

## Small Free-Living Heterotrophic Flagellates from Marine Intertidal Sediments of the Sydney Region, Australia

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**Abstract.** A total of 155 species and 75 genera were found at marine sediments in Sydney region (Australia) and are described using light microscopy: 117 species at Port Botany, 111 species at Kogarah Bay, 94 species at Woolooware Bay, 126 species at Quibray Bay, 74 species at Avoca beach, 48 species at Watsons Bay. The records include accounts of 15 unidentified taxa and two new taxa: *Eoramonas jungensis* sp. nov. (*Eoramonas* gen. nov.), *Protaspa flexibilis* sp. nov. Most flagellates described here have been found at other locations worldwide, but many species not reported from any other locations. I am unable to assess if these species are endemic because of the lack of intensive studies elsewhere. However, these results suggest that the flagellate communities from Botany Bay are distinctive.

**Keywords:** Protista, heterotrophic flagellates, intertidal sediment, endemic.

### INTRODUCTION

Heterotrophic flagellates are of importance as consumers or prey in aquatic ecosystems (e.g. Sherr and Sherr 1994; Hondeveld et al. 1995; Starink et al. 1996a, b; Epstein 1997a, b; Dietrich and Arndt 2000; Lee and Patterson 2000). As a contribution to the study of marine heterotrophic flagellates, a number of studies on their diversity and geological distribution have been conducted (e.g. Larsen and Patterson 1990; Vørs 1992a, b; Patterson et al. 1993; Ekebom et al. 1996; Patterson and Simpson 1996; Tong 1997a, b, c; Lee and Patterson 2000; Al-Qassab et al. 2002; Lee 2002b, 2006a, b, 2008, 2012, 2015; Lee et al. 2003; Tikhonenkov et al.

2006; Aydin and Lee 2012). Most of these studies have not been carried out intensively – they represent studies over a period of a few days. Our current insights into large-scale patchiness of previous studies may be questioned on the basis of under-reporting which, if the taxa usually reported are common and widespread, will lead to an erroneous conclusion that flagellates have a widespread distribution (Lee and Patterson 1998, Patterson and Lee 2000). This article reports the diversity of heterotrophic flagellates revealed in a long-term, intensive study at Botany Bay and in the studies at marine sediments near the Sydney region, and then to use that information to address issues of endemism in this group.

Previous taxonomic studies of marine benthic flagellates in Australia are Ruinen (1938), Post et al. (1983), Larsen and Patterson (1990), Ekebom et al. (1996), Patterson and Simpson (1996), Lee et al. (1999), Bernard et al. (2000), Lee and Patterson (2000), Al-Qassab et

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al. (2002), Lee et al. (2003) and Lee (2006a, b, 2008, 2012, 2015). Among them, the studies of Lee and Patterson (2000), Bernard et al. (2000) and Lee (2012) involved Botany Bay in Sydney, Australia. This study is complemented by a concurrent study of heterotrophic euglenids at Botany Bay (Lee 2012).

## MATERIALS AND METHODS

This study was carried out mainly at Botany Bay (Port Botany, Kogarah Bay, Quibray Bay, Woolooware Bay), and also carried out at Avoca Beach and Watsons Bay in the Sydney region from February 1998 to February 2000. Samples were collected on 126 occasions from Botany Bay. Details of Botany Bay were reported by Lee and Patterson (2000) and Lee (2012). Avoca beach is about 90 km north of Sydney (151° 26' 40" E; 33° 28' 30" S). The beach is 99 % sandy sediments and mean grain size is about 258 µm. Samples were collected on 4 occasions (28 Feb., 2 May, 24 Jul. 1999, Jan. 2000). Watsons bay is located on north Sydney (151° 16' 90" E; 33° 50' 80" S) and 99 % sandy sediment. There are some restaurants and a ferry wharf near the sampling site. Samples were collected on 5 occasions (1 Apr., 2 May, 30 May, 27 Jul. 1999, 30 Jan., 2000).

Samples were collected from marine intertidal sediments to a depth of about 1 cm from a 0.25 m<sup>2</sup> quadrat. After collecting sediments, the sediments were placed in plastic trays in 1cm deep layers. The sediments were covered with lens tissue and coverslips (No.1 22 x 22 mm) were placed on the lens tissue. After 18–24 hours the coverslips were removed and flagellates were observed using an Axiophot microscope (Zeiss) equipped with photographic facilities as similar to those described by Patterson (1982). The flagellates were also recorded on U-MATIC videotapes and records were also made with video prints. Specimens were also drawn. The samples were maintained at room temperature (~ 22°C) for 5–7 days.

## RESULTS

Nomenclature here follows the ICZN (International Commission on Zoological Nomenclature 1999).

Detailed accounts of the taxonomy of heterotrophic flagellates have already been published by Larsen and Patterson (1990), Lee and Patterson (2000), Al-Qassab et al. (2002), Lee et al. (2003) and Lee (2008, 2012, 2015). Therefore, only taxa not described by these authors, or which are different from previous descriptions, are described in detail and commented on.

It was difficult to establish the identities of some species (e.g. *Cercomonas* sp.1, Protist 1) due to not enough information, but their presences are recorded to establish the occurrences in marine sediments.

Lists of species encountered during this study are presented in Table 1. The lists include the results of Lee and Patterson (2000) and Lee (2012).

**Archamoeba** Cavalier-Smith, 1983

*Mastigamoeba psammobia* Larsen et Patterson, 1990

Figs 1a–b, 2a–b

**Description:** Cells are 12–14 µm long with very flexible amoeboid cell body. The anterior part of the cell is hyaline. Somewhat sharp pseudopodia are produced around the body and mostly from the posterior parts of the cell. One thickened flagellum emerges at the end of the cell, is about 20 µm long, and appears to be acronematic. When the cell is moving, the body is more spherical, and the flagellum is directed forwardly and beats slowly. The nucleus is located anteriorly and appears to be connected to the flagellum. Three cells were observed at Quibray Bay.

**Remarks:** The identities of taxa within the pelobionts are unclear because of the considerable polymorphism, which occurs in this group. An unpublished revision (Walker 1998) recommended a ten fold reduction in the number of recognized taxa. Species of *Mastigamoeba* Schulze, 1874 have been distinguished from species of *Mastigella* Frenzel, 1897 by the close association between the nucleus and flagellar apparatus in *Mastigamoeba* and the separation of the nucleus from the flagellar apparatus in *Mastigella* (Goldschmidt 1907, Simpson et al. 1997). In a recent study (Bernard et al. 2000) it was noted that, although in *M. simplex* the bulk of the nucleus between them. Bernard et al. (2000) determined that mastigamoebid pelobionts should be assigned to *Mastigamoeba* if there was any connection between the nucleus and the base of the flagellum and to *Mastigella* if there is no such connection. This interpretation was adopted here.

This species is assigned to *Mastigamoeba psammobia* Larsen et Patterson, 1990 because the cell lengths are similar and the cells described here produced long posterior pseudopodia. It has been found at marine sites in tropical Australia and Brazil, and reported to be 11–14 µm long (Larsen and Patterson 1990). According to Walker (1998), there are currently about 85 species in the genus. *Mastigamoeba psammobia* can be distinguished from *Mastigamoeba simplex* by producing long posterior pseudopodia. This species is distinguished from *Mastigamoeba punctachora* by the absence of the inclusion. It is distinguished from *M. schizophrenia* Simpson et al., 1997 because *M. schizophrenia* has adhering paired nuclei (Simpson et al. 1997).

**Table 1.** Lists of species encountered at each site. +: species reported from this study, \*: name changed by Lax et al. (2019). PB: Port Botany, KB: Kogarah Bay, WB: Wooloware Bay, QB: Quibray Bay, AB: Avoca beach, WatB: Watsons Bay.

SPECIES	PB	KB	WB	QB	AB	WatB
<i>Actinomonas mirabilis</i> Kent, 1880	+	+	+	+	+	+
<i>Amastigomonas debrynei</i> de Saedeleer, 1931	+	+	+	+	+	+
<i>Amastigomonas griebensis</i> Mylnikov, 1999	+	+	+	+	+	
<i>Amastigomonas mutabilis</i> (Griessmann, 1913) Molina et Nerad, 1991	+	+	+	+	+	+
<i>Ancyromonas impulsiva</i> Lee, 2015	+	+	+	+	+	+
<i>Ancyromonas melba</i> Patterson et Simpson, 1996				+		
<i>Ancyromonas sigmoides</i> Kent, 1880	+	+	+	+	+	+
<i>Ancyromonas sinistra</i> Al-Qassab et al., 2002	+	+	+	+		
<i>Anehmia exotica</i> Ekebom et al., 1996	+		+	+		
<i>Anisonema acinus</i> Dujardin, 1841	+	+	+	+	+	+
<i>Apusomonas proboscidea</i> Aléxiéeff, 1924				+	+	
<i>Barthelona vulgaris</i> Bernard et al., 2000	+	+		+	+	
<i>Bicosoeca conica</i> Lemmermann, 1914						+
<i>Bicosoeca gracilipes</i> James-Clark, 1967				+		
<i>Bodo cygnus</i> Patterson et Simpson, 1996	+			+		+
<i>Bodo platyrhynchus</i> Larsen et Patterson, 1990	+	+	+	+	+	+
<i>Bodo saltans</i> Ehrenberg, 1832		+	+	+		
<i>Bordnamonas tropicana</i> Larsen et Patterson, 1990	+	+	+	+	+	+
<i>Caecitellus parvulus</i> (Griessmann, 1913) Patterson et al., 1993	+	+	+	+	+	
<i>Cafeteria minuta</i> (Ruinen, 1938) Larsen et Patterson, 1990	+					
<i>Cafeteria roenbergensis</i> Fenchel et Patterson, 1988	+	+	+	+	+	+
<i>Cantina marsupialis</i> (Larsen et Patterson, 1990) Panek et al., 2015	+	+	+	+	+	+
<i>Carpediemonas membranifera</i> (Larsen et Patterson, 1990) Ekebom et al., 1996	+	+	+	+	+	+
<i>Cercomonas granulatus</i> Lee et Patterson, 2000	+	+		+		
<i>Cercomonas parva</i> (Hartmann et Chagas, 1910) Mignot et Brugerolle, 1975	+	+	+	+	+	
<i>Cercomonas</i> sp.1	+	+		+		
<i>Cercomonas</i> sp.2	+	+				
<i>Cercomonas</i> sp.3	+	+		+	+	+
<i>Chasmostoma nieuportense</i> Massart, 1920				+		
<i>Chilomastix cuspidata</i> (Larsen et Patterson, 1990) Bernard et al., 1997	+	+		+		
<i>Ciliophrys infusionum</i> Cienkowski, 1876	+	+	+			
<i>Clautriavia cavus</i> Lee et Patterson, 2000	+	+	+	+	+	+
<i>Cyranomonas asutralis</i> Lee, 2002	+			+		
<i>Dinema litorale</i> Skuja, 1939	+	+	+			
<i>Dinema platysomum</i> (Skuja, 1939) Lee et Patterson, 2000	+	+	+	+	+	
<i>Dinema validum</i> Larsen et Patterson, 1990	+	+	+			
<i>Discocelis punctata</i> Larsen et Patterson, 1990	+					
<i>Discocelis saleuta</i> Vørs, 1988		+		+	+	+
<i>Developayella elegans</i> Tong, 1995	+	+	+	+	+	+
<i>Dolium sedentarium</i> Larsen et Patterson, 1990		+	+	+		
<i>Eoramonas jungensis</i> nov. spec.	+	+		+	+	
<i>Glissandra innuerende</i> Patterson et Simpson, 1996	+				+	

