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Small Free-Living Heterotrophic Flagellates from Marine Sediments of Gippsland Basin, South-Eastern Australia

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Abstract. A total of 85 morpho-species of heterotrophic flagellates are reported from sediments at depths from 25–3000 m in the Gippsland Basin (Australia). They are drawn from the apusomonads, cercomonads, cryptomonads, euglenids, heteroloboseids, stramenopiles, thaumatomonads, and groups of uncertain taxonomic affinities (Protista incertae sedis). Three new species, *Ancyromonas impluvium* nov. spec., *Kurnaimonas celeris* nov. spec., *Sinistermonas sinistrorsus* nov. spec., one combination, *Psammosa unguis* nov. comb., and one unidentified species are described. The biogeography of the species seen in Gippsland Basin is discussed with reference to studies in other localities. It appears that many heterotrophic flagellates are cosmopolitan.

Key words: Protist, heterotrophic flagellates, *Ancyromonas impluvium*, *Kurnaimonas celeris*, *Sinistermonas sinistrorsus*, *Psammosa unguis*, Gippsland Basin, cosmopolitan distribution.

INTRODUCTION

Heterotrophic flagellates are unicellular protists, are generally less than 20 µm in length and typically have one or more flagella for motility. They are significant members of the microbial communities in aquatic ecosystems. They function as predators on bacteria and small algae, and as prey for larger ciliates and metazoa (e.g., Sherr and Sherr 1984, 1988; Pace and Vaqué 1994). Additionally they facilitate remineralization and recycling of elements essential for algal and microbial growth (e.g., Sherr and Sherr 1984, 1988; Jürgens and Güde 1990; Caron 1994; Kirchman 1994). For that reason, they are recognised as an important component of natural aquatic ecosystems functioning as an energy transporter in microbial food webs.

Heterotrophic flagellates are often found in similar habitats around the globe. Patterson and Lee (2000) has suggested that probably there are no more than 3000 species of free-living marine heterotrophic flagellates excluding dinoflagellates and haptophytes, and that most have a cosmopolitan distribution. The number of morphospecies reported from marine habitats in this group to date is about 600 (Patterson and Lee 2000) and is considerably fewer than might be expected given the fact that over half of the different kinds of eukaryotes are flagellates (Patterson 1999). Additional

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species continue to be described, but at a slow rate (e.g., Hausmann *et al.* 2002b, 2006; Park *et al.* 2006; Lee 2008). The low number reported to date may result from under-reporting from various environmental conditions such as deep-sea, hypersaline and anoxic habitats, and the absence of discriminating species concepts to circumscribe taxa that have discrete ecological identies.

The aims of this study are to document the diversity of free-living heterotrophic flagellates from bottom sediments of deep-sea and to address issues of cosmopolitanism in this group. There are very few studies in deep-sea sediments or bottom sediments (e.g., Patterson *et al.* 1993; Atkins *et al.* 2000; Hausmann *et al.* 2002a, b; Arndt *et al.* 2003; Lee 2008). This study is complemented by the concurrent study of heterotrophic euglenids (Lee 2008) at the same sites.

MATERIALS AND METHODS

This study was carried out from 18th of September to 5th of October 1998 at 43 stations in Gippsland Basin, south-eastern Australia (37°59′S–38°49′S; 147°21′E–148°42′E) as a part of the R/V Franklin Cruise (Fr 11/98) by CSIRO. Bottom sediment samples were collected with a Smith-McIntyre grab sampler from 43 sampling stations and the sampling depths varied from 25 m to 3,100 m. After collecting, the samples were transported in cool condition (~ 4°C) to the University of Sydney for analysis. The sediment samples were placed in plastic trays in 1 cm deep layers, allowed to settle for several hours, and excess water drained off. The material was covered with a sheet of lens tissue upon which coverslips (22 × 22 mm No.1) were placed. The samples were maintained at room temperature (~ 20°C). After 12 hrs and for up to 2 weeks, the coverslips were removed and the associated flagellates were observed using a Zeiss Axioplan microscope equipped with photographic facilities as described by Patterson (1982). The micrographs were taken at different magnifications (63 × and 100 ×). The flagellates were drawn and recorded on U-MATIC video tapes, and records were also made with video prints. Sediment types and sampling locations for each station are shown in Lee (2008).

Analysis of similarities among heterotrophic flagellate 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). The community from Gippsland Basin was compared with the communities from other sites. For this analysis, the species list of heterotrophic euglenids studied in Gippsland Basin by Lee (2008) was included.

RESULTS

The ICZN (International Code of Zoological Nomenclature 1999) is used for the nomenclature of the groups represented in this study. Lists of species are presented in Table 1.

Retortamonadida Grassé 1952

Chilomastix cuspidata (Larsen and Patterson 1990) Bernard *et al.* 1997 (Figs 1a, 2a)

Observation: Cells are drop-shaped with a long posterior spike and are 20 to 32 μm long (including the spike) with a groove extending from the apex to the posterior end of the untapered part of the cell. The cells have 4 flagella inserting subapically and directed anterior laterally; one is shorter than the cell and the other three are about the cell length. The short flagellum beats and lies within the ventral groove. The nucleus is situated subapically. Food vacuoles occur throughout the cell. The cells move slowly by swimming while rotating and may attach to the substrate by the tip of the spike. Rarely observed.

Remarks: This species was transferred from *Percolomonas* to *Chilomastix cuspidata* by Bernard *et al.* (1997). It has been found at marine sites in Australia, Demark and Ireland (Fenchel *et al.* 1995; Larsen and Patterson 1990; Weerakoon 1999; Bernard *et al.* 1997, 2000) and previously reported range of cell lengths is 14 to 33 μm. There are two free-living species in *Chilomastix* including this species, but the other species, *Chilomastix undulata* Skuja 1956 was described as having an undulating membrane and may be transferred to *Trimastix* (Bernard *et al.* 2000).

Cryptophyceae Pascher 1913

Goniomonas amphinema Larsen and Patterson 1990 (Fig. 2b)

Observation: Cells are almost triangular in shape and 3 to 4.5 μm long. Pellicle stripes and anterior row of ejectisomes may be not seen. Two flagella of unequal length insert in an anterior lateral pocket; one directed anteriorly, the other one posteriorly. Commonly observed.

Remarks: This species is one of the most widely reported taxa from diverse sites worldwide (Patterson and Lee 2000). *Goniomonas amphinema* has been distinguished from *G. pacifica* Larsen and Patterson, 1990 by having two flagella of unequal length and by the

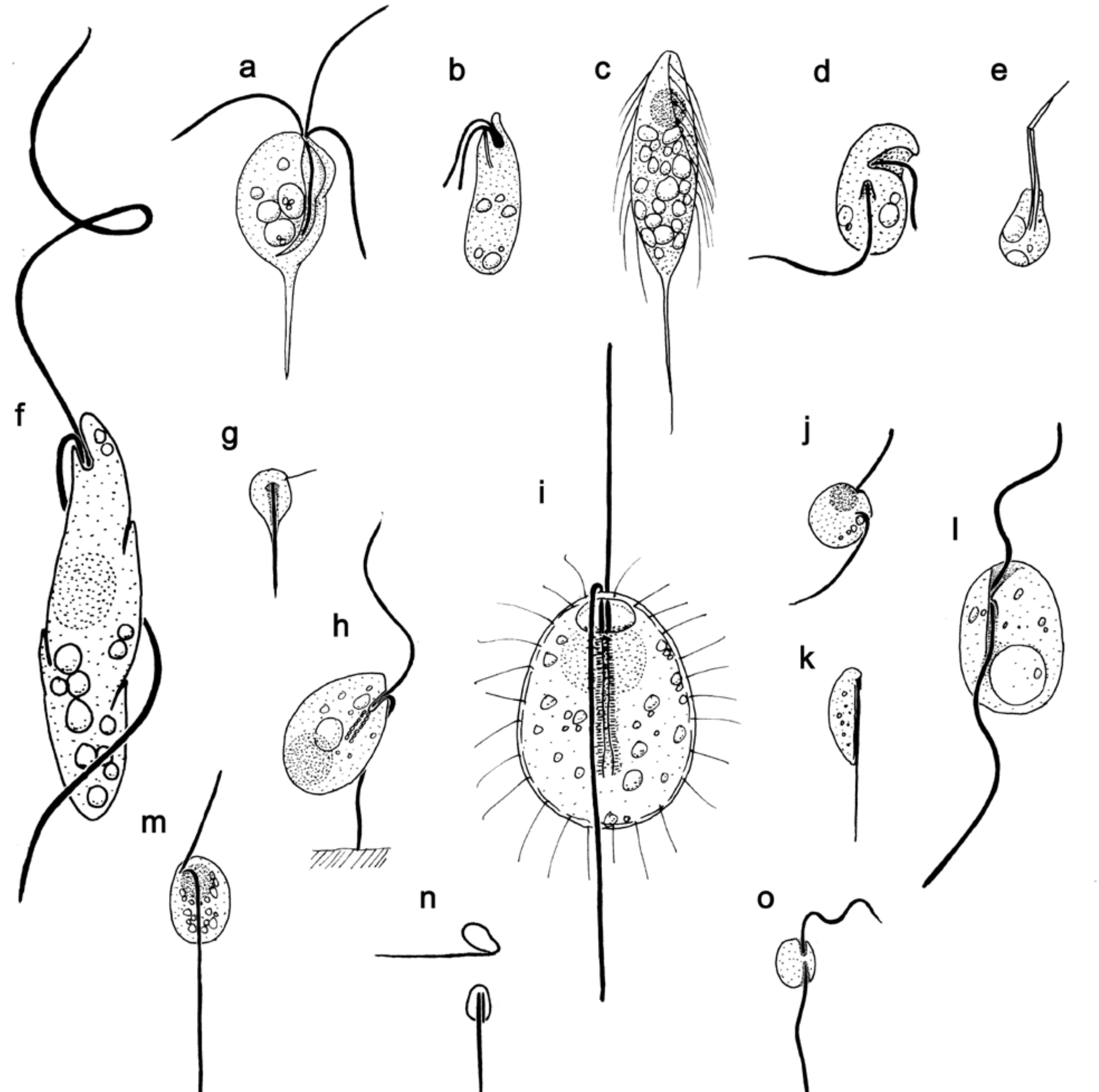

Fig. 1. a – *Chilomastix cuspidata*, **b** – *Rhynchopus amitus*, **c** – *Spironema multiciliatum*, **d** – *Psammosa unguis* nov. comb., **e** – *Apusomonas* sp., **f** – *Rhynchobodo formica*, **g** – *Mantamonas plastica*, **h** – *Roombia truncata*, **i** – *Thaumatomastix setifera*, **j** – *Ancyromonas impluvium* nov. spec., **k** – *Helkesimastix faecicola*, **l** – *Kurnaimonas celeris* nov. spec., **m** – *Sinistermonas sinistrorsus* nov. spec., **n** – *Kiitoksia kaloista*, **o** – Protist '1'. Scale bar: 10 µm for all figures.

flagellar orientation according to previous observers. Recently one more species (*Goniomonas avonlea*) was reported from marine sandy sediment by Kim and Archibald (2013). *Goniomonas avonlea* is different from *G. amphinema* in having two subequal flagella and a larger cell body (Kim and Archibald 2013).

