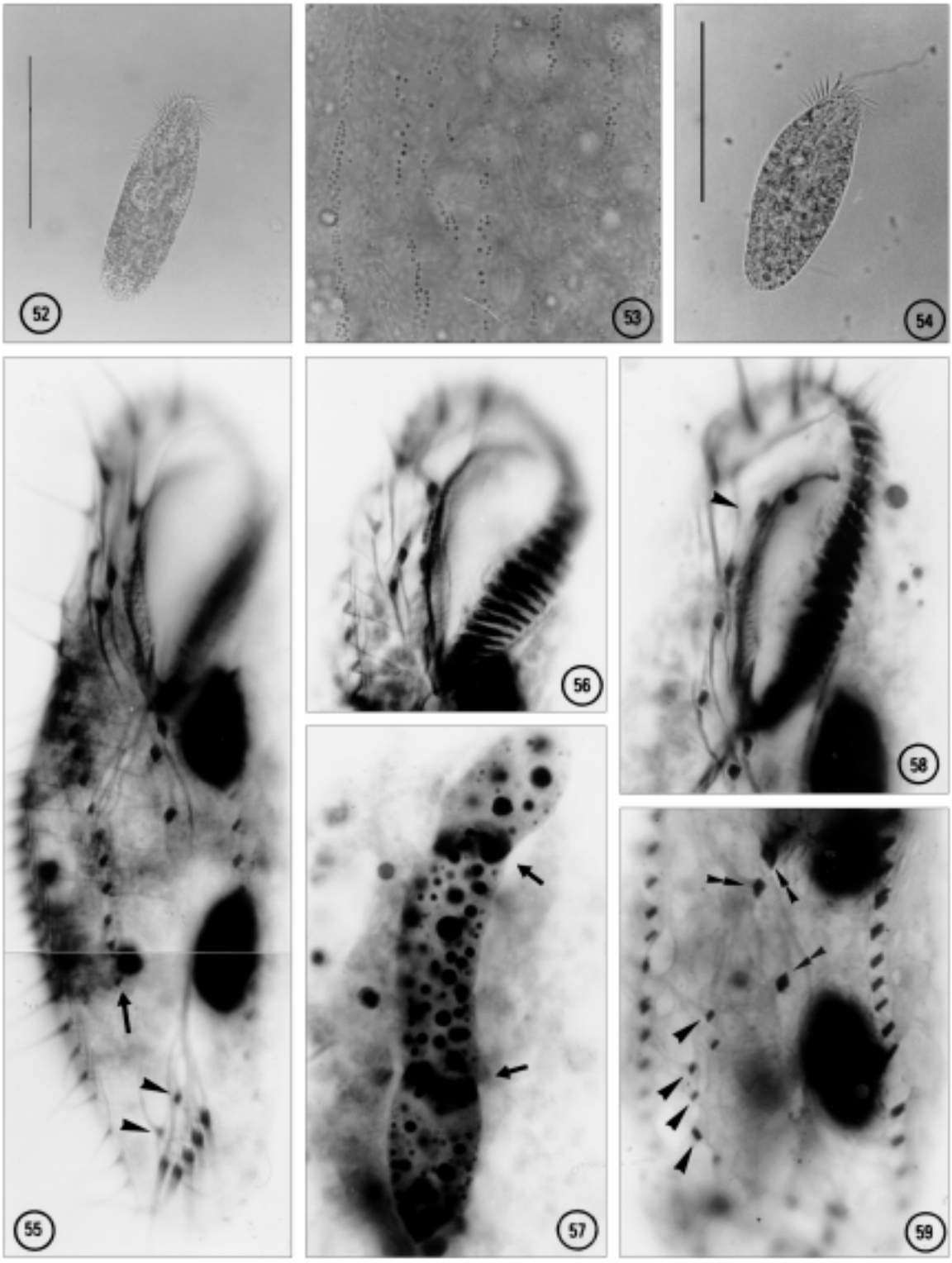
Characters	*H. diademata*	*H. diademata*	*H. pullaster*	*H. foissnseri*	*H. spindeleri*	*H. heterofoissneri*
Cell size *in vivo*	80-90 x 25-50	50-70 x 20-26	60-90 x 20-30	130-170 x 40	100-115 x 45	110-150 x 30-60
Size after impregnation	51-65 x 38-48	36-59 x 14-28	-	93-174 x 29-55	86-208 x 25-84	90-133 x 30-56
Habitat	marine	marine	freshwater	marine	marine	marine
Cortical granules	present, sparsely distributed	absent ?	absent	absent ?	absent ?	present, sparsely distributed
Elongate posteriormost adoral membranelle	absent	absent	absent	absent	present	absent
Position of contractile vacuole	behind mid-body	behind mid-body	behind mid-body	behind mid-body	behind mid-body	behind mid-body
Number of membranelles	24-30	10-21	20-28	26-36	22-43	41-49
Number and distribution of macronuclear nodules	2	2	2	5-11, in a lineal pattern	1-4	14-21, in a circular pattern
Number of dorsal kineties	4	4-5	4	4	4-5	5
Number of cirral pairs in midventral rows	*ca* 8	10-11	9-15	16-20	9-17	12-15
Number of transverse cirri	6-10	6-8	7-12	9-17	7-14	12-17
Number of cirri in left marginal row	8-12	9-12	10-15	20-26	19-26	19-25
Number of cirri in right marginal row	10-14	9-11	ca 15	21-27	11-26	27-33
Geographic region	Yellow Sea, China	Antarctica	Europe	Antarctica	Antarctica	Yellow Sea, China
References	Hu and Song (1999)	Petz *et al*. (1995)	Foissner *et al*. (1991)	Petz *et al*. (1995)	Petz *et al*. (1995)	present paper



Figs 46-51. Photomicrographs of *Holosticha heterofoissneri* from life (46-49) and after protargol impregnation (50, 51). **46** - ventral view of a typical individual; **47** - to show the extrusome-like cortical granules (arrowheads); **48, 49** - details of cortex, to show the cortical granules (arrowheads); **50** - ventral view of infraciliature; arrow marks the transverse cirri, while arrowhead indicates the gap between the anterior and posterior parts of the adoral zone of membranelles; **51** - general view of infraciliature on ventral side; double-arrowhead mark the midventral rows which extend completely to the posterior end of the body; note the gap in the adoral zone of membranelles (arrowhead)

when observed at high magnification. Granules of type 2 (possibly mitochondria) are colourless, blood-cell-shaped, *ca* µm across, densely packed and positioned somewhat deeper beneath the cell surface than type 1 granules (Figs 10, 12, 44).

Cytoplasm often containing numerous light-reflecting globules (3-6 µm), which are colourless or slightly yellowish. Contractile vacuole small, located on left in anterior 1/3 to 2/5 of cell length (Figs 16, 17). Food vacuoles not observed. About 100 macronuclear nod-



Figs 52-59. Photomicrographs of *Bakuella agamalievi* (52, 53) and *Cyrtohymena marina* (54-59) from life (52-54) and after protargol impregnation (55-59). **52** - a typical individual; **53** - to show the cortical granules on the dorsal side; **54** - a slender form; **55** - ventral view of infraciliature; arrow marks the "extra" marginal row in an abnormal individual; arrowheads indicate the posterior ventral cirri; **56** - to show the buccal structure; **57** - macronuclear apparatus during morphogenesis, arrows show replication bends; **58** - ventral view of anterior portion of cell; arrowhead indicates buccal cirrus; **59** - middle portion of ventral side, double-arrowheads indicate the 3 anterior ventral cirri; note that some extra cirri may be present during interphase (arrowheads). Scale bars - 100 μm

Table 5. Morphometric data of *Cyrtohymena marina* (Kahl, 1932). All data are based on protargol impregnated specimens. Measurements in μm

Character	Min	Max	Mean	SD	SE	CV	n
Length of body	133	170	152.8	12.19	3.52	8.0	12
Width of body	53	75	62.8	7.46	2.15	11.9	12
Length of buccal field	55	70	61.4	5.72	1.81	9.3	10
Number of membranelles	32	42	37.0	3.28	0.95	8.9	12
Number of frontal cirri*	8	8	8	0	0	0	>30
Number of ventral cirri	5	5	5	0	0	0	>30
Number of transverse cirri	5	5	5	0	0	0	>30
Number of left marginal cirri	17	21	18.1	1.45	0.46	8.0	12
Number of right marginal cirri	19	25	21.9	2.18	0.69	10.0	12
Number of caudal cirri	2	3	2.6	0.54	0.20	20.8	7
Number of macronuclei	2	2	2	0	0	0	>30
Length of macronuclei	19	29	23.3	3.32	0.89	14.2	14
Width of macronuclei	13	19	15.5	2.01	0.64	12.9	14
Number of micronuclei	2	5	2.8	1.09	0.36	39.3	9
Number of dorsal kineties**	4	4	4	0	0	0	12

* Including buccal cirrus; ** short fragment on anterior-right cell margin not included (see text)

ules, oval to elongate in shape, *ca* 5 x 3 μm in size, scattered within cytoplasm and difficult to observe *in vivo*; micronuclei ovoid, several in number (Fig. 15).

Locomotion relatively slow, crawling without pause on debris or on bottom of Petri dish.

Buccal apparatus as shown in Fig. 13. Adoral zone of membranelles (AZM) extending to about 1/4 of body length in fixed specimens, bases of membranelles up to 8-12 μm long, cilia *ca* 15 μm long *in vivo*. Distal end of AZM extends to right margin of cell and bends considerably posteriad. Paroral membrane short (arrow in Fig. 13), lies parallel to long endoral membrane. Pharyngeal fibres conspicuous after protargol impregnation, about 50 μm long (Fig. 14).

Most somatic cirri relatively fine. Bicorona consisting of about 7 pairs of slightly enlarged frontal cirri, which curve to left margin of cell. Two small frontoterminal cirri often difficult to recognize (FTC, Fig. 13). Single buccal cirrus (double-arrowhead in Fig. 13) small, situated beside mid-point of paroral membrane. Midventral rows terminating subcaudally about 20 μm anterior to transverse cirri, giving the appearance of a narrowed longitudinal furrow at low magnification. Usually 4 transverse cirri, rather fine and inconspicuous, positioned at posterior end of cell (Fig. 14), but projecting only slightly beyond posterior body margin when viewed *in vivo* (Fig. 9). Cirri in both marginal rows rather densely distributed; anterior portions of right marginal row extending to about distal end of adoral zone of membranelles. Posterior end of marginal rows clearly separated (Figs 9, 14).

Usually 4 (seldom 5) dorsal kineties in both populations, each comprising *ca* 30 pairs of basal bodies; cilia about 3-8 μm long. Caudal cirri absent (Fig. 15).

Comparison and discussion. Several morphotypes of marine *Pseudokeronopsis* with yellow, yellow-brownish or orange-red colour and slender body shapes have been previously reported. These include: *P. carnea, P. rubra, P. flava* and *P. flavicans* (Cohn 1866, Entz 1884, Möbius 1888, Morgan 1926, Kahl 1932, Rühmekorf 1935, Foissner 1984, Wirnsberger *et al.* 1987). Three of these, *P. carnea, P. rubra* and *P. flava,* have previously been redefined using modern methods (Foissner 1984, Wirnsberger *et al.* 1987).

Several workers have noted that species identification in *Pseudokeronopsis* is often difficult (Kahl 1932, Jerka-Dziadosz and Janus 1972, Borror and Wicklow 1983, Foissner 1984, Wirnsberger *et al.* 1987, Hu and Song 2000). This is because the body shape, the morphogenetic pattern, the infraciliature and the cell colour (even the arrangement of pigments) of many taxa are very similar. In the light of the body shape and size, general ciliary pattern, the cell colour as well as most of the morphometric data, *Pseudokeronopsis flavicans* is extremely difficult to distinguish from *P. flava* as described by Wirnsberger *et al.* (1987) (Fig. 20). We do not

consider them conspecific mainly because, according to the redescription of Wirnsberger *et al.* (1987), the latter seems to have no specialisation of the "cortical granules" as observed in *P. flavicans*, i.e. blood-cell-like granules and the colour of "pigments" (bright yellow-brownish *vs.* yellow). Other differences include the fact that *P. flava* is smaller (140-250 *vs.* 200-300 µm long), has fewer frontal cirri in the bicorona (*ca* 9 *vs. ca* 14) and transverse cirri (1-3 *vs.* 3-6) (Table 3), and is less slender (ratio of length to width about 3-5:1 *vs.* 5-6:1) than *P. flavicans*. In addition, Wirnsberger *et al*. (1987) emphasized that there is always a conspicuous gap (*ca* 30 µm) between the posterior end of midventral rows and the transverse cirri in *P. flava*, while in *P. flavicans* the gap is less conspicuous (<20 µm) (Fig. 14).

Pseudokeronopsis carnea can be easily separated from *P. flavicans* because it is smaller (140-200 *vs.* 200-300 µm long), has an orange-red (*vs.* yellow-brown) colour, a plumper body shape without a conspicuously narrowed caudal region, and a higher number of dorsal kineties (5-8 *vs.* usually 4) (Table 3).

Kahl (1932) emphasized that *Pseudokeronopsis flavicans* has inconspicuous pigments on dorsal side ("die um die Drsb. liegenden Prtrc. Kränze sind äusserst zart, bei manchen Indiv. kaum erkennbar; sie liegen aber so eng, dass sie auf der vorderhäfte des Infusors einander fast oder ganz berühren und sind auch hinter der Mitte nur *ca* 4 µm entfernt"). Since this character can only be described in qualitative terms, and especially when one considers that the original description of *P. flavicans* was made using classical microscopy 70 years ago (Kahl 1932), it does not seem unjustifiable to exclude our organism based on this distinction alone. In his diagram, Kahl (1932) depicted the contractile vacuole as being positioned rather near the posterior end of the AZM (Fig. 18), though he did not mention this in his description. We assume that this character is either population-dependent or was incorrectly illustrated by Kahl (1932).

In conclusion, *P. flavicans* is characterized by its body size and shape (i.e. with slightly narrowed caudal region), marine habitat, about 5 transverse cirri, yellowish colour and the midventral rows, which extend posteriorly and terminate only slightly above the transverse cirri (Kahl 1932).

Holosticha heterofoissneri Hu & Song, 2001 (Figs 21-29, 46-51, Table 1)

Some new data have been obtained from the populations studied here, thus we present an up-dated description and an improved diagnosis based on our current observations.

Improved diagnosis. Marine *Holosticha,* about 110-150 x 30-60 µm *in vivo*; adoral zone of membranelles comprising *ca* 50 membranelles and with a distinct gap between anterior and posterior parts of AZM; one anteriorly positioned buccal cirrus; 4 frontal, 2 frontoterminal and 12-17 transverse cirri; midventral rows comprising about 13 pairs of cirri, which extend almost to posterior end of cell; 5 dorsal kineties; cortical granules small and sparsely distributed on dorsal side; one post-equatorially located contractile vacuole. 14-21 macronuclear segments connected to each other by thread-like structure (or funiculus) and forming an elongated U-shape.

Morphological description. Body usually *ca* 140 x 50 µm *in vivo*, length to width ratio about 3-4:1. Cells slender and slightly flexible, somewhat contractile when stimulated. Body shape generally fusiform, with both ends slightly narrowed; anterior portion often slightly bent to left (Figs 21, 22). When contracted, left side more convex than right (Figs 22, 27). Dorsoventrally flattened about 2:1 (Fig. 23). Oral field narrow, extending about 1/3 body length.

Pellicle thin, cortical granules colourless to slightly greenish, more or less ellipsoid in shape, about 0.8 µm long and sparsely distributed (never grouped) on dorsal side (Fig. 27); if cell is disrupted, granules give appearance of extrusomes, becoming pear-shaped, each with a long (about 1-2 µm) thread at one end (Fig. 26). Mitochondria *ca* 1.5 µm long and grouped together forms several lines on dorsal side, conspicuously recognizable within cortex when viewed at high magnification (Fig. 24).

Cytoplasm colourless to slightly greyish, always containing numerous granular inclusions (3-5 µm across). Food vacuoles large, often containing diatoms and flagellates. Contractile vacuole positioned in posterior 1/3 - 2/5 of cell length (Figs 21, 23, 27). Macronuclear segments ovoid, about 9 x 7 µm in size, each with several large nucleoli; number of macronuclear segments relatively constant (17-19), connected to each other by an inconspicuous thread-like funiculus, and forming an elongated U-shape with a large space in the central region (Figs 22, 29). Micronuclei 3-5 in number, ovoid in shape, about 3 µm across, close to macronuclear segments (Fig. 29).

Locomotion typified by continuous crawling on substrate or on bottom of Petri dish, reacting to disturbance

by contracting and remaining motionless for a short while.

Infraciliature as shown in Figs 28 and 29. Adoral zone of membranelles about 2/5 of cell length after fixation and of *H. diademata*-pattern, i.e. distal end reaches right margin of cell and extends posteriorly, always in two parts with a small gap between them (arrowheads in Fig. 28). Cilia of anterior part of adoral zone of membranelles prominent and considerably longer (about 20 μm long) than those of posterior part. Bases of membranelles about 8-15 μm long, those in distal portion distinctly shorter than those in proximal portion (Fig. 28). Paroral membrane long and lies parallel to endoral membrane (Fig. 28).

Frontal and transverse cirri strong, about 15-20 μm in length, other cirri *ca* 12-15 μm long. Of the 4 frontal cirri, 3 anteriormost ones significantly enlarged and positioned on anterior cell margin. Buccal cirrus medium in size, conspicuously shifted anteriad. Two small, relatively weak frontoterminal cirri positioned close to distal end of adoral zone of membranelles. Midventral cirral rows continuous with frontal cirri and extending close to posterior transverse cirri (Fig. 28); cirri arranged in typical zig-zag pattern. Transverse cirri highly developed and arranged in a long row, which extends anteriorly almost half cell length (Fig. 28). Marginal rows distinctly separated at posterior end; anterior end of left marginal row characteristically bent towards central region of body (double-arrowhead in Fig. 28).

Consistently with 5 dorsal kineties extending over entire length of body, 1 or 2 of which are usually situated on lateral sides (Figs 28, 29). Dorsal cilia about 3-5 μm long.

Comparison and discussion. With reference to the body shape and basic pattern of infraciliature, especially the structure of buccal apparatus, *Holosticha heterofoissneri* should be compared with *H. diademata, H. pullaster, H. foissneri* and *H. spindeleri*.

Both *Holosticha diademata* and *H. pullaster* are smaller than *H. heterofoissneri* (70-140 and 60-90 μm long respectively *vs.* 140 μm long) and consistently have only 2 macronuclear segments (*vs.* 14-21), hence they can be clearly separated from the latter (Tucolesco 1962, Foissner 1980, Wilbert and Kahan 1981, Wilbert 1986, Foissner *et al.* 1991, Petz *et al.* 1995, Hu and Song 1999) (Table 4).

