Korean J. Syst. Zool. Vol. 23, No. 1: 1-7, May 2007

Choanoflagellates (Protist) from Marine Sediments of South-Eastern Australia

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ABSTRACT

Choanoflagellates were encountered in marine sediments of Gippsland Basin (Australia) and were classified into 8 species, 5 genera in 2 families. The species rarely found in this study were *Acanthocorbis unguiculata, Acanthoeca spectabilis, Polyoeca dichotoma* and *Saepicula pulchra* of the family Acanthoecidae; *Salpingoeca amphoridium, Salpingoeca infusionum, Salpingoeca megacheila* and *Salpingoeca tuba* of the family Salpingoecidae. Their descriptions were based on living specimens. Their morphological characters and geographic distribution are presented.

Key words: Choanoflagellates, protist, Acanthoecidae, Salpingoecidae, Gippsland Basin

INTRODUCTION

The significance of free-living heterotrophic flagellates in microbial food webs of aquatic environments has received much attention and been well established (Azam et al., 1983). Despite of this, the diversity of heterotrophic flagellates is less well studied. Only few studies on the taxonomic composition of heterotrophic flagellates in marine ecosystems, especially in deep-sea sediments or bottom sediments (Patterson et al., 1993; Atkins et al., 2000; Hausmann et al., 2002a, b; Arndt et al., 2003) have been conducted. In this study, among the benthic heterotrophic flagellates, choanoflagellates (collared flagellates) are focused and 8 species are reported from Gippsland Basin, south-eastern Australia.

Choanoflagellates are a relatively well defined and easily identifiable group of free-living heterotrophic flagellates (Leadbeater, 1991; Leadbeater and Thomsen, 2000). This group is characterised by the uniform appearance of the protoplast with a single anterior flagellum. The flagellum is surrounded by a funnel shaped collar consisting of several tentacles. The following references are useful guides to many species descriptions of choanoflagellates: James-Clark (1867), Kent (1880-1882), Lemmermann (1910), Ellis (1929), Hollande (1952), Norris (1965), Bourrelly (1968), Leadbeater (1981, 1991), Thomsen and Buck (1991), Thomsen et al. (1991, 1997), Tong (1997b), Vørs (1992).

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MATERIALS AND METHODS

Gippsland Basin is located in south-eastern Australia (37° 59′S-38° 49′S; 147° 21′E-148° 42′E). A large wedge of sediments has been accumulated in the last 30 million years in the Basin (Wilson and Poore, 1987). This study was conducted from 18th of September to 5th of October 1998 in Gippsland Basin as a part of the R/V Franklin Cruise (Fr 11/98) by CSIRO. Samples were collected from bottom sediments with a Smith-McIntyre grab sampler and sampling depths vary from 25 m to 471 m. After collecting, the samples were kept cool (~4°C) and then were transported to the University of Sydney for analysis. The samples were processed as described by Lee and Patterson (2000) in that bottom sediment was collected and placed in layers 1 cm deep in trays, allowed to settle for several hours, excess water drained off, and the material was covered with a sheet of lens tissue upon which 22 × 22 mm No.1 coverslips were placed. Flagellates tended to concentrate under the coverslips after 12-72 hrs. The coverslips were then removed and the associated flagellates were observed using a Zeiss (Axioplan) microscope equipped with photographic facilities. The flagellates were also recorded on U-MATIC video tapes and records were also made with video prints. Specimens were also drawn. The technique is designed for consistency between different sampling sites. The samples were maintained at room temperature (~20°C) for 2 weeks.

The nomenclature of some of the groups represented in this study has been in compliance with the ICZN (International Code of Zoological Nomenclature, 1999). The scheme of classification by Leadbeater and Thomson (2000) was adopted.

