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Redescription of the Rare Heterotrophic Flagellate (Protista) - *Phyllomitus undulans* Stein, 1878, and Erection of a New Genus - *Pseudophyllomitus* gen. n.

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Summary. *Phyllomitus undulans* Stein 1878 is redescribed by light-microscopy from live material found in marine sediments of Botany Bay, New South Wales, Australia. This species is 11-21 μm long, is somewhat flexible, has no gullet or pocket and has two flagella that adhere to each other and which arise at the anterior end of the cell. To accommodate the taxa without adhering flagella, which had previously been assigned to this genus, a new genus (*Pseudophyllomitus* gen. n.) is erected and contains 4 new combinations; *Pseudophyllomitus apiculatus* comb. n., *Pseudophyllomitus granulatus* comb. n., *Pseudophyllomitus salinus* comb. n., *Pseudophyllomitus vesiculosus* comb. n. One more new combination *Hemistasia amylophagus* comb. n. is introduced for one other species, *Phyllomitus amylophagus*. The evolutionary affinities of these genera (*Phyllomitus* and *Pseudophyllomitus*) cannot be established on the basis of present information, and they are placed among the protists as *incertae sedis*.

Key words: flagellate, kathablepharids, *Phyllomitus*, protist, Protista *incertae sedis, Pseudophyllomitus.*

INTRODUCTION

Heterotrophic flagellates are important numerically and ecologically in aquatic ecosystems both in the water column and in the benthos (e.g., Azam *et al*. 1983, Lee and Patterson 2002). Despite their importance, their taxonomy has until recently been little studied. Recently, the marine species have been the subject of a number of taxonomic studies (e.g., Vørs 1992a, b; Ekebom *et al*. 1996; Larsen and Patterson 1990; Thomsen *et al.* 1991, 1997; Patterson and Simpson 1996; Tong 1997a, b, c; Tong *et al*. 1997, 1998; Al-Qassab *et al*. 2002; Bernard *et al*. 2000; Lee and Patterson 2000; Lee 2002).

Many species of heterotrophic flagellates have been badly described and some species were introduced without descriptions or drawings (e.g., Lackey 1961, Lackey and Lackey 1970). Early descriptions may lack reference to characteristics, which have since proved necessary to distinguish species. Many species of heterotrophic flagellates are rare (Lee 2001) and have not been reported since their original descriptions. *Phyllomitus* is one such taxon. It was erected by Stein (1878) to accommodate *Phyllomitus undulans* from a freshwater site in Germany. This species was described as 21-

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30 µm long, is flexible and has two flagella that adhere to each other and emerge from a small depression (Figs 1a-f). Since then, 6 species have been included in *Phyllomitus* by Klebs (1893), Ruinen (1938), Lackey (1940), Skuja (1948) and Larsen and Patterson (1990). Later species do not have the adhering flagella mentioned by Stein (1878). This may be a useful diagnostic character for the genus (Larsen and Patterson 1990, Patterson and Zölffel 1991) and the assignment of these 6 species is therefore questionable.

Phyllomitus undulans is redescribed here to provide this species with a clear morphological identity, and a new genus, *Pseudophyllomitus,* is proposed to accommodate the taxa without adhering flagella which had previously been assigned to this genus.

MATERIALS AND METHODS

Phyllomitus undulans was encountered three times from September to November 1999, during a survey of the diversity of heterotrophic flagellates from marine sediments at Botany Bay in New South Wales, Australia (151°7' E; 33°59' S). The surface water temperature was 15-19.5°C, pH was 7.55-7.73 and salinity was 33-35 ‰. Sediments were taken from intertidal sandy sediments to a depth of about 1 cm from 1 m^2 quadrat using a flat spoon. The sediments were placed in plastic trays in 1 cm deep layers. Coverslips (No.1 22 x 22 mm) were placed on lens tissue laid on the sediments. After 12-24 h, flagellates were observed using a Zeiss Axiophot microscope equipped with photographic and video facilities (Lee and Patterson 2000).