Holosticha spindeleri is characterized by having an extremely elongate membranelle at the posteriormost end of the adoral zone of membranelles (*vs.* absent in *H. heterofoissneri*) and a maximum of only 4 (*vs.* 14-21) macronuclei (Petz *et al.* 1995).

Holosticha foissneri is evidently related to *H. heterofoissneri.* The former differs from the latter mainly in the number and distribution pattern of macronuclear segments (5-11 arranged in a linear pattern *vs.* 14-21 forming an elongated U-shaped pattern around margin of cell). Additional differences include the numbers of adoral membranelles (26-36 *vs.* 41-49), dorsal kineties (4 *vs.* 5) and cirral pairs in midventral rows (16-20 *vs.* 12-15) (Table 4).

Cyrtohymena marina (Kahl, 1932) (Figs 30-37, 54-59; Table 5)

Syn. *Steinia marina* Kahl, 1932

To the best of the authors' knowledge, no reinvestigations on this species have been conducted using modern methods. We hence suggest a new diagnosis based on our observations.

Improved diagnosis. Medium-size marine *Cyrtohymena* 100-150 x 30-50 μm *in vivo*, body elongate oval in outline shape. 32-42 adoral membranelles; consistently 8 frontal, 5 ventral, 5 transverse and 2 to 3 caudal cirri; 17-21 left and 19-25 right marginal cirri; 2 to 3 caudal cirri; 4 complete and one fragment-like dorsal kineties; 2 macro- and 2-5 micronuclei; single contractile vacuole in anterior body half; cortical granules fine, inconspicuous, very sparsely distributed.

Morphological descriptions. Cells flexible, *ca* 120 x 40 μm *in vivo*, body usually ellipsoid and distinctly broader than depicted by Kahl (1932) (Figs 30, 33); length to width ratio usually 2.5-3:1 but in slender forms about 3-4:1; posterior end often conspicuously narrowed (Figs 32, 35). Left margin slightly to considerably convex, right margin straight; highly flattened dorsoventrally about 3:1 (Fig. 31). Buccal field prominent, almost half cell length (Fig. 30).

Pellicle thin. Cortical granules colourless, usually fine (*ca* 0.8 μm across) and sparsely distributed (Fig. 32), but at least two other atypical forms were observed: in some specimens, granules were undetectable, while in others, numerous tiny granules (*ca* 0.2 μm) were observed beneath pellicle, grouped in closely-spaced lines, giving cell a cloudy appearance when viewed from dorsal side (not figured).

Endoplasm colourless to greyish, in well-fed individuals with many large food vacuoles containing small ciliates, flagellates and diatoms, which often render the

cell completely opaque; numerous crystals of varying size and shape distributed throughout entire cytoplasm (Fig. 35). Contractile vacuole rather large, positioned in upper 2/5 of cell.

Two large ovoid macronuclear nodules located in equatorial region near left cell margin, each containing numerous large spherical nucleoli. Micronuclei 2 to 5 in number, about 2-3 μm across, lying close to macronuclei (Fig. 37).

Locomotion medium fast, crawling on substrate.

Infraciliature as shown in Figs 36 and 37. Adoral zone of membranelles evenly curved with distal end bending strongly towards right. Bases of membranelles *ca* 10-12 μm long. Paroral and endoral membranes long and strongly curved towards left margin, endoral membrane possibly single-rowed (Fig. 36).

Cirri generally strong, about 10-15 μm long. All frontal and buccal cirri located within frontal area; buccal cirrus positioned at about mid-level of buccal field. Three anteriormost frontal cirri enlarged, the other 4 being smaller and arranged in 2 pairs; one pair on anterior right margin, the other pair in more posterior position (Fig. 36). Five ventral cirri typical of genus, three immediately posterior to cytostome, two anterior to transverse cirri. Five transverse cirri each about 20-25 μm in length, closely arranged forming a J-pattern near posterior end of cell (Fig. 36). Occasionally, cells with extra cirri were also observed (Figs 55, 59). Right and left marginal rows with densely arranged cirri, posterior ends clearly separated (Fig. 36).

Dorsal cilia about 3-5 μm long arranged in 4 complete, densely ciliated kineties that extend entire length of cell; on anterior-right margin there is occasionally one additional very short kinety fragment comprising only 2-4 pairs of kinetosomes (not included in Table 5). Usually 3 (occasionally two) fine caudal cirri situated at posterior end of cell (Fig. 37).

Comparison and discussion. Our organism exhibits almost all of the characters listed in the original description by Kahl (1932): flexible medium-size body, marine biotope, 2 macronuclei and sparsely distributed cortical granules (" …Ectpl. mit spärlichen zarten Protrc. perlen in Reihen…"), hence the two are considered conspecific.

As mentioned previously, there appears to be some variation in the appearance, or even the presence of, the cortical granules in this species. It is possible that this variability is related to the physiological status of the cell. Nevertheless, the cortical granules in the abnormal cells are so atypical that a detailed study of them would seem justified.

Kahl (1932) described the German form of *C. marina* as having a distinctly slender body shape (ratio of length to width about 4-5:1), whereas the Qingdao population is usually much plumper (2.5-3:1). However, body shape can show considerable variation in the "flexible" hypotrichs (even within the same population in many cases). Indeed, there was some variability within our population, i.e. in some slender forms the length to width ratio was about 3-4:1 (see Figs 32, 35), therefore we do not believe that this character is particularly reliable for species separation within the genus *Cyrtohymena*.

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ACTA
PROTOZOOLOGICA

Notes on the Poorly-known Marine Peritrichous Ciliate, *Zoothamnium plumula* Kahl, 1933 (Protozoa: Ciliophora), an Ectocommensal Organism from Cultured Scallops in Qingdao, China

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Summary. The living morphology and the infraciliature of a poorly-known marine ectocommensal peritrich ciliate, *Zoothamnium plumula* Kahl, 1933 (pro *Zoothamnium plumosum* Perejaslawzewa, 1886) have been investigated based on specimens collected from within the mantle cavity and the shell surface of the cultured scallop, *Chlamys farreri,* off the coast of Qingdao, China. The Qingdao-population measures about 50-100 x 30-50 µm *in vivo* and is characterized by having one apically located contractile vacuole, one-layer peristomial collar, slender body shape and alternatively arranged colony branches. The C-shaped macronucleus is horizontally oriented. Number of silverlines between oral area and aboral ciliary wreath, *ca* 70; between aboral ciliary wreath and scopula, 24-28. Unlike most other congeners, zooids of this species are often enlarged at the distal end of branches.

Key words: ectocommensal, marine peritrich, morphological studies.

INTRODUCTION

Colonial peritrichs are commonly found in marine biotopes usually as ectocommensals on aquatic animals including on some cultured animals of economical importance (Kahl 1933; Precht 1935; Sommer 1951; Lom 1961; Dietz 1964; Stiller 1971; Jankowski 1976; Song 1986, 1997; Schödel 1987; Hu and Song 2001). Compared with those in fresh water habitats, however, most of the marine forms, remain poorly know or only insufficiently described in terms of their morphological features.

Zoothamnium plumula Kahl, 1933 was originally described from the Black Sea and has been only very superficially described as *Zoothamnium plumosum* (Perejaslawzewa, 1886). Since then, over 100 years have passed, but no detailed morphological studies have been conducted though in Kahl's comprehensive monograph (Kahl 1935), confirmed its taxonomical validity.

In the summer of 2000, ciliate communities in mollusc culture waters near the Qingdao coast were examined. During the survey, a population of *Zoothamnium* was isolated and investigated using silver impregnation methods, which revealed that it might represent a population of the poorly-known *Zoothamnium plumula*. The re-

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sults of the present study thus represents a new contribution for this marine peritrich.

MATERIALS AND METHODS

Scallop mollusc (*Chlamys farrer*) were collected from the mollusc-culture waters (salinity about 32-36 ‰) off the coast of Qingdao. Ciliates were found as ectocommensal forms within the mantle cavity or on the shell surface and then removed to a Petri dish. The raw culture was kept at room temperature for several days.

Cells were examined *in vivo* using a high-power oil immersion objective and differential interference contrast microscopy. The following silver methods were used to reveal the infraciliature and cytological details (about 20 zooids examined): the "dry" silver nitrate method as described by Foissner (1976) and protargol impregnation according to Wilbert (1975).

Drawings of impregnated specimens were made with a camera lucida at 1250-fold magnification. Systematics and terminology are mainly according to Corliss (1979), Foissner *et al.* (1992) and Warren (1986).

RESULTS

Class: Oligohymenophora de Puytorac et al., 1974
Order: Peritrichida Stein, 1859
Family: Vorticellidae Ehrenberg, 1838
Genus: *Zoothamnium* Bory de St. Vincent, 1826
Zoothamnium plumula Kahl, 1933 (pro *Zoothamnium plumosum* Perejaslawzewa, 1886) (Figs 1-21)

Since this species was only very superficially described from observations made in vivo and no clear definition was given, a detailed redescription is here provided based on observations of the Qingdao population.

Improved diagnosis

Marine *Zoothamnium* with alternatively branched colony; zooids slender, *in vivo* about 50-100 x 30-50 μm with thin, one-layer peristomial collar; one apically located contractile vacuole; macronucleus C-shaped and horizontally oriented; zooids often enlarged at distal end of branches. Number of silverlines: from oral area to aboral ciliary wreath *ca* 70; from aboral ciliary wreath to scopula: 24-28.

Deposition of new types

Since no information concerning the deposition of types was given in original description (Perejaslawzewa 1886), two slides as protargol and silver nitrate impregnated cells have been deposited as voucher specimens in the Laboratory of Protozoology, Ocean University of Qingdao, China.

Host and site

Scallop (*Chlamys farrer*), off the coast of Qingdao (36°08'N, 120°43'E), China.

Morphology

Body highly form-constant, slender and elongated vase-shaped, only slightly constricted below peristomial collar with maximum width at oral border; peristomial disc large and flattened (Fig. 1). Zooids often in two different sizes: mostly ("normal" ones) about 50-60 μm long, but zooids at distal end of branches often considerably larger (*ca* 80-100 μm in length) (Figs 5, 14, arrowheads). Nevertheless, this size diversion is unlikely to represent physiological differentiation of macro- and microzooids for zooids of both types seem to be able to form the telotroch independently while exhibiting the same morphological features (except the size) during "nutritional period".

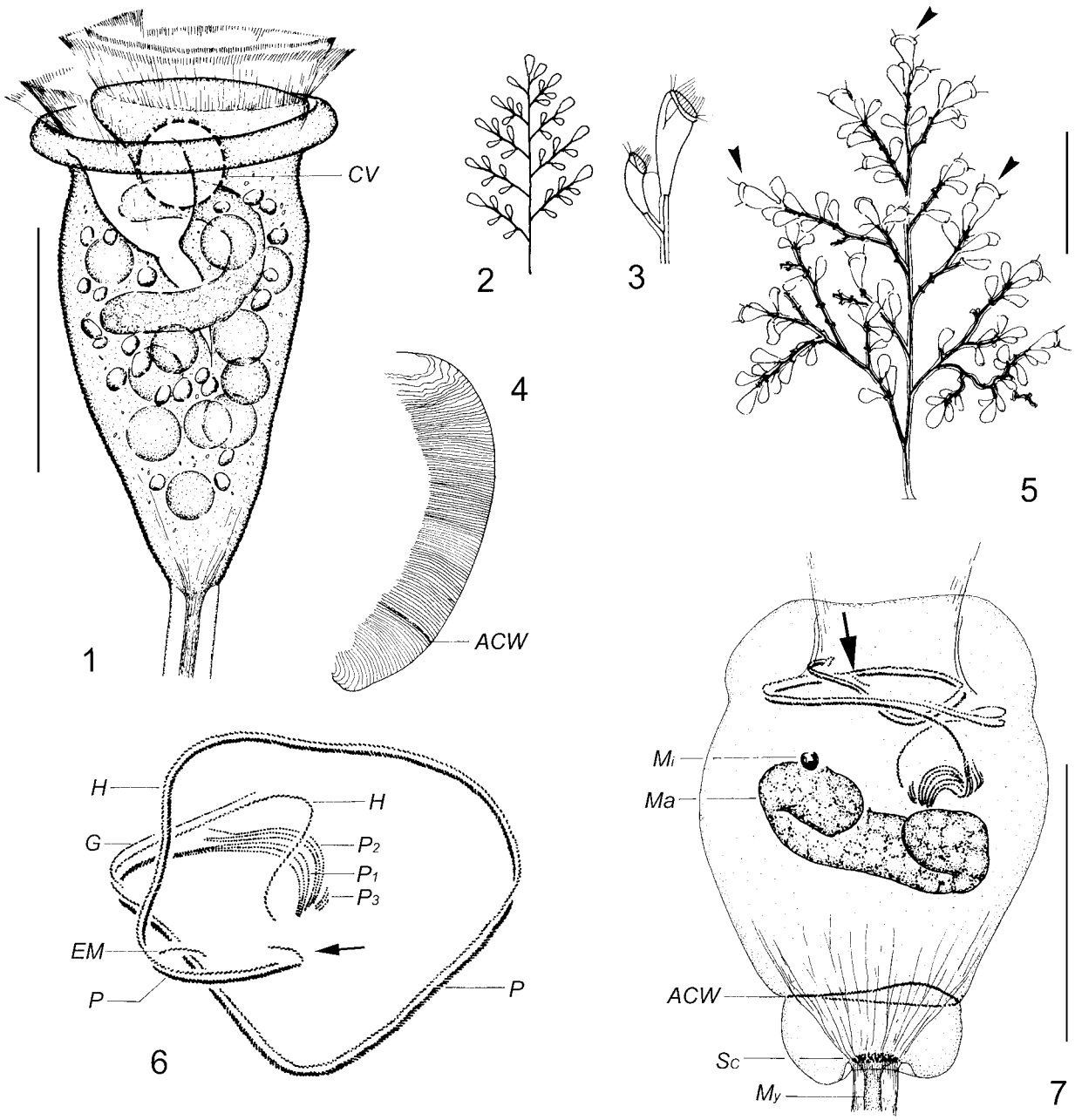
Cells not very sensitive to stimuli. When contracted, zooids usually elongated triangle-shaped but does not contact as strongly as in many of its congeners. Pellicle smooth when observed at low magnification, fine striations recognizable only under high magnification (x 400 or higher), on which no granules or any other pellicular structure are visible.

Cytoplasm colourless or slightly greenish, usually containing several large food vacuoles (about 5-8 μm across) and many "light-reflected" granules, which are oval or irregularly shaped and measure about 3 μm in length (Fig. 1). One large, apically located contractile vacuole (CV) lying slightly below peristomial disc, which contracts at a rate of about 30 s. Macronucleus (Ma) band-like and C-shaped, transversely oriented; micronucleus (Mi) small, anteriorly positioned (Fig. 7).

Stalk with smooth surface, about 8 μm thick. Colony branching alternatively with zooids located regularly in pairs, which consists of over 100 zooids and reaches a total length of over 1 mm (Fig. 5).

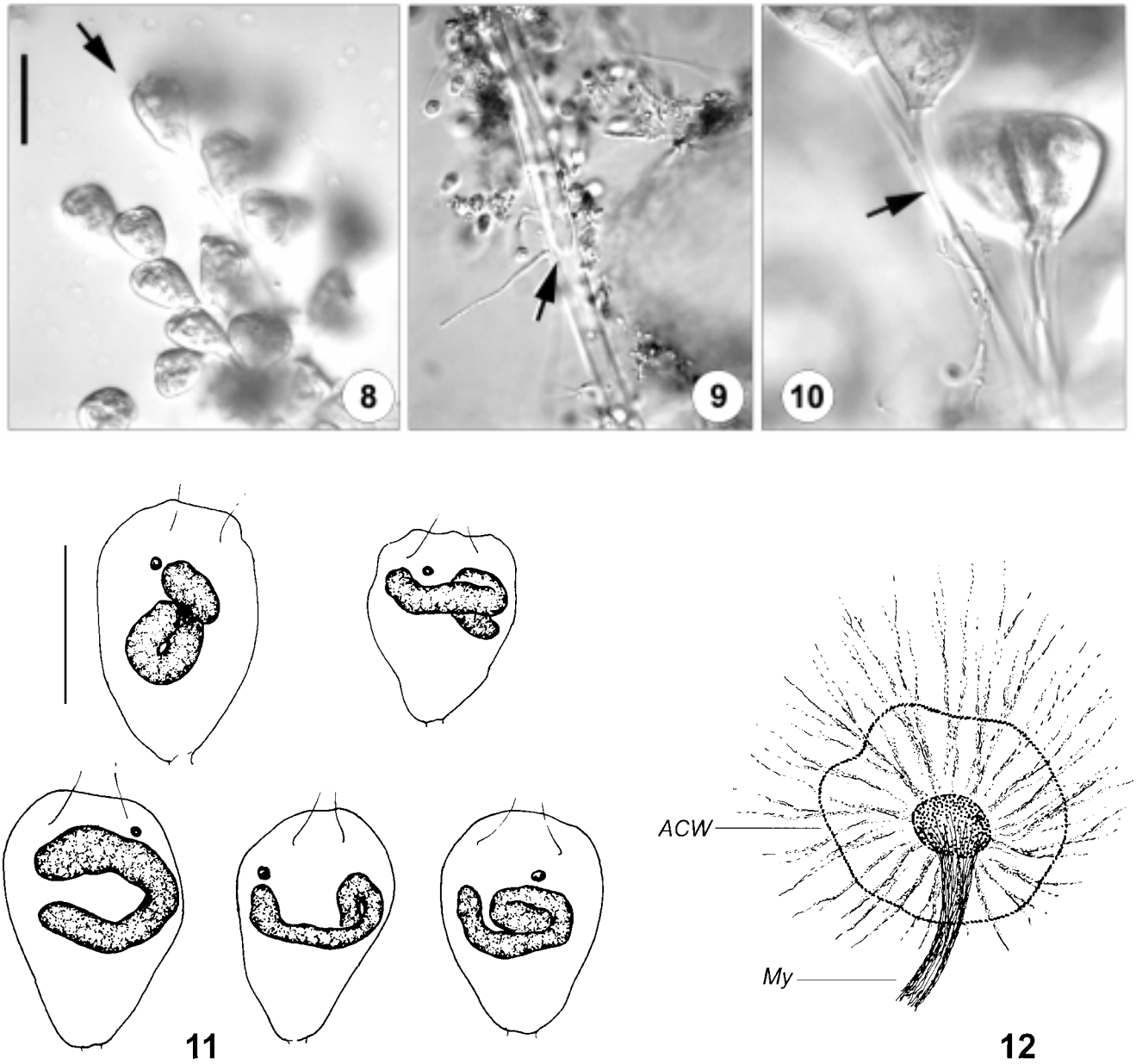
Infraciliature and silverline system

Infraciliature as shown in Figs 6 and 7. Structure of both haplokinety (H) and polykinety (P) basically similar to those in other congeners, which circle about one and half turns around peristomial disc before enter the vestibulum. At distal end of haplo- and polykinety often one kinety fragment forming hook-like structure (Fig. 6, arrow). Polykinety forming three peniculi in lower half of