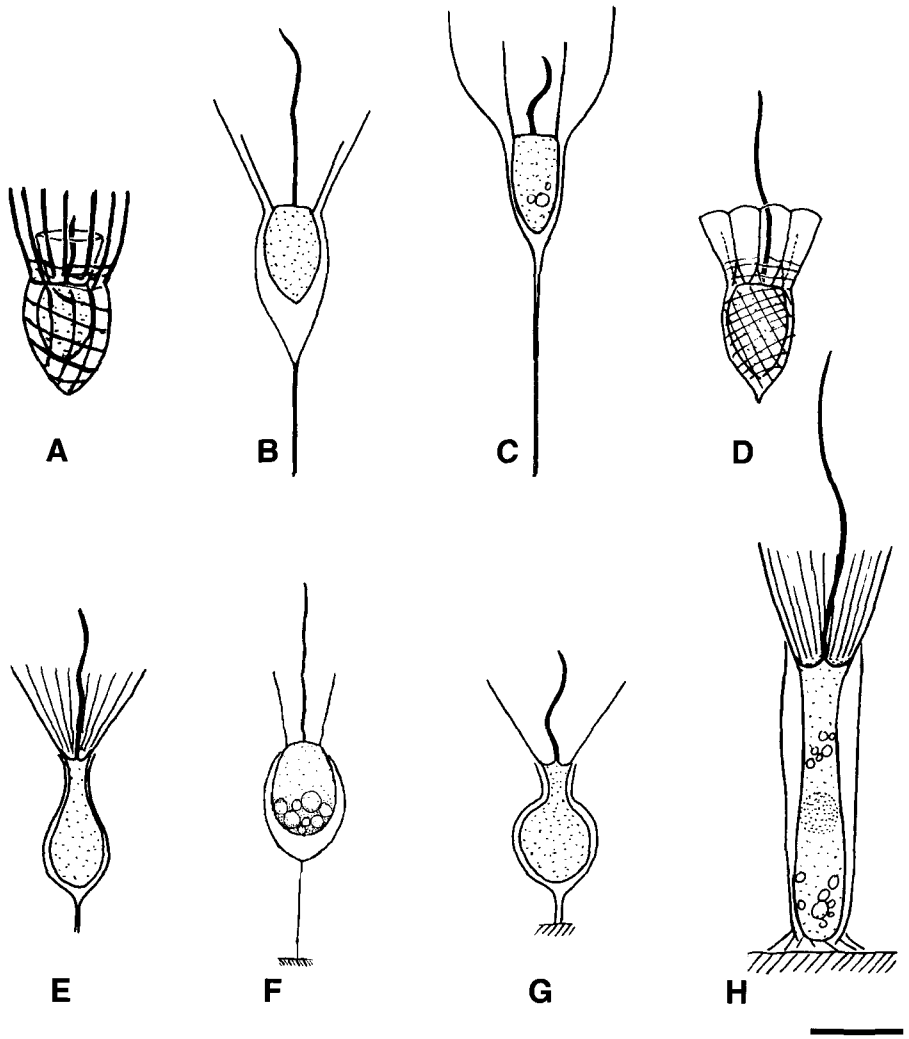

Fig. 1. A, *Acanthocorbis unguiculata*; B, *Acanthoeca spectabilis*; C, *Polyoeca dichotoma*; D, *Saepicula pulchra*; E, *Salpingoeca amphoridium*; F, *Salpingoeca marina* after Lee and Patterson (2000) under the name of *Salpingoeca infusionum*; G, *Salpingoeca megacheila*; H, *Salpingoeca tuba*. Scale bar=5 μm.

RESULTS AND DISCUSSION

Order Choanoflagellida Kent, 1880
Family Acanthoecidae Norris, 1965
Genus *Acanthocorbis* Hara and Takahashi, 1984
***Acanthocorbis unguiculata* (Thomsen, 1973)**
(Figs. 1A, 2A-B)

Acanthoecopsis unguiculata Thomsen, 1973, p. 3, figs. 3-5.
Acanthocorbis unguiculata: Hara and Takahashi, 1984, p. 826.

Description. Protoplast 5 μm long, flagellum surrounded by pseudopodial tentacles. The protoplast enclosed within a lorica consisting of two chambers. Anterior chamber with few obliquely oriented transverse costae and 12 strong logitudinal costae (Fig. 2B). Longitudinal costae free at anterior end of lorica. Posterior chamber with several transverse costae. Rarely found in depth of 173 m.