RESULTS

Phyllomitus undulans Stein, 1878 (Figs 2a-e)

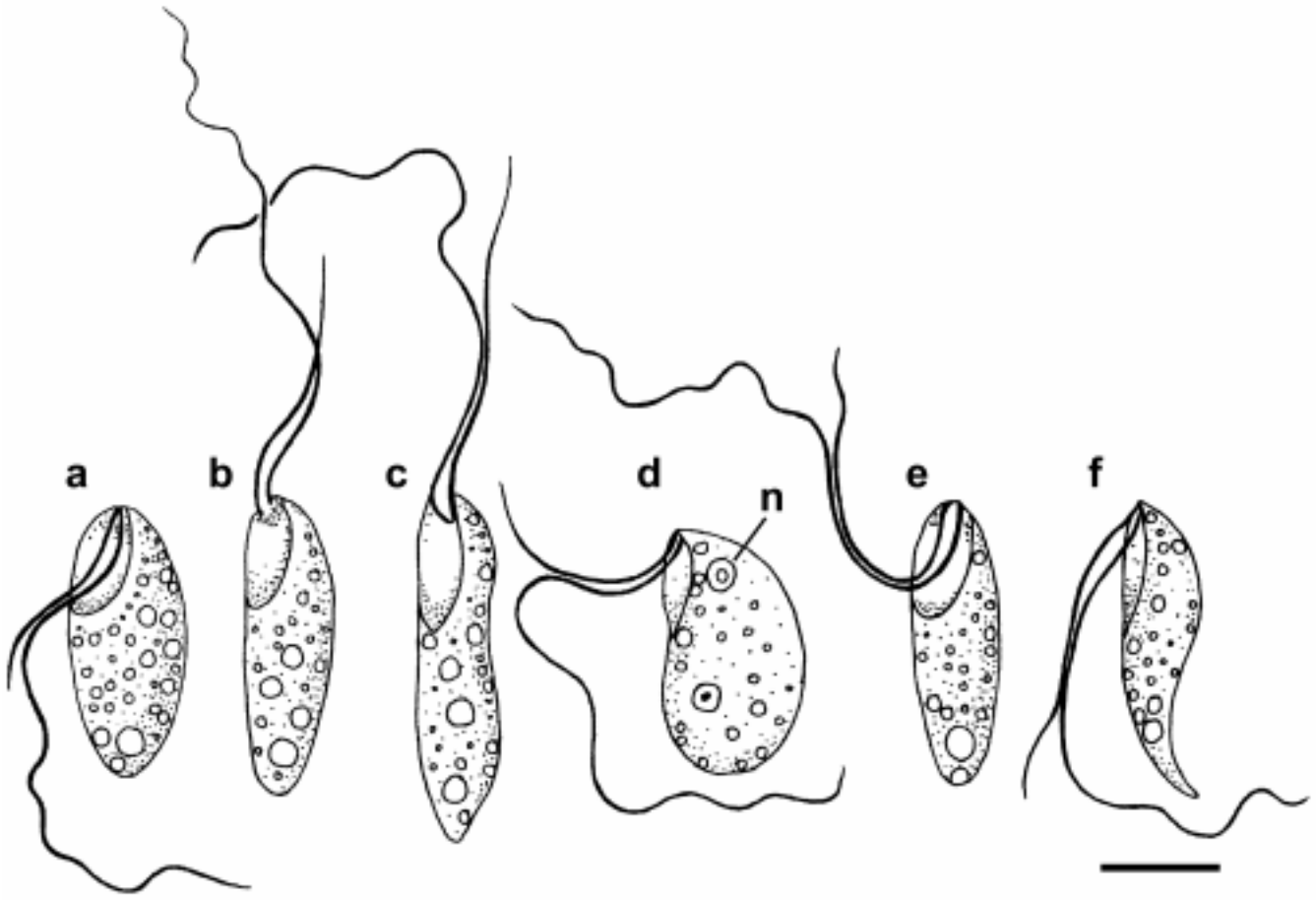
Cells are 11-21 µm long, slightly flexible with two flagella that adhere to each other. The cells are elongate and are pointed at both ends. The cells appear to be convex dorsally and concave ventrally. The flagella arise at the anterior end of the cell, appear to be similar in length about 3 times longer than the cell, and have an undulating beat. No anterior depression or pocket was observed. The cell surface is smooth and the nucleus is located on the mid-anterior part of the cell. Cytoplasmic strands may arise from the posterior end of the cell. The cells contained some food materials. The cells may attach to the substrate by the posterior end of the cell. Three cells were observed.

This species was also occasionally found at Moreton Bay Prawn Farm (Queensland, Australia) with cell lengths from 13 to 21 µm and reported without a name (Blackmore 1997). Only one record (Rico 1985) of the type species has been made. This was without a description or photos and was from soil samples in the San Juan Tezompa (Mexico).