Figs 1-7. *Zoothamnium plumula* from life (1-3, 5), after silver nitrate (4) and protargol (6, 7) impregnations. **1** - general view of a typical zooid; **2, 3** - colony and 2 zooids of the Black Sea population (redrawn after Perejaslawzewa 1886); **4** - silverline system; **5** - colony of the Qingdao-population, arrowheads mark the enlarged zooids; **6** - apical view of the oral apparatus, note that the peniculus 3 is much shorter than the other two peniculi (P_1, P_2); arrow marks the hook-like distal fragment; **7** - general infraciliature, arrowhead indicates the epistomial membrane. Abbreviations: ACW - aboral ciliary wreath, CV - contractile vacuoles, EM - epistomial membrane, G - germinal kinety, H - haplokinety, Ma - macronucleus, Mi - micronucleus, My - myoneme (= spasmoneme), P - polykinety, P_{1-3} - peniculus 1-3, Sc - scopula. Scale bars: 1, 7 - 40 µm; 5 - 300 µm

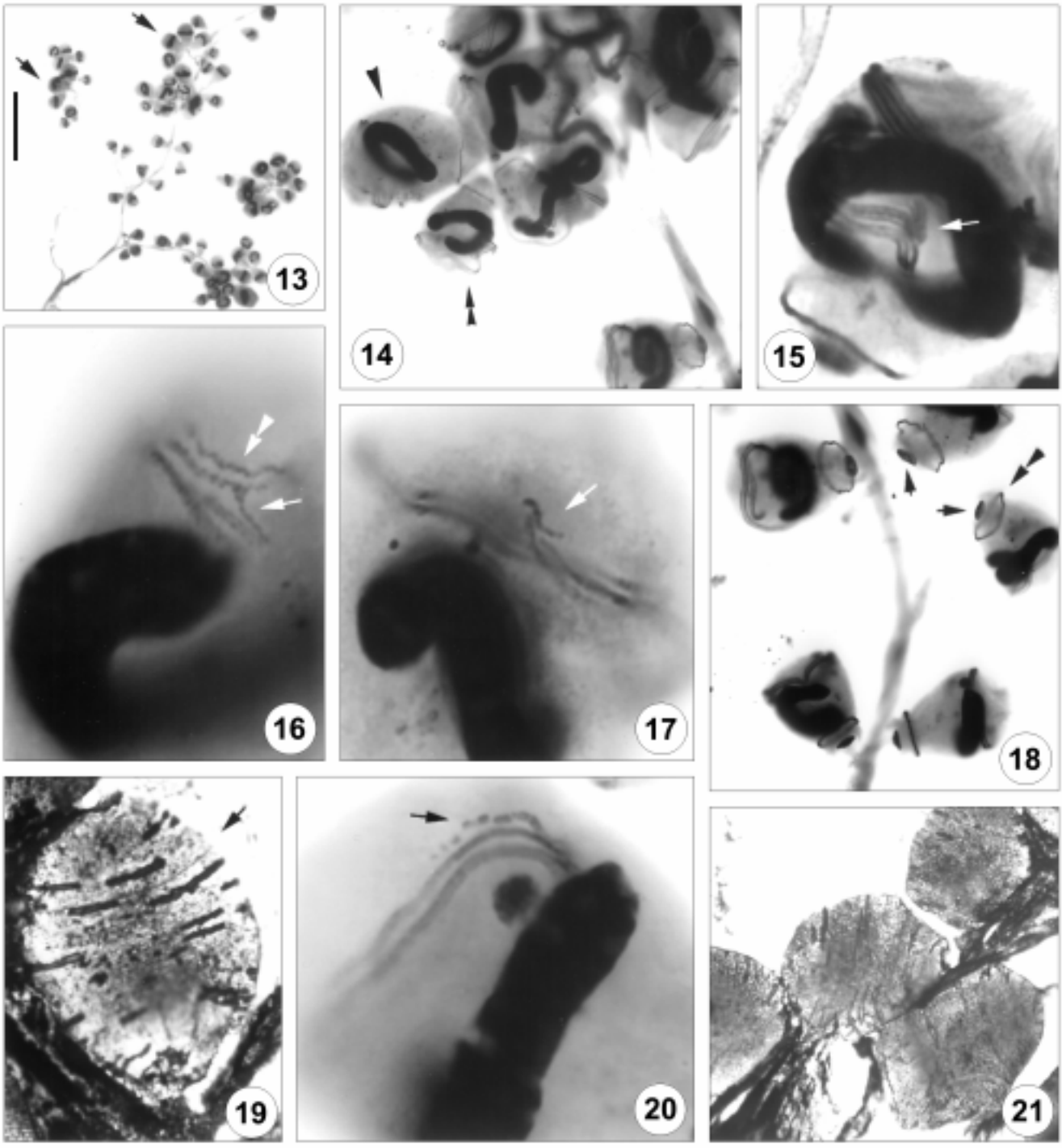
vestibulum: peniculus 1 (P_1) and 2 (P_2) about equal length, reaching end of vestibulum and converging with short peniculus 3 (P_3). Haplokinety passing around vestibulum on opposite wall to peniculi. Germinal kinety (G) consisting of densely arranged kinetosomes, extending to upper 1/2 of vestibulum where it passes parallel to the



Figs 8-12. *Zoothamnium plumula* from life (8-10) and after protargol (11-12) impregnations. **8** - to show the distal end of branches, arrow marks the enlarged zooid; **9** - stalk of a colony, arrow indicates the thick myoneme within the main stalk, which ends somehow away from the attaching end; **10** - to show the myoneme (arrow) and the young zooids, which are usually small and plum in body shape; **11** - to demonstrate the different shaped macronucleus; **12** - aboral end of cell, to show the scopula and the aboral ciliary wreath. Abbreviations: ACW - aboral ciliary wreath, My - myoneme. Scale bar - 50 μm

haplokinety. Epistomial membrane (EM) short, located away from distal end of polykinety (Fig. 7, arrow). Aboral ciliary wreath (ACW) composed of zig-zag structure of kinetosomes (Figs 7, 12).

Myoneme system consisting of thick spasmoneme within stalk (My), which is generally thicker in main stalk than in distal branches (4-5 *vs.* 2-3 μm), while in some old colonies often becomes irregularly shaped: broken or



Figs 13-21. Photomicrographs of *Zoothamnium plumula* after silver nitrate (19, 21) and protargol (13-18, 20) impregnations. **13** - to show the branching form of a colony, note that some zooids at the distal end of branches are enlarged (arrows); **14** - to show the shapes of macronucleus and different size of the enlarged (arrowhead) and "normal" zooids (doruble-arrowheads); **15** - oral apparatus, arrow indicates the peniculi; **16** - detailed part of buccal apparatus, arrow marks the "branched" kineties of the peniculus 2, while the doubel-arrowheads exhibit the germinal kinety; **17** - to show the epistomial membrane (arrow); **18** - general appearance, to show the scopula (arrows) and the aboral ciliary wreath (double-arrowheads); **19, 21** - silverline system, arrow in Fig. 19 marks the aboral ciliary wreath; **20** - to show the germinal kinety. Scale bar - 200 μm

thinned (Figs 9, 10). Around scopula (Sc), myoneme extending anteriorly towards central region of cell (Figs 7, 12).

Silverline system as shown in Figs 4, 19 and 21, striations conspicuously close-set and no conspicuous pellicular pores associated with silverlines.

Comparison

Compared with original descriptions, the population studied here is identified basically due to the following points: (1) the same branching pattern of the colony, i.e. alternatively branched stalk with zooids located in pairs; (2) zooids at the distal end of branches often considerably enlarged, (3) cells having the similar body shape and size (slender bell-shaped with thin peristomial collar), and (4) similar habitat (both found from marine biotopes).

In the author's previous description (Song 1991), this organism was incorrectly identified as *Zoothamnium thiophilum* Stiller, 1946. The latter resembles the former in the similar body shape and the form of colony branching but differs from *Z. plumula* in considerably larger size (90-95 *vs.* 50-60 μm in normal zooids while 150-180 *vs. ca* 80-100 μm in large forms) and different habitats (freshwater, "solfataras" vs. marine).

Based on some morphological features, i.e. the position of contractile vacuole, the appearance of the peristomial lip and the branching pattern of colony, comparison should be also made with *Zoothamnium commune* and *Z. sinense*, which were often found from the same habitat (Song 1991). However, according to the original and subsequent reinvestigations (Kahl 1935; Precht 1935; Küsters 1974; Song 1986, 1991), these two species have much smaller colony size (about 20-30 zooids), short and "plump" body shape (i.e. typically bell-like *vs.* elongated and slender in *Z. plumula*) and considerably smaller cell size (30-60 *vs.* 50-100 μm in length). In addition, both of them have "identically" sized zooids rather than enlarged ones at the distal end of branches as in *Z. plumula.*

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The spore of the Unicellular Organism *Nephridiophaga blattellae*: Ultrastructure and Substances of the Spore Wall

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Summary. Since many aspects of nephridiophagids are still unknown, we investigated their most typical life cycle stage, the spore, using different light and electron microscopic techniques. Cytochemical and fluorescence labelling seemed to indicate the presence of chitin, protein, and calcium as components of the spore wall. Organic solvents, alone or in combination with proteases and chitinases, influenced the staining properties. This led to the assumption that hydrophobic substances at the spore surface and a close association of protein and chitin impede staining and enzymatic actions. Freeze-fracture studies and thin sections showed details of the spore surface and depicted the different wall layers of the spores. The innermost electron-transparent layer bound WGA (indirectly labelled with gold) and thus may contain chitin. A central cap-like structure was revealed to be an opening through which an infectious sporoplasm may emerge. Ultrastructural aspects of spore hatching and hatched sporoplasms were demonstrated for the first time. Spore wall features are reminiscent of microsporidian spores but the similarities are too few to assign the nephridiophagids to any of the known groups of unicellular organisms.

Key words: *Blattella germanica,* chitin, cockroach, Nephridiophagidae, spore, ultrastructure.

INTRODUCTION

The family Nephridiophagidae Sprague (1970) (or Coelosporidiidae, Purrini and Weiser 1990) embraces a group of unicellular spore-forming organisms. It is a fairly unknown group of parasites; therefore the most important features should be briefly mentioned (for more details see Woolever 1966, Toguebaye *et al*. 1986, Purrini and Weiser 1990, Lange 1993, Radek and Herth 1999). Nephridiophagids (a collection of species as defined in Radek and Herth 1999) mainly infect the Malpighian tubules of insects, especially of cockroaches and beetles. Infection is oral *via* uptake of spores. The earliest stages of reproduction are found in the epithelial cells of the Malpighian tubules or in the tubule lumen. Oligo- and multinucleate plasmodia arise, then disintegrate into unicellular merozoite-like stages or undergo sporogony. During the process of sporogony, cisterns of the endoplasmic reticulum (ER) demarcate a portion of cytoplasm around each nucleus, thus generating sporoblasts. Some vegetative nuclei remain in the common cytoplasm and degenerate. A spore wall is formed between the two spore membranes that originate from the fused ER cisterns. Developmental stages with two nuclei [*Nephridiophaga blattellae* (see Radek and Herth 1999)*, N. schalleri* (see Purrini and Rhode 1988)] or even four nuclei [*N. apis* (see Ivanić 1937)*, N. periplanetae* (see Ivanić 1926 and Swarczewsky 1914)] have (controversially) been reported, while the

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mature spores are generally uninucleate (except for *C. binucleata*, see Gibbs 1959). The presence of a spore opening has often been debated, but in some species central, cap-like structures have been suggested to be such an orifice for hatching [*N. blattellae* (see Radek and Herth 1999), *N. blaberae* (see Fabel *et al*. 2001)].

The spore wall (of *N. blattellae*) is resistant to many chemicals and enzymes (Woolever 1966). None of these probes nor mechanical stress (alternate freezing and thawing, pressure on the cover slip) destroyed the spore or caused extrusion of its sporoplasm. The reasons for this enormous resistance are not clear. Results of tests for the presence of chitin were inconclusive (Woolever 1966).

The systematic position of the nephridiophagids is controversial. Due to their appearance under the light microscope, they have been assigned to microsporidia (Perrin 1905, Kowaljowa and Issi 1973), and later, after examining ultrastructural data, to haplosporidia (Woolever 1966, Purrini and Weiser 1990). Other investigators, however, have stated that they do not belong to either taxon (Toguebaye *et al*. 1986, Purrini and Rhode 1988, Purrini *et al*. 1988, Lange 1993, Radek and Herth 1999).

Our main aims were to gain more information on the chemical composition and morphology of the spore wall and hatching spores. Molecular studies were initiated but are not the subject of the present study.

MATERIALS AND METHODS

Specimens of the German cockroach *Blattella germanica* were obtained from cultures of the Federal Health Institute in Berlin. Decapitated specimens were processed for light and routine electron microscopy as recently described (Radek and Herth 1999). Fresh mounts, Giemsa-stained smears, DAPI-stained smears after acid hydrolysis, and paraffin sections stained with Weigert´s iron hematoxylin were used for light microscopy. For transmission electron microscopy, the Malpighian tubules were fixed in 2.5 % glutaraldehyde, rinsed, post-fixed in a 1:1 mixture of 2 % OsO_4 and $K_4[Fe(CN_6)]$, and embedded in Spurr´s medium (Spurr 1969), while the material for freeze-etched preparations was lightly fixed in 0.5 % paraformaldehyde with 0.5 % glutaraldehyde and stored in 20 % glycerol prior to shock freezing in nitrogen slush.

WGA-gold label

For lectin-gold labelling, the Malpighian tubules were fixed in 4 % paraformaldehyde with 2.5 % glutaraldehyde in a 0.1 M phosphate buffer of pH 7.2, dehydrated and embedded in LR-White. Indirect gold labelling of thin sections was performed as follows: rinse in TBS (0.1 M Tris-HCl pH 7.2, 0.15 M NaCl, 2 mM $MnCl_2$, 2 mM $MgSO_4$, 2 mM $CaCl_2$); incubate in 100 µg/ml WGA (lectin from *Triticum vulgaris*, Sigma) in TBS for 1 h; rinse in TBS 2 x 2 min; incubate in fetuin-gold (from fetal calf serum, 10 nm gold particles, Sigma) 1:50 diluted with TBS; rinse in TBS 2 x 2 min; shortly rinse in aqua dest.; routinely stain with uranyl acetate and Reynold´s lead citrate.

Light microscopic detection of chitin, cellulose, protein and calcium in untreated spores

When not otherwise stated, the Malpighian tubules from five cockroaches were collected for each experiment and torn with tweezers to release the spores.

Protein

Detection of α-amino acids by the Ninhydrin-Schiff-reaction (Böck 1989). Paraffin sections (fixation with 4 % formaldehyde) were used instead of native material. Aldehyde groups, which emerged from amino acids by oxidative desamination with Ninhydrin, are stained red-violet with Schiff´s reagent. Negative control: no Ninhydrin.

Calcium

Formaldehyde-fixed paraffin sections and native spore-containing material were used for the detection of calcium.

Silver method according to von Kossa (Böck 1989). Native material was centrifuged at 1000 g for 10 min at each step. Principal of detection: calcium ions in carbonates and phosphates are substituted by silver ions; those are reduced to metallic silver by light resulting in brown-black deposits.

Fura-2. Incubate deparaffinized sections or native spores with 100 µM Fura-2 (Sigma) in 0.1 M phosphate buffer and rinse. The fluorescent marker Fura-2 interacts with Ca^{2+}; the complex thus formed emits visible blue-white light (400-440 nm) after irradiation with ultraviolet light (340-460 nm).

Chitin

Cytochemical assay (Roelofsen and Hoette 1951 in Aronson and Bertke 1987): deacetylation of chitin by cooking in 23 M KOH leads to the formation of chitosan; chitosan transforms to a red-violet substance. Negative control: no KOH treatment.

Fluorescence: add 0.01 % Calcofluor White M2R (fluorescent brightener, Sigma) or 0.01 % FITC-wheat germ agglutinin (FITC-WGA, a fluorescent lectin, Sigma) in a 50 mM phosphate buffer of pH 7.2 to native material for 15 min. Both chemicals interact with β-linked polysaccharides; WGA has a high binding specificity for N-acetylglucosamine, the monomer of chitin (Goldstein and Poretz 1986).

Mannans

0.01 % FITC-Concanavalin A (FITC-Con A) in a 50 mM phosphate buffer of pH 7.2 was given to native material and paraffin sections. The lectin Concanavalin A has a nominal binding specificity for mannose and glucose (Goldstein and Poretz 1986).

Cellulose

Cytochemical method (Jensen 1962 in Aronson *et al*. 1987): Cellulose should be coloured blue-violet. Positive control: Some cellulose fibres of a paper towel processed in the same way.

Cellulose or chitin

Polarisation microscopy: Birefringence of objects may be caused by structure birefringence (e.g. fibres oriented in parallel) and/or inherent birefringence (e.g. uniform orientation of links within the molecules) (Gerlach 1976). While cellulose has a positive structure birefringence and a positive inherent birefringence, while chitin has a positive structure birefringence but negative inherent birefringence. Structure birefringence should disappear if the surrounding medium has the same refraction index. Native spores were suspended in 0.1 M phosphate buffer or glycerol (higher refraction index) and observed with a Reichert Austria microscope equipped with polarization filters and a Brace Köhler compensator.

Application of enzymes, HCl or organic solvents

Selective solubilization of spore wall substances was presumed to result in changed staining properties with fluorescent markers. The following treatments were performed: (1) Control: 0.1 M phosphate buffer pH 7.2, 30 min at room temperature. (2) 0.1 % chitinase (from *Streptomyces griseus*, Sigma) in 0.1 M citrate buffer pH 5.2 overnight at 37 °C. (3) 0.2 % proteinase K (from *Titirachium album*, Sigma) in 0.1 M phosphate buffer pH 7.6 for 60 min at 37 °C. (4) 100 % acetone. (5) 100 % chloroform. (6) 100 % isopropanol. (7) 100 % methanol. (8) 100 % xylene. (9) 1 N HCl. Substances 4 to 9 were left for 30 min at room temperature.