SPECIES	PB	KB	WB	QB	AB	WatB
<i>Glissandra similis</i> Lee, 2006	+	+	+	+		
<i>Goniomonas amphinema</i> Larsen et Patterson, 1990	+	+	+	+	+	+
<i>Goniomonas pacifica</i> Larsen et Patterson, 1990	+	+	+	+	+	+
<i>Gweamonas unicus</i> Lee, 2002		+		+		
<i>Hemiolia</i> (= <i>Anisonema</i> ) <i>trepidum</i> (Larsen, 1987) Lax et al., 2019*	+	+	+	+	+	+
<i>Heterochromonas opaca</i> Skuja, 1948	+			+	+	
<i>Harpagon descissus</i> (Perty, 1852) Pánek et al., 2012	+	+		+	+	
<i>Hemistasia phaeocysticola</i> (Scherffel, 1900) Elbrächter et al., 1996	+		+	+	+	
<i>Heteronema globuliferum</i> (Ehrenberg, 1838) Stein, 1878	+	+	+	+		
<i>Heteronema splendens</i> Larsen et Patterson, 1990		+				
<i>Heteronema vittatum</i> Larsen et Patterson, 1990			+			
<i>Heteronema</i> sp.1			+	+		
<i>Heteronema</i> sp.2			+			
<i>Hexamita inflata</i> Dujardin, 1838	+	+		+	+	
<i>Jenningsia fusiforme</i> (Larsen, 1987) Lee et al., 1999	+	+	+	+		
<i>Jenningsia macrostoma</i> (Ekeboom et al., 1996) Lee et al., 1999	+			+		
<i>Kathblepharis remigera</i> (Vørs, 1992) Clay et Kugrens, 1999	+	+	+	+		
<i>Kiitoksia ystava</i> Vørs, 1992					+	
<i>Kipferlia bialata</i> (Ruinen, 1938) Kolisko et al., 2010	+	+		+	+	+
<i>Kurnaimonas celeris</i> Lee, 2015		+	+	+		
<i>Lentomonas azurina</i> (Patterson et Simpson, 1996) Cavalier-Smith, 2016			+	+		
<i>Lentomonas corrugata</i> (Larsen et Patterson, 1990) Cavalier-Smith, 2016	+	+	+	+	+	+
<i>Malawimonas jakobiformis</i> O'Kelly et Nerad, 1999	+	+	+	+	+	
<i>Massisteria marina</i> Larsen et Patterson, 1990	+	+	+	+	+	+
<i>Mantomonas plastic</i> Glücksman et Cavalier-Smith, 2011		+	+			
<i>Mastigamoeba psammobia</i> Larsen et Patterson, 1990				+		
<i>Mastigamoeba simplex</i> Kent, 1880	+	+	+	+		
<i>Metopion fluens</i> Larsen et Patterson, 1990	+	+	+	+	+	+
<i>Metromonas grandis</i> Larsen et Patterson, 1990	+	+	+	+	+	+
<i>Metromonas simplex</i> (Griessmann, 1913) Larsen et Patterson, 1990	+	+	+	+	+	+
<i>Neobodo curvifilus</i> (Griessmann, 1913) Moreira et al., 2004	+	+				
<i>Neobodo designis</i> (Skuja 1948) Moreira et al., 2004	+	+	+	+	+	+
<i>Neobodo saliens</i> (Larsen et Patterson, 1990) Moreira et al., 2004	+	+	+	+	+	+
<i>Neometanema exaratum</i> (Larsen et Patterson, 1990) Lee et Simpson, 2014	+	+	+	+	+	+
<i>Neometanema larseni</i> (Lee et Patterson, 2000) Lee et Simpson, 2014	+	+		+	+	+
<i>Neometanema ovale</i> (Kahl, 1928) Lee et Simpson, 2014	+	+	+	+	+	
<i>Neometanema strenuum</i> (Skuja, 1948) Lee et Simpson, 2014	+					
<i>Notosolenus adamas</i> Lee et Patterson, 2000	+					
<i>Notosolenus apocamptus</i> Stokes, 1884	+	+	+	+	+	
<i>Notosolenus brothernis</i> Lee et Patterson, 2000	+					
<i>Notosolenus hemircularis</i> Lee et Patterson, 2000	+	+	+	+		
<i>Notosolenus lashue</i> Lee et Patterson, 2000	+					
<i>Notosolenus ostium</i> Larsen et Patterson, 1990	+	+	+	+		
<i>Notosolenus</i> cf. <i>papilio</i> Skuja, 1939				+		



<i>Notosolenus pyriforme</i> Lee et Patterson, 2000	+				+	
<i>Notosolenus scutulum</i> Larsen et Patterson, 1990	+	+	+	+		
<i>Notosolenus similis</i> Skuja, 1939	+	+	+	+	+	
<i>Notosolenus</i> cf. <i>tamanduensis</i> Larsen et Patterson, 1990	+					
<i>Notosolenus urceolatus</i> Larsen et Patterson, 1990	+	+	+	+	+	+
<i>Notosolenus</i> sp.		+				
<i>Pendulomonas adriperis</i> Tong, 1997	+	+	+	+		
<i>Percolomonas cosmopolites</i> (Ruinen, 1938) Fenchel et Patterson, 1986	+	+	+	+	+	
<i>Peranema dolichonema</i> Larsen et Patterson, 1990	+	+				
<i>Peranema trichophorum</i> (Ehrenberg, 1830) Stein, 1878	+	+	+	+		
<i>Petalomonas abscissa</i> (Dujardin, 1841) Stein, 1859	+	+	+	+	+	
<i>Petalomonas gini</i> Lee, 2012	+	+	+	+	+	
<i>Petalomonas intortus</i> Lee et Patterson, 2000	+	+	+	+	+	
<i>Petalomonas iugosus</i> Lee et Patterson, 2000	+					
<i>Petalomonas labrum</i> Lee et Patterson, 2000	+	+	+	+		
<i>Petalomonas marginalis</i> Larsen et Patterson, 1990		+				+
<i>Petalomonas minor</i> Larsen et Patterson, 1990	+	+	+	+	+	
<i>Petalomonas minuta</i> Hollande, 1942	+	+	+	+	+	+
<i>Petalomonas planus</i> Lee et Patterson, 2000	+	+	+	+		
<i>Petalomonas poosilla</i> Larsen et Patterson, 1990	+	+	+	+	+	+
<i>Petalomonas raiula</i> Larsen et Patterson, 1990	+	+	+	+	+	
<i>Petalomonas spinifera</i> (Lackey 1962) Lee et Patterson, 2000	+					
<i>Petalomonas virgatus</i> Lee et Patterson, 2000	+	+	+	+		
<i>Petalomonas</i> sp.	+	+	+	+		
<i>Phyllomitus undulans</i> Stein, 1878		+		+		
<i>Platychilomonas psammobia</i> Larsen et Patterson, 1990	+	+		+	+	+
<i>Ploeotia adhaerens</i> Larsen et Patterson, 1990	+					
<i>Ploeotia discoides</i> Larsen et Patterson, 1990	+	+	+	+		
<i>Ploeotia longifilum</i> Larsen et Patterson, 1990	+			+		
<i>Ploeotia oblonga</i> Larsen et Patterson, 1990	+		+	+		
<i>Ploeotia plumosa</i> Ekeboom et al., 1996	+			+	+	
<i>Ploeotia pseudoanisonema</i> Larsen et Patterson, 1990	+		+	+		
<i>Ploeotia vitrea</i> Dujardin, 1841	+	+	+	+	+	+
<i>Ploeotia</i> sp.1				+		
<i>Ploeotia</i> sp.2	+					
<i>Ploeotia</i> sp.3				+	+	+
<i>Polyoeca dichotoma</i> Kent, 1880	+	+		+		
<i>Protaspa flexibilis</i> nov. spec.		+		+		
<i>Protaspa gemmifera</i> (Larsen et Patterson, 1990) Cavalier-Smith, 2011	+	+		+	+	+
<i>Protaspa obliqua</i> (Larsen et Patterson, 1990) Cavalier-Smith, 2011	+	+	+	+	+	+
<i>Protaspa simplex</i> (Vørs, 1992) Cavalier-Smith, 2011	+	+	+	+	+	+
<i>Protaspa tegere</i> (Larsen et Patterson, 1990) Cavalier-Smith, 2011	+	+	+	+	+	+
<i>Protaspa verrucosa</i> (Larsen et Patterson, 1990) Cavalier-Smith, 2011		+		+	+	
<i>Psammosa unguis</i> (Patterson et Simpson, 1996) Lee, 2015	+	+	+	+	+	
<i>Pseudobodo tremulans</i> Griessmann, 1913	+	+	+	+		+

SPECIES	PB	KB	WB	QB	AB	WatB
<i>Pseudophyllomitus granulatus</i> (Larsen et Patterson, 1990) Lee, 2002	+	+	+	+	+	+
<i>Pteridomonas danica</i> Patterson et Fenchel, 1985	+	+	+	+	+	+
<i>Rhynchobodo longiciliatus</i> (Skuja, 1948) Bernard et al., 2000		+	+	+		
<i>Rhynchobodo simius</i> Patterson et Simpson, 1996	+	+	+	+	+	+
<i>Rhynchomonas nasuta</i> (Stokes, 1888) Klebs, 1893	+	+	+	+	+	+
<i>Rhynchopus amitus</i> Skuja, 1948	+	+		+		
<i>Roombia truncata</i> Okamoto et al., 2009	+	+	+	+	+	
<i>Saepicula pulchra</i> Leadbeater, 1980		+	+	+		
<i>Salpingoeca amphoridium</i> James-Clark, 1867			+	+		
<i>Salpingoeca marina</i> James-Clark, 1867	+	+		+		+
<i>Sinistermonas sinistrorsus</i> Lee, 2015	+	+	+	+		+
<i>Sphenomonas angusta</i> Skuja, 1956	+	+	+	+	+	
<i>Sphenomonas elongata</i> Lackey, 1962		+	+	+		
<i>Sphenomonas</i> sp.				+		
<i>Spironema multiciliatum</i> Klebs, 1893		+	+	+		
<i>Stephanoeca diplocostata</i> Ellis, 1929	+					
<i>Stephanopogon</i> sp.				+	+	
<i>Telonema subtilis</i> Griessmann, 1913			+			
<i>Thaumatomastix</i> sp.	+	+		+	+	
<i>Trepomonas agilis</i> Dujardin, 1841		+				
<i>Urceolus cornutus</i> Larsen et Patterson, 1990	+	+	+	+	+	+
<i>Urceolus costatus</i> (Stein, 1878) Lemmermann, 1910		+	+	+		
Protist 1			+	+		
Protist 2				+		
Total	117	111	94	126	74	48