DISCUSSION

Phyllomitus undulans is a distinctive species of flagellate because it has two flagella, which adhere to each other. The cells described by Stein (1878) and observed here have two adhering flagella and somewhat a pointed posterior end. The cell length ranges overlap, although I note the magnification given by Stein (1878) appears to be inaccurate, and common organisms reported by him are 1.5-2 times bigger than reported by others. This species was originally reported with an anterior depression but this could be an inaccurate description of the concave ventral side. Consequently, the cells described here are regarded as *P. undulans.*

The following species have been included in *Phyllomitus* (Fig. 3): *Phyllomitus amylophagus,* Klebs, 1893, *P. apiculatus* Skuja, 1948, *P. granulatus,* Larsen et Patterson, 1990, *P. salinus* Lackey, 1940, *P. vesiculosus* Larsen et Patterson, 1990, *P. yorkeensis,* Ruinen, 1938. None of these species have two adhering flagella. *Phyllomitus yorkeensis* was transferred to *Palustrimonas* by Patterson and Simpson (1996) because the body of *P. yorkeensis* is not very plastic and has two opposed flagella, which insert subapically and close together in separate grooves. *Phyllomitus amylophagus* (Fig. 3a) is transferred to *Hemistasia* (*H. amylophagus* comb. n.). It does not have two adhering flagella and it shares cell shape, cell length, flexibility and having a spiral groove with *Hemistasia*. The spiral groove in *H. amylophagus* was not shown in Klebs (1893)'s drawings (Fig. 3a), but he noted it in the text. Additionally, Elbrächter *et al*. (1996) noted that *P. amylophagus* needed re-investigation and may be transferred to *Hemistasia,* and Myl'nikov (personal communication) is also of the view that *Phyllomitus amylophagus* belongs to *Hemistasia* on the basis of ultrastructural evidence (Myl'nikov *et al*. 1998). *Hemistasia amylophagus* most closely resembles *Hemistasia phaeocysticola* (Scherffel, 1900) Elbrächter *et al.* 1996 and these species are difficult to distinguish by light-microscopy. They can be distinguished by electron-microscopy (Myl'nikov *et al*. 1998) because *H. phaeocysticola* has no microtubular prism



Figs 1 a-f. *Phyllomitus undulans* (after Stein 1878), nucleus (n). Scale bar -10 μm

(nemadesm), it has distinct glycocalyx-like coat on the plasmalemma and has more than 11 extrusomes. *Hemistasia amylophagus* has no cysts or cyst-like bodies, no division cyst (Myl'nikov *et al*. 1998), and the cytoplasm of *H. phaeocysticola* has swollen peripheral lacunae (see Elbrächter *et al*. 1996, p127, Fig. 5).