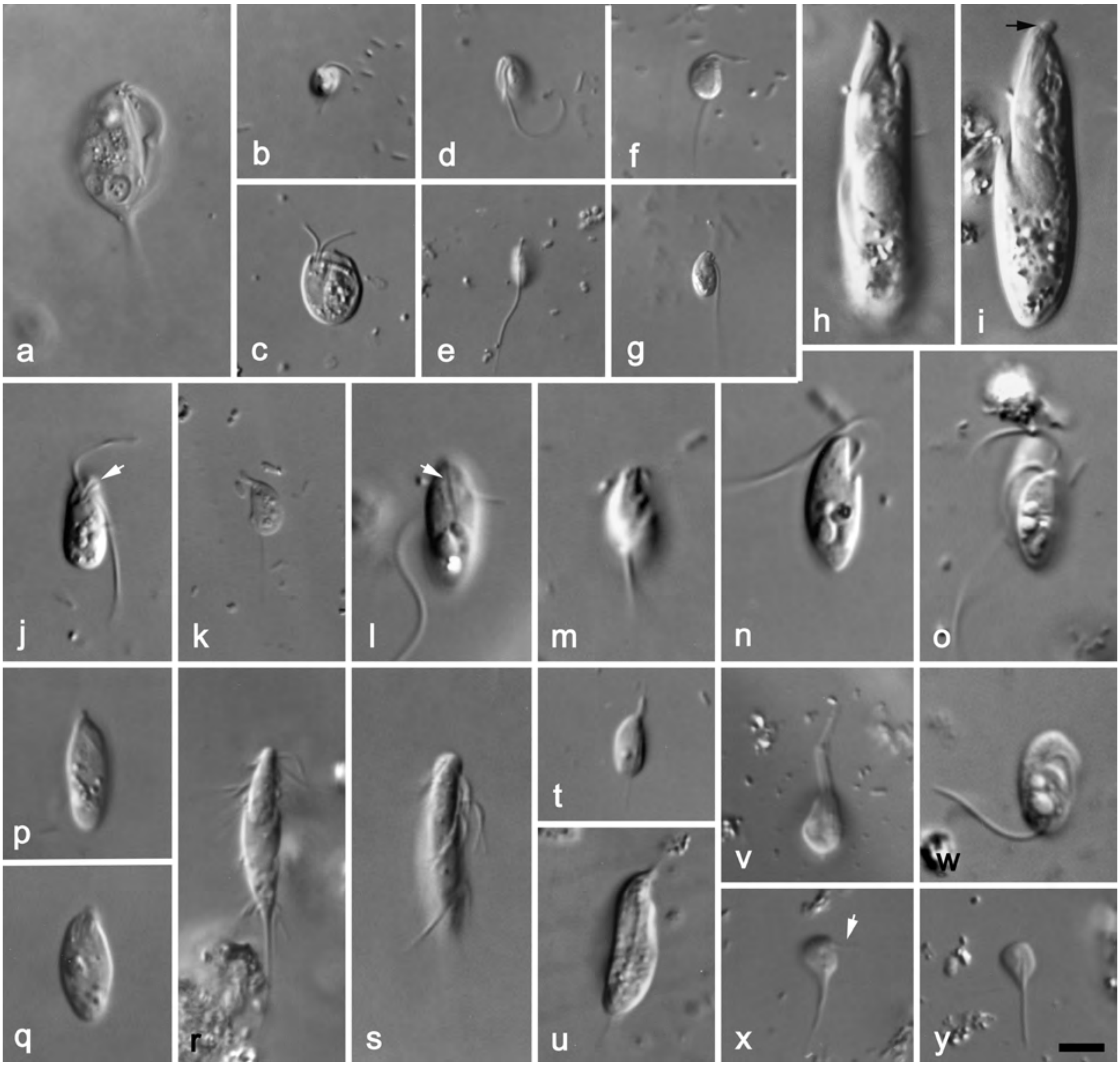

Fig. 2. **a** – *Chilomastix cuspidata*, showing general appearance of cell; **b** – *Goniomonas amphinema*, showing general appearance of cell; **c** – *G. pacifica*, note two flagella diverging; **d** – *Percolomonas similis*, note ventral groove; **e** – *Bodo platyrhynchus*; **f** – *Neobodo curvifilus*; **g** – *N. saliens*; **h–i** – *Hemistasia phaeocysticola*, note a papillum (arrow); **j** – *Bordnamonas tropicana*, showing general appearance and mouth (arrow); **k** – *Rhynchomonas nasuta*; **l–o** – *Rhynchobodo simius*; **l** – tubular ingestion organelle (arrow); **m, o** – showing groove; **n** – general appearance of cell; **p–q** – *Rhynchopus amitus*; **r–s** – *Spironema multiciliatum*; **t** – *Amastigomonas debruynei*, general appearance of cell; **u** – *Amastigomonas mutabilis*, note anterior flagellum projecting from sleeve and recurrent flagellum; **v** – *Apusomonas* sp.; **w** – *Psammosa unguis* nov. comb., general appearance of cell; **x–y** – *Mantamonas plastica*; **x** – note anterior flagellum (arrow) and **y** – ventral depression. All micrographs are DIC images. Scale bar: 5 µm for all figures.

Goniomonas pacifica Larsen and Patterson 1990 (Fig. 2c)

Observation: Cells are 4 to 10 µm long with several distinct longitudinal ridges on both sides of the cell. There is a row of about seven to nine ejectisomes near the anterior end of the cell, which is truncated with the posterior end rounded. Two flagella of similar length emerge from a small anterior depression and are directed anteriorly. When the cells are swimming, two flagella diverge in different directions.

Remarks: This species is one of the most widely reported taxa from diverse sites worldwide (Patterson and Lee 2000). *Goniomonas pacifica* is similar to a freshwater species (*G. truncata*) in cell shape and having ridges. Also, the length ranges of the two species overlap (*G. pacifica*, 4 to 15 µm; *G. truncata*, 3 to 25 µm). Further study is required to establish the identities of these species. Skvortzov (1932) described *Monas abrupta* Skvortzov 1932, which is oval and 7 to 12 µm long, and has ejectisomes near the anterior end of the cell. In the drawings of Skvortzov, two flagella arise from the anterior end of the cell and diverge in different directions. The species is certainly not from the stramenopile genus *Monas*, and he may have observed a species of *Goniomonas*. Given the lack of details, ambiguity and absence of appropriate comparisosn in Skvortzov's descriptions, it is not clear precisely what taxon he observed. The taxon is therefore regarded as a nomen nudum (e.g., *Goniomonas abrupta*).

Heterolobosea Page and Blanton 1985

Percolomonas similis Lee *et al.* 2003 (Fig. 2d)

Observation: Cells are oval in shape, 5 to 7 µm long, and laterally compressed. Two flagella emerge from the end of a ventral depression. One long flagellum is 3 to 3.5 times the cell length, is slightly thinner than the shorter flagellum and is non-acronematic. It is used for adhesion to the substrate and lies underneath the shorter flagellum. The thickened short flagellum is slightly shorter than the cell length, usually lies in the groove and may beat fast in the groove to creat a current of water from which suspended particles are extrated. The cells move by skidding or gliding. Relatively often observed. Description based on records of 20 cells.

Remarks: *Percolomonas* has been known as having four flagella, but Tong (1997a) included *Percolomonas denhami* with three flagella and Lee *et al.* (2003) included *Percolomonas similis* with two flagella. *Percolomonas similis* is most similar to *P. denhami* in cell shape and length, but can be distinguished by the number of flagella and because *P. denhami* appears to be more flattened laterally. This species can be easily distinguished from the other common species, *P. cosmopolitus*, because *P. cosmopolitus* has four flagella: one long flagellum and three short flagella. The short flagella of *P. cosmopolitus* may be easily overlooked when they beat fast. Further studies are needed to clarify the identities of these three species. The general appearance and moving pattern of the cells are similar to *Carpediemonas* species, but can be distinguished by the beating pattern of the anterior flagellum which in *Carpediemonas*, and because the flagella in *Percolomonas* are directed posteriorly.

Kinetoplastida Honigberg 1963

Bodo platyrhynchus Larsen and Patterson 1990 (Fig. 2e)

Observation: Cells are 5 to 9 µm long, dorso-ventrally flattened and slightly flexible. The anterior margin of the cell is flattened. Cell outline is ovoid and two flagella of unequal length insert subapically. The anterior flagellum beats stiffly from side to side and the posterior flagellum is about twice the cell length, acronematic and trails. The cells glide slowly with a waggling movement, but may more rarely swim with a slow rotating movement.

Remarks: This species has been found at marine sites in Australia, Brazil, Hawaii and Korea with lengths of 3.5–11 µm (Larsen and Patterson 1990; Lee and Patterson 2000; Al-Qassab *et al.* 2002; Lee 2002b, 2006b). Generally, the observations presented here are in good agreement with the descriptions of previous observers. *Bodo platyrhynchus* can be easily distinguished from *Neobodo designis* by its flattened body and because it usually glides with a waggling behaviour. The acronematic trailing flagellum is suggestive that this taxon is a kinetoplastid, but a kinetoplast has not been confirmed. It has been distinguished from species of *Cercomonas* which are more flexible and strands of cytoplasm may be drawn out from the posterior end of the cell.

Hemistasia phaeocysticola (Scherffel 1900) Elbrächter *et al.* 1996 (Figs 2h–i)

Observation: Cells are oblong, 27 to 33 µm long, less metabolic, and are not flattened with a spiral groove. The cells form a constant shape (oblong) rather than highly metabolic, and form a flexible anterior papillum containing a tubular ingestion organelle. The nucleus is located in the centre of the cell. There are small particles throughout the cell. The posterior flagellum is slightly longer than the anterior flagellum and the cell. During swimming the cells rotate. Two cells were observed.

Remarks: This species has been reported from marine sites in Australia, Germany, Wadden Sea and Korea, with cell lengths of 12–25 µm (Scherffel 1900; Griessmann 1913; Elbrächter *et al.* 1996; Tong *et al.* 1998; Lee and Patterson 2000; Al-Qassab *et al.* 2002;

Lee 2002b, 2006b). The genus *Hemistasia* resembles *Entomosigma*, *Rhynchobodo* and *Cryptaulax*. The latter two genera have been synonymised (Bernard *et al.* 2000). *Hemistasia* is very similar to *Rhynchobodo*, but can be distinguished by its anterior papillum. The cells observed here are similar to *Rhynchobodo thiophila sensu* Skuja 1956 in cell length and general appearance, but can be distinguished by the presence of the flexible anterior papillum.

Neobodo curvifilus (Griessmann 1913) Moreira *et al.* 2004 (Fig. 2f)

Observation: Cell outline is oval or bean-shaped. The cells are 5 to 8 µm long, flattened, and pliable. The two flagella of unequal length insert subapically to one side in a small pocket. The anterior flagellum is about the cell length, hook shaped, and beats slowly in a broad curve in front of the gliding cell. The trailing posterior flagellum is acronematic and 2.5 to 3 times the cell length. The cells normally glide but may have a squirming movement, and rotate when swimming. Often observed.

Remarks: This species has been described under the name *Bodo curvifilis* from marine sites in Antarctica, Arctic, north Atlantic, northeast Atlantic, Australia, Denmark, West Greenland and Norway with cell lengths of 4–12 µm (Griessmann 1913; Throndsen 1969; Turley and Carstens 1991; Vørs 1992a, b, 1993a; Patterson *et al.* 1993; Tong *et al.* 1997; Lee and Patterson 2000; Lee *et al.* 2003). The description is in accord with Vørs (1992a). *Neobodo curvifilus* is characterized by the curved shape and the paddling beat of the anterior flagellum, thus it is easily distinguished from other species of *Neobodo* because of these characters.