### Diplomonadida Wenyon, 1926

#### *Hexamita inflata* Dujardin, 1838

Figs 1c, 2c

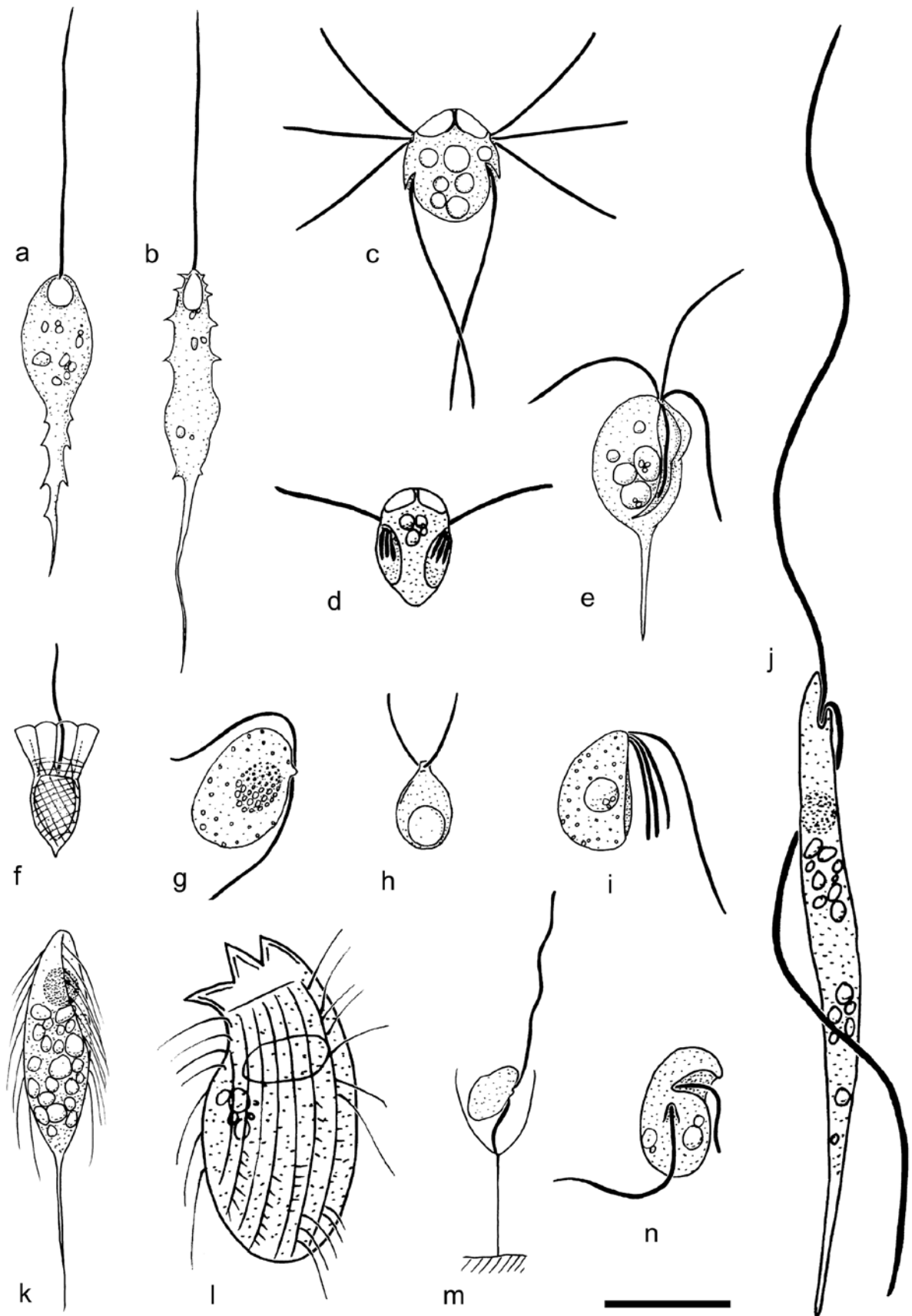
**Description:** Cells are roundish to ovoid and 6–10 µm long. Several contractile vacuoles are seen. The posterior end of the cell may be pointed or rounded. Two nuclei are located anteriorly, and two longitudinal cytostomal tubes are visible below the equator of the cell and on the ventral side of the cell. There are two sets of 4 flagella (total eight flagella); two medium flagella are 1.3–1.5 times the cell length, four short flagella are about the cell length. These flagella insert

anterio-laterally into a small depression on the anterior part of the cell. Two long flagella emerge from the cytostomal tube and are about 2 times the length of the cell. The long flagella normally cross each other. The cells move by skidding or swimming. Relatively common.

**Remarks:** There are about 15 nominal species in this genus: *Hexamita angusta* (Klebs, 1893) Starmach, 1968, *H. caudata* (Skuja, 1939) Starmach, 1968, *H. crassa* Klebs, 1893, *H. eurykephale* Skuja, 1956, *H. fissa* Klebs, 1893, *H. fusiformis* Klebs, 1893, *H. furcata* Zhukov et al., 1978, *H. gyrans* Stokes, 1887, *H. inflata* Dujardin, 1838, *H. mutabilis* Zhukov et al., 1978,



**Fig. 1.** Archamoeba, Trepomonadea, Retortamonadida, Choanoflagellata, Kathablepharidae, Telonemida, Heterolobosea, Kinetoplastida, Hemimastigida, Stephanopogonidae, Colpodellida and Stramenopiles. (a)–(b) *Mastigamoeba pasmmobia*, (c) *Hexamita inflata*, (d) *Trepomonas agilis*, (e) *Chilomastix cuspidata*, (f) *Saepicula pulchra*, (g) *Roombia truncata*, (h) *Telonema subtilis*, (i) *Harpagon descissus*, (j) *Rhynchobodo longiciliatus*, (k) *Spironema multiciliatum* (from Lee 2015), (l) *Stephanopogon* sp., (m) *Bicosoeca conica*. (n) *Psammisia unguis*. Scale bar = 10 µm for all figures.



*H. minor* (Tschernov, 1950) Mylnikov, 1985, *H. pusilla* Klebs, 1893, *H. tremellorans* Skuja, 1939, *H. truncata* Stokes, 1888, *H. rostrata* Stein, 1878. *Hexamita inflata* (10–25 µm) resembles *H. crassa* (24–35 µm), *H. fissa* (20–26 µm), *H. gyrans* (9 µm), *H. minor* (18–20 µm), *H. pusilla* (10–13 µm), *H. tremellorans* (11–19 µm) and *H. truncata* (11 µm) in cell length and shape. It is not possible to distinguish unambiguously any cells reported in the literature as having a rounded or oval body and measuring from 9 to 35 µm long. I therefore adopt the view that *H. inflata* is a species of variable size, that *H. crassa*, *H. fissa*, *H. pusilla*, *H. tremellorans* are synonyms, and because the cells observed here are distinguishable only by size, I assign the cells reported above to *H. inflata*.

*Hexamita inflata* is quite common in freshwater sites (Mylnikov 1985), but is not often found at marine sites. This species was found at a marine site in Denmark with cell length about 10 µm (Fenchel et al. 1995). Previously reported cell length is from 10 to 25 µm (e.g. Mylnikov 1985, Fenchel et al. 1995). The observations extend the cell length range.

***Trepomonas agilis* Dujardin, 1841**

Figs 1d, 2d

**Description:** Cell is ovoid, but S-shaped in cross section and is about 10 µm long. Two nuclei are located anteriorly. Two groups of flagella are inserted laterally at the end of each groove: two long flagella and six short flagella. The length of the long flagella was not measured, but the short flagella are less than half the cell length and lie in the grooves. The cell moves by swimming. Contractile vacuoles are seen. One cell was observed at Kogarah Bay.

**Remarks:** This species has been found at a marine site in Australia (Bernard et al. 2000), and also has been reported widely from freshwater, wastewater and anoxic (e.g. Playfair 1921, Lavier 1936, Calaway and Lackey 1962, Brugerolle 1973, Eyden and Vickermann 1975, Zhukov and Mylnikov 1983, Fenchel et al. 1995). Previously reported cell length ranges are from 8 to 22 µm. This genus was reviewed by Mylnikov (1985) to include 7 nominal species: *Trepomonas agilis* Dujardin, 1841, *T. angulatus* Klebs, 1893, *T. communis* Klebs, 1893, *T. latecapitata* Skuja, 1956, *T. rotans* Klebs, 1893, *T. simplex* Klebs, 1893, *T. steini* (Stein, 1878) Klebs, 1893. *Trepomonas agilis* is hard to distinguish *T. angulatus*, *T. communis* and *T. simplex*, which have similar features such as cell appearance and having two long flagella and six short flagella (*T. agilis*,

8–22 µm; *T. angulatus*, 30 µm; *T. communis*, 13–25 µm; *T. simplex*, 7–8 µm). *Trepomonas communis* was regarded as a junior synonym of *T. agilis* (Bernard et al. 2000). Here, other two species (*T. angulatus* and *T. simplex*) are regarded as junior synonyms of *T. agilis*.

**Retortamonadida** Grassé, 1952

***Chilomastix cuspidata* (Larsen et Patterson, 1990) Bernard et al., 1997**

Figs 1e, 2e

**Description:** Cells are drop-shaped with a long posterior spike and 20–32 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 four flagella inserting subapically and are directed anterior laterally. 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, Denmark and Ireland (Larsen and Patterson 1990; Fenchel et al. 1995; Bernard et al. 1997, 2000; Weerakoon et al. 1999; Lee 2015) and previously reported range of cell lengths is from 14 to 33 µm.