After the incubation all samples were centrifuged at 1000 g for 10 min and washed twice with a 0.1 M phosphate buffer of pH 7.2. Some portions of the pellet were stained with either 0.01 % Calcofluor White or with FITC-WGA. Other portions were first incubated with 0.1 % chitinase in a 0.1 M citrate buffer of pH 5.2 overnight at 37 °C and then stained. The latter test was performed to see whether treatment with proteinase or organic solvents could enhance the attack of chitinase. Both the staining intensity with Calcofluor or FITC-WGA and the percentage of fluorescent spores were estimated using fresh samples.

RESULTS

General appearance of the spores

Since the light and electron microscopic morphology of the life cycle stages of *N. blattellae* have been recently described in detail (Radek and Herth 1999), this study only briefly reports on the general features of the spores. Ten to 40 spores (generally about 25-30) are endogenously formed within a sporogenic plasmodium (= pansporoblast; Fig. 1). Mature spores have an oval, biconcave form and measure about 5.5 x 3.2 µm. Giemsa stain or DAPI cannot diffuse into the intact spores and stain their nuclei, but they do stain the residual, vegetative nuclei in the plasmodial cytoplasm (Fig. 2). The spore nuclei may only be stained after hydrolysis with hot 0.1 N HCl (not shown) or if the spores are broken open as was the case, for example, by the paraffin sections (Fig. 3).

Detection of protein, calcium, chitin, and cellulose in chemically untreated spores

Protein. Application of Ninhydrin-Schiff-reagent stained the spore walls red-violet, meaning that α-amino acids are probably present (Fig. 4).

Calcium. Using the silver method according to von Kossa, the spores had slightly brown-black walls (not shown). Fura-2, however, resulted in a strong fluorescence of the spore walls (Fig. 5). Results were comparable using native spores or paraffin sections.

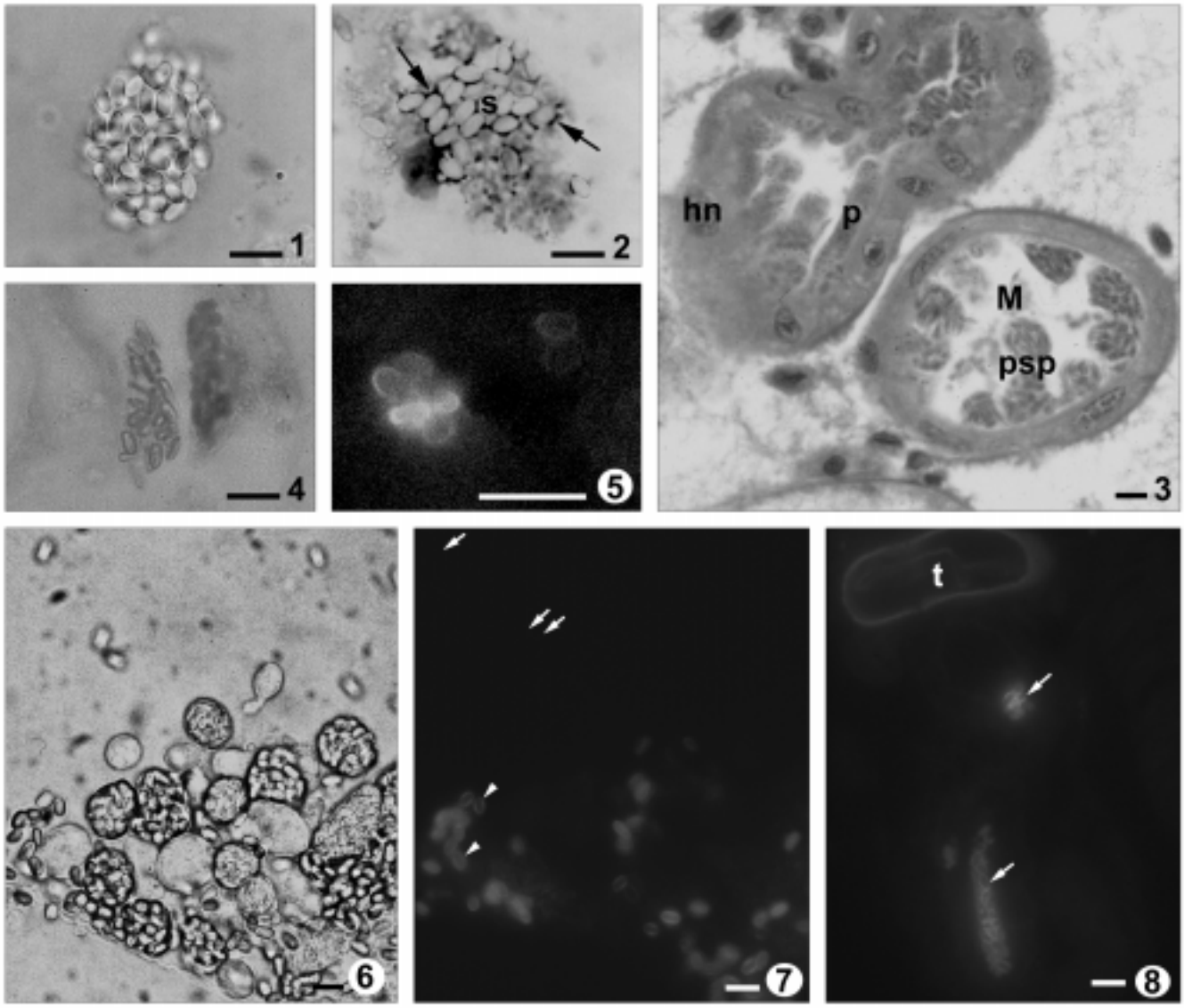
Chitin. The cytochemical method according to Roelofsen and Hoette (1951, in Aronson and Bertke 1987) did not give a positive result with spores. The controls looked the same as the test specimens did.

Fluorescent labelling with Calcofluor White or the fluorescent lectin FITC-WGA stained spores of native preparations to a variable degree. Spores stained heavily, weakly or not at all occurred at the same time, often depending on their position/life cycle stage, such as either single spores free in the medium, or spores within pansporoblasts or in the lumen of Malpighian tubules (Figs 6, 7; Tables 1, 2, controls). Only about 20 % of the spores were stained. Incubation of paraffin sections instead of native smears led to a more intense and complete staining of the spore walls (Fig. 8). Nevertheless, native preparations were used for the further detection methods and experiments (chemical treatments prior to staining) since it cannot be ruled out that spore wall materials are lost or chemically changed during the embedding procedures.

Mannans. FITC-Con A led to a very faint staining of spores in paraffin sections (not shown). The presence of α-mannans remains speculative.

Cellulose. To rule out the possibility that the positive result of the Calcofluor-staining was due to the presence of cellulose instead of chitin, a histochemical test for cellulose was performed. Cellulose could not be detected in the spores; control tests on cellulose fibres, however, were positive.

Chitin/cellulose in polarisation microscopy. A further proof that chitin but not cellulose is present in nephridiophagid spores was given by the use of polarisation microscopy. The relative optical character of the spore walls is positive, meaning that they are birefringent. Birefringence disappeared after the addition of glycerol. The positive structural birefringence



Figs 1-8. Light micrographs of *Nephridiophaga blattellae*. **1.** Mature spores of a pansporoblast in a squash preparation. **2.** Smear stained with Giemsa. The small, residual nuclei of the pansporoblast cytoplasm are stained (arrows), the spore nuclei are not stained. s- spore. **3.** Paraffin section stained with hematoxylin. The lumina of the Malpighian tubules (M) contain pansporoblasts (psp) with spores of different maturity, and multinucleate plasmodia (p) which adhere to the epithelium. hn- host cell nucleus. **4.** The red-violet staining (dark in the black and white photo) of the spore walls after application of Ninhydrin-Schiff-reagent reveals the presence of α-amino acids. **5.** Fura-2-fluorescence depicts Ca^{2+} in the spore walls. **6.** In the fresh squash preparation several pansporoblasts and single spores are visible. **7.** Same preparation as in Fig. 6 showing Calcofluor-fluorescence. Most spore walls of single spores were stained (arrowheads) but not all (arrows). Spores within pansporoblasts did not fluoresce. **8.** Spores in paraffin sections exhibited a more intense and complete Calcofluor-fluorescence (arrows). The chitin-containing tracheas (t) also fluoresced. Scale bars-10 μm

seemed to have been lowered to such a degree that the birefringent nature was abolished by an equivalent negative inherent birefringence. Thus, it seems plausible that chitin is present. If cellulose had been present, a residual positive structural birefringence together with a positive inherent birefringence should have resulted in a fainter but still detectable birefringence.

Staining properties of the spore wall after various chemical treatments

Calcofluor- and FITC-WGA-staining were used to check the influence of different agents on the spore wall material. It was difficult to interpret the results since only a portion of the spores was labelled. Estimation of the

Table 1. Quantitative and qualitative results of staining spores with Calcofluor White M2R or FITC-WGA after treatment with different agents. Quantitative values in %; qualitative data: +++ (intensive), ++ (medium), + (weak), + - (variable), - (none)

	Calcofluor White		FITC-WGA	
	qualitative	quantitative	qualitative	quantitative
0.1 M phosphate buffer pH 7.2 (control)	20	+ -	10	+ -
0.2 % proteinase K	50	+ -	20	+ -
1 N HCl	70	+	50	+ -
acetone 100 %	50	+ -	80	+
chloroform 100 %	70	+ -	50	++
isopropanol 100 %	50	+ -	90	+ -
methanol 100 %	50	+ -	100	+++
xylene	60	+ -	50	+

Table 2. Quantitative and qualitative results of staining spores with Calcofluor White M2R or FITC-WGA after treatment with different agents and subsequent incubation with chitinase. Quantitative values in %; qualitative data: +++ (intensive), ++ (medium), + (weak), + - (variable)

	Calcofluor White		FITC-WGA	
	quantitative	qualitative	quantitative	qualitative
0.1 M phosphate buffer pH 7.2 (control)	20	+ -	20	+ -
0.2 % proteinase K	20	+ -	20	+
acetone 100 %	50	+ -	10	+
chloroform 100%	50	+ -	10	+
isopropanol 100 %	20	+ -	20	+ -
methanol 100 %	50	+ -	10	+
xylene	50	+ -	10	+ -

percentages and fluorescence intensities of marked spores are given (see Tables 1, 2).

Enzymes (proteinase K, chitinase) or 1 N HCl (Tables 1, 2). Incubation of spores with proteinase K prior to Calcofluor-labelling resulted in a higher percentage of stained spores (50 % instead of 20 %, Table 1). A preceding hydrolysis with HCl increased the positive spore yield to 70 %, which demonstrates that Calcofluor can bind to the wall material much better if proteins are removed (at least partially) before staining.

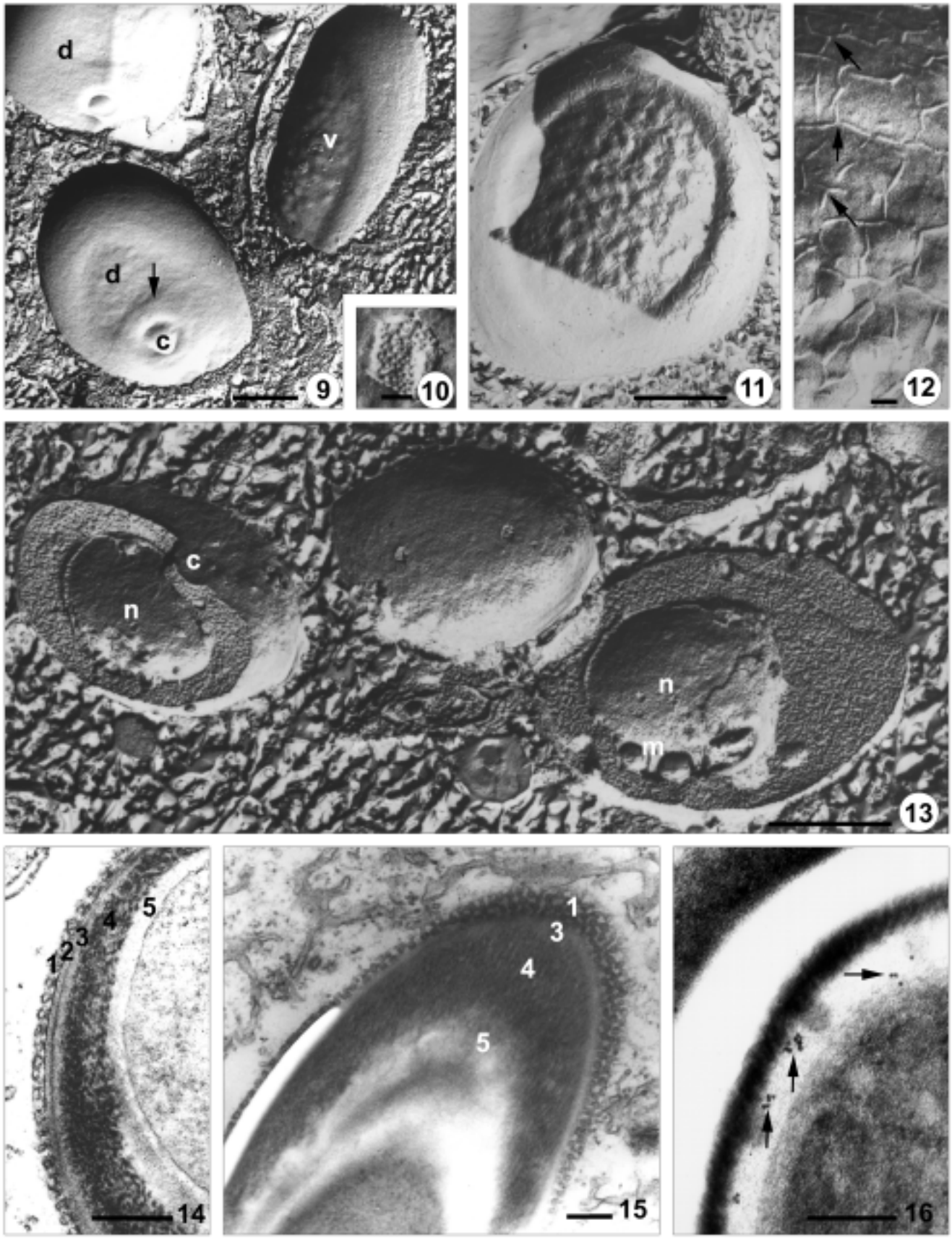
Incubating with chitinase (Table 2, control), however, did not lower the percentage of Calcofluor-stained spores (20 %) compared to untreated specimens.

Proteinase treatment followed by chitinase (Table 2) led to a reduction of stained spores (to 20 %) compared to their number after proteinase incubation alone (50 %).

Organic solvents (Table 1). In order to show whether diffusion barriers such as hydrophobic surface layers (e.g. waxes, lipids) or close physical aggregation and chemical interactions of wall material hampered the diffusion of stains, *Nephridiophaga*-spores were treated with different organic solvents and then stained with Calcofluor or FITC-WGA.

The application of alcohols (methanol, isopropanol) or acetone elevated the percentage of spores stained with Calcofluor to 50 % (control 20 %). Xylene treatment delivered 60 % and chloroform treatment 70 % fluorescent specimens. Labelling with FITC-WGA resulted in an even higher percentage of positive spores (control 10 %) in most cases. Especially after incubation in acetone (80 %), isopropanol (90 %) and methanol (100 %), most spores were stained. The staining intensity was also remarkably strong, particularly in the methanol and chloroform assays.

Organic solvents followed by chitinase treatment (Table 2). The goal of these experiments was to find out whether removal of hydrophobic surface layers could allow chitinase to attack chitin in the spore walls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more effectively. Using Calcofluor, the percentage of positive spores was either the same (acetone and methanol, 50 %) or lower (for chloroform 50 % instead of 70 %; isopropanol 20 % instead of 50 %; xylene 50 % instead of 60%) compared with staining after treatment with solely organic solvents. Labelling with FITC-WGA, however, resulted in a very distinct decline of positive spores in all treatments, and staining intensities also declined (see Table 2). This means that chitinase would function much better if hydrophobic substances were removed from the spore surfaces; the increased degradation of chitin resulted in a fainter staining.