Neobodo saliens (Larsen and Patterson 1990) Moreira *et al.* 2004 (Fig. 2g)

Observation: Cells are usually elongate elliptical and somewhat inflexible, 4 to 12 µm long. Two flagella of unequal length emerge subapically from a shallow pocket. The anterior flagellum is as long as the cell and held forwards with a single anterior curve, held perpendicular to the substrate. The posterior flagellum is acronematic, typically directed straight behind the cell and 2.5 to 3.5 times the cell length. The cells swim in rapid darts in straight lines. Frequently observed.

Remarks: This species appears to be cosmopolitan. Generally, the observations presented here are in accord with those of previous observers under the name *Bodo saliens*. *Neobodo saliens* is characterized by the rapid darting movement, the anterior flagellum held forwards with a single anterior curve, held perpendicular to the substrate and the posterior flagellum directed in a straight line when the cell is swimming.

Rhynchobodo formica (Skuja 1948) Bernard *et al.* 2000 (Figs 1f, 3a–b)

Observation: The cell is spindle-shaped, about 40 µm long, and highly metabolic. The cell body appears to be twisted with a shallow longitudinal groove. Two flagella emerge from the subapical flagellar pocket and are longer than the cell. The anterior flagellum beats freely forwards and the posterior flagellum appears to wrap the cell body. The nucleus is located near the centre of the cell. Large granular bodies are seen below the centre of the cell and small particles are seen above the centre. The cell moves by swimming. One cell observed.

Remarks: The cell observed here is assigned to *Rhynchobodo formica* (Skuja 1948) Bernard *et al.* 2000. Skuja (1948) described *R. formica* (under the name of *Cryptaulax formica*) as 20–35 µm long, very metabolic, with a twisted body, usually retains a spindle shape and has a shallow longitudinal groove. The cell observed is slightly larger but otherwise similar. *Rhychobodo formica* is distinguished from other *Rhynchobodo* species by its larger size and by greater flexibility. This species is similar to *R. longiciliatus* (Skuja 1948) Bernard *et al.* 2000 in cell length (20–45 µm), but can be distinguished because *R. longiciliatus* has a narrow shape.

Bernard *et al.* (2000) synonymised *Cryptaulax* with *Rhynchobodo*. The genus *Rhynchobodo* contains 11 species of kinetoplastid flagellates with an apical ingestion apparatus, no papilla, a stiff anterior part of the body, and in some cases a spiral groove on the body. The distinctions are not clear, and many synonyms will probably be found.

Rhynchobodo simius Patterson and Simpson 1996 (Figs 2l–o)

Observation: Cells are fusiform and 10 to 16 µm long with a slightly acute or broad rostrum containing a tubular ingestion apparatus openning at the tip of the cell. Two flagella of unequal length: the anterior flagellum is about 2 times the cell length and the posterior one is 2.5 to 3 times the cell length. Both flagella are thickened and insert in conspicuous pocket at the base of the rostrum. There is a spiral groove from the flagellar pocket to near the posterior end of the cell. The cells swim with undulating rotation.

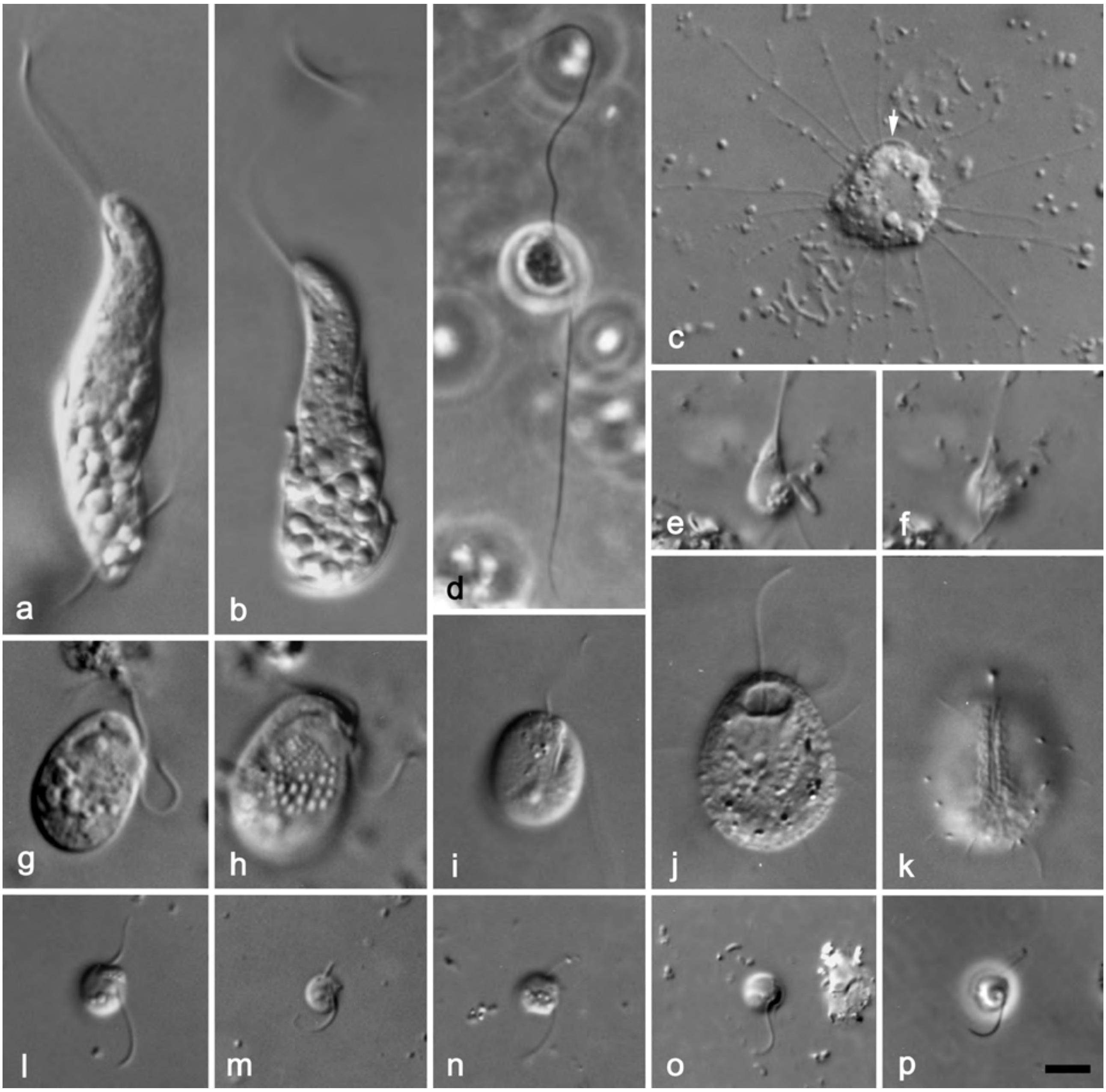

Fig. 3. a–b – *Rhynchobodo formica*; **c** – *Massisteria marina*, general appearance of cell showing pseudopodia and flagella (arrow); **d–f** – *Cercomonas* sp.; **d** – note two acronematic flagella; **e** – general appearance; **f** – note flagellar orientation; **g–h** – *Roombia truncata*, note cell attached to the substrate by the tip of the posterior flagellum and note extrusomes; **i** – *Protaspa obliqua*, note anterior protrusion; **j–k** – *Thaumatomastix setifera*, note spines around the body; **j** – general appearance of cell; **k** – ventral face showing a deep groove and pseudopodia; **l–m** – *Ancyromonas sigmoides* of different cells; **l** – note slightly thick anterior flagellum with acronematic tip and broad rostrum; **m** – note thick anterior flagellum and acute rostrum; **n–p** – *Ancyromonas impluvium* nov. spec., showing general appearance of cell and note flagellar insertion. All micrographs are DIC images with the exceptions of (d) and (p) which are phase contrast images. Scale bar: 5 µm for all figures.

Remarks: *Rhynchobodo simius* has been previously reported from hypersaline sites in the Shark Bay area (Australia) by Patterson and Simpson (1996) and Al-Qassab *et al.* (2002) with lengths of 9–16 µm. Most *Rhynchobodo* are more or less metabolic whereas *R. simius* is inflexible (Patterson and Simpson 1996). *Rhynchobodo simius* can be distinguished from *R. taeniata* (Skuja 1956) Vørs 1992 which is smaller (13–20 µm) and because *R. taeniata* has a more conspicuous spiral groove. This species resembles *Rhynchobodo conoidea* (Skuja 1956) Bernard *et al.* 2000, *R. agilis* Laekey 1940, *Rhynchobodo vulgaris* (Skuja 1948) Bernard *et al.* 2000 and *R. armata* Brugerolle 1985 in cell shape and length, and in having an acute anterior end. Further studies are needed to distinguish these species.

Rhynchomonas nasuta Klebs 1893 (Fig. 2k)

Observation: Cells are 3.5 to 6 µm long and flattened. The cells are flexible with 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 to 2.7 times the cell length, and acronematic. The cells consume attached bacteria and cells move by gliding. Commonly observed.

Remarks: This species is one of the most widely reported taxa from diverse sites worldwide (Patterson and Lee 2000). It can be distinguished from other small gliding species by the bulbous snout.

Euglenozoa *incertae sedis*

Bordnamonas tropicana Larsen and Patterson 1990 (Fig. 2j)

Observation: Cells are 7 to 11 µm long, flattened and flexible, particularly when squashed. The cells are anteriorly narrow and posteriorly broad. Two slightly thickened flagella insert subapically in the right ventral side of the cell, are about 1.5 times the cell length and not acronematic. The anterior flagellum is held in a sigmoid arc and beats stiffly, and the posterior one curves near its flagellar insertion and is directed towards the rear of the cell. An apical mouth is visible by light microscopy. The cells move by gliding or rapidly by skidding close to the substrate. Often observed.

Remarks: Previously reported size ranges are 5 to 20 µm (Lee and Patterson 1998; Patterson and Lee 2000; Al-Qassab *et al.* 2002; Lee 2002b, 2006b; Lee *et al.* 2003; Aydin and Lee 2012). This species appear to be cosmopolitan. The cell appearance is entirely consistent with the description of Larsen and Patterson (1990). *Bordnamonas tropicana* is similar to *Neobodo curvifilus* in having an anterior flagellum held anteriorly in a curve, the thicker nature of the anterior flagellum, but can be distinguished by its swimming pattern, non-acronematic flagellar thickness and the anterior mouth. This species resembles *Klosteria bodomorphis* Nikolaev *et al.* 2003 in cell length and cell shape, but it may be distinguished by its non-acronematic flagella. Further studies are required to clearly distinguish these two species.

Rhynchopus amitus Skuja 1948 (Figs 1b, 2p–q)

Observation: Cells are 11 to 18 µm long, sac shaped, but markedly and actively flexible and contractile. There is a fine apical papilla, which has slightly thickened margin and is an ingestion organelle. A conspicuous flagellar pocket, about 2 µm deep, opens immediately below the papilla and is directed anterio-laterally. Most cells have very short flagella, which do not emerge from the pocket, but a few cells observed had two thickened flagella, 0.5 to 1 times the cell length. These flagella are directed posteriorly and usually inactive. The posterior half of the cell often contains large food vacuoles. The cells move by gliding. Rarely observed.