**Choanoflagellata** Kent, 1880

***Acanthoecida* Cavalier-Smith, 1997**

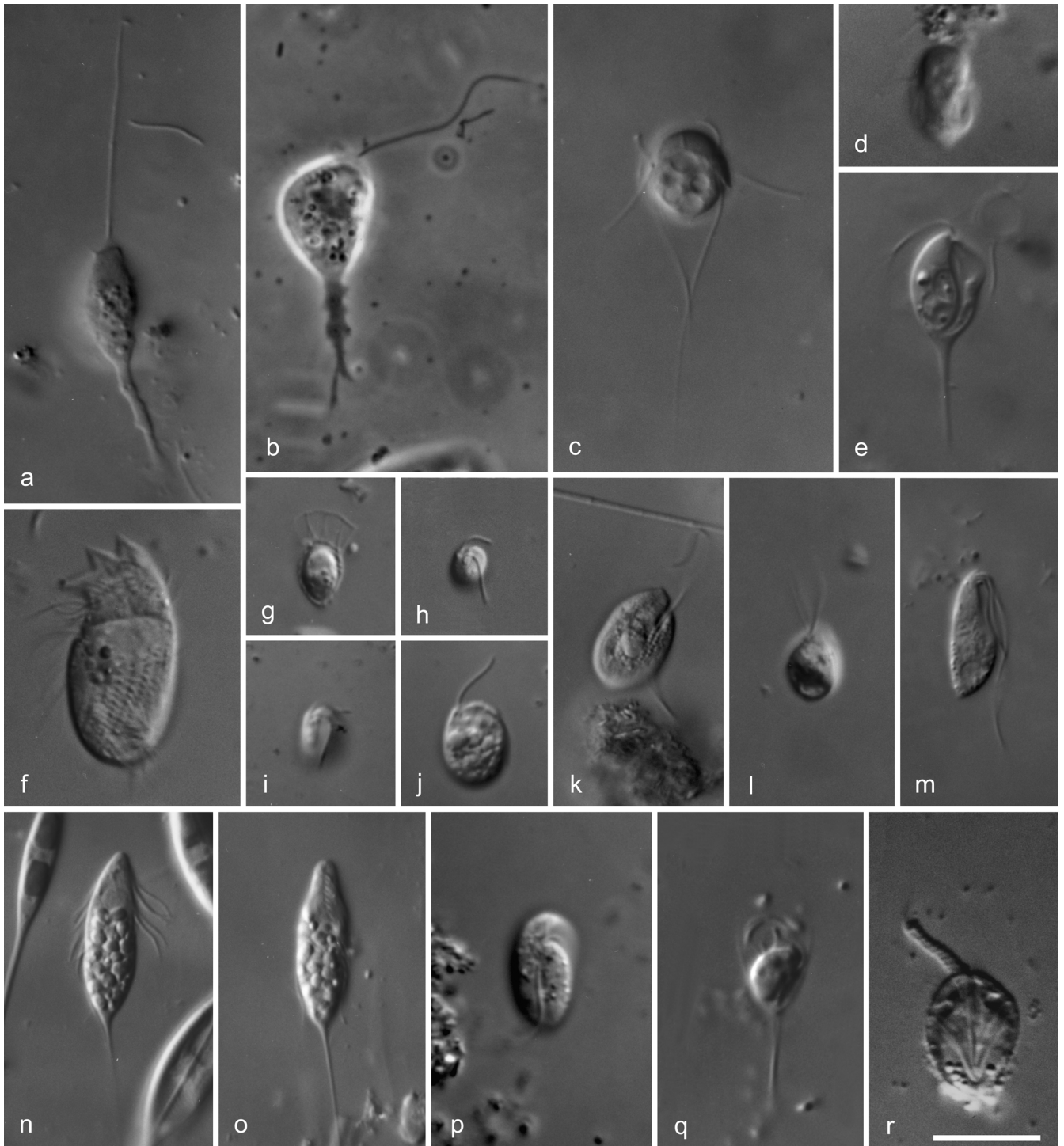
***Saepicula pulchra* Leadbeater, 1980**

Figs 1f, 2g

**Description:** Cell body is 4–6 µm long. The flagellum is about 1.5 times the cell body and its tip is thin. The lorica consists of several transverse costae and 10 longitudinal costae. The anterior of the lorica is everted and the posterior of the lorica is conical. The anterior end of the lorica is surmounted by a single transverse costal band, which is connected to the longitudinal costae, and the lower part composes of several transverse and longitudinal costae. Cells attach with the end of the posterior to the substrate.

**Remarks:** This species has been described from subtropical Australia, Denmark, England, Gulf of Finland and France (Leadbeater 1980; Thomsen 1992; Vørs 1992a; Tong 1997a, c; Lee 2015) and previously reported length is 2.5–5.5 µm (Tong 1997a, c). According to previous authors, two chambers can be seen when using an electron microscope. This genus contains two species, *Saepicula pulchra* Leadbeater, 1980 and *S. leadbeateri* Takahashi, 1981 (Thomsen et al. 1997).





**Fig. 2.** (a)–(b) *Mastigamoeba psammobia*, (a) general appearance of cell, note cytoplasm drawn from the posterior end, (b) general appearance of different cell and note pseudopodia. (c) *Hexamita inflata*, general appearance, note the nucleus. (d) *Trepomonas agilis*, general appearance of cell. (e) *Chilomastix cuspidata*, showing general appearance. (f) *Stephanopogon* sp., showing general appearance. (g) *Saepicula pulchra*, showing general appearance of cell. (h)–(j) *Goniomonas amphinema*. (h) Form I, (i) Form II, (j) Form III, note flagellar arrangement. (k) *Roombia truncata*, note attached cell to the substrate by the tip of the posterior flagellum, note extrusomes, surface striations and deep gullet. (l) *Telonema subtilis*, showing general appearance. (m) *Harpagon descissus*, general appearance of cell. (n)–(o) *Spironema multiciliatum*, (n) general appearance of cell, (o) note the kinetiks. (p) *Psammosa unguis*, general appearance of cell. (q) *Bicosoeca conica*, general appearance of cell. (r) *Apusomonas proboscidea*, general appearance of cell, note the V-shaped structure on dorsal face. All micrographs are DIC images except for (b), which is phase contrast images. Scale bar in (r) = 10  $\mu$ m for all figures.

**Cyathomonadacea** Pringsheim, 1944***Goniomonas amphinema*** Larsen et Patterson, 1990

Figs 2h–j

**Description:** *Goniomonas*, cells are 3–9 µm long. Two flagella insert in an anterior lateral pocket; one directed anteriorly, one posteriorly. The cells are flattened with anterior row of ejectisomes and with or without several longitudinal ridges. Three forms of the cells are described.

*Form I* (Fig. 2h): the cells are almost triangular in shape and 3–4.5 µm long. Pellicle stripes may be not seen. The two flagella are unequal in length. The long flagellum usually trails over the body and is slightly longer than the cell. The cells swim fast with the short flagellum beating.

*Form II* (Fig. 2i): the cells are oblong and 4.5–7.5 µm long with several delicate stripes on both sides. The row of ejectisomes is at times difficult to observe.

*Form III* (Fig. 2j): the cells are wide (anteriorly truncate and posteriorly rounded) and 7–9 µm long. The flagella are similar in length or the trailing flagellum is slightly longer than the flagellum directed anteriorly.

**Remarks:** *Goniomonas amphinema* has previously been found in marine sites in Australia, North Atlantic, Denmark, England, Fiji, Gulf of Finland, Panama, Turkey and White Sea, and reported to be 4 to 8 µm (Larsen and Patterson 1990; Vørs 1992a, b; Patterson et al. 1993; Tong et al. 1998; Lee and Patterson 2000; Tikhonenkov et al. 2006; Aydin and Lee 2012). *Goniomonas amphinema* has been distinguished from *G. pacifica* Larsen et Patterson, 1990 by having two flagella of unequal length and by the flagellar orientation according to previous observers. This species can be also distinguished from *Goniomonas avonlea* Kim et Archibald, 2013 by the cell shape and cell length.

In this study, three forms were observed. These three forms have similar characters such as flagellar length and orientation, but have minor differences in cell shape and cell length. Because of the overlap in dimensions and appearance, the forms may not be unambiguously separated. More work is needed to establish if these represent three separate species or ecomorphs of the same species. The form III is similar to *Goniomonas pacifica*, but is distinguished because when *G. pacifica* is swimming two flagella diverge in different directions. Food ingestion takes place near the flagellar insertion.

**Kathablepharidae** Skuja, 1939***Roombia truncata*** Okamoto et al., 2009

Figs 1g, 2k

**Description:** Cells are oblong, 11–14 µm long, 6–7 µm wide, 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 are 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 has been reported at marine sites in Canada (Nova Scotia), Korea, tropical Australia and Gippsland Basin (Australia) with a length of 11–17 µm (Lee 2002b, 2006b, 2015; Okamoto et al. 2009).

**Telonemia** Shalchian-Tabrizi, 2006***Telonema subtilis*** Griessmann, 1913

Figs 1h, 2l

**Description:** 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 are acronematic and are 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. Only found once at Woolooware Bay.

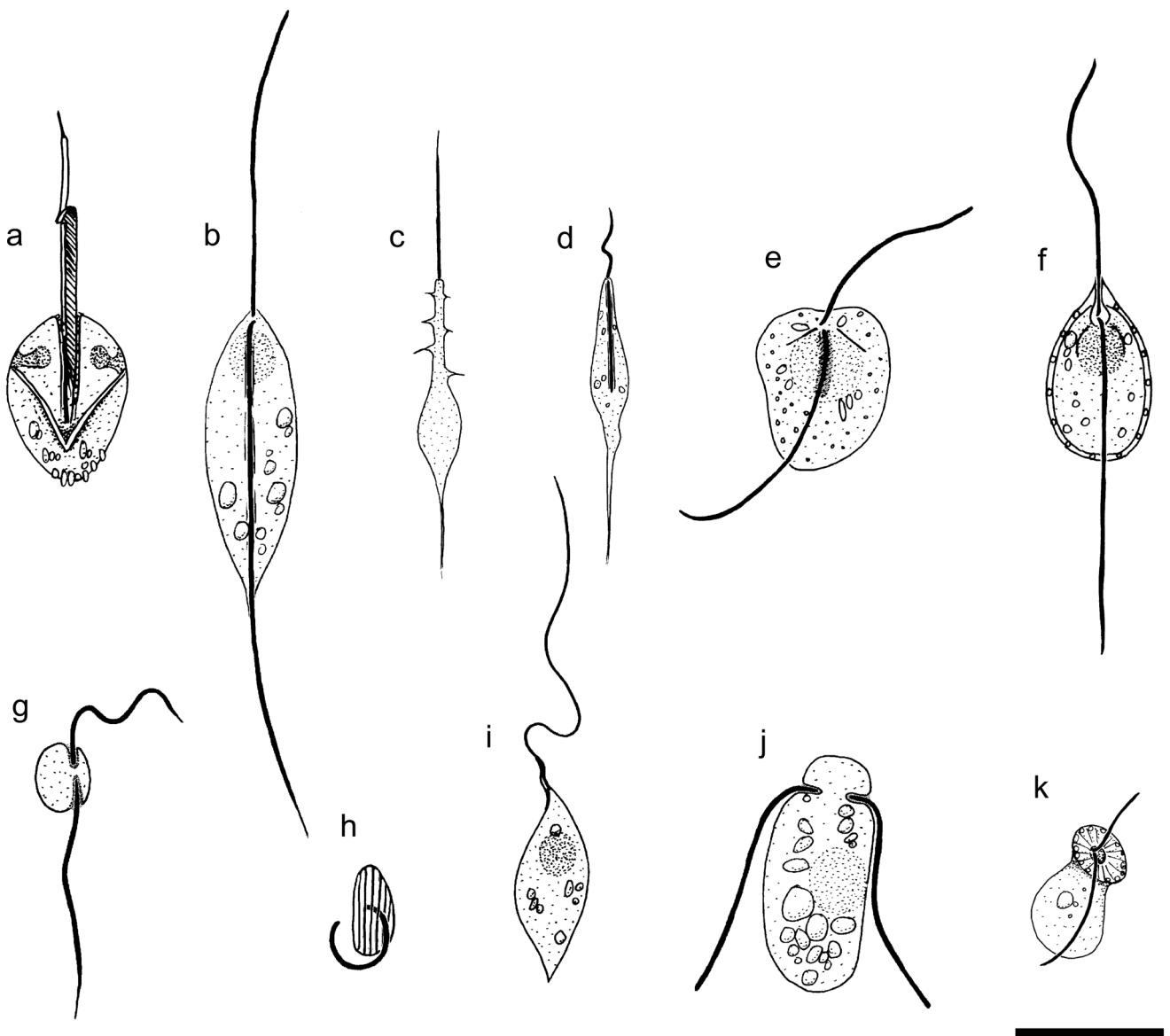
**Remarks:** The observation is in accord with Griessmann (1913). This species has been found at marine sites in France, Mediterranean, Arctic Norway, Antarctica, Australia, Arctic Canada & Greenland, Japan, Belize, Norway and Gulf of Finland (e.g. Vørs 1992a, b, 1993a, b; Patterson et al. 1993; Tong et al. 1997, 1998; Vørs et al. 1995; Yabuki et al. 2013; Lee 2015). It has not been reported from freshwater sites. Previously cell lengths were reported to be from 4 to 18 µm. This species is distinguished from *Telonema antarctica* Thomsen, 1992 by the cell shape. *Telonema antarctica* is reniforme.