Remarks. The identity of most acanthoecid choanoflagellates can only be confirmed through electron microscopical observation. The distinguishing characters include the form of dimension of the lorica, the shape, number and types of joints of the costal strips and whether or not there are anterior or posterior projections (Thomsen and Buck, 1991; Thomsen et al., 1997). *Acanthocorbis* includes 10 nominal species: *A. apoda, A. asymmetrica, A. camarensis, A. campanula, A. haurakiana, A. nana, A. prolongata, A. tintinnabulum, A. unguiculata* and *A. weddellensis. Acanthoecopsis* was transferred to *Acanthocorbis* by Hara and Takahashi (1984). *A. unguiculata* was first described by Thomsen

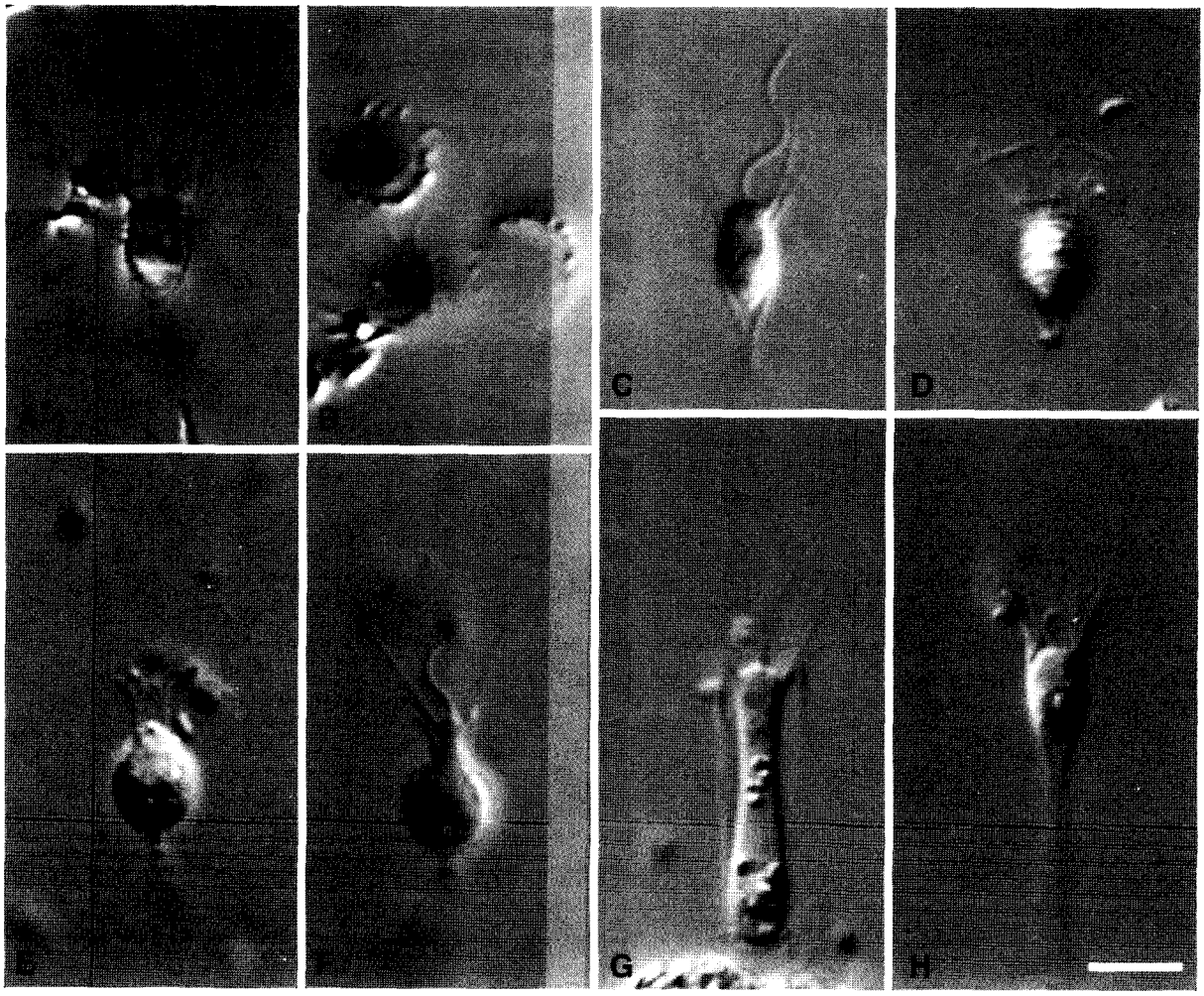

Fig. 2. A, *Acanthocorbis unguiculata*, general appearance of the cell; B, *Acanthocorbis unguiculata*, showing longitudinal costae; C, *Acanthoeca spectabilis*, general appearance of the cell; D, *Saeicula pulchra*, general appearance of the cell; E, *Salpingoeca amphoridium* retracted in the lorica; F, *Salpingoeca megacheila*, general appearance of the cell; G, *Salpingoeca tuba*; H, *Polyoeca dichotoma*, general appearance of the cell. All micrographs are DIC images. Scale bar=5 μm.