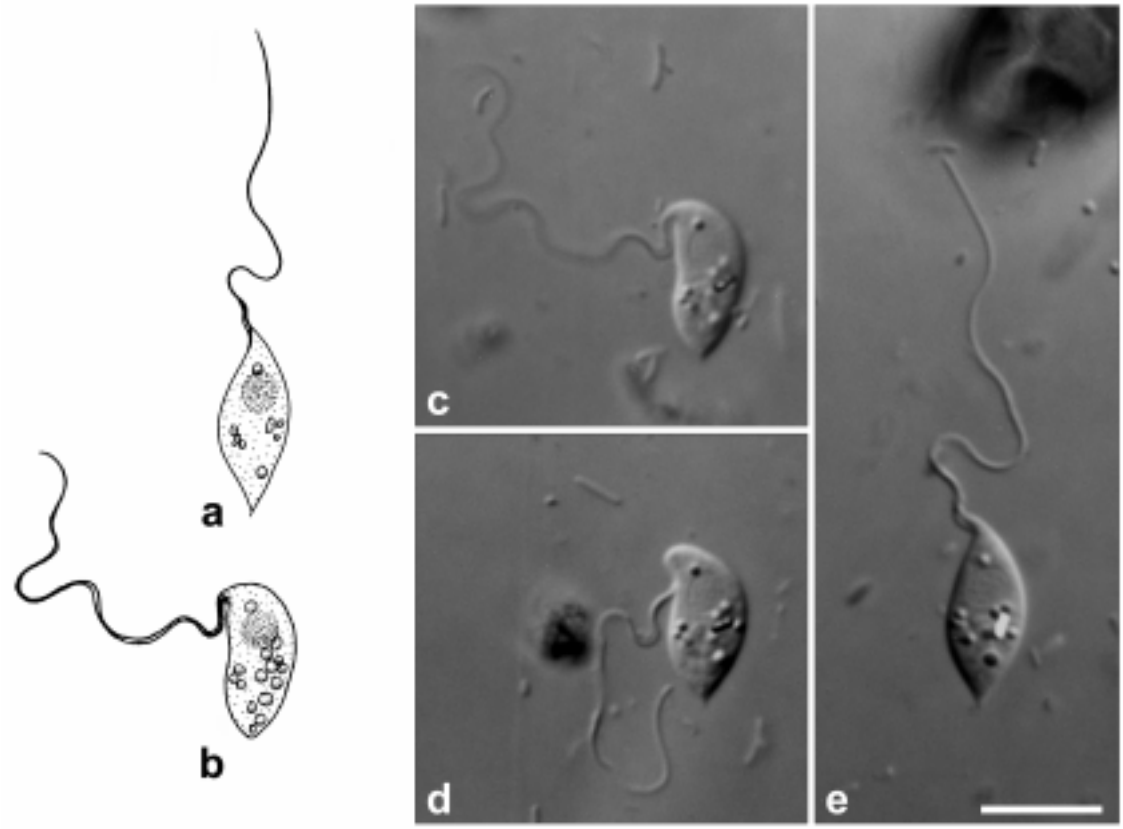
Larsen and Patterson (1990) and Patterson and Zölffel (1991) noted that the adhesion of the two flagella mentioned by Stein (1878) might be a good character to distinguish the genus *Phyllomitus*. On this basis, the remaining species, other than *P. undulans* currently placed in the genus would be best located in a different genus. I adopt their view and erect the new genus *Pseudophyllomitus* to accommodate the taxa without adhering flagella. This genus may contain unrelated taxon (see *P. apiculatus*), but I believe that it is appropriate to retain it in a single genus until more information is available.

***Pseudophyllomitus* gen. n.**

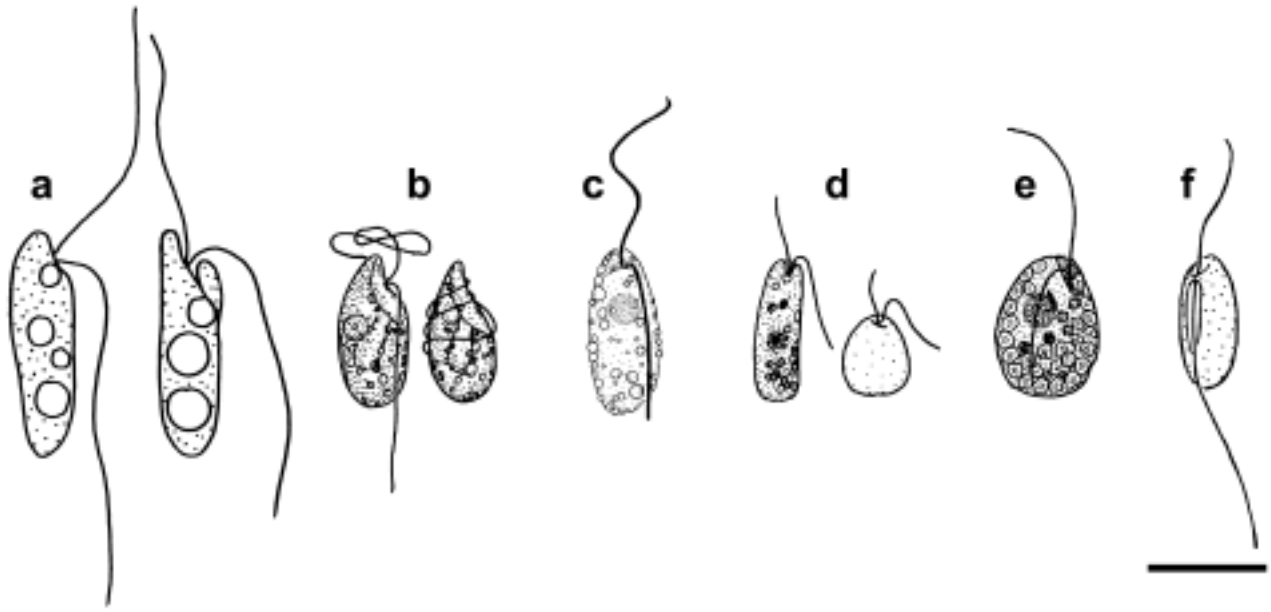
Diagnosis: free-living heterotrophic, flexible, sac-shaped protists with two flagella, which do not adhere to each other and which insert subapically into a gullet or pocket. One flagellum is directed anteriorly and the other one trails posteriorly. Cells swim and may glide. The genus contains 4 nominal species.