Ultrastructure of spore walls

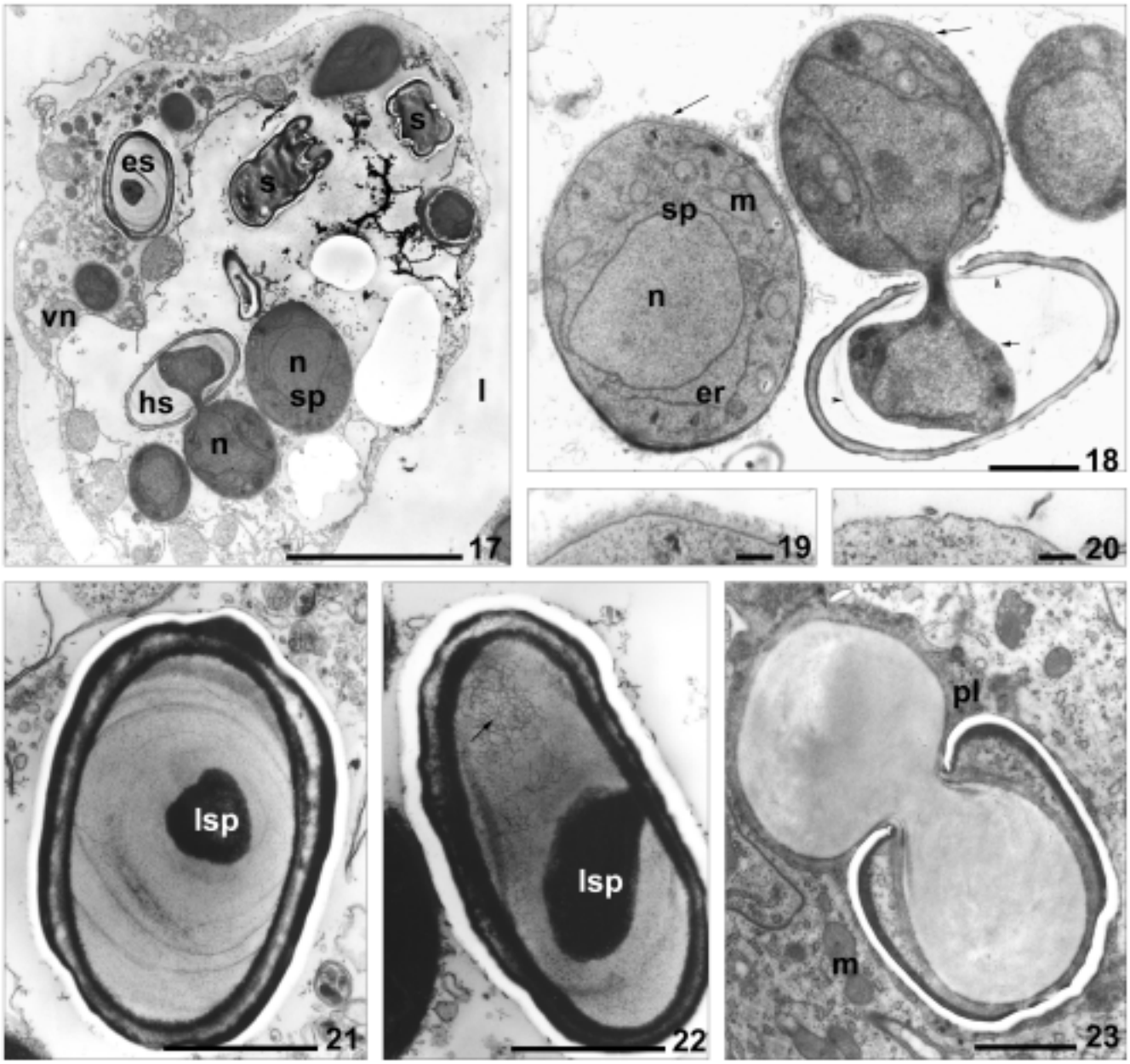
Spore wall morphology in freeze-etched specimens. The oval spores had a thickened rim (resembling an erythrocyte) and two different flattened sides (Fig. 9). One side possessed small swellings as the sole surface structures, while the other side (defined as dorsal) was characterized by a central cap-like structure. The cap lay in the middle of a bridge-like elevation connecting the lateral rims and measured about 0.5 µm in diameter. We obtained only very few freeze-fracture views of internal wall layers. In nearly all cases the fracture plane ran within the bi-layer of the plasma membrane, exhibiting a smooth spore surface with integral membrane proteins (Figs 9, 13). Occasionally residual plaques of a vesicular layer remained at the spore surface (Fig. 10). One spore revealed a fracture plane with conspicuous rods that were arranged in a loose network (Figs 11, 12). Their length varied from about 80 nm to 150 nm (mean value 115 nm) and their diameter was about 20 nm. Most of the strands participated in an association of three strands forming a kind of triskelion with star-like protruding arms (Fig. 12). These triskelions were loosely arranged in a network. Each strand belonged to either two triskelions or serially contacted a strand of a neighbouring triskelion. The small swellings at the ventral side of the spore protruded through the meshes of the network. They had the same dimensions as the swellings, which could be seen in superficially fractured spores. In a few cases spores were broken open, revealing the nucleus and membranous organelles (possibly mitochondria) within the cytoplasm (Fig. 13). Different spore wall layers as were depicted in ultra-thin sections could not be recognized nor could the wall be clearly differentiated from the cytoplasm. The region of the central cap obviously had divergent fracturing properties, since the fracture plane took its course around that structure and not straight through it (Fig. 13).

Spore wall fine structure and immunogold labelling of chitin. Mature spores possess different wall layers (Fig. 14), which may be described as follows (viewed from outside to inside): (1) an outermost complete cover of small vesicles, 20 to 30 nm thick; (2) a unit-membrane or remnants of it; (3) several thin, indistinct homogenous layers of different electron density; (4) a thick layer (about 50 nm) of electron-dense fibres that is extremely thick at the lateral rims of the spore (about 100 nm) and surrounding the dorsal cap (about 150 nm); (5) an inner electron-transparent layer of medium thickness that makes contact to the plasma membrane. The fibrous nature of layer 4 becomes especially clear in grazing sections (Fig. 15). The diameter of the fibres is difficult to measure; they seem to be 8-13 nm thick.

Indirect post embedding labelling for chitin, using wheat germ agglutinin (WGA) and fetuin-gold on LR-White thin sections, resulted in a rather low quantity of bound gold particles. The gold particles were mainly found at the inner electron-transparent layer, i.e. layer 5 (Fig. 16).

Spore hatching (TEM). Different stages of spore hatching could be observed in a section of a sporogenic plasmodium lying in the lumen of a Malpighian tubule (Fig. 17). In addition to one spore in the process of

Figs 9-16. Ultrastructure of the spore and spore wall of *Nephridiophaga blattellae* 9-13. Freeze-fractured and freeze-etched spores. **9.** Surface views of three spores. The flattened, oval spores possess a thickened rim and two different sides. The so-called dorsal side (d) possesses a central cap-like structure (c) that is surrounded by a further elevation (arrow) while the ventral side (v) shows numerous small swellings. **10.** Residual plaques of a vesicular layer occasionally remained on the spores. **11.** This fracture reveals a network of short rods, probably close to the inner margin of the spore wall. **12.** A higher magnification of Fig. 11 better reveals the arrangement of the rods in a rough triskelion pattern (arrows). **13.** Two of the three spores are broken open, revealing nuclei (n) and mitochondria (m). Spore wall layers cannot be recognized but the cap-like structure (c) has divergent fracturing properties; the fracture goes around the cap 14-16. Thin sections of spore walls embedded either in Spurr (Figs 14, 15) or in LR-White (Fig. 16), followed by WGA and fetuin-gold labelling. **14.** The spore wall consists of five layers: 1- outermost cover of small vesicles; 2- unit-membrane; 3- several thin, homogenous zones of different electron density (are not always visible); 4- thick layer of electron-dense fibres; 5- electron-transparent layer of medium thickness. **15.** Numbers as in Fig. 14. The grazing section depicts the vesicular nature of layer 1 and the fibres of layer 4 quite well. **16.** Indirect gold labelling for chitin. Most gold particles (arrows) are found at the inner electron-transparent layer, i.e. layer 5. Scale bars: 9, 11, 13 - 1 µm; 10, 14-16 - 0.2 µm; 12 - 0.1 µm



Figs 17-23. Stages of *Nephridiophaga blattellae* spore hatching depicted in thin sections of infected *Blattella germanica* Malpighian tubules (Figs 17-22) or male accessory glands (Fig. 23). **17.** A sporogenic plasmodium laying in the lumen (l) of a Malpighian tubule contains several stages of spore hatching. The plasmodial cytoplasm is degenerated; some vegetative nuclei (vn) and other cell organelles are left. es - empty spore, hs - hatching spore, n - nucleus, s - spore, sp - sporoplasm. **18.** Serial section to accompany Fig. 17 showing a spore during the process of hatching and a completely hatched sporoplasm (sp). The hatching sporoplasm leaves the spore shell through a small, central aperture, i.e. penetrating the cap-like structure. The cytoplasm including the nucleus is squeezed through the opening. Residues of material remain in the spore shell (arrowheads). The spore wall is of relatively even thickness and consists of only one thick layer with darker borders. While the membrane of the extruding sporoplasm is smooth at the cell portion inside the spore shell (short arrow), it carries a fuzzy coat at the outer cell portion (long arrow). The completely hatched sporoplasm is totally covered by the fuzzy coat (long arrow). Its cytoplasm is not as electron-dense as it is when a sporoplasm lies within a spore, therefore its cell organelles such as nucleus (n), mitochondria (m) and endoplasmic reticulum (er) can be seen clearly. **19.** Plasma membrane of an emerged sporoplasm covered with the fuzzy coat at higher magnification. **20.** Compared to the sporoplasm membrane, the plasma membrane of a merozoite is smooth. **21.** Horizontal section through a nearly emptied spore. Concentric material remnants remain in the spore shell. lsp - leaving sporoplasm. **22.** Material remnants have a network-like appearance (arrow) in a (nearly) median section of a hatching spore. lsp - leaving sporoplasm. **23.** Empty spore inside a sporogenic plasmodium (pl) within the male accessory gland; the sporoplasm left the spore; medial section. The spore wall is thick close to the region of the spore opening. A hollow space remains in the plasmodial cytoplasm where the sporoplasm crept through. m - lamellar mitochondria of a gland cell. Scale bars: 17 - 5 μm; 18, 21-23 - 1 μm; 19, 20 - 0.2 μm

hatching, completely hatched sporoplasms and empty spores were also present. The sporoplasm left its spore shell through a narrow aperture (diameter 0.5 μm) at the centre of one spore side (Figs 17, 18). The spore opening corresponds to the round, cap-like structure of the dorsal surface, as it was seen in the freeze-etched specimens. The shape and dimensions of the spore changed during the hatching process. In median, longitudinal sections, the dormant, mature spores have thickened rims and concave dorsal and ventral sides, measuring about 4.6 to 1.2 μm. Equally sectioned hatching spores showed an oval outline of 4.0 to 2.1 μm. This means that the spore swelled; it became slightly shorter and about twice as thick. At the spore opening, spore wall portions seem to be shifted aside (Figs 17, 18). Apparently, the cap was not folded open but penetrated.

The layered structure of the spore wall was changed in the hatching spore. The outer vesicular layer disappeared, and of the three layers of different electron density (layers 3-5), only one layer with electron-dense borders remained; it had a roughly uniform thickness of about 150 nm and could not unambiguously be assigned to layer 4 or 5 (Figs 18, 21, 22). Fine remnants of material were found between the spore wall and hatching sporoplasm (Fig. 18). A horizontal section through a nearly empty spore revealed concentric material remnants, as if showing consecutive stages of the sporoplasm leaving (Fig. 21). In a nearly median section of a hatching spore, material remnants have a network-like appearance (Fig. 22). Heavily infected cockroaches may harbour sporogony stages intracellularly in male accessory glands. An empty spore was also found here (Fig. 23). Its wall layers appeared to be more intact and were of different thickness in different regions. Obviously the sporoplasm had already left its enclosure and penetrated the host cell where an electron-transparent space remained.

The emerging sporoplasm had to press its total cell mass through the narrow spore opening. Its nucleus seemed to be extremely flexible; it was heavily deformed during hatching (Fig. 18). The cytoplasm of the extruding sporoplasm was lighter than that of the dormant, mature spores, where cell organelles were hidden in the dense cytoplasm. Now the nucleus and other cell organelles such as mitochondria, endoplasmic reticulum, and ribosomes could be easily seen (Fig. 18). Completely hatched sporoplasms were even more electron-transparent, resembling the vegetative stages (Fig. 18). Their sizes seemed to be larger than the volumes of the spores. Sporoplasms closely resembled merozoites but there was at least one difference: sporoplasms possessed a fuzzy, thick membrane coat (Figs 18, 19), whereas the plasma membrane of merozoites appeared to be nearly naked (Fig. 20). The fuzzy coat appeared during the process of hatching. While the portion still inside the spore shell possessed a smooth membrane, its already extruded portion exhibited the fuzzy coat (Fig. 18).

DISCUSSION

Chemical composition of the spore wall

Woolever (1966) performed experiments to cause extrusion of the sporoplasm, but despite incubation in many different chemicals and application of physical stress no hatching took place. Methods known to cause emergence of microsporidian and myxozoan infectious stages also failed. Resistance by the spore wall is probably evoked by the presence of chemically inert and pressure-resistant materials. We tried to identify the main chemical components of the nephridiophagid spore walls.

Spores of microsporidia (Vávra 1976, Perkins 1991) and myxosporidia (Lukeš *et al*. 1993) are known to contain chitin, like the cyst or lorica walls of many ciliates, flagellates and amoebae (Mulisch 1993). Further substances have been reported from cyst and lorica walls of protists such as proteins, glycoproteins, (acid) mucopolysaccharides, silica, iron, pigments, and so on (Mulisch 1993). Woolever´s (1966) tests for chitin in the spores of *N. blattellae* did not give unequivocal results. Our experiments were also difficult to interpret. The cytochemical method (deacetylation of chitin to chitosan followed by J_2KJ - 1 % H_2SO_4) did not reveal chitin. According to Ericson and Blanquet (1969), however, although considered valid when positive, a negative test for chitosan need not be a proof for the absence of chitin. Fluorescent labelling with Calcofluor White and FITC-WGA did stain the spores, meaning that β-linked polysaccharides or more precisely N-acetylglucosamine is present.

A point of criticism when attempting to demonstrate chitin by staining with Calcofluor and WGA is that these agents are not exclusively specific for chitin, although they are often used to label it (Lukeš *et al*. 1993). Calcofluor also interacts with other β-linked D-glucopyranose polysaccharides, especially cellulose (Pringle *et al*. 1989). The interaction with β-1,3-linked polymers is, however, significantly weaker than that with β-1,4-linked

polymers, so that e.g. the glucan of the *S. cerevisiae* cell wall is weakly stained or totally undetectable (Pringle *et al*. 1989). However, the presence of cellulose as the major β-1,4-linked polymer besides chitin, which binds to Calcofluor, was ruled out by the negative histochemical test and by the specific birefringent properties of the spores in the polarizing microscope. The relative optical character of chitin in water is positive, but is negative in highly refractive embedding media (the same applied to the spores), whereas cellulose is birefringent in both media (Schüβler *et al*. 1994). The lectin WGA has a nominal specificity for N-actetylglucosamine, the main component of chitin. However, N-actetylglucosamine may also be part of other saccharides when in its monomeric form, and WGA also has a low binding affinity to other sugars, such as sialic acid residues. In agreement with Lukeš *et al*. (1993), we think that the fact that both stains (Calcofluor and FITC-WGA) label the spore walls strongly supports the hypothesis that chitin is present.

One fact making staining results difficult to interpret is that staining did not follow an "all-or-nothing" pattern; the intensity and the percentage of stained spores varied. We believe that there are several possible factors, which may account for this fact: 1. The maturation status of the developing spores; young spores may not contain enough chitin to be labelled; spores of medium age may be permeable for stains in contrast to impermeable, fully developed spores. 2. Slight injuries of resistant, mature spore layers might be necessary to allow stains to penetrate so that only injured stages can be positively stained. 3. Diffusion of stains may be hampered within sporogenic plasmodia, leading to negative results of intracellular stages. A less intense staining of immature spores was also shown for microsporidian spores (Vávra *et al*. 1993). The same authors showed that staining intensity is dependent on the composition of the Calcofluor-containing medium, on alkaline pre-treatment, and on the age and formulation (fresh, fixed, smear) of the spores. There is also evidence that chitin is not always accessible to WGA (Barkai-Golan and Sharon 1978). In our experiments, judging the percentages of stained spores seemed to deliver clearer results than estimation of fluorescence intensity. FITC-WGA-staining was always fainter than Calcofluor-fluorescence. Maybe the larger size of the lectin [molecular weight of WGA 36000 D (Sigma); WGA dimer measures 4 x 4 x 7 nm (Reeke and Becker 1988)] compared with the smaller Calcofluor-molecule (molecular weight 917 D) makes diffusion of the lectin into the spore wall more difficult.

Chitin is generally associated with other substances, such as extensively cross-linked proteins in animals, or with other polysaccharides in fungal cell walls (Muzzarelli 1977, Blackwell 1982, Giraud-Guille and Bouligand 1986). The protein is thought to protect against attacks by chitinases (Blackwell 1982), and after sclerotization of the proteins, also to give hardness and rigidity to the spores (Muzzarelli 1977). These close associations of chitin with other substances may impede access of the stain to the spore wall and the action of added enzymes on its components, as was the case for example in our chitinase experiments in which the percentage of Calcofluor stained spores was not lowered. Different treatments were performed in order to solubilize obstructing substances and thus ameliorate chitin staining. Protein has histochemically been shown to be a spore wall component (positive Ninhydrin-Schiff-reaction) and, in fact, the removal of at least part of the protein by the application of proteinase K doubled the percentage of stained spores (Table 1). Further treatment with chitinase lowered the amount of positive spores, suggesting that chitinase was then able to degrade part of the chitin (Table 2). In addition to chitin and proteins, β-glucan might also be a component of the matrix as in some fungal cell walls, e.g. oomycetes (Muzzarelli 1977). In this case, treatment with glucanase would have been essential before chitinase could effectively attack the chitin. Alpha-mannans are probably either not present in the spore wall at all or are only a minor component, since the spores stained only very faintly with FITC-Con A (Barkai-Golan and Sharon 1978).

In addition to a close association of proteins with chitin conferring a certain staining resistance to the spores, diffusion of stains may be hampered by hydrophobic substances on the surface of the spores. Deposition of substances such as waxes or lipoproteins on the chitin-protein complexes can make the structures impermeable (Muzzarelli 1977). In order to remove these hydrophobic substances, different organic solvents were applied to spores prior staining and incubation in chitinase. Although these solvents all improved staining, the effect of a subsequent chitinase treatment was ambivalent. Labelling with FITC-WGA was less intense, suggesting that chitinase had acted, but labelling with Calcofluor was rather strong. The explanation could be that the effect of partial chitin degradation was compensated by a better accessibility of remaining molecules to Calcofluor.

Going a step further, we tried to assign the presumed chitin-label to a certain layer of the spore wall. Thin sections indirectly labelled with WGA and fetuin-gold showed a rather faint staining, but most gold particles were found at the inner electron-transparent layer (layer 5), which thus seemed to be the chitin-containing structure. This situation is similar to that found for microsporidia, which possess an outer proteinaceous layer (exospore) and an inner, electron-transparent chitin-containing layer (endospore) (Larsson 1986a, Perkins 1991). In general, chitin fibrils show no contrast in thin sections; the nephridiophagid layer 5 was equally electron-transparent.

Besides chitin and protein, calcium was also demonstrated to be a component of the spores. It probably contributes to the mechanical stability of the spore. The chitinous exoskeleton of invertebrates also possesses a calcified matrix, e.g. in crustacean shells (Blackwell 1982).

Electron microscopy of spore walls

Thin sections revealed different wall layers of the spores including a thick, fibrous layer and a more homogenous, electron-transparent layer reminiscent of the exo- and endospore of microsporidia (Vávra 1976, Larsson 1986a). Spore walls of nephridiophagids are only briefly described in the literature as being an electron-dense granulo-fibrous layer (Toguebaye *et al*. 1986) or a double-layered wall (Purrini *et al*. 1988, Purrini and Weiser 1990). The chemical nature and especially the presence of chitin could not be assigned to any specific layers by routine electron microscopy. Chitin fibres are generally thought to be electron-transparent. For example, microfibrils in the cuticle of arthropods (Giraud-Guille and Bouligand 1986) or the spores of fungi (Schüßler *et al*. 1994) appear as transparent rods surrounded by opaque material, a pattern that according to some authors seems to be evoked by a central chitin crystallite surrounded by protein. However, according to Giraud-Guille and Bouligand (1986), chitin covalently linked with protein *via* aspartic acid (polar amino acid) is incorporated in the dark phase. The irregularly arranged fibres of the *Nephridiophaga*-spores (layer 4) are completely electron-dense. This means that if chitin is contained in these fibres, hydrophilic (contrastable) substances must be associated. The fibre diameter of 8-13 nm fits to the size spectrum of chitin microfibrils: insect cuticles 2.5-3 nm, crustacean and fungi 20-25 nm, lorica of the ciliate *Eufolliculina uhligi* 15-20 nm (Muzzarelli 1977, Mulisch 1993), microsporidian spores 8-9 nm (Vávra *et al*. 1986). Another idea is that the nephridiophagid fibres are proteinaceous. WGA-gold staining results seem to indicate the presence of chitin in the homogenous, electron-transparent wall layer (layer 5) of the spores. The electron-transparent appearance of that wall layer may be due to a high amount of chitin.