**Heterolobosea** Page and Blanton, 1985***Psalteriomonadidae*** Cavalier-Smith, 1993***Harpagon descissus*** (Perty, 1852) Pánek et al., 2012

Figs 1i, 2m

**Description:** Cells are ovoid to pyriform and 9 to 15 µm long with a big ventral groove, which extends





**Fig. 3.** Apusomonadida, Cercomonadida, Protaspidae, Thaumatomonadidae and Protista *incertae sedis*. (a) *Apusomonas proboscidea*, (b) *Cercomonas parva*, (c) *Cercomonas* sp.1, (d) *Cercomonas* sp.2, (e) *Protaspa flexibilis* sp. nov., (f) *Thaumatomastix* sp., (g) *Eoramona jungensis* sp. nov., (h) *Gweamonas unicus*, (i) *Phyllomitus undulans* (from Lee 2002a), (j) Protist 1, (k) Protist 2. Scale bar = 10  $\mu\text{m}$  for all figures.

from the anterior end to half or three quarters the way down the cell. There are four flagella, which insert subapically and are directed ventrally and posteriorly. Three flagella are similar in length and one long flagellum is 1.5–2 times the length of the cell. All flagella usually beat together and close to the groove. The longest flagellum appears to be acronematic. The nucleus is situated behind the apex of the cell. The cells swim by rotating movement and may attach to the substrate by the longest flagellum. The cells have typically many

food vacuoles. Cytoplasmic strands are seen in the posterior end of the cell. Observed frequently under anaerobic conditions.

**Remarks:** This species has been reported under the name of *Percolomonas descissus*. It has been found at marine sites in Australia (Bernard et al. 2000) and the range of cell lengths reported to date is 7–30  $\mu\text{m}$ . This genus resembles species of *Percolomonas*. Bernard et al. (2000) regarded *Percolomonas elephas* (Klug, 1936) Larsen et Patterson, 1990 as the same species

of *H. descissus* (= *P. descissus*). *Harpagon descissus* is distinguished from *Percolomonas sulcatus* (Stein, 1878) Larsen et Patterson, 1990 by the much narrower anterior end, and from *P. denhami* Tong, 1997 by having four flagella and granular appearance. It differs from *P. cosmopolitus* (Ruinen, 1938) Fenchel et Patterson, 1986 because it does not glide or skid using the posterior flagellum and because it has a granular appearance.

#### **Kinetoplastea** Honigberg, 1963

***Rhynchobodo longiciliatus*** (Skuja, 1948) Bernard et al., 2000

Figs 1j, 4a–b

**Description:** Cells are long and thin with a pointed posterior end, 25–50  $\mu\text{m}$  long, flexible, not flattened, and with no pellicular striations. The anterior part of the cell appears to be more flattened than other parts of the cell. The cells have a spiral groove. Two flagella emerge subapically from a shallow flagellar pocket and are thickened. The anterior flagellum appears to attach to the anterior part (?rostrum) of the cell, is longer than the cell and beats freely. The posterior flagellum is about the cell length or may be shorter. When the cells swim, they rotate and the posterior flagellum appears to wrap around the body. When squashed, the cells may squirm. The nucleus is situated in the posterior part of the cell. Food materials are seen throughout the cell. Commonly observed at Woolooware Bay.

**Remarks:** This species is assigned to *Rhynchobodo longiciliatus* (Skuja, 1948) Bernard et al., 2000, although the posterior flagellum of the cell observed here is shorter than the original description of the species. Skuja (1948) described *R. longiciliatus*, which is 20–45  $\mu\text{m}$  and its posterior flagellum is about 2.5 times the cell length, but did not mention about the flagellar pocket, which is difficult to observe. Larsen and Patterson (1990) recorded this species about 22  $\mu\text{m}$  long from a marine site in tropical Australia and tentatively put into *Heteronema acutissimum* Lemmermann, 1910. However it is different to *Heteronema* because it has no pellicle strips and there is no deep flagellar pocket – as in euglenids. This species is generally in accord with the descriptions of *Bodo faccatus* Skuja, 1956 (25–35  $\mu\text{m}$  long) and *Cercomonas draco* (Skuja, 1956) Mignot et Brugerolle, 1976 (35–57  $\mu\text{m}$  long) which have a long body shape and are similar in length. These species are also flexible. Here *Bodo faccatus* is regarded as a junior synonym of *R. longiciliatus*, but *Cercomonas draco* may be different because it produces pseudopodia. *Rhynchobodo longiciliatus* is distinguished from *Heteronema*

*fusiformis* Skvortzov, 1957 which appears to have no pellicle striations and is about 37  $\mu\text{m}$  long, by having a spiral groove and because the body is not fusiform.

#### **Spironemidae** Doflein, 1916

***Spironema multiciliatum*** Klebs, 1893

Figs 1k, 2n–o

**Description:** Cells are lanceolate, relatively flattened and flexible. The cells have a spiral groove, long kinetics and a tail, which tapers posteriorly, and are 15–21  $\mu\text{m}$  without the tail. 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. Frequently observed at Woolooware Bay. Description based on records of 5 cells.

**Remarks:** This species has been reported at marine sites in Gippsland Basin, Australia and Canada (Lee 2015, Lax et al. 2018). It resembles *Stereonema geiseri* Foissner et Foissner, 1993 in general appearance of cell. *Stereonema geiseri* was regarded as a junior synonym of *Spironema multiciliatum* (see Lee 2015 for more details).

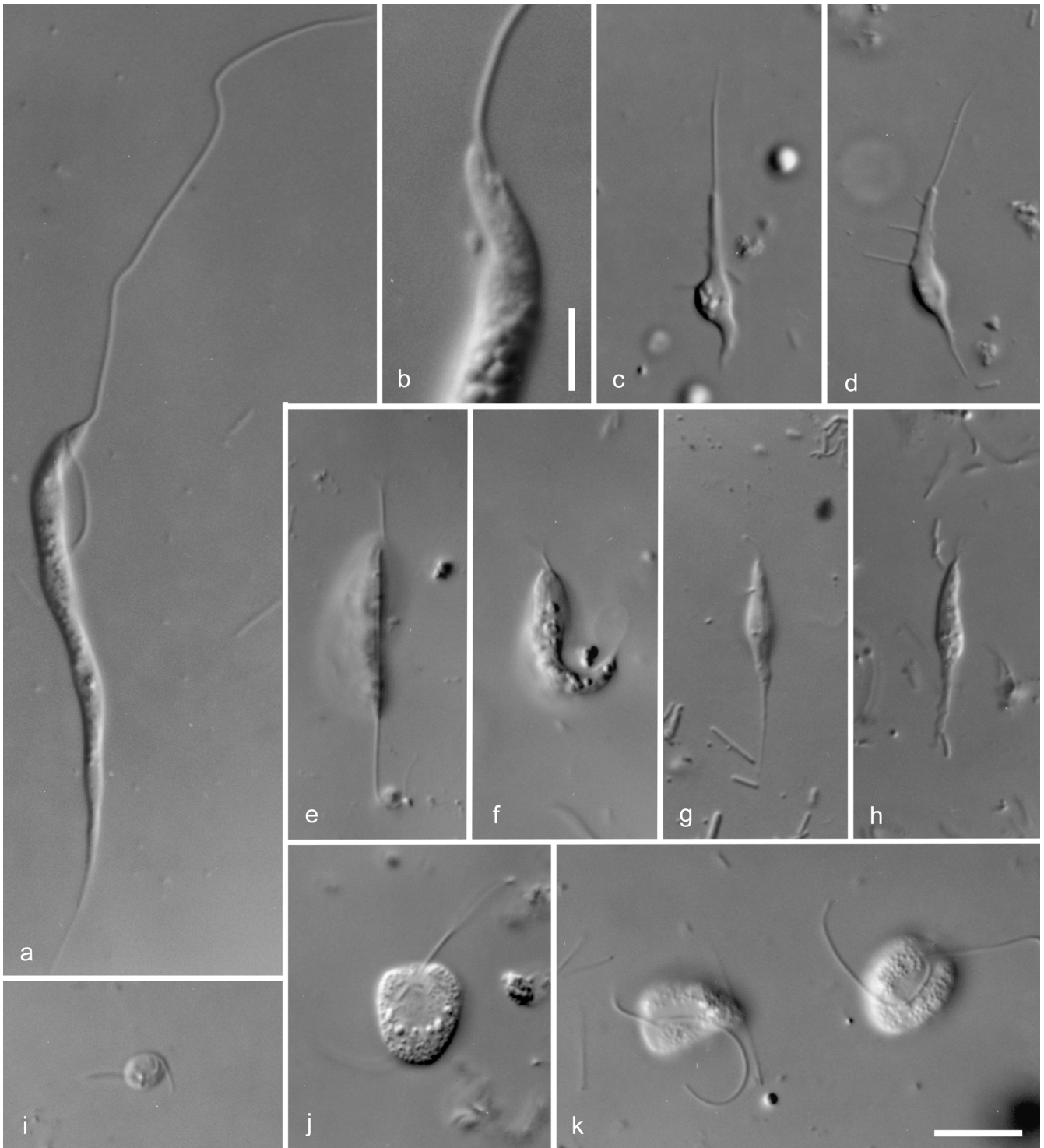
#### **Stephanopogonidae** Corliss, 1961

***Stephanopogon* sp.**

Figs 1l, 2f

**Description:** Cells are 16–30  $\mu\text{m}$  long and dorsoventrally flattened with several rows of kinetics. The ventral surface terminates anteriorly as three barbs. The cells move by crawling or swimming with flagella. Rarely observed.

**Remarks:** This genus contains 7 nominal species: *S. apogon* Borror, 1965, *S. colpoda* Entz, 1884, *S. mesnili* Lwoff, 1923, *S. minuta* Lei et al., 1999, *S. mobilensis* Jones et Owen, 1974, *S. paramesnili* Lei et al., 1999 and *S. pattersoni* Lee et al., 2014. This species is distinguished from *Stephanopogon apogon* by the barbs at the anterior end of the cell and is substantially smaller than *Stephanopogon colpoda*, *Stephanopogon mesnili* and *Stephanopogon paramesnili*. It can not be distinguished morphologically from other three species (*S. minuta*, *S. mobilensis*, *S. pattersoni*) under the light microscopy because all of them have barbs at the anterior end of the cell and they are similar in cell length. Recently, this genus was well documented by Yubuki and Leander (2008) and Lee et al. (2014).