(1973) as a species of *Acanthoecopsis*, but was subsequently transferred to *Acanthocorbis* for nomenclatural reasons by Hara and Takahashi (1984). This species is mostly similar to *A. apoda* and *A. camarensis*, but can be distinguished from *A. apoda* and *A. camarensis* by having anterior projections with claw-formed tips (Hara et al., 1996). It is also distinguished from *A. tintinnabulum* by the length of longitudinal costae extending beyond the anterior chamber.

Distribution. Marine habitats in Antarctica, Atlantic, Arctic Canada, Denmark, England, west Greenland, Norway and USA (Gold et al., 1970; Thomsen, 1973, 1977, 1979, 1982; Tangen, 1974; Thordsen, 1974; Espeland and Throndsen, 1986; Van den Hoff and Franzmann, 1986; Marchant et al., 1987; Marchant and Perrin, 1990; Patterson et al., 1993; Thomsen et al., 1997; Tong, 1997b; Tong et al., 1997).

Genus *Acanthoeca* Ellis, 1929

***Acanthoeca spectabilis* Ellis, 1929 (Figs. 1B, 2C)**

Acanthoeca spectabilis Ellis, 1929, p. 77, fig. 5.

Description. Protoplast 4-6 μm long, elongated with pointed posterior end. Acronematic flagellum 4-12 μm. Pseudopodial collar shorter than cell. Lorica conical shaped, tapering posteriorly with stalk. Frequently observed and found in depths of 55-471 m.

Remarks. A. spectabilis is common in marine. The previously reported cell length ranges from 3.5 to 9 μm (Tong, 1997a, b; Tong et al., 1998). The genus contains only 2 species; *A. brevipoda* and *A. spectabilis*. *A. spectabilis* can be distinguished from *A. brevipoda* Ellis, 1929 because *A. spectabilis* has a long stalk, which usually attaches to the

substrate.
Distribution. Marine habitats in Antarctica, Australia, Canada, Denmark, England, Gulf of Finland, France, Norway and USA (e.g., Ellis, 1929; Lee and Patterson, 1998; Al-Qassab et al., 2002; Lee et al., 2003).

Genus *Polyoeca* Kent, 1880

***Polyoeca dichotoma* Kent, 1880 (Figs. 1C, 2H)**

Polyoeca dichotoma Kent, 1880, p. 360, Pl. V. fig. 20, Pl. III. figs. 27, 28.

Description. Cells solitary. Protoplast about 5 μm × 2.5 μm, flagellum slightly shorter than protoplast, surrounded by pseudopodial tentacles. Protoplast within lorica, 15 μm long. Lorica widely opened at anterior part, pointed posteriorly. Two cells found in depths of 25-55 m.
Remarks. The cells observed here are assigned to *P. dichotoma* Kent, 1880 because the observations are agreement with those of Hara and Takahashi (1984). This genus is usually distinguished from other species of Acanthoecidae by electron microscopy. The genus has equally spaced transverse costae (two or more) encircling the lorica chamber, which constructed of numerous rod-shaped costal strips with anterior projections, and have numerous longitudinal costae (30-40) being continuous with an aggregated stalk (Thomsen and Buck, 1991). The genus contains only one species, *P. dichotoma* Kent, 1880. *P. dichotoma* is mostly similar to *A. spectabilis* Ellis, 1929 in cell length and general appearance of lorica, but can be distinguished by overall length of lorica and the long stalk by light microscopy (Hara and Takahashi, 1984). Additionally, these species are well distinguished by electron microscopy because the lorica in *Polyoeca* is encircled by two or more transverse costae while the lorica in *Acanthoeca* is encircled by numerous spiral costae.
Distribution. Marine habitats in Antarctica, Australia, Canada, England, France, Japan and USA (Wailes, 1929, 1939; Norris, 1965; Boucaud-Camou, 1967; Thomsen, 1977, 1992; Buck, 1980, 1981; Hara and Takahashi, 1984; Tong, 1997a, b).