The outermost layer of the spores consists of a single layer of small vesicles (20-30 nm in diameter), which appears when the spore coat begins to develop, and disappears when the spores are fully mature (Radek and Herth 1999). The vesicles may transport the spore wall material to the growing spore wall as was also assumed for *N. ormieresi* (Toguebaye *et al*. 1886) and *N. periplanetae* (Lange 1993). In fungi, chitin synthetases are bound to the plasma membrane to which they are transported by small vesicles (diameter less than 100 nm), the so-called chitosomes (Kamada *et al*. 1991); perhaps the nephridiophagid vesicles have a similar function.

Freeze-fracture studies did not reveal many details of the different spore wall layers. Interestingly, one specimen showed rods measuring 80-150 x 20 nm that were arranged in a triskelion-like network. They could represent a sub-membrane skeleton stabilizing the shape of the spore. Rod-like structures of a similar size range (120 x 50 nm) were described from the inner limiting layer (unit-membrane) of a microsporidian spore coat. Different functions have been suggested: anti-slipping device to prevent the spore content from spinning (Liu and Davies 1973); synthetic activity or material transport during spore-coat deposition (Liu 1975).

Spore hatching

Generally, the nephridiophagids are believed to have no spore opening (Toguebaye *et al*. 1986) or lid (Undeen and Vávra 1997). However, Gibbs (1959) saw sporoplasms of *Coleospora binucleata* hatching through a central opening with a plug while empty spores with polar openings were reported for *Nephridiophaga periplanetae* (Ivanić 1926) and *Oryctospora alata* (Purrini and Weiser 1990).

We were able to demonstrate spore hatching at the ultrastructural level and prove the existence of a defined spore opening for the first time. The infectious sporoplasm can emerge at the thin-walled central cap. A zone with thinner or missing wall layers through which an infectious stage can break seems to be a general feature of spores of organisms of different systematic groups [e.g., haplosporidians (Desportes and Nashed 1983),

apicomplexans (Perkins 1991)]. In addition, microsporidian sporoplasms hatch through a thin spore wall region at the anchoring disk of the polar filament (Weiser und Purrini 1985, Larsson 1986b). According to Weidner and Halonen (1993), the outer spore envelope of the microsporidian *Spraguea lophii* completely disassembles at the time of spore activation and the authors suggested that keratin of the spore envelope disassembles and phosphorylates. The spore wall of *N. blattellae* also lost part of its layers during hatching.

The infectious stage of *N. blattellae* seems to incorporate water during hatching; the sporoplasm swells and becomes less electron-dense. In microsporidia a rise in internal osmolarity leads to water uptake and pressure increase, resulting in the emergence of the polar filament through which the infectious sporoplasm exits (Weidner 1976, Perkins 1991).

The hatched sporoplasms of *N. blattellae* possessed a thick, fluffy surface layer at their plasma membranes. At the emerging sporoplasm, this layer was only recognized at the portion that had already left the spore shell. It is uncertain whether this layer is a glycocalyx. A quick synthesis of glycocalyx material during the passage through the spore opening seems unlikely.

Outlook

New information concerning the structure and chemical composition of the nephridiophagid spores and spore hatching could be gathered, but some questions remain. Nothing is known about the triggers of the spore hatching, the mode of movement of the sporoplasm and other stages, and the way in which the infectious sporoplasm takes in the insect body to infect the Malpighian tubules is speculative. The components of the spore walls also need be further studied to finally characterize the substances and their distribution within the wall.

Nephridiophagids share some features with the microsporidia, for example spore wall characteristics (exo-, endospore, presence of chitin and protein) and parts of the life cycle (merogony, sporogony leading to several spores stemming from one mother cell, oral infection). The fine structure of the two spore types, however, is completely different. Unless molecular data is gathered, the systematic affiliation of the Nephridiophagids will remain uncertain.

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An Analysis of the Abundance, Diversity and Patchiness of Terrestrial Gymnamoebae in Relation to Soil Depth and Precipitation Events Following a Drought in Southeastern U.S.A.

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Summary. The objectives of this research were to examine the abundance, diversity and patchiness of gymnamoebae sampled at a distance of 3.0 cm apart and at three soil depths (surface, 5.0 cm and 10.0 cm) during a drought and after a significant precipitation event. The observed gymnamoebae were categorized into four morphotypes using a standard categorization scheme, but genera of the family Cochliopodiidae were categorized separately since their abundance patterns were different from the other gymnamoebae, and taxonomically they possess characteristics of both naked and testate amoebae. Gymnamoebae abundance was significantly correlated with soil moisture ($r = 0.75$, $n = 18$, $p < 0.001$). The mean abundance of gymnamoebae (combining all the data) after the precipitation event was significantly larger than the abundance during the drought based on a univariate ANOVA test ($F(2, 15) = 12.8$, $p < 0.001$). Furthermore, the mean gymnamoebae abundance of the triplicate samples taken at the surface and at 5.0 cm after the precipitation event was significantly greater than the mean abundance during the drought $F(1, 4) = 18.3$, $p < 0.013$ (surface), $F(1, 4) = 15.0$, $p < 0.018$ (5.0 cm). There was no significant difference in the mean gymnamoebae abundances at a depth of 10 cm, $F(1, 4) = 6.4$, $p = 0.064$. Within triplicates assays, diversity coefficients were generally more similar following the precipitation event than in samples taken during the drought, and there was evidence of patchy distribution in most of the triplicate samples taken at 3.0 cm apart.

Key words: Cochliopodiidae, gymnamoeba abundance, microbial ecology, precipitation events, soil biology, testate amoebae.

INTRODUCTION

Relative to global climate issues, considerable research has been done on the importance of precipitation events in arid and semiarid regions on the ecology of macrobiota (e.g., Montana *et al*. 1995, Lin Guanghui and Ehleringer 1996, Gebauer and Ehleringer 2000), but less on terrestrial protozoa (e.g., Parker *et al.* 1984) and gymnamoebae (e.g., Rodriguez-Zaragoza and Garcia 1997, Anderson 2000) where water availability is considered the primary limiting factor.

Studies investigating how changes in precipitation patterns in arid and semiarid regions affect terrestrial gymnamoebae populations are especially important considering the ecological role gymnamoebae play in terrestrial systems including the cycling of nutrients (Stout 1980, Foissner 1997, Treonis and Lussenhop 1997), the regulation of bacterial populations (Darbyshire and Greaves 1967, Habte and Alexander 1977, Clarholm

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1981), and as a possible food source for earthworms and other soil-dwelling invertebrates (Bonkowski and Schaefer 1997, Anderson and Bohlen 1998).

Although it has been established that moisture is a significant environmental factor affecting soil gymnamoebae abundance, little is known about the effects of rain bursts on gymnamoebae abundance at varying depths especially in semi-arid or marginally arid regions in relation to global climate change. The study investigated the following research questions:

(1) What are the effects of precipitation after an extended drought on the abundance and diversity of gymnamoebae sampled at three soil depths (surface, 5 cm and 10 cm)? (2) What are the differences in abundance and diversity of gymnamoebae at the three sample depths? (3) To what extent is their evidence of patchiness in gymnamoebae abundance in three closely spaced samples (within 3 cm apart) taken at each of the three depths?

MATERIALS AND METHODS

Study location and soil characteristics

The study site was located in Americus, Georgia on the Georgia Southwestern State University Campus (33° 3' 7.79" N, and, 89° 13' 1.83" W) at *ca* 935 m above sea level. Soil samples were taken in an area of the campus shaded by pine trees and sparse grasses that had not been fertilized or cultivated for at least 30 years. A spade was used to search for and identify a loosely compressed soil with a surface horizon that continued for at least 15 cm. This kind of soil was chosen to more clearly investigate the effects of depth on gymnamoebae community structure while minimizing the effects of soil type associated with horizon changes. The sampling site was covered by a very thin layer (< 1.0 cm) of pine needles and leaf litter that were brushed away before each sampling. A homogeneous appearing surface horizon extended beyond 20 cm.

Data of measured soil characteristics and precipitation trends are shown in Table 1. Triplicate samples were prepared at the surface, 5.0 cm and 10.0 cm during an extended drought and two months later during a series of brief rain bursts. To collect soil samples, three cork borers were simultaneously inserted laterally into the soil in a 3 cm space and the cores were removed.

The sample was immediately taken to the lab in a zip-lock bag, removed and cut in half lengthwise. Half was used to determine soil characteristics and half was used for the enumeration technique (explained below). Enlarging the trench with a hand trowel and exposing fresh soil prevented contamination of later-date samples. Soil moisture was determined by measuring weight loss after drying in an oven at 100 °C and % organic matter was calculated by measuring additional weight loss after ashing the dried core at 500 °C overnight. The pH of each sample (n = 18) was determined using a pH meter (Orion SA520, Orion Research Inc. Boston, MA), and the soil temperature was recorded *in situ* by inserting a centigrade thermometer into the soil. The soil at each depth was tested once for water retention using a combination permeameter, (Gilson model HM-80, Lewis Center, OH) and precipitation data were obtained from the Plains Experimental Station, located 10 km from the study site.

Enumeration technique

Numbers of gymnamoebae/g of dry soil were determined using a laboratory culture enrichment method (Anderson and Rogerson 1995, Anderson 2000). The half of the core sample designated for enumeration was suspended in 10 ml of 0.45 µm pore size -filtered pond water in a graduated 15 ml centrifuge tube, shaken to flocculate attached amoebae and allowed to stand for a period of *ca* 0.5 min to sediment large particles. A 2 ml aliquot of the diluted suspension was removed and further diluted with 10 ml of 0.45 µm pore size filtered pond water in a 100-ml beaker. Using a micropipette, 10-µl aliquots were placed into each well of a 24-well sterile Falcon culture dish, where each well already contained 2 ml of 0.45 µm filtered pond water and a small piece (*ca* 1 mm^3) of malt-yeast agar (Page 1983) to support bacterial growth as a food for the gymnamoebae. Two culture plates were used for each sample, totaling 48 wells. The plates were wrapped in plastic film to avoid moisture loss and placed in an incubator at 19 °C for 14 to 16 days to allow proliferation of the gymnamoebae for counting and morphotype categorization. Cultures were observed with a Nikon inverted microscope using phase contrast optics, a 40x objective lens and a 15x eyepiece.

Because gymnamoebae have few definitive diagnostic characteristics at the light microscopic level, a morphotypic categorization scheme was used that has been extensively applied in recent ecological studies (e.g. Anderson and Rogerson 1995, Bischoff and Anderson 1998, Bass and Bischoff 2001). Morphotype 1 (mt-1) are amoebae with extended lobose or fine sub-pseudopodia during locomotion such as *Acanthamoeba* sp., *Mayorella* sp., *Vexillifera* sp., *Thecamoeba* sp., *Echinamoeba* sp., and *Rhizamoeba* sp. Type 2 are limax non-eruptive gymnamoebae. Genera of this type include *Hartmannella* sp., *Glaeseria* sp., and *Saccamoeba* sp. Type 3 are limax amoeba exhibiting eruptive locomotion by extrusion of a hyaline cap. Genera of type 3 include *Vahlkampfia* sp. and *Naegleria* sp. Type 4 are flattened or discoid, circular or fan-shaped, only sporadically with protruding elongated sub-pseudopodia. Representative genera of type 4 are *Platyamoeba* sp. and *Vannella* sp. Genera of the family Cochliopodiidae (Page 1988) were categorized separately because some genera are partially covered by microscales, characteristic of testate amoebae, but are surrounded by a pseudopodial base that is more typically associated with naked amoebae. Hence, they are of uncertain taxonomic status as reported by Page (1988).

The frequency of occurrence of each morphotype was tallied in the 48 wells of the culture dishes. This frequency count was converted to number of individuals per ml of original sample suspension by correcting for the dilution steps described above. The total number in the original volume of the suspension was calculated and divided by the soil sample dry weight to obtain the number/g soil for each morphotype.

Statistical analyses

Statistical data were computed using SPSS 10.0™ software. A univariate ANOVA was used to analyze the effect of precipitation on total abundance while controlling for depth as a covariate. A linear

Table 1. Mean data for soil characteristics at each sampling depth. Precipitation is expressed as millimeters of rain 14-days prior to sampling and the number in parentheses is the number of days it rained during the 14-day period

	Surface - 4/17/01		Surface - 6/21/01	
	Mean	Range	Mean	Range
% H_20	7.3	6.0 - 9.1	24.1	23.6 - 26.8
% Organic	4.8	4.0 - 5.9	8.6	4.0 - 13.0
pH	6.4	6.3 - 6.5	6.3	5.9 - 6.7
Temp °C	18	-	24	-
H_2O Retention[a]			51.1 ml/100cm^3 dry soil	
Precipitation	20 (2)	-	130 (11)	-
	5 cm Depth - 4/26/01		5 cm Depth - 7/4/01	
% H_2O	5.3	3.7 - 7.2	11.3	9.6 - 13.3
% Organic	4.9	3.3 - 7.1	4.6	2.3 - 5.7
pH	6.3	6.2 - 6.4	6.6	6.5 - 6.7
Temp °C	21	-	29	-
H_2O Retention			43.8 ml/100cm^3 dry soil	
Precipitation	13 (1)	-	144 (7)	-
	10 cm Depth - 5/12/01		10 cm Depth - 7/4/01	
% H_20	1.5	1.0 - 3.6	7.3	4.8 - 9.7
% Organic	3.0	1.2 - 5.0	2.2	1.6 - 2.7
pH	6.3	6.1 - 6.5	6.6	6.6 - 6.7
Temp °C	22	-	28	-
H_2O Retention			37.0 ml/100cm^3 dry soil	
Precipitation	25 (1)	-	144 (7)	-

[a]Water retention was measured once for each soil depth.

regression was used to calculate the correlation between abundance and soil moisture. Diversity (H) was calculated using the Shannon-Wiener formula ($H = - S\ p_i\ \log_2\ p_i$), where H is the diversity coefficient, and pi is the proportion of each type of gymnamoeba relative to the total numbers. Since it is not always possible to identify individuals to species level, morphotypic categories or "type of gymnamoebae" were determined by size (5 - µm increments), locomotion pattern, and morphology.

RESULTS

Soil characteristic data measured before and after the precipitation events are shown in Table 1. During the drought the soils at all three depths were very dry and none contained water near their water retention capacities. The surface soil had a mean moisture content of 7.3 % and the soils at 5.0 and 10.0 cm had mean moisture contents of only 5.3 and 1.5 %. After the precipitation events the mean moisture content of the surface soil was 24.1%. The mean moisture content for the soil at 5.0 cm was 11.3 %, and the mean moisture content of the soil at 10.0 cm was 7.3 %. The soils at 5.0 and 10.0 cm depth were wetter after the precipitation events, but still did not retain water near their full potential.

Precipitation events and gymnamoeba abundance

Addressing the first research question, precipitation events had a significant effect on the abundances of gymnamoebae. A univariate ANOVA with depth as a covariate showed a significant difference $F(2, 15) = 12.8$, $p < 0.001$ in the mean total abundance of gymnamoebae (combining all sampling depths) between the dry, drought condition soil and the moist, post precipitation soil. The mean total abundance for the dry soil (sampled between April 17th and May 12th) was 1114 gymnamoebae/g dry soil, and the mean total abundance for the moist soils was 4118/g. Further ANOVA tests using abundance of gymnamoebae as the dependent variable and moisture as the independent variable compared the mean differences in gymnamoebae abundances for the dry soil and the moist soil at each depth. There were significant differences in the mean gymnamoebae abundances at the surface $F(1, 4) = 18.3$,

Table 2. Abundances (numbers/g soil dry weight) and diversity coefficients for the three replicate samples (3 cm apart) at each depth before and after substantial precipitation events

				Surface sample (4/17/01) prior to precipitation			
Sample No.	Type 1	Type 2	Type 3	Type 4	Cochliopodiidae	Total	Diversity
1	0	0	0	1766	0	1766	1.3
2	0	0	0	507	0	507	1.4
3	0	0	0	578	0	578	1.8
Mean	-	-	-	950	-	950	1.3
S.D	-	-	-	707	-	707	0.2
				Surface sample (6/21/01) following the precipitation events			
Sample No.	Type 1	Type 2	Type 3	Type 4	Cochliopodiidae	Total	Diversity
4	0	391	391	6652	196	7630	1.8
5	417	556	0	5417	0	6390	2.7
6	229	344	0	3094	229	3896	2.5
Mean	215	430	130	5054	141	5972	2.3
S.D	209	111	225	1806	124	1901	0.5
				Soil at 5 cm (4/26/01) prior to precipitation			
Sample No.	Type 1	Type 2	Type 3	Type 4	Cochliopodiidae	Total	Diversity
7	65	0	0	588	0	652	1.3
8	40	40	40	370	40	530	2.4
9	0	0	0	858	50	908	1.9
Mean	35	13	13	605	30	697	1.8
S.D.	33	23	23	244	26	192	0.5
				Soil at 5 cm (7/4/01) following the precipitation events			
Sample No.	Type 1	Type 2	Type 3	Type 4	Cochliopodiidae	Total	Diversity
10	0	0	0	193	2759	2952	2.9
11	206	206	0	0	4535	4947	3.3
12	0	0	0	0	2655	2655	2.9
Mean	69	69	-	64	3316	3518	3.0
S.D	118	118	-	111	1056	1246	0.2
				Soil at 10 cm (5/12/01) prior to precipitation			
Sample No.	Type 1	Type 2	Type 3	Type 4	Cochliopodiidae	Total	Diversity
13	0	0	0	284	805	1089	3.2
14	0	0	0	1107	842	1947	2.1
15	0	0	99	999	954	2052	2.7
Mean	-	-	33	797	867	1696	2.6
S.D.	-	-	57	447	77	528	0.5
				Soil at 10 cm (7/4/01) following the precipitation events			
Sample No.	Type 1	Type 2	Type 3	Type 4	Cochliopodiidae	Total	Diversity
16	120	0	0	179	2453	2752	3.4
17	0	0	69	0	2260	2329	2.6
18	0	0	180	180	3780	3510	2.9
Mean	40	-	83	119	2621	2863	2.9
S.D	69	-	91	103	468	598	0.4

$p < 0.013$, and at a depth of 5.0 cm $F(1, 4) = 15.0$, $p < 0.018$. However, there was no significant difference at the 10.0 cm depth $F(1, 4) = 6.4$, $p = 0.064$. Furthermore, there was a strong correlation between total gymnamoebae abundance and moisture ($r = 0.75$, $n = 18$, $p < 0.001$). The regression equation for the correlation between abundance and moisture was $Y' = 3.022X + 2.188$.