**Fig. 4.** (a)–(b) *Rhynchobodo longiciliatus*, (a) general appearance of cell, (b) flagellar insertion into pocket. (c)–(d) *Cercomonas* sp.1, general appearance, note slender anterior part and hyaline cell body. (e)–(f) *Cercomonas parva*, (e) general appearance showing a posterior flagellum attached to the body, (f) flexible body. (g)–(h) *Cercomonas* sp.2, general appearance, note both short flagella and cytoplasm drawn from the posterior part. (i) *Cyranomonas australis*, showing general appearance of different cells, note flagellar insertion. (j)–(k) *Protaspa flexibilis* sp. nov., general appearance of different cells, (j) nuclear caps around nucleus. All micrographs are DIC images. Scale bar in (k) = 10  $\mu$ m for all figures with the exception of (b). Scale bar in (b) = 5  $\mu$ m.



**Perkinsidae Levine 1978 *Incertae sedis***

***Psammosa unguis*** (Patterson et Simpson, 1996)  
Lee, 2015

Figs 1n, 2p

**Description:** Cells are 8–13  $\mu\text{m}$  long, bean-shaped in profile 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. It is directed laterally and posteriorly. The posterior flagellum inserts at the top of a shallow longitudinal groove, is nearly two times the length of the cell, and is directed posteriorly. Some cells have large vacuoles containing eukaryotic prey material. 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:** This species has been reported from hypersaline sites and marine sediments in Shark Bay with cell length from 7 to 10  $\mu\text{m}$  (Patterson and Simpson 1996, Al-Qassab et al. 2002, Lee 2002b, Aydin and Lee 2012) under the name of *Colpodella unguis*. *Copodella unguis* was transferred to *Psammosa* by Lee (2015) because their cell shape and flagellar orientation are very similar. *Psammosa unguis* and *P. pacifica* share similar characters such as cell shape (elongated reniform), flagellar orientation and cell length (*P. unguis* 7–10  $\mu\text{m}$ , *Psammosa pacifica* 7–8  $\mu\text{m}$ ). Only the difference is the proportion of anterior left part (*P. unguis* about 2/3 CL, *P. pacifica* about 1/3 CL) (Okamoto et al. 2012). Later Cavalier-Smith (2018) created a genus *Colpovora* for *Colpodella unguis*. However, I regard *Colpovora unguis* as a junior synonym of *Psammosa unguis*. The distinctive division cysts of *P. unguis* have not been observed to date.

**Bicosoecida Grassé, 1926**

***Bicosoeca conica*** Lemmermann, 1914

Figs 1m, 2q

**Description:** Cell is about 6  $\mu\text{m}$  long located in a conical lorica, which is about 7  $\mu\text{m}$  long. Two flagella of unequal length arise near the anterior end of the cell. The flagellum is about 3 times longer than the cell and beats with a sine-wave, and the posterior flagellum is slightly longer than the cell and attaches to the lorica. The lorica attaches to the substrate using a stalk, which is slightly longer than the lorica. One cell was observed at Watsons Bay.

**Remarks:** Generally, the observation is agreement with those of Larsen and Patterson (1990). *Bicosoeca*

*conica* has been found at marine sites in Atlantic, Brazil and Turkey and previously reported lorica length is 2.5–5  $\mu\text{m}$  (Larsen and Patterson 1990, Patterson et al. 1993, Aydin and Lee 2012). This species is similar to *B. epiphytica* Hillard, 1971, but can be distinguished by the dimension of the lorica (20–27.5  $\mu\text{m}$  long).

**Apusomonadida Karpov et Mylnikov, 1989**

***Apusomonas proboscidea*** Aléxéieff, 1924

Figs 2r, 3a

**Description:** Cells are 4–12  $\mu\text{m}$  long and dorso-ventrally flattened with a V-shaped structure on the ventral side of the cell. Cell outline is roundish to slightly ovate. The mastigophore emerges from a cavity about two thirds of the cell length from the anterior end, is highly flexible and may be withdrawn into the sheath when the cell is stressed – the mastigophore may become wrinkled. The anterior 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 than the cell. On the dorsal side, there is no structure. The cells glide closely to the substrate. Rarely observed. Description based on records of 4 cells.

**Remarks:** *Apusomonas proboscidea* has been usually found from freshwater sites and soils (e.g. Vickerman et al. 1974; Karpov and Zhukov 1980, 1986) with the ranges of cell length from 9 to 14  $\mu\text{m}$ . This species has a junior synonym, *Rostromonas applanta* Karpov et Zhukov, 1980. This is the first observation for the species in marine habitats. *Apusomonas proboscidea* is similar to *Apusomonas australiensis* Ekelund et Patterson, 1997 reported from a cultivated garden soil with cell length from 6 to 10  $\mu\text{m}$  (Ekelund and Patterson 1997), but is distinguished by having a V-shaped structure on the ventral side of the cell and by the lack of the conspicuous pointed bulge that is a diagnostic character of *A. australiensis* (Ekelund and Patterson 1997).

**Cercomonadida** Poche, 1913, emend. Vickerman, 1983

***Cercomonas parva*** (Hartmann et Chagas, 1910)  
Mignot et Brugerolle, 1975

Figs 3b, 4e–f

**Description:** Cells are about 20  $\mu\text{m}$  and flexible. A structure is seen on the left ventral side of the cell. Two flagella emerge subapically. The anterior flagellum is about the cell length and beats freely. The posterior flagellum is 1.3–1.8 times the cell length and appears to adhere tightly to the cell body in a ventral groove. Neither flagellum is acronematic. The cyto-

plasm contained many small particles. The nucleus is located in the anterior part of the cell. Pseudopodia and strands of cytoplasm were not seen under the cell or behind the posterior end of the moving cell. When the cell is moving, the anterior part of the cell slightly wobbles from side to side. Not often observed.

**Remarks:** Previously reported cell length is 6.5–20 µm (Hartmann and Chagas 1910, Lemmermann 1914). The description here is well consistent with the description of Hartmann and Chagas (1910) who described it under the name *Cercobodo parva*, which was transferred once to *Cercomastix* by Lemmermann (1914). This species is similar to *Cercomonas granulifera* sensu Hollande, 1952 which has granules around the nucleus and is 12–15 µm long, but can be distinguished by the posterior flagellum tightly adhered to the cell body and lack of granules around the nucleus.

***Cercomonas* sp.1**

Figs 3c, 4c–d

**Description:** *Cercomonas*. Cells are 7–15 µm long and flexible. The cells are slender; narrow towards the posterior end which is somewhat elliptical. The cells have one flagellum; the second flagellum is not seen. The flagellum arises from the anterior end of the cell and is about the cell length, and its tip is thin at two third from its base. The cells have a ventral groove. When moving, pseudopodia move backwards and the strands of cytoplasm are drawn out from the posterior end of the cell. Some food materials are seen under the cell surface. The cells move by gliding.

**Remarks:** *Cercomonas* sp.1 is tentatively assigned to *Cercomonas* although it has only one flagellum. This species can be distinguished from the species in *Cercomonas* that have two flagella by having only one flagellum and the slender cell shape.

***Cercomonas* sp.2**

Figs 3d, 4g–h

**Description:** *Cercomonas*. Cells are 8–12 µm and flexible with two flagella. The anterior flagellum is 0.25–0.5 times the cell length and beat from side to side, and its tip is thin. The second flagellum is about 0.5 times the cell length and appears to adhere to the cell body. Long strands of cytoplasm are seen from the posterior end of the cell.

**Remarks:** This organism is similar to *Cercomonas* sp.1 in cell appearance and length, but can be distinguished by the shorter anterior flagellum and the presence of the posterior flagellum.

**Protaspidae** Cavalier-Smith, 1993

***Protaspa* *flexibilis* sp. nov.**

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Figs 3e, 4j–k

**Etymology:** The name refers to ‘flexible’.

**Diagnosis:** *Protaspa*, cell 10–14 µm long, somewhat flexible and dorso-ventrally flattened with an indistinctive ventral groove. Anterior flagellum directed to left and posterior flagellum to right trails posteriorly. Nucleus situated subapically with nuclear caps.

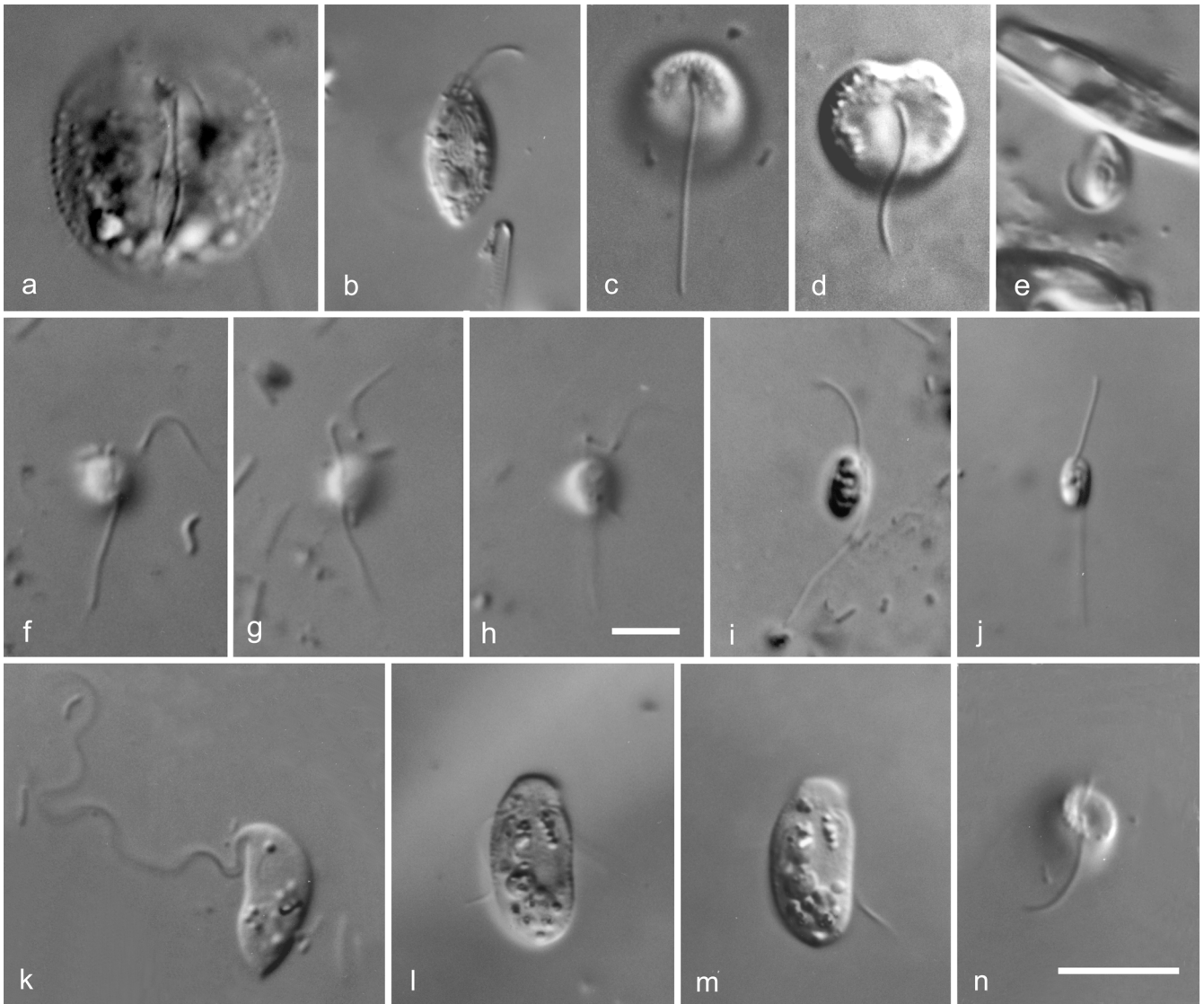
**Description:** Cells are 10–14 µm long, somewhat flexible and dorso-ventrally flattened with an indistinctive ventral groove. The cells vary in shape and are more flexible at the posterior part of the cell. Cell surface is warty. Two non-acronematic flagella of similar length emerge subapically and are not thickened. The anterior flagellum is directed to the left direction and the posterior flagellum to the right trails posteriorly. The nucleus is situated subapically with nuclear caps. Reserve materials are seen around the nucleus. Pseudopodia were not seen. The cells move by gliding. The cells were observed twice abundantly, but not common. Description based on records of 35 cells.