Remarks: This species was described from a freshwater site in northern Europe by Skuja (1948) and a marine site in Australia (Al-Qassab *et al.* 2002). *Rhynchopus conscinodiscivorus* was reported from a marine habitat by Schnepf (1994), but was regarded as a junior synonym of *R. amitus* by Al-Qassab *et al.* (2002). Al-Qassab *et al.* (2002) suggested that *Menoidium astasia* reported from a hypersaline habitat (Marion Bay, South Australia) is a senior synonym of *R. amitus* because it is flexible and has two long flagella or no flagella at times. *Rhynchopus* is difficult to distinguish from *Diplonema*, but trophic cells in *Diplonema* have emergent flagella of a 'normal' thickness. *Rhynchopus* only has emergent flagella in a 'dispersal phase' and these flagella are considerably thickened by paraxonemal rods (Simpson 1997). Further studies are needed to establish the identities of the genera, *Diplonema* and *Rhynchopus*.

Spironemidae Doflein 1916

Spironema multiciliatum Klebs 1893 (Figs 1c, 2r–s)

Observation: Cells are lanceolate, relatively flattened and flexible. The cells have a spiral groove, long kineties and a tail, which tapers posteriorly, and are 15 to 21 µm without the tail. Many flagella are arranged in the kineties. The nucleus is located anteriorly or near the centre of the cell. When the cells are squashed, the cells are more flexible. Food materials are seen under the cell surface.

Remarks: *Spironema multiciliatum* was first found at a freshwater site in Germany (Klebs 1893) and the previously reported range of cell length is 14 to 18 µm. The observations here extend the range of the cell length. This species resembles *Spironema terricola* Foissner and Foissner 1993 and *S. goodeyi* Foissner and Foissner 1993, but can be distinguished by its smaller size and cell shape and by the length of kineties. Kineties are restricted to only the anterior part of the cell in *S. terricola* and *S. goodeyi* while kineties in *S. multiciliatum* extend to near the posterior end of the cell. *Spironema multiciliatum* is very similar to *Stereonema geiseri* reported from a freshwater site in Germany by Foissner and Foissner (1993) who separated *S. multiciliatum* from *Stereonema geiseri* because *S. multiciliatum* has the inability to perform euglenoid movement. However, both species are flexible and may be more flexible when cells aresquashed. The cell length ranges of these two species overlap and both species havethe same shape. Thus, *Stereonema geiseri* is regarded here as a junior synonym of *Spironema multiciliatum*.

Alveolates

Psammosa unguis (Patterson and Simpson 1996) nov. comb. (Figs 1d, 2w)

Observation: Cell outline bean-shaped, 8 to 13 µm long and somewhat flattened. The cells may have longitudinal lines of granules. 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, and is directed laterally and posteriorly. The posterior flagellum inserts at the top of a shallow longitudinal groove and is about 2 times the cell length. The cells typically swim rapidly, usually in contact with the substrate. The cells erratically flip from one side to the other when swimming. Not common.

Remarks: Patterson and Simpson (1996) described *Colpodella unguis* from the marine sediment of Shark Bay, Australia and assigned to *Colpodella* with some uncertainty. In respect of general appearance, size and flagellar orientation, the organisms from the present site agree well with Patterson and Simpson's description. Body form and flagellar orientation of this species agree well with the genus *Psammosa*, and thus *Colpodella unguis* is here transferred to *Psammosa*. This species has been reported from marine sites in Australia and Korea (Patterson and Simpson 1996; Al-Qassab *et al.* 2002; Lee 2002b, 2006b), with cell length from 7 to 10 µm, under the name *Colpodella unguis*. It resembles *Psammosa pacifica* Okamoto *et al*. 2012 in general appearance, cell length and shape. Further study is required to establish the identities of these species.

Apusomonadida Karpov and Mylnikov 1989

Amastigomonas debruynei de Saedeleer 1931 (Fig. 2t)

Observation: Cells are 5 to 6.5 µm long, dorso-ventrally flattened and flexible. The anterior flagellum is shorter than the cell, emerges from the tip of a laterally directed sleeve and beats in a small angle. The posterior flagellum is slightly longer than the cell, lies in a groove along the margin of the cell, trails under the cell and occasionally protrudes behind the cell. Strands of cytoplasm may be drawn out behind the cell. The nucleus is situated in the anterior left of the cell. The cells move by gliding. Rarely observed.

Remarks: In this study the criteria for identifying morpho-species within *Amastigomonas* given by previous authors (Molina and Nerad 1991, Mylnikov 1999, Lee and Patterson 2000) is used. According to Ekebom *et al.* (1996) and Lee and Patterson (2000), *Amastigomonas debruynei* has synonyms; *Thecamonas trahens*, *A. borokensis* and *A. caudata*. This species has been reported from marine sites in Australia, North Atlantic, Brazil, Arctic Canada, Denmark, England, Gulf of Finland, Greenland, Hawaii, Korea and Panama with lengths of 3–7.5 µm (Larsen and Patterson 1990; Vørs 1992a, b, 1993a, b; Patterson *et al.* 1993; Ekebom *et al.* 1996; Tong 1997a, b; Tong *et al.* 1998; Lee and Patterson 2000; Al-Qassab *et al.* 2002; Lee *et al.* 2003; Lee 2002b, 2006b). It appears to be cosmopolitan. Further works are required to clarify the identities of species in this genus.

Amastigomonas mutabilis (Griessmann 1913) Molina and Nerad 1991 (Fig. 2u)

Observation: Cells are elliptical, 11 to 16 µm long, dorso-ventrally flattened and flexible. There is a flexible sleeve around the base of the anterior flagellum. The anterior flagellum is about 0.5 times the cell length and the same thickness as the posterior one. The recurrent posterior flagellum is slightly longer than the cell and trails under the body, to which it attaches loosely in a slight groove. The nucleus is situated subapically near the right margin of the cell. Some cells have granules along side the recurrent flagellum. Relatively rare than *Amastigomonas debruynei*.

Remarks: This species has synonyms: *Rhynchomonas mutabilis* and *Thecamonas mutabilis*. It has been reported from marine sites in Agean Sea (Turkey), Australia, Brazil, Denmark, England, France, Greenland, Korea and North Atlantic with lengths of 7–16 µm (Griessmann 1913; Ruinen 1938; Larsen and Patterson 1990; Vørs 1992b, 1993a; Patterson *et al.* 1993; Patterson and Simpson 1996; Tong 1997b; Tong *et al.* 1998; Lee and Patterson 2000; Al-Qassab *et al.* 2002; Lee 2002b, 2006b; Lee *et al.* 2003; Aydin and Lee 2012). It seems to be cosmopolitan. This species was characterised by the line of granules along side the posterior flagellum (Larsen and Patterson 1990), but some cells lack the granules. *Amastigomonas bermudensis* Molina and Nerad 1991 is very similar to *A. mutabilis* in general appearance and their cell length. Further work is required to clarify the identities of these two species.

***Apusomonas* sp.** (Figs 1e, 2v)

Observation: Cell is about 7 µm long and dorso-ventrally flattened with a single flagellum. Cell outline is ovate. The mastigophore emerges from a cavity about half of the cell length from the anterior end, is flexible. The flagellum is partly in a sleeve, which arises at the distal end of the mastigophore. The length of the flagellum plus the mastigophore is longer the cell. No structure seen on the dorsal and ventral sides. The cell glides closely to the substrate. One cell was observed at St. 38.

Remarks: The cell observed here is assigned to the genus *Apusomonas* because of its mastigophore and its cell appearance. It was identified to genus because of lack of information. This genus has been usually found from freshwater sites and soils (e.g., Vickerman *et al.* 1974; Karpov and Zhukov 1980, 1986; Ekelund and Patterson 1997), but recently *A. proboscidea* was reported from a marine sediment in tropical Australia (Lee 2006b).

Cercomonadidae Mylnikov and Karpov 2004

***Cercomonas* sp.** (Figs 3d–f)

Observation: Cell outline is pyriform. Cells are 7 to 15 µm long and flexible. Two flagella are of similar thickness and appear to be acronematic. The anterior flagellum is about 3.5 times the cell length, beats from side to side with entire length, and the basal part of the flagellum adheres to the anterior part of the cell. The posterior flagellum is about 4 times the cell length, may adhere to the body surface for part of its length and beats slowly from side to side as the cells glide. The cells glide with the flagella in contact with the substrate, and during gliding the anterior part of the cell is elongated. Rarely observed.

Remarks: This species is similar to *Cercomonas granulatus*, but it lacks a row of refractile bodies on the ventral side, which is one of the diagnostic characters for *C. granulatus*. It is also similar to the cells found in Cape Tribulation under the name *Cercomonas granulatus* (Lee 2006b). Most similar nominal taxa to *Cercomonas granulatus* have been compared and discussed by Lee and Patterson (2000) and Lee (2006b). Recently Bass *et al.* (2009) described 66 species from cercomonads, and they also said that it is difficult to describe any particular species adequately because of their morphological plasticity and behavioural diversity. That is, it is not easy to distinguish cercomonad species by light microscopy. Probably the composition of the cercomonads is still uncertain and there are many synonyms in this group.

Granofilosea Cavalier-Smith and Bass 2009

Massisteria marina Larsen and Patterson 1990 (Fig. 3c)

Observation: Cells are 3 to 6.5 µm and dorso-ventrally flattened irregular body with delicate pseudopodia bearing extrusomes. The pseudopodia 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. Rarely observed.

Remarks: Generally, the observations are in good agreement with those of Larsen and Patterson (1990) and Lee and Patterson (2000). Previously reported size ranges are 2 to 12 µm and this species was found at marine sites in Agean Sea (Turkey), subtropical and tropical Australia, Brazil, Denmark, Gulf of Finland, equatorial Pacific, Korea and Panama (Larsen and Patterson 1990; Patterson and Fenchel 1990; Vørs 1992a, b; Vørs *et al.* 1995; Ekebom *et al.* 1996; Tong 1997a; Tong *et al.* 1998; Lee and Patterson 2000; Al-Qassab *et al.* 2002; Lee 2002b, 2006b; Aydin and Lee 2012).

Mantamonadida Cavalier-Smith 2011

Mantamonas plastica Glücksman and Cavalier-Smith 2011 (Figs 1g, 2x–y)

Observation: Cell outline is rhombic to oval. Cells are 3 to 4 µm long, flexible and flattened. The very fine anterior flagellum arises at the anterior end of the cell, is directed to the left and about the cell length. The posterior flagellum arises from a depression about one third of the cell length from the anterior end, appears to lie in a ventral groove or tightly adhere to the cell body and is 2 to 2.5 times the cell length. At times, cytoplasmic strands appear behind the posterior end of the cell. The cells glide slowly on the substrate. Not common and observed at Sts 31 and 41.

Remarks: Recently, this species was found from a marine site in England (Glücksman *et al.* 2011). It can be confused from some other species in *Cercomonas* because it has a flexible body, strands of cytoplasm from the posterior end of the cell and gliding movements, but can be distinguished by its smaller size, cell shape and flagellar orientation.

Kathablepharidae Vørs 1992

Roombia truncata Okamoto *et al.* 2009 (Figs 1h, 3g–h)

Observation: Cells are oblong, 11 to 16 µm long, laterally flattened, and rigid. There are no surface structures on both faces of the cell. Two flagella arise near the anterior-lateral side of the cell separately by a small protrusion. Both flagella are of similar length and not acronematic. The anterior flagellum beats backwardly in a small excursion and the posterior flagellum is usually held down. The nucleus is located in the centre of the cell. The cells appear to move by skidding. When the cells rest, the anterior flagellum lies along the margin of the cell. Rarely observed.