Gymnamoebae abundance and diversity related to soil depth

The results of the second research question addressing differences in gymnamoebae abundance and diversity at the three sample depths are shown in Table 2. There was a general trend of increasing abundance with increasing depth for the dry soil samples. The mean total abundance (1696/g) of gymnamoebae in the dry soil at 10.0 cm was substantially larger than the mean abundance at the surface (950/g), and about twice as dense as the dry soil at 5.0 cm (697/g). This is particularly interesting because the soil at 10.0 cm depth on May 12th contained a mean moisture content of only 1.5 %. Mean diversity indices also increased with depth in the dry soils ranging from H = 1.3 in the soil at the surface to H = 2.6 at 10.0 cm. This was due to the presence of large numbers of individuals of the family Cochliopodiidae (Page 1988) with lengths ranging from 25-97 μm and mt-4 species (Platyamoeba and Vannella) with a mean length of 9.4 ± 3.4 μm.

By contrast, gymnamoeba density decreased with depth after the precipitation events. The mean density of gymnamoebae in the moist soils was respectively: surface soil (5972/g), 5.0 cm depth (3518/g), and 10.0 cm depth (2863/g). Unlike the dry surface soil that was completely dominated by mt-4 gymnamoebae, the moist, post-precipitation surface soil contained all four morphotypes and species in the family Cochliopodiidae. The mean diversity coefficients of the morphotypes observed in the post precipitation soils sampled at the surface, 5.0 and 10.0 cm depths were similar (H = 2.3, 3.0 and 2.9). However, the mean diversity coefficients of the samples taken at the three depths in the dry soils (H = 1.3, 1.8 and 3.0) were more varied and may indicate greater variation in community composition with depth following precipitation events.

Patchiness of gymnamoebae morphotypes

Addressing question 3, there were six sample collections where triplicate samples were taken on the same day within a horizontal distance of 3.0 cm, and there is evidence of patchy distribution for the total abundance and for each morphotype of gymnamoebae within the triplicate samples for each of the sample collections.

Evidence of patchy distribution included variations in populations of mt-4. For example, mt-4 densities in sample 1 (Table 2) were three-times larger than samples 2 and 3. The abundance of mt-4 in sample 4 is double that of sample 6, and the abundance of mt-4 in sample 9 is double that of sample 8. The total abundance of sample 11 is almost double that of samples 10 and 12 due to a localized high occurrence of Cochliopodiidae. The abundance of gymnamoebae in sample 13 is about half that of sample 14 and 15 because far fewer mt-4 were observed. Samples 16, 17 and 18 (the moist soil at 10.0 cm) showed the least amount of patchiness. There is no evidence of a relationship between patchiness and soil moisture. Thus, rain bursts while increasing overall abundances and diversity of gymnamoebae in the more moist uppermost samples had no apparent effect on the patchiness of gymnamoebae.

DISCUSSION

This report confirms findings of previous studies that protozoan abundances increase following major precipitation events (e.g. Darbyshire 1976) and gymnamoebae (e.g., Clarholm 1981, Rodriguez-Zaragoza and Garcia 1997, Anderson 2000, Bass and Bischoff 2001). This report advances our knowledge of the ecology of soil gymnamoebae by providing a more detailed analysis of the changes in abundances of morphospecies and by describing the effects of precipitation events following an extended drought. This situation more closely approximates the conditions that prevail in arid and semi-arid environments and therefore has significance in elucidating how precipitation in arid regions can affect terrestrial microbial communities and their productivity. The data, moreover, go beyond abundance by including variations in diversity and patchiness with depth in relation to precipitation events.

The drought and post precipitation gymnamoebae density data are useful in estimating how rapidly trophonts can emerge and proliferate. Based on a linear regression analysis, Anderson (2000) reported that as much as 20 % of the gymnamoebae could be active in soil with 10 % moisture, 50 % active with soil moisture near 20%, and the proportion of active amoebae could be as high as 70-80 % with soil moisture content above 25 %. However, these data were for a site in northeastern U.S.A.

and there may be significant differences for other soil types. The mean water content of all three drought soils in the current study was low ranging from 7.3 % at the surface to a very dry 1.5 % at 10.0 cm. Considering Anderson's linear regression model as a reasonable estimate of likely active stages, all three dry soils should have contained very few, if any, active gymnamoebae. Even if there was a very small proportion of trophonts present in the dry soils, it is unlikely that they would have been observed since only a 10 µl aliquot is deposited in each well of the culture plate. Hence, the enumeration process would favor the transfer of encysted forms to the culture-plate wells, thus the active gymnamoebae observed would be, with few exceptions, those individuals that excysted in the culture wells. It is reasonable to infer that the gymnamoebae densities reported in Table 2 for the dry soils were encysted in the natural environment.

The post precipitation soils were wetter and had higher gymnamoebae densities than the dry soils presumably due to the emergence and proliferation of trophozoites. At the surface the mean density of gymnamoebae increased 6-fold from 950/g during the drought (mean soil moisture 7.3 %) to 5972/g following the precipitation events (24.1 % soil moisture). The density increase at the surface following precipitation was due mostly to a burst in mt-4. This is consistent with other studies (Anderson 2000, Bass and Bischoff 2001) that reported large increases in the density of mt-4 (Vannella and Platyamoebae) with increased moisture. At 5.0 cm, the mean water content increased from 5.3 to 11.3 % following precipitation. Both of these moisture levels are low and we would expect almost complete encystations at 5.3 % moisture and only about 10 % of the gymnamoebae to be active at 11.3 %, yet the density of Cochliopodiidae increased from a mere 30/g during the drought to a mean of 3316/g following precipitation. A similar pattern of large Cochliopodiidae density increases with a small moisture increase was observed at 10.0 cm. At this depth soil moisture increased from a very dry 1.5 to 7.3 %, but the density of Cochliopodiidae tripled from a mean of 867/g to 2621/g. The cause for the high numbers of Cochliopodiidae is not clear, but perhaps the morphology of Cochliopodiidae, including the presence of microscales often surrounded by a pseudopodial base enables them to conserve cytoplasmic moisture and survive in the smallest of water films.

The significant differences in total mean abundance for the post precipitation soils, and the strong correlation between soil moisture and abundance support the conclusion that the gymnamoebae in this soil possessed a tenacious capacity to proliferate when environmental conditions improved. Although no significant difference in total mean abundance was found for the gymnamoebae community at 10.0 cm comparing the drought samples to those after the precipitation events, the total mean gymnamoebae abundance at that depth increased by 60 %. The capacity of soil gymnamoebae to withstand drought conditions and proliferate soon after precipitation events is encouraging.

Overall, the mean density of gymnamoebae in the wet soil was 4 times greater than the dry soil. Similarly, Anderson (2000) conducted a four-year study in Northeastern U.S.A and reported mean gymnamoebae abundances were four times larger during the warm and wet El Niño winter of 1997-1988 than the mean of the non El Niño years, and Rodriguez-Zaragoza and Garcia (1997) reported a doubling of gymnamoebae densities following precipitation in a Mexican desert at depths of both 10 and 30 cm.

The trend of increased gymnamoebae abundance and diversity following precipitation was more extensive at the surface than at depth. For example, the mean density of gymnamoebae in the wet surface soil was six times greater than the dry soil and the drier soil contained only mt-4 species (*Platyamoeba* and *Vannella*). After a substantial series of rain events, all four morphotypes and genera of the family Cochliopodiidae (most likely *Cochliopodium bilimbosum*) were observed in the moist surface samples demonstrating the capacity of soil gymnamoebae to flourish, probably due to excystment and proliferation when conditions improved.

An interesting pattern in the soil at 5-cm was the substantial increase in the abundance of Cochliopodiidae and the concomitant decline of mt-4 gymnamoebae following the rain events. The data suggest the possibility of a predator prey relationship, or the ability of Cochliopodiidae to competitively exclude mt-4 in the microbial community. This observation needs verification and could be an interesting future study.

At 10-cm depth, mt-4 gymnamoebae and Cochliopodiidae dominated the drier soil. Only sample 15 contained Vahlkampfiid-type, mt-3 gymnamoebae and these were present in relatively small densities. Even though 144 mm of precipitation occurred during the 14-days preceding sampling, the soil moisture only increased from a very dry 1.5 % to 7.3 %. This increase in moisture, however small, seemed sufficient to triple the mean abundance of Cochliopodiidae and drive down the populations of mt-4, following a pattern very similar

to the data from soil at 5.0 cm. Additionally, mt-1 which were not detectable in the drier soil at 10.0 cm were found in sample 16, and mt-3 abundances increased slightly.

There was evidence of patchy abundance patterns in almost all of the triplicate samples (Table 2). Surface samples 4, 5 and 6 were the wettest soils sampled and they are the best data to look for patterns of reduced patchiness with increased moisture. The abundance patterns of mt-2 display only a small amount of patchiness and that could be attributed to error in the sampling method and not true patchiness in the soils. There is, however, evidence of patchy abundance patterns for morphotypes 1, 3, 4 and Cochliopodiidae in samples 4, 5 and 6.

With some of the triplicate data points, two of the assays yielded similar abundance points and a third identified either a growth hot spot, like sample 11 or a cold spot, like sample 13 where mt-4 abundances were low compared to samples 14 and 15. This patchy pattern of spatial distribution across a space of only 3.0 cm is of interest, but the causes are not known. It could occur due to heterogeneous distribution of prey, variations in nutrient concentrations, unevenness in moisture, varied physical characteristics of the soil, etc.

Overall, the data demonstrating the remarkable capacity of soil gymnamoebae to withstand severe drought and resume their role in the microbial food web in response to a series of brief rain bursts are reassuring. More research integrating our knowledge of gymnamoebae ecology with global climate issues is needed so that we may more fully understand the dynamics of our changing environment and its likely influence on terrestrial microbial communities.

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Short Communication

Effect of Oxytocin and its Analogues on the Phagocytosis of *Tetrahymena*: Outstanding Impact of Isotocin

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Summary. The influence on the phagocytosis of *Tetrahymena pyriformis* of oxytocin and its analogues (derivatives) tocinoic acid, isotocin and the tyrosin supplemented tail part (Tyr-Pro-Leu-Gly=Tyr) as well, as the impact of oxytocin and its analogues on the phagocytosis of the populations of *Tetrahymena* selected to itself and to the three latter molecules were studied in the experiments. The molecules tested did not influence phagocytosis in the random populations. However, populations selected to isotocin have a higher phagocytotic activity (without further stimuli) and also reacted to oxytocin or isotocin with an increased phagocytosis. Also isotocin was the only molecule the selection of which resulted in a "size-altered" population (smaller cells), and produced minimal number of non-phagocytizing "0" cells. Populations selected to tocinoic acid or Tyr reacted with a decrease of phagocytosis to oxytocin treatment. The experiments calls attention to the possible evolutionary role of (chemotactic) selection to signal molecules, to the differentiating ability of *Tetrahymena* between signal molecules and to the advantage of phylogenetically older molecules from this point of view.

Key words: chemotactic selection, evolution, oxytocin analogues, phagocytosis, *Tetrahymena*

INTRODUCTION

Hormone receptors, signal transduction pathways and hormones, characteristic to higher vertebrates are also present at unicellular level (LeRoith *et al*. 1983; Csaba 1985, 2000; Christopher and Sundermann 1995). *Tetrahymena* can react to histamine and serotonin with increased phagocytosis (Csaba and Kovács 1994, Hegyesi *et al*. 1998) , to thyroxin and its precursors with enhanced growth (Csaba and Németh 1980), to insulin, epinephrine and glucagon with altered sugar metabolism etc (Csaba and Lantos 1975, 1976; Csaba 1994). However, in addition to the identical response, these hormones can develop other physiological reactions of protozoa. At the same time, the binding sites (receptors) sometimes are very sensitive to differences in the hormone molecule given. Precursors of hormones are more effective in provoking response than the vertebrate hormone itself and some amino acids of peptides are preferred in a signal molecule - binding site connection, which could have some importance in the evolution of signalization (Csaba 1994, 2000).

In earlier experiments, when the effect of the 9 amino acid containing peptide hormones, oxytocin and vasopressin were studied to the function of contractile vacuole, the phylogenetically older oxytocin showed the more prominent influence, in contrast to the fact that in higher

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animals the regulator of water "management" is the vasopressin (Csaba and Kovács 1992). In an other experiment, studying the effect of oxytocin and its five analogues to a basic index, chemotaxis, the consistent, repellent response was developed only by the two "matured" hormone, oxytocin and vasopressin (Csaba *et al*. 2000). This made reasonable to study the effect of oxytocin and its analogues to an other basic physiological index, the phagocytosis of *Tetrahymena*. The study has been combined with chemotactic selection of *Tetrahymena* by previous encounter with the hormone or hormone like molecule, and the study of the selected populations.

MATERIALS AND METHODS

Cells and Culturing. Cultures of *Tetrahymena pyriformis* GL were used in the logarithmic phase of growth. The cells were sustained in a medium containing 1% tryptone (Difco, Michigan, USA) and 0.1% yeast extract (Difco, Michigan, USA) at 28 °C.

Chemicals. Oxytocin and its derviatives were obtained from Sigma Ltd. (St. Louis, USA). Substances were diluted in fresh culture medium immediately before the experiments.

Chemotactic selection. For this purpose chemotaxis assays were carried out according to Kőhidai *et al.* (1995), the test containing two-chambers: the outer chamber was filled with the cells to be tested, the inner one contained the test substance. In this setup tips of multi-8-channel micropipette served as inner chambers, while wells of 96-well microtitration plate were the outer chambers. Capillaries of tips served as connecting junctions between the inner and outer chambers. The concentrations of the oxytocin derivatives for these assays were chosen according to their most effective concentration of the concentration course (Csaba *et al.* 2000). In this set-up, cells of the outer chamber were considered as "mixed population", and the drive of chemotactic substances, filled into the inner chamber, were applied to select populations on the basis of their chemotactic preference. In control groups fresh culture medium was applied as chemoattractant. Experiments were done under sterile air-flow, the time of incubation was 20 min.

The following groups were formed (see Table 1).

After each run, selected cells (of the inner chamber) were transferred into fresh culture media. The cultures formed this way were transferred every third day.

Phagocytosis assay. After a week of the chemotactic selections the phagocytotic activity of cultures were tested. Three hours prior to the assay the cells were transferred to Losina-Losinsky solution (hereafter LL solution - containing 1% NaCl, 0.1% $MgCl_2$, 0.1% $CaCl_2$, 0.1% KCl and 0.2% $NaHCO_3$) in the aim to have starved model cells with particle free cytoplasm.

Volumes of starved cultures, suspension of Chinese ink and agonists were mixed (v/v/v=1:1:1) After 5 min incubation the cells were fixed with 4% formaldehyde containing LL solutions. The phagocytosis assays were done in the following groups: cultures provided by chemotactic selection were treated (i) with the solvent (fresh culture medium); (ii) with oxytocin and (iii) with the identical "selector" oxytocin derivative. The applied concentrations were: oxytocin 10^{-12} M; tocinoic acid 10^{-10} M, isotocin 10^{-7} M, Tyr-Pro-Leu-Gly 10^{-7} M. The test particle number was determined by light microscope in 200 cells/group.

Table 1. Chemoattractants used. The first word indicates the type of selection / the second indicates the one applied week later

Oxytocin:	**Tocinoic acid:**
Control/Control	Control/Control
Control/Ox1-9	Control/Toc
Ox1-9/Control	Toc/Control
Ox1-9/Ox1-9	Toc/Toc
Isotocin:	**Tyr-pro-leu-Gly:**
Control/Control	Control/Control
Control/Iso	Control/Tyr
Iso/Control	Tyr/Control
Iso/Iso	Tyr/Tyr

The distribution of particle quantity in the cells was also determined (Figs 1-4). Fig. 5 shows the number of cells containing no test particle ("0 cells").

Morphometry. Effect of chemotactic selelction upon formation new subpopulations was investigated in respect of morphological characteristics of cells. For this purpose cells of the chemotactically selected cultures (Ox, Toc, Iso, Tyr) without any further treatment were investigated by fluorescent activated cell sorter (FACS-Calibur, Becton-Dickinson). The number of evaluated cells was 10000/ sample. The FSC-H values provided us to describe the subpopulations (Fig. 6).

Statistical evaluation of data. Each assay was repeated in five independent experiments, in three replica of each. Groups treated with culture medium or groups selected with culture medium and tested with plain medium were considered as "absolute control" groups. In phagocytosis assay for each group/experiment mean values of number of particles were calculated. Data-points of figures were calculated from the mean values of identical groups. In phagocytosis assays and morphometry values of geo-mean are also given. Other data were evaluated by using statistical tests (ANOVA and two tailed t-test) of Microcal Origin 4.0.