Genus *Saepicula* Leadbeater, 1980

***Saepicula pulchra* Leadbeater, 1980 (Figs. 1D, 2D)**

Saepicula pulchra Leadbeater, 1980, p. 346, Pl. I. figs. 1-6.

Description. Cells solitary, protoplast 5 μm long, flagellum 7.5 μm. Lorica consisting of two chambers delimited by waist, and consisting of several transverse costae and 10 longitudinal costae. Anterior chamber everted, surmounted by single transverse costal band connected to longitudinal costae. Posterior chamber abconical, composing of several transverse and longitudinal costae. Found in depth of 58m.
Remarks. The prevously reported cell length is 2.5-5.5 μm (Tong, 1997a, b). According to previous authors, two chambers can be easily seen when using an electron microscope. This genus currently contains two species, *S. pulchra* Leadbeater, 1980 and *S. leadbeateri* Takahashi, 1981 (Thomsen et al., 1997). These two species can be easily distinguished by the presence/absence of thick costal strips and lorica shape.
Distribution. Marine habitats in subtropical Australia, Denmark, England, Gulf of Finland and France (Leadbeater, 1980; Thomsen, 1992; Vørs, 1992; Tong, 1997a, b; Lee et al., 2003).

Family Salpingoecidae Kent, 1882
Genus *Salpingoeca* James-Clark, 1867

***Salpingoeca amphoridium* James-Clark, 1867 (Figs. 1E, 2E)**

Salpingoeca amphoridium James-Clark, 1867, p. 322, Pl. IX. figs. 37, 37[d].
Salpingoeca ampullacea Stein, 1878, Taf. XI. figs. 1-5.
Salpingoeca ampulloides Bicudo and Bicudo, 1983, p. 16, figs. 2, 3.

Description. Cells solitary, protoplast 6-7 μm, flagellum 6-7 μm. Lorica about 10 μm long, with narrow neck. Protoplast may be retracted in lorica (Fig. 2E). Lorica attaching to substrate by short pedicel or posterior end of lorica. Two cells found in depth of 121 m.
Remarks. The cells observed here are assigned to *Salpingoeca* because the cells have a single theca without silicified costae, are sedentary and do not form colonies. Many previously reported species assigned to *Salpingoeca* appear to be very similar. The species taxonomy is unclear because the species are mainly distinguished by the thecal morphology (Vørs, 1992). *Salpingoeca* is regarded as synonyms of *Lagenoeca* Kent, 1880 and *Pachysoeca* Ellis, 1929 (Bourrelly, 1957; Norris, 1965). This genus is mostly similar to *Choanoeca* Ellis, 1929 in general appearance of cells, but is distinguished because *Choanoeca* have no flagellum in sedentary cells. Generally, the observations are in accordance with Vørs (1992). The cells of Griessmann (1913) appear to be different from those of other observers. *S. amphoridium* has two synonyms with *S. ampullacea* Stein, 1878 and *S. ampulloides* Bicudo and Bicudo, 1983 (Vørs, 1992). The previously reported lorica length of *S. amphoridium* is from 5 to 16 μm (James-Clark, 1867; Kent, 1880; Starmach, 1985; Autio et al., 1990; Vørs, 1992; Tong, 1997b).
Distribution. Marine habitats in Baltic Sea, Denmark, England, Gulf of Finland, USA (James-Clark, 1867; Autio et al., 1990; Vørs, 1992; Tong, 1997b).

***Salpingoeca marina* James-Clark, 1987 (Fig. 1F)**

Salpingoeca marina James-Clark, 1867, p. 320, Pl. IX. figs. 28-32[a].

Salpingoeca longipes Kent, 1880, p. 353, Pl. VI. fig. 7.

Salpingoeca infusionum Kent, 1880, p. 356, Pl. VI. figs. 8-16.

Description. Loricate choanoflagellate. Protoplast 6 μm long with pedicel. Flagellum thickened, about 1.5 times cell length, surrounded by pseudopodial tentacles. Lorica ovoid or droplet-shaped, with short neck, connected to pedicel (~ 2 times cell length). Lorica attaching to substrate by pedicel. Relatively rarely found in depth of 78 m.

Remarks. Like Griessmann (1913) and Boucoud-Camou (1967), *S. infusionum* is regarded as a junior synonym of *S. marina* because they are indistinguishable when the cell body of *S. marina* is retracted into the lorica (Lee, 2006). *S. longipes* Kent, 1880, which were regarded as a synonym of *S. infusionum* (Boucaud-Camou, 1967; Tong, 1997b).