**Remarks:** This organism is related to *Protaspa* because of anteriorly located nucleus with caps. It is distinguished from other species of *Protaspa* by having a flexible body and because two flagella of this species point in different directions – like in *Neometanema* species (see Lee and Simpson 2014). This species can be characterised by its flexibility and the orientation of the flagella, which make it different from the other *Protaspa* species listed in Hoppenrath and Leander (2006). It is similar to *P. gemmifera* in having reserve materials and in cell length, but is distinguished by its flexible body and because *P. gemmifera* moves quickly.

***Protaspa verrucosa*** (Larsen et Patterson, 1990)  
Cavalier-Smith, 2011

Fig. 5a

**Description:** Cell outline is oval to roundish. Cells are 15–22 µm long and dorso-ventrally flattened. Cell surface is warty. The cells have a prominent ventral groove or slit, which extends from the subapical end almost to the posterior end of the cell. Two flagella emerge from the widely opened anterior depression. The anterior flagellum is about the cell length and beats from side to side. The posterior flagellum is about 1.3 times the cell length and trails. The nucleus is situated anteriorly be-



**Fig. 5.** (a) *Protaspa verrucosa*, note ventral groove. (b) *Thaumatomastix* sp., general appearance of cell and showing a layer of body scales. (c)–(d) *Clautriavia cavus*, general appearance of different cells, (c) cell from Lee and Patterson (2000). (e) *Gweamonas unicus*, note coiled flagellum and flagellar insertion. (f)–(h) *Eoramonas jungensis* sp. nov., showing general appearance of different cells, note flagellar insertion and beating pattern of anterior flagellum. (i)–(j) *Glissandra similis*, showing general appearance of different cells, note the ventral groove. (k) *Phyllomitius undulans*, note two flagella adhering each other, from Lee (2002a). (l)–(m) Protist 1, showing general appearance, note flagellar insertion. (n) Protist 2, showing general appearance, note collar and two emergent flagella. All micrographs are DIC images. Scale bar in (h) = 5  $\mu\text{m}$  for (c)–(h) and in (n) = 10  $\mu\text{m}$  for other figures.

low the flagellar insertion with anterior caps, which are not distinctive. Several materials are seen in the posterior part of the cell. The cells glide slowly. Rarely observed.

**Remarks:** Generally the description here is accordance with the original description of Larsen and Patterson (1990). This species has been found at marine sites in Australia, Arctic Canada, Denmark, England, Gulf of Finland, Fiji and White Sea (Larsen and Patterson 1990;

Tong et al. 1998; Vørs 1992a, b, 1993a; Tikhonenkov et al. 2006) and reported cell length is from 9 to 18  $\mu\text{m}$ . It was quite common at Avoca Beach and co-occurred with *Protaspa gemmifera* Larsen et Patterson, 1990. This species is distinguished from other species in *Protaspa* by the strongly developed ventral groove and wide flagellar pocket. *Protaspa verrucosa* is very similar to *Thaumatomastix setifera* Lauterborn, 1896, *T. tripus* (Takahashi et



Hara, 1984) Beech et Moestrup, 1986 and *T. splendida* Thomsen et al., 1995, but is distinguished by the lack of the visible body scales and spines.

#### **Thaumatomonadidae** Hollande, 1952

##### ***Thaumatomastix*** sp.

Figs 3f, 5b

**Description:** Cells are spindle-shaped, anteriorly and posteriorly pointed. The cells are 8–15 µm long, flattened and rigid, and appear to be covered by a layer of body scales. The spines on the scales are present, but are only visible when the cells become stressed. Two thickened flagella emerge from the widely opened flagellar canal or the anterior end of the cell. The anterior flagellum is about the cell length and beats slowly from side to side. The posterior flagellum is about 2 times longer than the cell, is non-acronematic and trails. The nucleus is located subapically with caps, which are easy to overlook. Some extrusomes are symmetrically distributed around the margin of the cell. The cells glide slowly and may have a slow jerking movement. When the cell is gliding, the anterior part of the cell is adpressed to the substrate and the posterior end is directed away from it. Food materials are seen throughout the cell. Relatively common.

**Remarks:** This species is assigned to *Thaumatomastix* because it is a gliding flagellate, with two flagella inserting into a subapical depression and has scales. It could not be identified because ultrastructural features of the silicified scales were not recorded. There are about 13 nominal species in *Thaumatomastix*; *T. bipartita* Beech et Moestrup, 1986, *T. dybsoeana* Thomsen et al., 1993, *T. formosa* Thomsen et al., 1993, *T. fragilis* Thomsen et al., 1995, *T. fugiformis* Thomsen et al., 1995; *T. patelliformis* (Takahashi et Hara, 1984) Beech et Moestrup, 1986, *T. salina* (Birch-Andersen, 1973) Beech et Moestrup, 1986, *T. sagittifera* (Conrad, 1920) Beech et Moestrup, 1986, *T. setifera* Lauterborn, 1899, *T. spinosa* Thomsen et al., 1993, *T. splendida* Thomsen et al., 1995, *T. triangulata* (Balonov, 1980) Beech et Moestrup, 1986, *T. tripus* (Takahashi et Hara, 1984) Beech et Moestrup, 1986. Of the 13 species, plate scales, spine scales and flagellar scales of 9 species were summarised by Thomsen and co-workers (Thomsen et al. 1993). *Thaumatomastix* Lauterborn, 1899, *Thaumatomonas* de Saedeleer, 1931 and *Protaspa* Cavalier-Smith, 2011 (= *Protaspis* Skuja, 1939) have similar characters; a similar shape, a ventral groove, anteriorly located nucleus, they glide, produce pseudopodia and have two flagella. *Thaumatomastix* and *Thaumatomonas* have been known to have scales

visible by electron microscopy or light microscopy, but *Protaspa* has not been known whether it has scales or not. Further studies are needed to establish the identity of these genera.

##### **Variglossida** Cavalier-Smith, 2014

##### ***Clautriavia cavus*** Lee et Patterson, 2000

Figs 5c–d

**Description:** Cell outline is oval to oblong. Cells are 7.5 to 12 µm long, flattened and rigid. One flagellum directed posteriorly emerges from a ventral subapical depression, is 1.5 to 2 times the length of the cell and makes close contact with the substrate when the cell is gliding. The cells have a shallow, wide ventral groove, which is easy to overlook. The ventral face of the cell appears to be slightly concave. The cell surface may be rather warty and food particles are seen in the posterior part of the cell. The cells glide slowly and smoothly with the posterior part slightly raised above the substrate. Often observed, but not in large numbers. Description based on the observations of 8 cells.

**Remarks:** This species was reported from marine sites in subtropical Australia, England, Korea and White Sea (Tong 1994; Lee and Patterson 2000; Al-Qassab et al. 2002; Lee 2002b, 2006b; Tikhonenkov et al. 2006) and reported cell lengths are 5–12 µm. *Clautriavia* is reminiscent of *Protaspa* and *Allantion*, but can be recognised from *Protaspa* by having only one flagellum. *Clautriavia cavus* is less easy to distinguish from *Allantion* because *Clautriavia* lacks a rostral prominence at the anterior end of the cell and has a concave ventral face. The genus *Clautriavia* to date contains four nominal species; *C. cavus* Lee et Patterson, 2000, *C. mobilis* Massart, 1900, *C. parva* Schouteden, 1907, *C. biflagellata* Chantangsi et Leander, 2010 (Chantangsi and Leander 2010). *Clautriavia cavus* differs from *C. mobilis* because *C. mobilis* is twice as large, has a relatively short flagellum and has a nucleus at the right posterior of the cell. *Clautriavia parva* may be the gliding stage of *Metromonas* (Lee and Patterson 2000). This species is not easily distinguished from *C. biflagellata* having two flagella because both species have a similar cell shape and the cell length ranges are overlapped. In addition, the shorter flagellum in *C. biflagellata* is very difficult to observe under the light microscopy because it is very short. *Clautriavia biflagellata* may be a junior synonym of *C. cavus*. Further studies are needed to distinguish these two species.

**Marimonadida** Cavalier-Smith et Bass, 2011

***Cyranomonas australis*** Lee, 2002

Fig. 4i

**Description:** Cells are ovoid, 4–7 µm long, dorso-ventrally flattened, and somewhat flexible. The anterior part is depressed or concave. Two thickened flagella emerge from the right side of the cell and are not acronematic. The anterior flagellum is about the length of the cell and flickers stiffly forwards. The posterior flagellum inserts to the left of the anterior flagellum, is 1.5–2 times the length of the cell, trails and appears to lie in the anterior groove at its base. The nucleus is located anteriorly. The cells contained small food materials. The cells glide slowly with the anterior flagellum. Observed commonly.

**Remarks:** This species has been found at marine sites in Canada, UK, Korea and Turkey (Tong 1994, Lee 2002b, Aydin and Lee 2012, Lee and Park 2016). It was well documented by Lee and Park (2016).

**Protista incertae sedis**

***Eoramonas*** gen. nov.

**Diagnosis:** Biflagellated colourless protists of unknown affinities. Body rigid with ventral depression subapically and two flagella inserting into ventral depression. Anterior flagellum beats to left direction. Cell glides.

**Type species:** *Eoramonas jungensis* sp. nov.

**Etymology:** The name ‘Eora’ is given by the earliest settlers to a group of indigenous people belonging to the clans along the coastal area of what is now known as the Sydney basin, New South Wales, Australia.

***Eoramonas jungensis*** sp. nov.

urn:lsid:zoobank.org:act:8D6AE46A-7679-4657-B09D-E12EC9AD110B

Figs 3g, 5f–h

**Diagnosis:** *Eoramonas*, cell 3–7 µm long and rigid. Two acronematic flagella insert separately by a small papilla or protrusion into small subapical depressions. Anterior flagellum beats to left direction. Moves by gliding.

**Description:** Cells are spherical to ovoid, 3–7 µm long, rigid and not flattened. The cells have two flagella, which are acronematic in similar length, 2–4 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:** *Eoramonas jungensis* was reported from Gippsland Basin by Lee (2015) as ‘Protist 1’. This species can be distinguished from other small gliding flagellates by the beating of the anterior flagellum to the left. Its anterior flagellum does not draw water towards the cell body, thus it does not belong to stramenopiles.