Remarks: This species was reported from a marine habitat in Nova Scotia, Canada by Okamoto *et al.* (2009). It is similar to *Platychilomonas*, but is distinguished by the flagellar orientation and the lack of the gullet, and because this species lacks rows of extrusomes. It can be distinguished from other small flagellates by the moving behaviour and the cell appearance such as flatness and lack of surface structure. This species was also observed at marine sediments in subtropical Australia and Korea (Lee unpublished data).

Protaspidae Cavalier-Smith 1993

Protaspa obliqua (Larsen and Patterson 1990) Cavalier-Smith 2011 (Fig. 3i)

Observation: Cells are slightly oval or roundish, 13 to 15 µm long, dorso-ventrally flattened and with thickened cortex. There is a ventral median groove; cell indented anteriorly and posteriorly where the groove meets margin. Subapically, the right margin of the groove forms a protrusion. With two flagella inserting under the protrusion; the anterior flagellum is about 0.5 times the cell length and the posterior flagellum is from 0.5 to 1.5 times the cell length. The rounded nucleus does not have nuclear caps, is located subapically in a median position. Commonly observed.

Remarks: Recently, *Protaspis* Skuja 1939 was replaced with *Protaspa* by Cavalier-Smith in Howe *et al.* (2011) because it was preoccupied by the Devonian fish *Protaspis* Bryant 1933. This species has been reported from marine sites in Aegean Sea (Turkey), Australia, England, Fiji and Korea (Larsen and Patterson 1990; Tong 1997b; Lee and Patterson 2000; Lee 2002b, 2006b; Aydin and Lee 2012) and a freshwater site in Tasmania (Lee *et al.* 2005). Cell length was reported to be 8 to 32 µm by previous authors. Generally, the observations are in accordance with those of Lee and Patterson (2000). The protrusion near the flagellar insertions makes this species easy to identify, but *Protaspa grandis* (Hoppenrath and Leander 2006) has a protrusion near the flagellar insertion. *Protaspa grandis* is elongated without the posterior indentation and generally bigger than *P. obliqua* (*P. grandis*, 32.5–55 µm).

Thaumatomonadidae Hollande 1952

Thaumatomastix setifera Lauterborn, 1896 (Figs 1i, 3j–k)

Observation: Gliding heterotrophic flagellate. Cells are oval, 14–17 µm long and slightly dorso-ventrally flattened. The cells have a layer of visible scales and delicate spines. A ventral groove extends from the anterior depression to the posterior end of the cell. Two flagella insert to the widely opened anterior depression on the ventral face of the cell. The anterior flagellum is 0.6 to 1 times the cell length and beats from side to side. The posterior flagellum is 1.2 to 1.6 times the cell length and trails. The nucleus is anteriorly located. The cells move slowly by gliding. Two cells were observed.

Remarks: The species described here is assigned to *Thaumatomastix setifera* although pseudopodia have not been seen. Subsequent to the original description (Lauterborn 1896), *T. setifera* was found at a marine site in Hawaii (Larsen and Patterson 1990) and at a freshwater site in Latvia (Skuja 1939). The previously reported cell lengths are 16–28 µm. Generally, the description here is in good agreement with previous observations. This species resembles *Protaspis verrucosa*, but can be distinguished by the layer of visible scales and the spines. However, it is difficult to distinguish from *T. salina* (7–12 µm), *T. splendida* (12–19 µm) and *T. tripus* (8–15 µm) because their size ranges overlap, and they can be easily identified on the basis of the shape of body scales visible by electron microscopy. They may not be distinguished by light microscopy.

Protista *incertae sedis*

Ancyromonas impluvium nov. spec. (Figs 1j, 3n–o)

Diagnosis: *Ancyromonas*, cell 4 to 6 µm long, ovoid to roundish, somewhat laterally compressed. Two thickend flagella emerging from wide snout at anterior end of cell insert apically and are thin at tip.

Etymology: The name refers to 'basin'.

Observation: Cells are ovoid to roundish, 3 to 6 µm long and somewhat laterally flattened. The cells have two thickened flagella, which insert separately by a shallow, wide snout at the anterior end of the cell. Both flagella are thin at the tip. The anterior flagellum is slightly longer than the cell and beats stiffly. The posterior flagellum is 2 to 3 times longer than the cell and is directed posteriorly. The nucleus is located subapically. Food materials are seen in the posterior end of the cell. Normally, the cells glide with the beating of the anterior flagellum, but at times glide slowly with the anterior flagellum directed to the rear (Fig. 3o). Commonly observed.

Remarks: This species is assigned to *Ancyromonas* because it glides, is laterally compressed, and has one short flagellum emerging from the anterior end and one long trailing flagellum emerging subapically from the ventral depression. *Ancyromonas impluvium* is distinguished from *Ancyromonas sinistra* by the lack of a rugose flange on the left anterior-lateral margin of *Ancyromonas sinistra*. This species can be distinguished from other *Ancyromonas* species (*A. melba, A. micra, A. sigmoides, A. sinistra*) because it glides with the beating of the anterior flagellum and at times glides in the anterior flagellum helding down. It is similar to *Heteromita globosa,* which glides in the anterior flagellum helding down, but both flagella in *H. globosa* insert close together subapically. *Ancyromonas impluvium* is similar to *Protaspa* species in that the nucleus is located anteriorly, but is distinguished by the orientation of the flagella and because *A. impluvium* is laterally compressed.

Ancyromonas sigmoides Kent 1880 (Figs 3l–m)

Observation: Gliding flagellate. Cell outline is oval. Cells are 3 to 5 µm long and dorso-ventrally flattened. The cells have a shallow groove ventrally near an anterio-lateral margin of the cell. The cells have a thin stiff anterior flagellum emerging from an anterior depression. The anterior flagellum can be easily overlooked and beats slowly. The posterior flagellum is about 1.5 times the cell length and may not be acronematic. The cells move by gliding with the posterior flagellum trailing. Commonly observed.

Remarks: Previously reported lengths range from 2 to 7.6 µm. This species has been reported from marine sites in Antarctica, Australia, Arctic Canada, Denmark, Fiji, Gulf of Finland, Greenland, Hawaii, Eastern Pacific (hydrothermal vent) and Panama (Larsen and Patterson 1990; Vørs 1992a, b; Ekebom *et al.* 1996; Tong 1997a; Tong *et al.* 1997; Tong *et al.* 1998; Atkins *et al.* 2000; Bernard *et al.* 2000). The observations are in agreement with those of previous authors. *Ancyromonas sigmoides* can be confused with *Metopion fluens*, but is distinguished by the anteriorly directed flagellum; the second flagellum in *M. fluens* is thicker and directed to the rear. It is similar to *A. melba* Patterson and Simpson 1996, found in hypersaline habitats of Shark Bay, subtropical Australia, but *A. melba* has an anterior flagellum which is as thick as the posterior flagellum, and the cell is slightly larger. Three cells had an anterior flagellum, which was basally thickened and the cells were included here (Fig. 3m) and these maybe refer to *Ancyromonas micra*. Further studies are needed to establish the identity of *A. melba, A. micra* and *A. sigmoides*. *Ancyromonas sigmoides* can be distinguished from *Ancyromonas sinistra* by the laterally flattened body and its cell shape (*A. sinistra* is spherical) and from *Ancyromonas impluvium* by its cell shape. *Ancyromonas sinistra* has a rugose flange on the left anterior-lateral margin of the cell and *Ancyromonas impluvium* has an anterior flagellum, which beats more actively.

Carpediemonas membranifera Ekebom *et al.* 1996 (Fig. 4a)

Observation: Cells are elliptical or obovate and 3 to 6 µm long with a longitudinal ventral groove, which extends most of the cell length. Two flagella of unequal length emerge from the anterior distal part of the cell. The anterior flagellum is bent over backwards and is

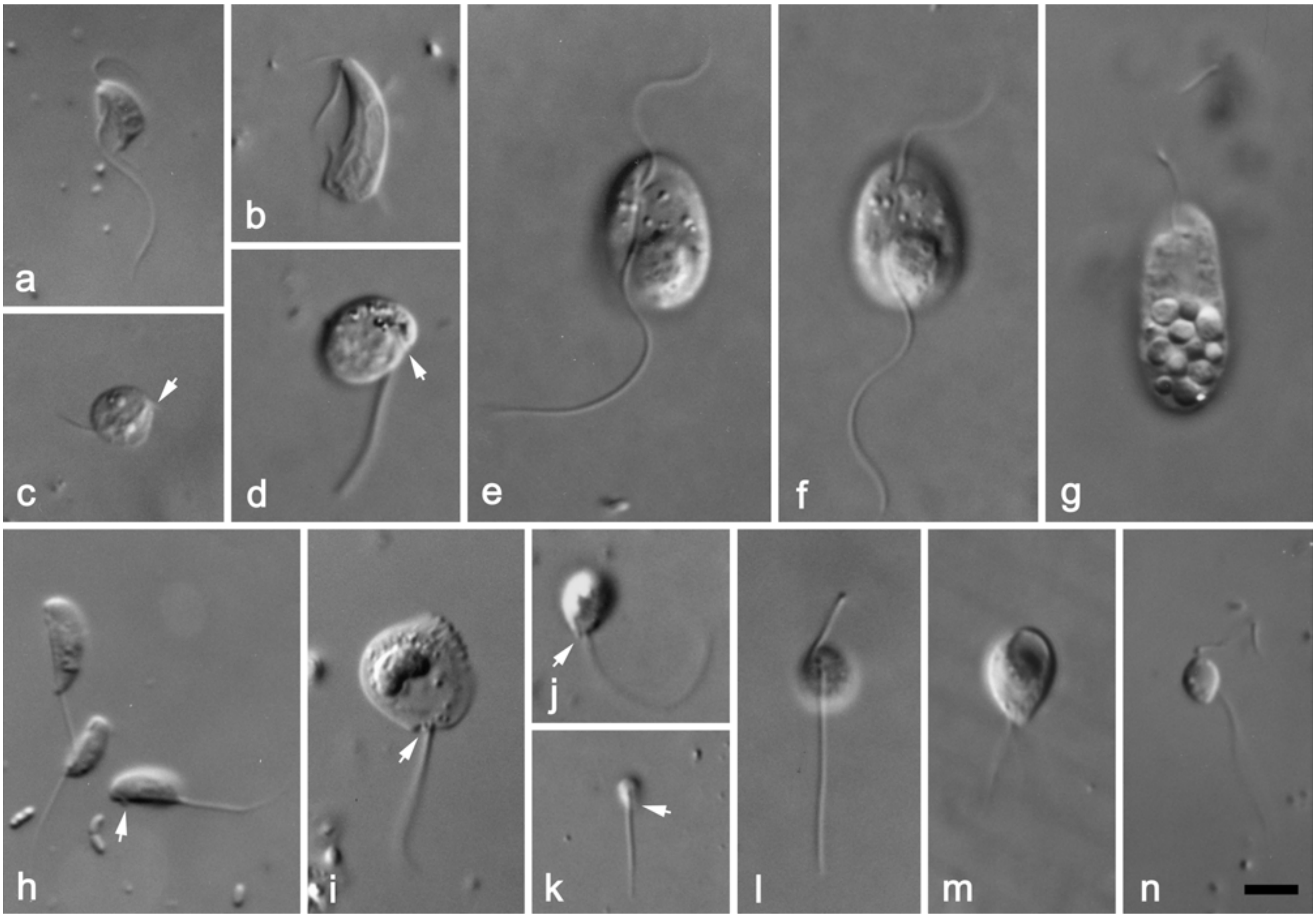

Fig. 4. a – *Carpediemonas membranifera*; **b** – *Kipferlia bialata*; **c** – *Discocelis saleuta*, note a short flagellum; **d** – *Metopion fluens*, showing general appearance of cell, note shorter flagellum (arrow); **e–f** – *Kurnaimonas celeris* nov. spec., showing general appearance of cell; **g** – *Pseudophyllomitus granulatus*; **h** – *Helkesimastix faecicola*, showing general appearance and note short flagellum (arrow); **i** – *Metromonas grandis*, showing general appearance of cell and note the folded margin on the left side and short flagellum (arrow); **j** – *Metromonas simplex*, showing general appearance of cell and note short flagellum (arrow); **k** – *Kiitoksia kaloista*, showing two flagella and note short flagellum (arrow); **l** – *Sinistermonas sinistrorsus* nov. spec., showing general appearance of cell, and note flagellar insertion and beating pattern of anterior flagellum; **m** – *Telonema subtilis*, showing general appearance; **n** – Protist '1'. All micrographs are DIC images. Scale bar: 5 µm for all figures.

as long as the cell and beats stiffly. The acronematic posterior flagellum is 2.5 to 4 times cell length, beats actively in the ventral depression and usually lies in the groove. The cells usually move by skidding with the anterior flagellum beating with a stiff paddling motion. The cells consume bacteria. Commonly observed in anoxic conditions.