Type species: *Pseudophyllomitus granulatus* (Larsen et Patterson, 1990) comb. n.

Discussion of similar taxa: *Pseudophyllomitus* differs from *Phyllomitus* in that its flagella emerge subapically from a gullet or pocket and because one flagellum is directed anteriorly and the other one trails posteriorly. This genus resembles *Colponema* Stein, 1878 in being highly flexible and in having two flagella emerging from a deep gullet of pocket, but is distinguished by the lack of a groove extending the length of



Figs 2 a-e. *Phyllomitus undulans*; **a-b** - drawings of the species; **c-e** - cells from Botany Bay, note the two adhering flagella, DIC images. Scale bar - 10 μm



Figs 3 a-f. **a** - *Hemistasia amylophagus* comb. n. (after Klebs 1893 under the name *Phyllomitus amylophagus*); **b** - *Pseudophyllomitus apiculatus* comb. n. (after Skuja 1948); **c** - *Pseudophyllomitus granulatus* comb. n. (after Lee and Patterson 2000); **d** - *Pseudophyllomitus salinus* comb. n. (after Lackey 1940); **e** - *Pseudophyllomitus vesiculosus* comb. n. (after Larsen and Patterson 1990); **f** - *Palustrimonas yorkeensis* (after Patterson and Simpson 1966 under the name *Phyllomitus yorkeensis*). Scale bar - 10 μm

the cell (Larsen and Patterson 1990). *Pseudophyllomitus* is similar to *Heterochromonas* Pascher, 1912 in being flexible and in cell shape and length, but can be distinguished because the flagella are directed anteriorly in *Heterochromonas*. This genus is also similar to *Palustrimonas* Patterson et Simpson, 1996 which contains only one species (*P. yorkeensis*), but can be distinguished because *Palustrimonas* is not highly flexible or metabolic and the two flagella insert separate prominent grooves.

***Pseudophyllomitus apiculatus* (Skuja, 1948) comb. n. (Fig. 3b)**

Basionym: *Phyllomitus apiculatus* Skuja, 1948

Cells are 11-15 μm long and 4-5.5 μm wide, flexible, somewhat flattened laterally, and narrowed anteriorly with a pocket. Two flagella emerge from the pocket. The anterior flagellum is about the cell length and winds around the anterior end of the cell, and the posterior flagellum is 1.5-2 times the cell length and extends posteriorly. In the resting stage, the two flagella wind spirally around the body. The contractile vacuole is located in the anterior end of the cell. The cells feed on detritus and small algae through the pocket. The cells move by swimming with rotating movements.

This species has been reported from freshwater sites in Germany and Sweden (Skuja 1948, 1956; Steinberg *et al*. 1983). The drawings of Steinberg *et al*. (1983, p308, Abb.1) appear to be different to the original drawing of Skuja (1948) and appear to be a species of *Rhynchobodo* (maybe *R. agilis*). *Phyllomitus apiculatus* mostly resembles *Rhynchobodo conoidea* (Skuja, 1956) Bernard *et al*. 2000 in general appearance, cell length (*R. conoidea* is 11-14 μm long), and in being flexible and in having a ventral pocket and a rotating movement. Both species were recorded in Skuja (1956), but found in different locations. There is one ultrastructural study on organisms with this name (Myl'nikov 1986), but according to Patterson and Zölffel (1991) the cells studied are *Rhynchobodo armata* of Brugerolle (1985). This species may belong to *Rhynchobodo*, sharing with that genus a lateral deep pocket, rotating movements, and its two flagella, which wrap around the body of resting cells. Elbrächter *et al*. (1996) noted that this species needs reinvestigation and may be transferred to *Hemistasia*. I retain this species as a member of the genus *Pseudophyllomitus* until further ultrastructural or molecular information becomes available.