RESULTS AND DISCUSSION

Of the four molecules studied, oxytocin is the "real" hormone which is present also in higher vertebrates regulating smooth muscle contraction and maternal behavior (Pedersen *et al*. 1982). Isotocin can be found only in bony fishes as a hormone, influencing a variety of physiological functions (Hausmann *et al*. 1995). Tocinoic acid, which contains the first six amino acids (the ring) of oxytocin is known as a molecule, which can inhibit

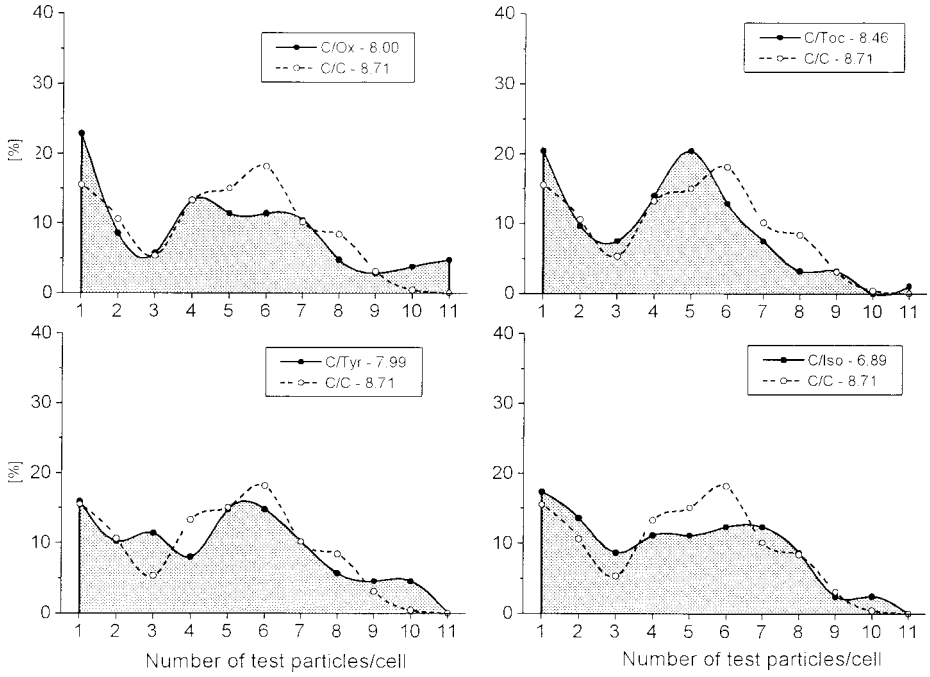

Fig. 1. Phagocytotic activity induced with 10^{-12} M oxytocin (C/Ox); 10^{-10} M tocinoic acid (C/Toc); 10^{-7} M Tyr-Pro-Leu-Gly (C/Tyr) and 10^{-7} M isotocin (C/Iso) of *Tetrahymena pyriformis* cultures selected with control culture medium. Dotted line represents the histogram of absolute control (C/C). Geo-means of histograms are given after the abbreviations in the boxes

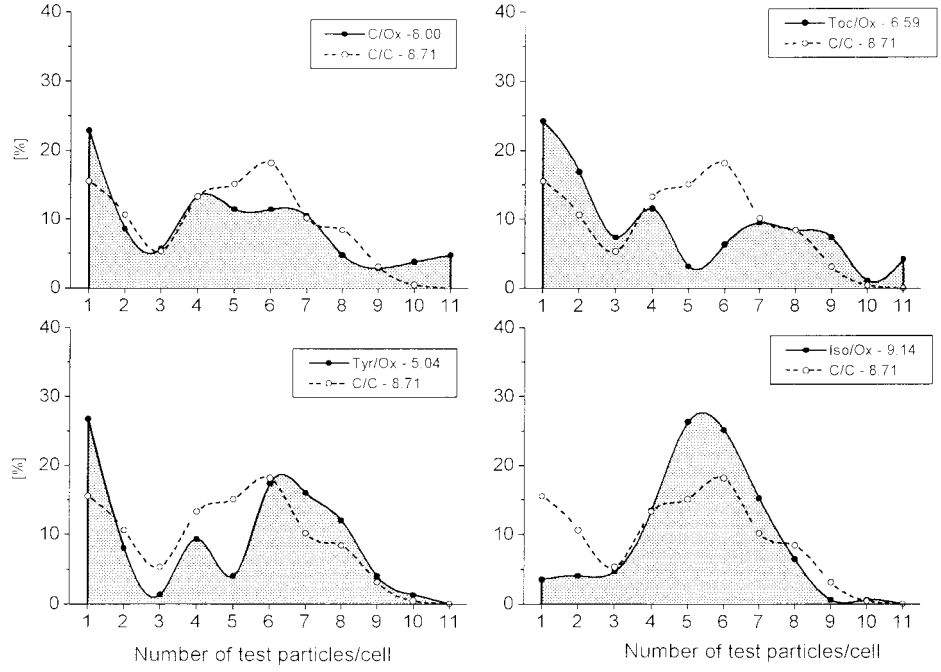

Fig. 2. Phagocytotic activity induced with 10^{-12} M oxytocin in *Tetrahymena* cultures selected with culture medium (C/Ox); 10^{-10} M tocinoic acid (Toc/Ox); 10^{-7} M Tyr-Pro-Leu-Gly (Tyr/Ox) and 10^{-7} M isotocin (Iso/Ox). Dotted line represents the histogram of absolute control (C/C). Geo-means of histograms are given after the abbreviations in the boxes

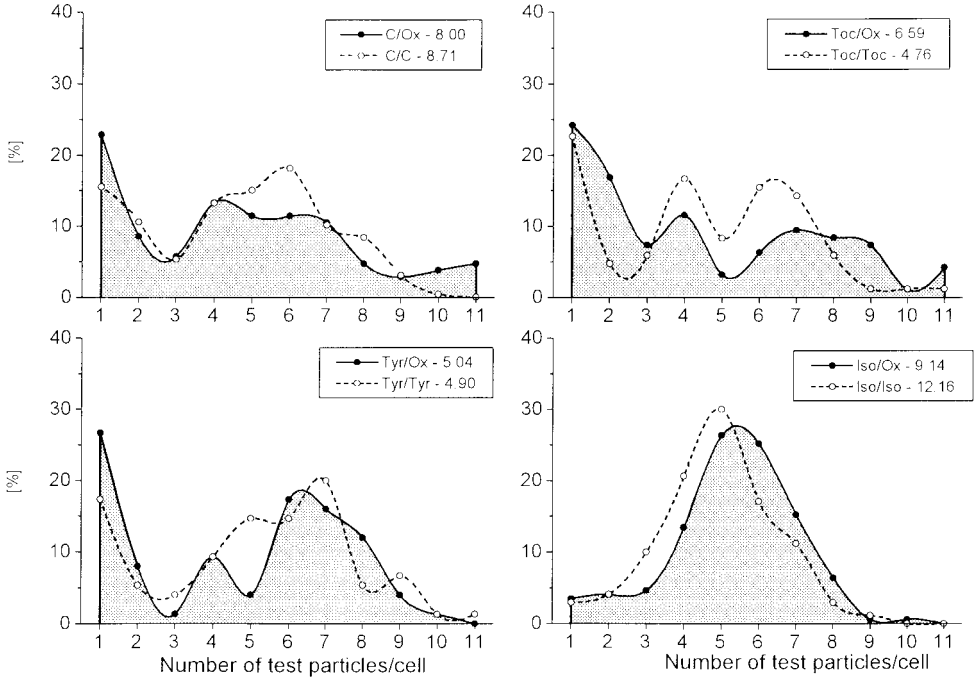

Fig. 3. Comparison of phagocytotic responsiveness to identical selector and oxytocin in cultures selected by oxytocin derivatives. Selections were made as it was described above. Solid lines represent effect of 10^{-12} M oxytocin; dotted lines represent histograms of identical derivative. Geo-means of histograms are given after the abbreviations in the boxes

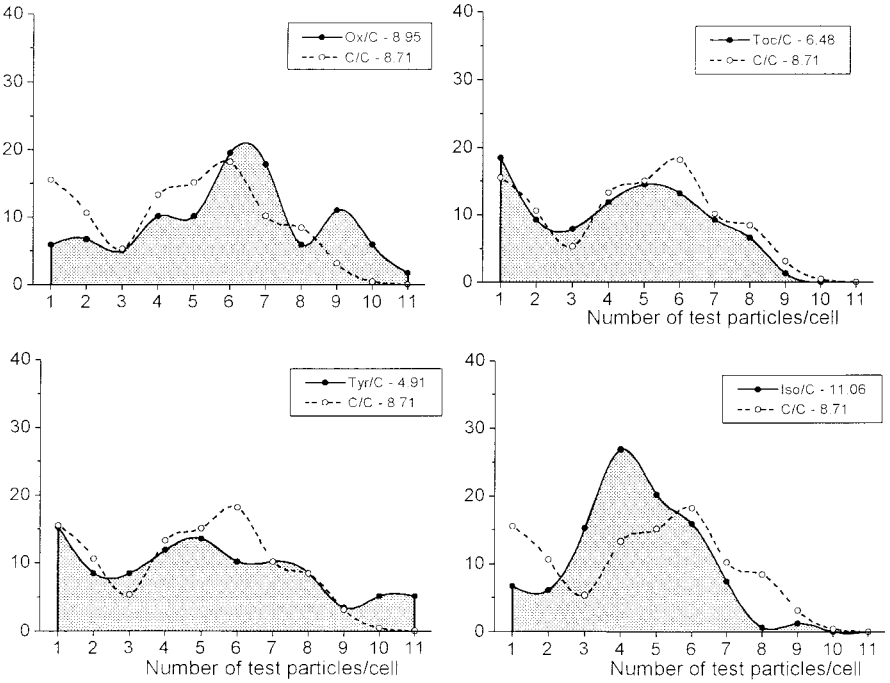

Fig. 4. Basic phagocytotic activity of *Tetrahymena* cultures selected with 10^{-12} M oxytocin (Ox/C); 10^{-10} M tocinoic acid (Toc/C); 10^{-7} M Tyr-Pro-Leu-Gly (Tyr/C) and 10^{-7} M isotocin (Iso/C). Dotted line represents the histogram of absolute control (C/C). Geo-means of histograms are given after the abbreviations in the boxes

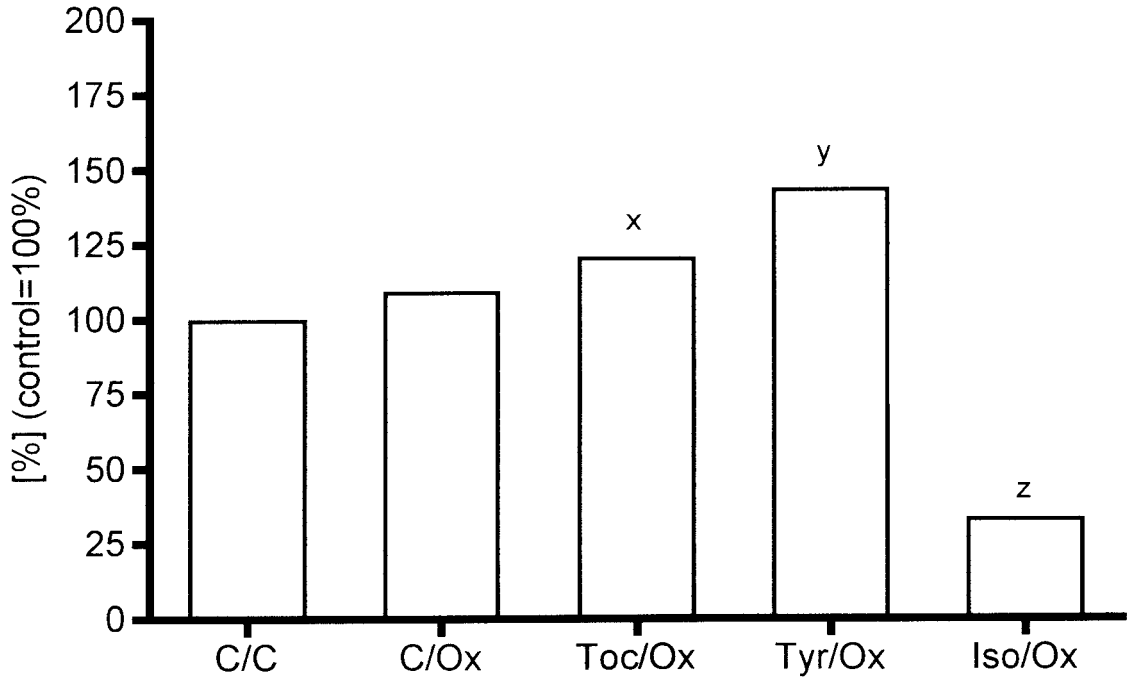

Fig. 5. Number of non-phagocytotic, „0-cells" in response of 10^{-12} M oxytocin treatment in *Tetrahymena* populations selected with control medium (C/C and C/Ox) and oxytocin derivatives (Toc/Ox; Tyr/Ox; Iso/Ox)

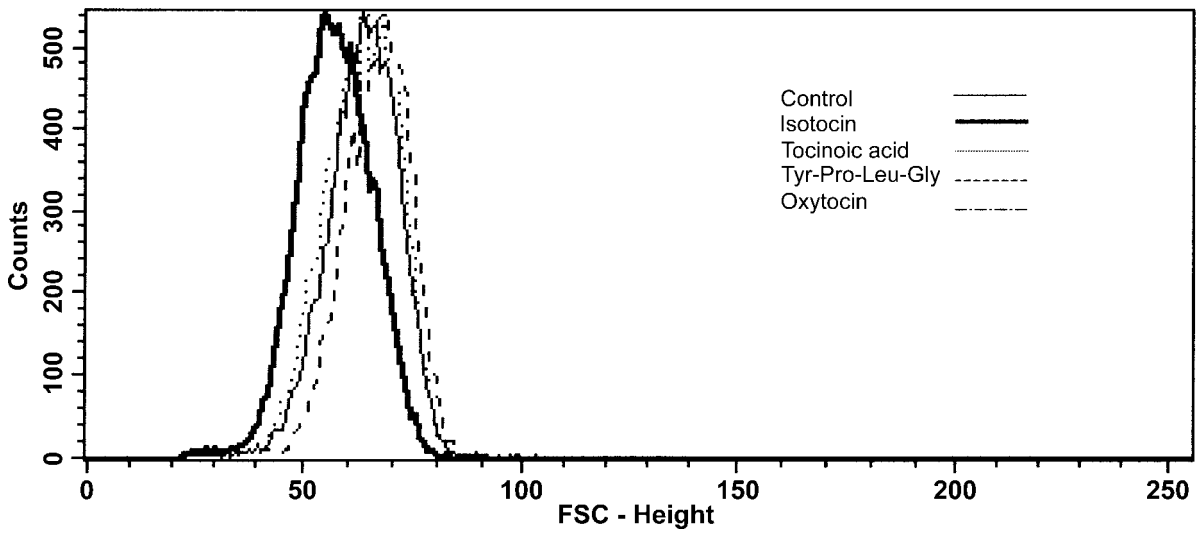

Fig. 6. Flow-cytometric results on the effect of selection with oxytocin derivatives on the morphometric properties of subpopulations

melanocyte stimulating hormone (MSH) release from the (rat) pituitary and also induces maternal behavior (Pedersen *et al*. 1982). The fourth molecule is a tyrosine containing variant of the "tail" of oxytocin (Tyr), the last three amino acids. There are no data in the literature on the effect of three of these four molecules influencing phagocytosis, however there are scarce data on the effect of vasopressin and oxytocin to the phagocytosis of macrophages (Block *et al*. 1981, Fernandez-Repollet *et al*. 1983), in higher animals.

There was no significant effect of molecules studied on the phagocytosis of *Tetrahymena* in case of random cell population (Fig.1). Though some-non significant-differences were observed in the number of test particles per cell, the mean values were near to each other. This means that oxytocin and its analogues are indifferent to phagocytosis in *Tetrahymena*. However selection of the cells changed the picture. Subpopulations gained by selection with Tyr or tocinoic acid the phagocytotic responsiveness to oxytocin was reduced (Tyr/Ox, Toc/Ox; Fig. 2), while it seemed to be indifferent after isotocin selection. This means that selection is working and the selected population is different -depending on the selector molecule- from the random population and because of this, hormonal effects are also differently manifested.

Comparing the phagocytic activity to oxytocin in the selected populations with the reaction of selected populations to the selector hormone itself, isotocin and tocinoic acid showed a considerable difference. In this case isotocin treatment of isotocin-selected population (Iso/Iso) significantly surpassed the value of isotocin-treated in control population (C/Iso) (Fig. 1 compared to 3) and also oxytocin treatment in isotocin selected population (Iso/Ox) (Fig. 3). In addition, isotocin selection alone considerably elevated phagocytosis (Fig. 4). while tocinoic acid- or Tyr-selection did not do the same (Fig. 4). If we also consider that Iso/Ox was significantly higher than C/Ox, which was similar to C/C, in addition only isotocin selection produced a "size-altered" population (Fig. 6), and produced the less "0" cells (Fig. 5), these facts point to the outstanding and special influence of isotocin. However, selection to tocinoic acid or Tyr also influenced (reduced) the sensitivity to oxytocin or to the selectors themselves, their effect confined to this single act (Fig. 3).

Isotocin has a hormonal function in teleosts (Hausmann *et al*. 1995) and, however its effect is manifested also in higher animals on oxytocin or vasopressin receptors, this effect is much lower than that of oxytocin (Meidan and Hsuch 1985). Nevertheless, isotocin preference by *Tetrahymena* is not surprising, in earlier experiments also the phylogenetically older signal molecules were more effective in oxytocin-vasopressin relation as well (Csaba and Kovács 1992), as in case of the thyroxin series (Csaba and Németh 1980). From this aspect the higher effectiveness of the "real" hormones (oxytocin, vasopressin in case of chemotaxis) seems to be the exception.

From the results of the experiments it can be concluded that 1) *Tetrahymena* can differentiate between related signal molecules; 2) selection to a signal molecule can change the functional state of the cells and this could have an evolutionary role; 3) of the molecules studied isotocin has a prominent role influencing phagocytosis in selected cell populations; 4) phylogenetically older (more ancient) signal molecules are preferred by *Tetrahymena*.

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Each table must be on a separate page. Figure legends must be in a single series at the end of the manuscript. References must be listed alphabetically, abbreviated according to the World List of Scientific Periodicals, 4th ed. (1963). Nomenclature of genera and species names must agree with the International Code of Zoological Nomenclature, third edition, London (1985) or International Code of Botanical Nomenclature, adopted by XIV International Botanical Congress, Berlin, 1987. SI units are preferred.

Examples for bibliographic arrangement of references:

Journals:

Häder D-P., Reinecke E. (1991) Phototactic and polarotactic responses of the photosynthetic flagellate, *Euglena gracilis*. *Acta Protozool.* **30:** 13-18

Books:

Wichterman R. (1986) The Biology of *Paramecium*. 2 ed. Plenum Press, New York

Articles from books:

Allen R. D. (1988) Cytology. In: *Paramecium*, (Ed. H.-D. Görtz). Springer-Verlag, Berlin, Heidelberg, 4-40

Zeuthen E., Rasmussen L. (1972) Synchronized cell division in protozoa. In: Research in Protozoology, (Ed. T. T. Chen). Pergamon Press, Oxford, **4:** 9-145

Illustrations

All line drawings and photographs should be labelled, with the first author's name written on the back. The figures should be numbered in the text as Arabic numerals (e.g. Fig. 1). Illustrations must fit within either one column (86 x 231 mm) or the full width and length of the page (177 x 231 mm). Figures and legends should fit on the same page. Lettering will be inserted by the printers and should be indicated on a tracing-paper overlay or a duplicate copy.

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