Remarks: This species has been described from marine sites in subtropical and tropical Australia, Brazil and Korea, and previously reported cell lengths are 3 to 9 µm (Larsen and Patterson 1990, Ekebom *et al*. 1996, Simpson and Patterson 1999, Bernard *et al*. 2000, Lee and Patterson 2000, Al-Qassab *et al*. 2002, Lee 2002b). This species is distinguished from *Kipferlia bialata* by its smaller size, the absence of the moving membrane and the relatively long posterior flagellum. It consumes bacteria (Larsen and Patterson 1990, Ekebom *et al*. 1996, Lee and Patterson 2000) and usually occurs in large numbers with *Cafeteria marsupialis* and *Kipferlia bialata* (Lee and Patterson 2000).

Discocelis saleuta Vørs 1988 (Fig. 4c)

Observation: Cells are 3.5 to 6.5 µm long, disc-shaped, flattened, anteriorly concave and posteriorly convex. Two flagella emerge from a depression on the anterior margin of the cell. The recurrent flagellum trails behind the gliding cell and is slightly longer than the cell. The shorter flagellum is less than 1 µm long, is hard to see and inactive. The nucleus is located anteriorly in the right half of the cell. The margin of the cell may be delicately punctate. The cells glide smoothly in closely contact with the substrate. Commonly observed.

Remarks: *Discocelis saleuta* has been described from marine sites in tropical Australia, Brazil, Finland and Panama (Vørs 1988, Larsen and Patterson 1990) and reported cell lengths are 3 to 6 µm. This species is similar to *Discocelis punctata* Larsen et Patterson 1990, but they can be distinguished by the cell length and by the presence/absence of the peripheral bodies (Vørs 1988, Larsen and Patterson 1990). However, the cell length ranges overlap, and according to Larsen and Patterson (1990) and Vørs (1988) the peripheral bodies may be seen in *D. saleuta*. Some cells from this study also had delicate peripheral bodies visible by light microscopy. Further studies are required to establish the identity of both species.

Helkesimastix faecicola Woodcock and Lapage 1914 (Figs 1k, 4h)

Observation: Cells are elliptical, 4 to 8 µm long, flexible, not flattened and with two flagella. The short flagellum (arrow, Fig. 4h) emerging subapically (almost apically) from a shallow depression directs posteriorly and is easy to overlook. The long flagellum is 1.5 to 2.5 times longer than the cell, attaches to the cell body and is acronematic. The cells glide closely contact to the substrate. Observed abundantly at one sampling station in Gippsland Basin.

Remarks: The cells described here are assigned to *Helkesimastix faecicola* Woodcock and Lapage, 1914 (6–7 µm) although *H. faecicola* had only one flagellum. The tiny flagellum in the cells described might be overlooked. This species was found at a marine site in England (Lackey and Lackey 1963). It appears to be slightly different from *H. faecicola* described by Zhukov (1971), Zhukov and Mylnikov (1983), and Tong *et al.* (1997). The cells described by Tong *et al.* (1997) have only one flagellum, and the cells by Zhukov (1971) and Zhukov and Mylnikov (1983) are more similar to *Bodo platyrhynchus* (Larsen and Patterson 1990). *Helkesimastix faecicola* is similar to *Prismatomonas limax* Massart 1920 in cell shape and cell size, and in having an acronematic flagellum attaching to the cell body. *Prismatomonas limax* may be a junior synonym of *H. faecicola*. *Helkesimastix faecicola* is distinguished from *Allantion* by having two flagella and by the long flagellum attached to cell body. This species is similar to *Bodo platyrhynchus* in cell length and having two flagella, but is distinguished by the beating of the short flagellum in *B. platyrhynchus*. The anterior flagellum of *B. platyrhynchus* beats forwardly while that of *H. faecicola* directing posteriorly does not beat. Recently, another species (*H. marina*, 4.5–7 µm) has been reported from marine sediment in Belize by Cavalier-Smith *et al.* (2009). According to Cavalier-Smith *et al.* (2009), there are two major differences from *H. faecicola*; the presence of a contractile vacuole and the shape of the cysts (~ 4 µm) which are non-spherical and dish-shaped (Cavalier-Smith *et al.* 2009, p. 455; Figs 1r, s). However, Woodcock and Lapage (1914) also mentioned that it has a contractile vacuole (p. 357), and its cysts are 3 to 3.5 µm and spherical or ovate (Plate 13: 1–3). When looking at the cysts in Figs 1r and 1s of Cavalier-Smith *et al.* (2009), they are almost spherical. In addition, these species overlap in cell length. Thus, these two species are indistingushiable from each other and here *H. marina* is regarded as a junior synonym of *H. faecicola*.

Kiitoksia kaloista Tong *et al.* 1997 (Figs 1n, 4k)

Observation: Cells are spherical or elongate, 2 to 3 µm long and 2 µm wide, rigid and with two flagella. The cells appear to be ventrally concave or have a shallow ventral depression. The flagella insert into the ventral depression: the longer one is 3 to 4 times the length of the cell and may be acronematic, and the short one is about the cell length. The anterior part of the cell is closely contacted to the substrate when the cell is gliding and the posterior part is raised. Three cells observed.

Remarks: Generally, observations are in accord with a previous data from a freshwater site in Antarctic (Tong *et al.* 1997). It is similar to *Kiitoksia yatava* Vørs 1992, but differs in possessing a second short flagellum. *Kiitoksia* resembles *Clautriavia* Massart 1900 having only one flagellum and gliding pattern, but is distinguished by the smaller size.

Kipferlia bialata (Ruinen 1938) Kolisko *et al.* 2010 (Fig. 4b)

Observation: Cell outline is kidney-shaped. Cells are 6 to 14 µm long (mostly 9 to 12 µm), not rigid, and with a longitudinal ventral groove. A membrane moves down along the groove. Two flagella emerge from the anterior part of the cell; the anterior flagellum bends backwards, is about the length of the cell and beats over the cell with a slow sweeping motion. The acronematic posterior flagellum beats asymmetrically and is about 1.5 times cell length. The posterior flagellum may vibrate actively in the groove when not beating. The cells consume bacteria, and food materials are transferred by the moving membrane to the back of the cell. The cells may have many food vacuoles and attach to the substrate with the tip of the posterior flagellum. The cells move slowly by skidding or gliding with the anterior flagellum beating with a flicking motion. Commonly observed in late cultures.

Remarks: This species was originally described by Ruinen (1938) as *Cryptobia bialata* from Australia with biflagellated protist having a ventral groove and an undulating membrane, but was transferred to *Carpedimonas* by Lee and Patterson (2000) because it has many similar characters to *Carpediemonas*. Recently Kolisko *et al.* (2010) created a genus (*Kipferlia*) for this taxon on the basis of molecular and ultrastructural studies. Previous authors reported the species to be 6–14 μm long. This species resembles *Ergobibamus cyprinoides* Park *et al.* 2010 and other *Carpediemonas*-like organisms presented by Kolisko *et al.* (2010) in that they are bean-shaped, have two flagella, and a conspicuous ventral groove. They may be not easily distinguished by light microscopy. For further discussion see Kolisko *et al*. (2010) and Park *et al.* (2010).

Kurnaimonas Lee nov. gen.

Diagnosis: Biflagellated colourless protists of unknown affinities. Body rigid and flattened with ventral depression subapically. Both flagella inserting into ventral depression. One flagellum directed anterior-laterally, the other trailing. Move by swimming.

Type species: *Kurnaimonas celeris* nov. spec.

Etymology: 'Kurnai' is a collective name for five aboriginal tribes in the Gippsland area.

Kurnaimonas celeris Lee nov. spec. (Figs 1l, 4e–f)

Diagnosis: Cell oblong, 14 to 15 μm long, dorso-ventrally flattened and rigid. With two flagella of unequal length into wide ventral depression. With small protrusion separating insertion of flagella. Moving by fast swimming.

Etymology: The name refers to 'fast'.

Observation: Cells are oblong, 14 to 15 μm long, dorso-ventrally flattened and rigid. The anterior part of the cell forms relatively a wide depression, which continues to a mid-ventral groove on the right side of the cell. The cells have a small protrusion, which is directed laterally and may separate the insertion of the flagella. The cells have two flagella, which are in the same strength and are not acronematic. The anterior flagellum inserts anterio-laterally into the ventral groove and is slightly longer than the cell. The posterior flagellum inserts just below the anterior flagellum, is about 2 times the length of the cell and appears to lie in the ventral groove. When the cells are moving, the anterior flagellum is directed to the front and beats in a sine-wave, and the posterior flagellum beats also in a sine-wave and directs posteriorly. The cells often contain large food particles in the posterior end of the cell. The cells move very fast by swimming. Rarely observed.

Remarks: This organism is similar to *Pseudophyllomitus granulatus*, but is distinguished because its posterior flagellum has an undulating beating pattern when swimming and because it is rigid and flattened. It is similar to *Platychilomonas psammobia* in being flattened and cell length, but *P. psammobia* has two rows of extrusomes and a coiled posterior flagellum when it is inmobile. This species may be distinguished from other swimming flagellates by its flagellar orientation. It was also reported from intertidal marine sediments in Korea under the name 'Protist 2' (Lee 2002b).

Metopion fluens Larsen and Patterson 1990 (Fig. 4d)

Observation: Cells are ovate, 4 to 9 μm long, laterally compressed and with a small rostrum anterior to the flagellar insertion. Small bodies are seen in the protrusion or at the proximal anterior part of the cell. Two flagella of unequal size emerge from a ventral groove located in the left side of the cell. The thickened long flagellum is about 1.5 times the cell length and is not tapered at the tip, and the shorter flagellum may be difficult to see. The nucleus is situated near the groove. The cells move by gliding. Rarely observed.