***Pseudophyllomitus granulatus* (Larsen et Patterson, 1990) comb. n. (Fig. 3c)**

Basionym: *Phyllomitus granulatus* Larsen et Patterson, 1990

Cell outline is sac-shaped. Cells are flexible, 7-21 μm long and slightly flattened being elliptical in cross section. Two flagella emerge subapically. The anterior flagellum beats with a sine-wave, is 1.0-1.5 times the cell length and is directed to the front and slightly to the right during swimming. The posterior flagellum inserts to the left of the anterior flagellum, varies in length from 0.5 to 2.5 times the cell length and trails behind the cell when swimming. Cytoplasm is drawn out at the posterior end. Refractile granules underlie the cell surface. The nucleus is located below the anterior pocket, near the centre of the cell and is roundish. The cells contain ingested eukaryotic algae. When eating, food materials are driven into the pocket, and the cell becomes very plastic (almost amoeboid) extending as a very thin layer around the food. Common at times.

This species has been reported from marine sites in subtropical and tropical Australia, Brazil, Denmark, Hawaii and Korea (Larsen and Patterson 1990, Vørs 1992b, Lee and Patterson 2000, Lee 2002). This species is similar to *P. salinus* in general appearance and cell length, but *P. salinus* has shorter flagella. Further studies are required to distinguish these two species.

***Pseudophyllomitus salinus* (Lackey, 1940) comb. n. (Fig. 3d)**

Basionym: *Phyllomitus salinus* Lackey, 1940

Cells are about 12 μm long and 5 μm wide. The cells are cylindrical, typically elongate, and metabolic with two flagella emerging from a subapical depression. The posterior flagellum is about two-thirds the cell length, the anterior one is about 0.5 times the cell length. The nucleus is located in the centre of the cell. Cytoplasm clear, granular, a few small spheres sometimes present. Nutrition and reproduction not ascertained. Very common at times.

This species was found in USA (Lackey 1940).

***Pseudophyllomitus vesiculosus* (Larsen et Patterson, 1990) comb. n. (Fig. 3e)**

Basionym: *Phyllomitus vesiculosus* Larsen et Patterson, 1990

Cells have a sac-shaped body, are 11-15 μm long, and are slightly dorso-ventrally flattened. The cells have two

flagella arising from large anterior depression; the anterior flagellum extends in front of the cell with a long sweeping curve and the posterior flagellum trailing behind. The nucleus is located near the centre of the cell, adjacent to the base of the flagella; a rod or bar lies against one anterior side of the nucleus. The outer region of the cytoplasm is highly vesiculate. The cells move by swimming, frequently in the immediate vicinity of the substratum, with the posterior flagellum dragging against the substratum.

This species has been found from tropical Australia (Larsen and Patterson 1990). It is easily distinguished from other species of *Pseudophyllomitus* because the cell surface is underlain with a layer of vesicles.

Taxonomic position of these genera

Many heterotrophic flagellates have no evident affinities with other types of protists (Patterson 1999). The affinities of most genera studied by light-microscopy only cannot be established without further ultrastructural or molecular studies (Patterson 1999). This situation applies to *Phyllomitus* and *Pseudophyllomitus*.

Early attempts were made to locate *Phyllomitus* on the basis of light microscopy alone, but they have not stood the test of time. Lemmermann (1914) placed *Phyllomitus* (*P. undulans* and *P. amylophagus*) in the family Bodonaceae, and Pringsheim (1944) placed *Phyllomitus* in the family Kathablepharidaceae Skuja 1939 (ICBN) = Kathablepharidae Vørs 1992 (ICZN) at that time erroneously treating this family as a component of the Cryptophyceae. None of the four genera included in this family (*Kathablepharis* Skuja, 1939, *Leucocryptos* Butcher, 1967, *Phyllomitus* Stein, 1878 and *Platychilomonas* Larsen et Patterson, 1990) have anything more than a superficial similarity with the true cryptomonads, leading to the conclusion that these taxa do not belong to the Cryptophyceae (Lee and Kugrens 1991; Lee *et al*. 1991a, b; Vørs 1992a, c; Clay and Kugrens 1999a). *Phyllomitus* should be removed from the kathablepharids and cryptomonads because it lacks the characteristics of both groups such as the rigid body and the ejectisomes (Clay and Kugrens 1999b). Although *Pseudophyllomitus apiculatus* may belong to Kinetoplastida (see above) and *P. granulatus* may belong to stramenopiles due to the beating pattern of the anterior flagellum (see Larsen and Patterson 1990, Lee and Patterson 2000, Lee 2002), *Phyllomitus* and *Pseudophyllomitus* cannot be confidently assigned to any major group of flagellates, and are regarded as Protista *incertae sedis* until ultrastructural or molecular information becomes available.

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