***Glissandra similis*** Lee, 2006

Figs 5i–j

**Description:** Cells are rigid, 5–6 µm long, elliptical and slightly flattened laterally. The cells have a small depression at the anterior part of the cell and a longitudinal ventral groove extending from the depression to the posterior end of the cell. Two flagella are thickened and slightly unequal in length, and insert subapically into the depression. The tip of the anterior flagellum moves back and forth, and the trailing posterior flagellum may lie in the ventral groove. During gliding, the cells usually appear to move obliquely. Sometimes common.

**Remarks:** This species was firstly reported from marine sediments in tropical Australia (Lee 2006b). It can be distinguished from *G. innuerende* because it is elliptical and somewhat laterally flattened while *G. innuerende* is ovoid and dorso-ventrally flattened. In addition, *Glissandra similis* has a longitudinal ventral groove extending from the anterior depression to the posterior end of the cell while *G. innuerende* has a small depression subapically and has a structure on the ventral side of the cell.

***Gweamonas unicus*** Lee, 2002

Figs 3h, 5e

**Description:** The cells are 4–7 µm long, rigid and not flattened with a ventral groove and with one flagellum emerging posterior-laterally from the mid-right side of the cell. The anterior part of the cell is somewhat concave and the left margin appears to be higher than the right side of the anterior part. The cells have prominent grooves on both sides of the cell. Rarely observed.

**Remarks:** This species was also found at a marine site in Korea (Lee 2002b).

***Phyllomitius undulans*** Stein, 1878

Figs 3i, 5k

**Description:** *Phyllomitius*. Cells are 11–12 µm long and have two flagella that adhere to each other. The cells are pointed anteriorly and posteriorly. The flagella arise

at the anterior end of the cell. The flagella appear to be similar in length, about 3 times longer than the cell, and undulate. The pellicle is smooth and the nucleus is located on the mid-anterior part of the cell. Cytoplasmic strands may be seen from the posterior end. The cells may attach to the substrate by the posterior end of the cell. Three cells were observed at Kogarah Bay and Quibray Bay.

**Remarks:** *Phyllomitus* Stein, 1878 has two flagella adhered together emerging from a depression which might be misinterpreted. No records of this type species have been made since its original description. Although the cell described here is somewhat smaller than that (18  $\mu\text{m}$ ) of Stein (1878) and do not have a depression, the cell is regarded as the same species of *P. undulans*. This species was also found at Moreton Bay Prawn Farm (Queensland, Australia) with the length ranges from 13 to 21  $\mu\text{m}$  (See Lee 2002a). The following species have been included to the genus; *P. amylophagus* Klebs, 1893, *P. apiculatus* Skuja, 1948, *P. salinus* Lackey, 1940, *P. granulatus* and *P. vesiculosus* (Larsen and Patterson 1990). However, those species do not have two adhering flagella and have been transferred to *Pseudophyllomitus* by Lee (2002a).

#### *Unidentified flagellates*

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

#### **Protist 1**

Figs 3j, 5l–m

**Description:** Cells are oblong, 14–16  $\mu\text{m}$  long, and rather dorso-ventrally flattened. Two flagella insert subapically into small depression separately and are about the length of the cell. The nucleus is located near the centre of the cell. The cells contained food materials up to 3.5  $\mu\text{m}$  long. The cells may swim backward with the flagella which point away from the direction of movement and the cells rotate. Also, the cells move forwards with the flagella beating up and down. At times, the cells rotate with the beating of the right flagellum and with the holding of the left flagellum. The cells move by swimming with the flagella. Two cells were observed.

**Remarks:** The cells described here may be related to *Telonema*. This organism is distinguished from *Telonema subtilis* Griessmann, 1913 and *T. antarctica* Thomsen in Vørs (1992a) by the cell shape and bigger size. It can be recognised from other small gliding or swimming flagellates by the cell shape and the backward movement.

#### **Protist 2**

Figs 3k, 5n

**Description:** Cell is about 7  $\mu\text{m}$  long with an anterior collar that is wide relative to the body size. Two emergent flagella; one is slightly shorter the cell and the other is about the cell length. The ingestion organelle was not seen. The cell moves by smooth gliding with the beating of the shorter flagellum. One cell was observed at Quibray Bay.

**Remarks:** This organism is similar to species in *Urceolus* in general appearance, but is easily distinguished from all species in *Urceolus* species by its smaller size and two emergent flagella. Maybe it is a dividing *Urceolus*. It is also distinguished from other small gliding species by the sac-shaped body and the anterior collar. This species was recorded on videotape, but it was unable to identify whether this species is rigid or flexible.

## DISCUSSION

This study sought to create the most extensive account from a single site (Port Botany) in Botany Bay and to document the diversity of heterotrophic flagellates from several marine sites around Sydney. In this section, the previous results (Lee and Patterson 2000, Lee 2012) at Botany Bay are included.

Intensive studies on the diversity of heterotrophic flagellates in marine sites are rare, and those that exist were generally conducted in planktonic ecosystems (e.g. Tong 1997a, b; Vørs 1992a) and benthic ecosystem (Lee and Patterson 2000). Lee and Patterson (2000) reported about 79 species from 35 sampling occasions from a marine sediment of Port Botany, one of sites, which were conducted in this study. During the present study, 155 morphospecies (76 genera) of heterotrophic flagellates were described, of which 140 were identified to species, 12 were identified to genus (e.g., *Notosolenus* sp., *Ploetia* sp.1) and 2 were given names as Protist 1 and Protist 2 under 'unidentified flagellates' from a total of 135 sampling occasions (72 sampling occasions at Port Botany; 18 at each site of Kogarah Bay, Quibray Bay and Wollooware Bay; 4 at Avoca Beach; 5 at Watsons Bay). The number of species encountered at each site is 115 at Port Botany, 110 at Kogarah Bay, 124 at Quibray Bay, 93 at Wollooware Bay, 73 at Avoca Beach and 49 at Watsons Bay (Table 1). The diversity at each site is higher than those (mean 28, ranges from 5 to 79) previously reported from marine sediments (e.g. Patterson and Lee 1998,



Lee 2015). Of the species, 134 species were previously reported in marine and freshwater habitats and 18 species have not been previously reported from marine or freshwater habitats.

### Common and rare taxa

During the present study, the most commonly encountered genera were *Amastigomonas*, *Ancyromonas*, *Neobodo*, *Bordnamonas*, *Goniomonas*, *Notosolenus*, *Petalomonas*, *Ploeotia* and *Rhynchomonas*. These taxa were encountered frequently in early culture samples (1–2 days old), and *Cafeteria* and *Carpediemonas* were commonly encountered in late culture samples (after 3–5 days). Many taxa were represented by fewer than ten cells during the study. These included *Ancyromonas sinistra*, *Chasmostoma nieuportense*, *Dinema validum*, *Gweamonas unicus*, *Heterochromonas opaca*, *Kurnaimonas celeris*, *Mastigamoeba simplex*, *M. psammobia*, *Notosolenus adamas*, *Notosolenus brothernis*, *Notosolenus tamanduensis*, *Petalomonas iugosus*, *Petalomonas labrum*, *Petalomonas spinifera*, *Protaspa verrucosa*, *Trepomonas agilis*, choanoflagellate species, Protist 1 and Protist 2. A large diversity of choanoflagellates has been found in water columns (e.g. Thomsen et al. 1991, 1997; Vørs 1992a; Tong 1997a). These species are common and are characteristics of water columns, but only 5 species of choanoflagellates were rarely found in this study (sediments).

Some species were frequently observed at only one site or two sites or on a few occasions during the study. These include; *Heteronema* sp.2, *Rhynchobodo longiciliatum* (Woolooware Bay), *Notosolenus adamas*, *N. brothernis*, *N. lashue*, *Ploeotia* sp.2 (Port Botany), *Notosolenus* sp. (Kogarah Bay), *Mastigamoebae psammobia*, Protist 2 (Quibray Bay). For examples, *Mastigamoebae* species were often observed in late culture samples (after 5–6 days) at Port Botany and Quibray Bay, but not at other sites. *Rhynchobodo longiciliatum* were frequently observed at Woolooware Bay. Some of these species may be restricted to a site, but I am unable to assess if these species are endemic because of the lack of intensive studies elsewhere.

### Morphospecies

A large number of the aloricated choanoflagellates (Codosigidae and Salpingoecidae) have been described and many species have been separated by minor differences in morphology because the distinguishing characters were not well defined, unlike loricated choanoflagellate (Acanthoecidae). In some previous studies (e.g.

Boucaud-Camou 1967, Lee and Patterson 2000), some species of *Salpingoeca* were synonymised because of being indistinguishable. About 227 species have been described in the Pelobionts (Walker 1998), but generic and specific boundaries are unclear because of polymorphism within species. The taxonomy of these taxa and some others (such as *Amastigomonas*, *Bico-soeca* and *Cercomonas*) is in need of clarification and revision. Recently, *Amastigomonas* and *Cercomonas* were revised and divided into several genera through the use of cultures and molecular technique (Bass et al. 2009, Cavalier-Smith and Chao 2010).

Three different shapes in *Goniomonas amphinema* are reported in this study. Additionally, three different shapes in *Metromonas grandis* with only minor differences among them have been showed in Lee and Patterson (2000, Fig. 24k–m). There are two different shapes in *Phyllomitus granulatus* with minor differences (see Lee and Patterson 2000, Fig. 26 h–k and l–m). These observations suggest that the present morphospecies concepts will be needed to be refined. Our options are to loosen the concept (lumpers' approach) or to tighten it (splitters' approach) (Lee 2015). A detailed survey of species requires the study of intraspecific variation and the identification of different morphotypes in the life cycle through the uses of cultures and molecular technique (e.g. Simpson et al. 1997 for *Mastigamoeba schizophrenia*; Lee et al. 2014 for *Stephanopogon pattersoni*).

Morphospecies about heterotrophic euglenids was discussed by Lee (2012). Many genera or species of heterotrophic flagellates (e.g., *Anisonema-Dinema-Ploeotia* and *Heteronema-Jenningsia-Peranema*) are similar in shape and behaviour. For example, *Metanema* resembles some species of *Heteronema* (*H. exaratum*, *H. larseni* and *H. ovale*) and the only difference is that *Metanema* do not possess an ingestion organelle visible by light microscopy. These species have merged into one new genus (*Neometanema*) through the use of a pure culture by Lee and Simpson (2014). In addition, Lax et al. (2019) demonstrated that the recent supra-familial systematics including ploetids (*Anisonema*, *Decastava*, *Entosiphon*, *Hemiolia*, *Keelungia*, *Lentomonas*, *Liburna*, *Olkasia*, *Ploeotia*, *Serpenomonas*) are not supported by SSU rRNA phylogenies. Thus, ultrastructural and molecular works (multigene phylogenetics) through the use of cultures are needed to solve these matters.

Lee (2015) showed that the distinguishing characters for most heterotrophic euglenids and small glid-

ing flagellates are cell shape, cell length, number of flagella, flagellar beating patterns, flagellar orientation, swimming/gliding behaviour, surface structure, cell flexibility, flatness of cell and presence/absence of ingestion organelle visible by light microscopy. These characters should be more defined to be not ambiguous. We need to develop more clear and detailed morphological characters with the use of ultrastructural and molecular techniques.

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