Remarks: This species has been found in marine sites in the world (Lee and Patterson 1998; Al-Qassab *et al.* 2002; Lee 2002b, 2006), with cell length of 3 to 9 μm. The observations are in accord with those of previous authors. This species is similar to *Ancyromonas sigmoides*, but is distinguished by its broad anterior end and by the position of the second flagellum – that of *M. fluens* is directed posteriorly, while that of *Ancyromonas sigmoides* is directed anteriorly.

Metromonas grandis Larsen and Patterson 1990 (Fig. 4i)

Observation: Cells are leaf shaped, 5 to 11 μm long and dorso-ventrally flattened. One side of the cell appears folded. The cells have two flagella of unequal length. The long flagellum is 1.5 to 2.5 times the cell length and trails behind the cell when gliding. The shorter flagellum is inactive, less than 2 µm long, and inserts to the right of the major flagellum and is always present. The nucleus lies near the flagellar insertion. The cells attach to the substratum with the longer flagellum and move with a nodding action – like a pendulum. Relatively common.

Remarks: This species was reported from marine sites in Australia, Brazil, Fiji, Hawaii and Turkey (Aegean Sea), and cell length reported was 5 to 12 µm (Larsen and Patterson 1990; Tong *et al.* 1998; Lee and Patterson 2000; Al-Qassab *et al.* 2002; Lee 2002b, 2006b; Aydin and Lee 2012). Generally, the current observations are in agreement with those of Larsen and Patterson (1990) and Lee and Patterson (2000; p. 552, Fig. 26f). *Metromonas grandis* is distinguished from *M. simplex* by its cell shape, slightly larger size and folded margin. The cell shape and folded margin may be good diagnostic characters for this species. This species usually co-occurs with *M. simplex*. According to Lee (2002b), Skvortzov (1957) reported 9 new *Ancyromonas* species with one long flagellum, but these species may be gliding stages of *Metromonas*. The short flagellum in *Metromonas* is easy to overlook.

Metromonas simplex (Griessmann 1913) Larsen and Patterson 1990 (Fig. 4j)

Observation: Cell outline is obovate. Cells are 3.5 to 7 µm long and dorso-ventrally flattened, and have smooth pellicle. Two flagella of very unequal length arise from the posterior part of the cell. The major flagellum is 1.5 to 3.0 times the cell length, the shorter flagellum is inactive and about 1 µm long, and inserts to the right of the major flagellum. It may be difficult to see sometimes. The cells normally attach to the substratum with the long flagellum and swing from side to side like a pendulum and the cells may also glide with the cell body in front of the flagellum.

Remarks: This species was found in various marine sites in the world and cell lengths from 3 to 9 µm were reported (Griessmann 1913; Larsen and Patterson 1990; Vørs 1992a, b, 1993a; Ekebom *et al.* 1996; Patterson and Simpson 1996; Tong 1997b; Tong *et al.* 1997, 1998; Lee and Patterson 2000; Al-Qassab *et al.* 2002; Lee 2002b, 2006b; Aydin and Lee 2012).

Pseudophyllomitus granulatus (Larsen and Patterson 1990) Lee 2002 (Fig. 4g)

Observation: Cell outline is sac-shaped. Cells are about 12 µm long, slightly flexible and flattened. Refractile granules underlie the cell surface. Two flagella emerge from the deep flagellar pocket or near half the way down. Both flagella are slightly longer than the cell and are similar in length. In non-motile cells, the posterior flagellum coils up. Cytoplasm drawn out at the posterior end was not seen. The nucleus is located below the anterior pocket, near the centre of the cell and is roundish. Rarely observed.

Remarks: This species has been reported under the name *Phyllomitus granulatus* from marine sites in Australia, Brazil, Demark, Hawaii, Korea and Turkey (Aegean Sea) (Larsen and Patterson 1990; Vørs 1992b; Lee and Patterson 2000; Lee *et al*. 2003; Lee 2002a, b, 2006b), and previously recorded cell length ranges from 7 to 21 µm. Generally, our observations are in accord with those of Larsen and Patterson (1990) and Lee (2002a). *Pseudophyllomitus granulatus* can be distinguished from all species of the genus *Pseudophyllomitus* by its granules. The organism is phagotrophic and may consume relatively large particles such as detritus and diatoms much larger than the flagellate (see Larsen and Patterson 1990, Lee and Patterson 2000).

Sinistermonas Lee nov. gen.

Diagnosis: Biflagellated colourless protists of unknown affinities. Body rigid and flattened with ventral depression subapically. With two flagella, both inserting into ventral depression. The anterior flagellum is directed anterior-laterally to the left, the other trailing. Move by gliding.

Type species: *Sinistermonas sinistrorsus* nov. spec.

Etymology: the name refers 'to the left'.

Sinistermonas sinistrorsus nov. spec. (Figs 1m, 4l)

Diagnosis: *Sinistermonas.* Cells ovoid, 5 to 8 µm long, rigid, slightly dorso-ventrally flattened. Two flagella of unequal length, beating always to left in small excursion. Move by gliding.

Observation: Cells are ovoid, 5 to 8 µm long, rigid, slightly dorso-ventrally flattened, and with two flagella of unequal length in the same strength. The anterior flagellum emerges sub-apically near the right margin of the cell and directs forwardly to the left direction. It is 1 to 1.5 times the length of the cell and beats always to the left direction in a small excursion. The posterior flagellum emerges near the anterior flagellum and trails behind the cell. It is 2.5 to 3.5 times the length of the cell. The nucleus is located anteriorly. The cells move fast by gliding with the beating of the anterior flagellum. Relatively rare. Description based on observations of five cells.

Remarks: *Sinistermonas* is similar to *Protaspa* in being flattened, and having two flagella emerging subapically and anteriorly located nucleus, but it lacks nuclear caps. This species is mostly similar to *Protaspa gemmifera*, but can be distinguished by its smaller size, and the lack of reserve materials and nuclear caps. It is also different in the position of flagellar insertion. *Sinistermonas sinistrorsus* is distinguished from *Protaspa simplex* 1992 because *P. simplex* lacks the distinguishing characters of *Protaspa*. It is similar to *Cyranomonas australis* (Lee 2002b) in general appearance and cell size, but can be easily distinguished by the flexible body of *Cyranomonas* and also the position of flagellar insertion. The anterior flagellum of *C. australis* emerges from the anterior end of the cell, and the posterior flagellum inserts just below the anterior flagellum.

Telonema subtilis Griessmann 1913 (Fig. 4m)

Observation: Cell outline is oval-ovoid. Cells are about 8 µm long, anteriorly narrow and posteriorly broad with a short anterior neck. Two flagella insert below the neck, and are acronematic and slightly longer than the cell. The nucleus is centrally located. The cells swim backward with the flagella, which point behind the swimming cells. Food materials are shown in the posterior end of the cell. Not common. Three cells were observed.

Remarks: The observations are in accord with Griessmann (1913). This species has been found at marine sites in France, Mediterranean, Arctic Norway, Australia, Arctic Canada, Greenland, Japan, Belize and Gulf of Finland (Grieessmann 1913; Hollande and Cachon 1950; Throndson 1969, 1983; Vørs 1992a, b, 1993a, b; Patterson *et al*. 1993; Tong *et al.* 1998; Vørs *et al.* 1995; Lee *et al.* 2003), and has also been reported from a freshwater site in the Antarctic (Tong *et al.* 1997). Previously cell lengths were observed to be 4 to 18 µm. Ultrastructural studies were conducted by Vørs (1992a) and Yabuki *et al.* (2013). This species is distinguished from *Telonema antarctica* Thomsen in Vørs 1992 by the cell shape. *Telonema antarctica* is reniforme.

Unidentified flagellate

A species was particularly distinctive and found on a number of occasions, so that it seems appropriate to mention here.

Protist '1' (Fig. 4n)

Observation: Cells are spherical to ovoid, 3 to 7 µm long, rigid and not flattened. The cells have two flagella, which are acronematic and similar in length, being 2 to 4 times the cell length. Both flagella insert into small subapical depressions or grooves. The flagella insert separately by a small papilla or protrusion about one third of the way down. The anterior flagellum slowly beats always to the left direction, and the posterior flagellum trails and beats very slowly. The nucleus is situated subapically. The cells move by gliding with the anterior flagellar beating. Frequently observed.

Remarks: This species is characterised by the beating of the anterior flagellum to the left. The character may distinguish this species from other small gliding flagellates. It does not appear to belong to stramenopiles because of the anterior flagellum, which did not draw water towards the body of the cell.

In addition to the species described above, some choanoflagellates and many small bacterivorus flagellates have been encountered from this area (Table 1).

The similarities of communities with others studied using the same techniques based on the cluster analysis in the PRIMER program, are shown in Figure 5. From the 41 habitats in the world, the first major separation at about 7% similarity separates communities from extreme saline and anoxic habitats from more normal habitats. The most remote community within the normal cluster is that from a brackish water Baltic Sea habitat. The next is that from a freshwater habitat in Antarctica, but its species list was derived from material that was shipped from Antarctica to Sydney for analysis (Patterson and Lee 2000). At the level of about 20% similarity, separates communities from freshwater habitats from marine habitats. At a level of about 24% similarity, marine benthic communities separate from marine planktonic communities. At a level of about 55% similarity, the community from Gippsland Basin (bottom sediments) separates from other benthic communities from the eastern board of Australia (intertidal sediments).

DISCUSSION

This study sought to create to document the diversity of heterotrophic flagellates from Gippsland Basin and to contribute to the understanding of geographical distribution of heterotrophic flagellates (e.g., Larsen and Patterson 1990; Ekebom *et al.* 1996; Patterson and Simpson 1996; Lee and Patterson 2000; Al-Qassab *et al.* 2002; Lee *et al.* 2003; Schroeckh *et al*. 2003; Lee

Table 1. Lists of species found at each station of Gippsland Basin.

SPECIES / STATIONS	St. 11	St. 12	St. 13	St. 14	St. 15	St. 16	St. 17	St. 18	St. 19	St. 20	St. 21	St. 22	St. 23	St. 24	St. 25	St. 26	St. 27	St. 29	St. 31	St. 32	St. 33	St. 34	St. 35	St. 36	St. 37	St. 38	St. 39	St. 40	St. 41	St. 42	St. 43	St. 44	St. 45	St. 46	St. 47	St. 49	St. 51	St. 53	St. 54	St. 55	St. 56	St. 57	St. 61
Acanthocorbis unguiculata																																										*	
Acronema sippewissettensis			*																																								
Acanthoeca spectabilis												*						*	*																				*	*			
Actinomonas mirabilis					*	*		*	*			*		*				*		*		*	*	*	*		*	*		*	*	*	*	*	*		*	*	*	*	*	*	
Amastigomonas debruynei	*	*	*	*	*	*		*	*		*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*		*		*	*	*	*	
Amastigomonas mutabilis						*		*	*							*			*							*														*			
Ancyromonas impluvium nov.spec.	*	*		*					*				*		*	*							*	*	*			*	*	*	*			*	*		*		*	*	*		
Ancyromonas sigmoides	*	*	*	*	*	*		*	*		*	*	*	*		*		*	*	*	*	*	*	*			*	*	*			*	*	*	*		*		*			*	
Anisonema trepidium					*	*			*												*																						
Apusomonas sp.																										*																	
Bicosoeca gracilis									*							*																											
Bodo cygnus	*											*																															
Bodo platyrhynchus				*	*						*	*	*			*		*		*	*	*	*	*				*	*	*		*	*	*		*	*			*	*	*	*
Bordnamonas tropicana	*	*	*	*	*	*		*	*		*	*	*	*		*		*	*	*		*					*		*	*	*	*	*	*					*				
Caecitellus parvulus								*																																			
Cafeteria minuta			*		*						*	*				*		*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*		*		*	*	*	*	
Cafeteria roenbergensis							*	*																																			
Carpediemonas membranifera																										*																	
Cercomonas sp.																																						*		*			
Chilomastix cuspidata																										*																	
Ciliophrys infusionum			*															*																									
Developayella elegans									*			*								*									*						*	*	*						
Discocelis saleuta	*		*	*	*	*		*	*		*	*		*		*		*									*		*		*	*	*								*		
Goniomonas amphinema	*	*	*	*	*	*		*	*		*	*	*	*		*		*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*		*		*	*	*	*	
Goniomonas pacifica	*	*	*	*	*	*		*	*			*	*	*	*	*		*			*	*	*		*	*	*	*	*	*	*	*		*	*		*		*	*	*	*	
Hemistasia phaeocysicola		*																																									
Heteronema exaratum	*																					*					*																
Kipferlia bialata				*										*										*				*						*				*					
Kittoksia yatava					*							*		*																													
Kurnaimonas celeris. nov. spec.	*																											*															

Mantamonas plastica																			*										*														
Massisteria marina	*	*	*	*	*	*		*	*		*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*		*	*	*	*	*
Metopion fluens					*				*				*						*															*			*		*				
Metromonas grandis	*								*																*																*	*	
Metromonas simplex	*		*	*	*	*			*				*				*					*	*		*	*	*	*	*	*	*		*	*	*				*	*		*	
Neobodo curvifilus							*																				*	*				*						*					
Neobodo designis	*	*	*	*	*	*		*	*		*	*	*	*	*	*	*	*		*	*	*	*	*	*	*	*	*	*	*	*	*	*	*			*		*		*	*	
Neobodo saliens	*	*	*	*	*	*		*	*		*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*		*	*	*	*	*	*			*		*	*		*	
Notosolenus apocamptus		*						*	*				*	*																													
Notosolenus caudatus													*										*																				
Notosolenus ostium															*																												
Notosolenus urceolatus						*												*					*	*						*									*				
Notosolenus sp.																							*																				
Paraphysomonas sp.			*	*		*		*			*	*	*	*	*	*	*	*			*		*		*	*	*	*	*	*	*	*	*	*		*		*	*	*	*	*	
Percolomonas similis	*		*	*	*	*	*	*	*	*	*	*		*	*	*	*	*		*		*	*	*	*	*	*	*			*	*		*	*	*		*		*	*	*	
Petalomonas abscissa					*								*				*																										
Petalomonas intortus		*	*	*	*	*		*	*					*								*															*						
Petalomonas minuta																						*			*					*													
Petalomonas poosilla			*						*			*	*					*			*									*													
Petalomonas raiula			*																																								
Petalomonas vigatus				*																																							
Petalomonas sp.1						*																																					
Petalomonas sp.2				*																																							
Ploeotia corrugata														*																													
Ploeotia oblonga													*																														
Ploeotia plana								*																																			
Ploeotia plumosa	*	*	*					*										*									*																
Ploeotia vitrea			*	*		*			*			*																															
Ploeotia sp.1								*	*												*																						
Ploeotia sp.2																*																											
Polyoeca dichotoma								*													*				*																		
Protaspa obliqua		*											*			*													*														
Protaspa tegere								*																																			
Psammosa unguis nov. comb.					*																		*																				

SPECIES / STATIONS	St. 11	St. 12	St. 13	St. 14	St. 15	St. 16	St. 17	St. 18	St. 19	St. 20	St. 21	St. 22	St. 23	St. 24	St. 25	St. 26	St. 27	St. 29	St. 31	St. 32	St. 33	St. 34	St. 35	St. 36	St. 37	St. 38	St. 39	St. 40	St. 41	St. 42	St. 43	St. 44	St. 45	St. 46	St. 47	St. 49	St. 51	St. 53	St. 54	St. 55	St. 56	St. 57	St. 61
Pseudobodo tremulans																			*		*							*									*		*			*	
Pseudophyllomitus granulatus													*																					*	*								
Pteridomonas danica					*	*		*	*			*		*				*		*		*	*	*	*		*	*		*	*	*	*	*	*		*	*	*	*	*	*	
Rhynchopus amitus																																	*						*				
Rhynchobodo formica																																	*										
Rhynchobodo simius			*		*																												*										
Rhynchomonas nasuta		*	*		*	*		*	*		*	*	*	*		*	*	*	*	*	*	*	*	*	*		*		*	*		*	*	*	*		*		*		*	*	
Roombia truncata				*																		*																	*				
Saepicular puchra				*																							*																
Salpingoeca marina													*															*															
Salpingoeca tuba																					*																						
Salpingoeca megacheila										*																																	
Salpingoeca. amphoridium																																							*				
Sinistermonas sinistrorsus nov. spec.																*									*				*														
Sphenomonas angusta												*																															
Spironema multiciliatum																																			*								
Spumella sp.			*	*		*		*			*	*	*	*	*	*	*	*			*		*		*	*	*	*	*	*	*	*	*	*		*		*	*	*	*	*	
Telonema subtilis																																										*	
Thaumatomastix setifera	*																											*															
Thaumatomastix sp.		*																																									
Protist '1'												*														*																	
Total	18	16	22	22	23	22	3	25	26	2	14	24	23	20	10	21	10	21	12	14	18	19	21	15	19	16	21	21	20	19	16	18	19	21	15	6	17	8	22	16	16	19	2

2002b, 2006b, 2008, 2012; Aydin and Lee 2012). These studies have found that most morpho-species of heterotrophic flagellates have a worldwide distribution.

The number of species from each station varies among the sampling stations and is in the range of 2–26 (avg. 17) (Table 1). It does not appear to vary in the sediment types or water depths (see Lee 2008 for the sediment types and depths), although their species composition varies according to those factors (e.g., Patterson *et al.* 1989; Lee and Patterson 2000). Station 61 is over 3000 m and had only 2 species, *Bodo platyrhynchus* and *Massisteria marina*. Probably, these species show cosmopolitan distribution at least at the level of the morphological species, as currently recognised. These are included in the 20 most common and widely distributed species by Patterson and Lee (2000).

The results of the cluster analysis showed that communities from freshwater habitats are distinctive from those from marine communities. Salinity appears to be a determining factor as the communities from freshwaters segregate from the communities from marine habitats with a normal salinity. Some species such as *Rhynchomonas nasuta* and *Neobodo designis* are remarkably widespread, but most species occur in certain habitats (marine or freshwater). About 10% of the species reported previously can be found in freshwater and marine habitats (Patterson and Lee 2000). The community from Gippsland Basin separates from other benthic communities, and is distinguishable from communities from normal salinity habitats of the eastern seaboard of Australia (Fig. 5). The Gippsland Basin community appears to be distinctive, despite the weakness of the data being from one sampling occasion.

Eighty five species from 53 genera (including the result of Lee (2008) for heterotrophic euglenids) were encountered in the Gippsland Basin. 71 species (about 83.5%) have been previously recorded from other localities, but 14 species (about 16.5%) have not been previously reported from marine or freshwater habitats. Of the 14 species, 4 species were new, 9 species were

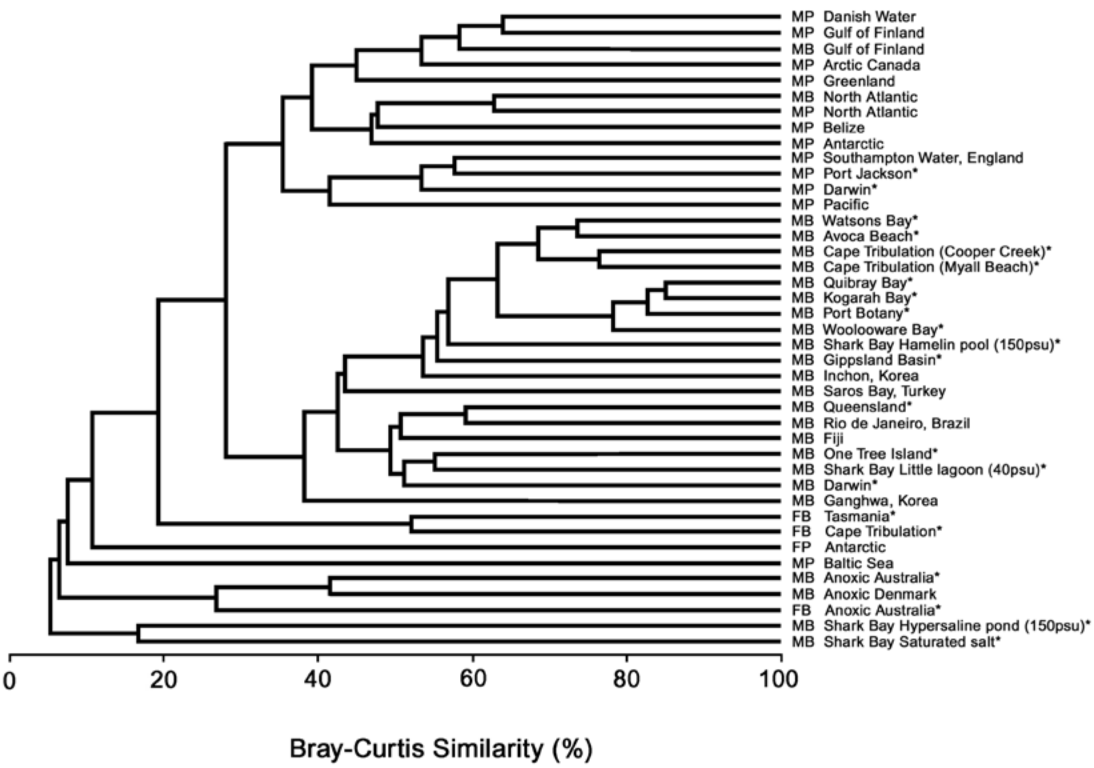

Fig. 5. Dendrogram showing the Bray-Curtis similarity (%) between the communities from 41 habitats; taxonomic information based on species. Species data used for this analysis were from Lee and Patterson (1998), Al-Qassab *et al.* (2002), Lee *et al.* (2003, 2005), Lee (2002b, 2006a, b, 2008, 2012), Schroeckh *et al.* (2003), Aydin and Lee (2012) and the present study. MB – marine benthic habitats, MP – marine planktonic habitats, FB – freshwater benthic habitats, FP – freshwater planktonic habitats. * indicates Australian sites.

identified to genus but not to species (e.g., *Notosolenus* sp., *Ploeotia* sp.1) and the remaining 1 species was given a name as Protist '1' under 'Unidentified flagellate'. Some more species were found, but not reported here because of lack of information. Two species (*Mantamonas plastica* and *Roombia truncata*) of the 71 species were first found at this area, but recently reported as new species from marine intertidal habitats (Okamoto *et al*. 2009, Glücksman *et al.* 2011). Two new species here (*Ancyromonas impluvium, Sinistermonas sinistrorsus*) were also found from marine intertidal habitats in subtropical Australia (Lee unpublished data). Probably these are rare species. According to the analysis by Patterson and Lee (2000) of the literature and field surveys, there may be many rare species in some habitats. It suggests that new or unidentified species here may be also found elsewhere if we carry out intensive studies in other localities or habitats. Despite this, most marine free-living heterotrophic flagellates appear to have a wide distribution, and also our insights about biogeography remain overly influenced by extrinsic factors such as incomplete sampling regimes, and incomplete or selective reporting.

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