Distribution. Marine habitats in Antarctica, north Atlantic, Australia, Denmark, England, France, Gulf of Finland, France, Germany, Korea and USA (e.g., James-Clark 1867; Lee and Patterson, 1998, 2000; Lee, 2002, 2006).

***Salpingoeca megacheila* Ellis, 1929 (Figs. 1G, 2F)**

Salpingoeca cardiforma Ellis, 1929, p. 82.

Salpingoeca cardiforma var. *tulepoda* Ellis, 1929, p. 82, fig. 17, 24a-e.

Salpingoeca desaedeleleeri Ellis, 1929, p. 82, fig. 19.

Salpingoeca megacheila Ellis, 1929, p. 81, fig. 16.

Description. Cells solitary, protoplast 6 μm long, single flagellum slightly shorter than cell length. Lorica 8-9 μm long, with short or long pedicel. Posterior part of lorica spherical, anterior part with narrow short neck flaring at anterior end of lorica. Pseudopodial collar 6 μm long. Three cells found in the depth of 62 m.

Remarks. The cells observed here are assigned to *Salpingoeca* because the cells have a single theca without silicified costae, are sedentary and do not form colonies. The previously reported range of lorica lengths of *S. megacheila* is 9-12 μm (Ellis, 1929; Boucaud-Camou, 1967; Tong et al., 1998). This species is similar to some species of *Salpingoeca* (such as *S. cardiforme* Ellis, 1929, *S. cardiforme* var. *tulepoda* Ellis, 1929, *S. desaedeleeri* Ellis, 1929, *S. napiforme* Kent, 1880) in cell length and in having a short neck and a spherical chamber of the lorica whose the posterior end is the stalk, but can be distinguished because the lorica of *S. megacheila* flares at its anterior end. Additionally, other four species (*S. cardiforme, S. cardiforme* var. *tulepoda*, *S. desaedeleeri* and *S. napiforme*) are indistinguishable. *S. napiforme* is regarded as a senior synonym of the other three species, and was regarded as a synonym of *S. desaedeleeri* (Boucaud-Camou, 1967).

Distribution. Marine habitats in Australia, England and France (Ellis, 1929; Boucaud-Camou, 1967; Tong et al., 1998).

***Salpingoeca tuba* Kent, 1880 (Figs. 1H, 2G)**

Salpingoeca cylindrica Kent, 1880, p. 348, Pl. VI. fig. 37.

Salpingoeca petiolata Kent, 1880, p. 349, Pl. VI. fig. 26.

Salpingoeca tuba Kent, 1880, p. 351, Pl. VI. fig. 38 .

Description. Cells solitary, protoplast 10-15 μm long, located in cylindrical lorica, single flagellum 10-15 μm. Lorica rounded at base, basal part of lorica with filamentous threads. Nucleus near centre of protoplast. Found in depth of 25 m.

Remarks. The cells observed here are assigned to *Salpingoeca* because the cells have a single theca without silicified costae, are sedentary and do not form colonies. In this genus, some species have a cylindrical lorica and include *S. cylindrica* Kent, 1880, *S. petiolata* Kent, 1880 and *S. vaginocola* Stein, 1878. *S. cylindrica* and *S. petiolata* were regarded as synonyms of *S. tuba* (Boucaud-Camou, 1967; Vørs, 1993). *S. tuba* is distinguished from *S. vaginocola* because the lorica of *S. vaginocola* has a pointed posterior end with a pedicel (Vørs, 1993). This species is distinguished from *S. camelopardula* Norris, 1965 by its cylindrical lorica and because *S. camelopardula* has a very slender cell body. It is also similar to *S. lefevrei* Bourrelly and *S. serpettei* Bourrelly in cell shape, but can be distinguished because the lorica of *S. tuba* is smaller (*S. lefevrei*, 26-46 μm long; *S. serpettei*, 28-30 μm) and attaches to the substrate by its posterior end (those of other two species attach by a stalk). The body of *S. tuba* is as long as the lorica, while the body of *S. lefevrei* and *S. serpettei* is shorter than the lorica. The cell length of *S. tuba* has been reported from 3.5 to 12 μm (Kent, 1880; Boucaud-Camou, 1967; Vørs, 1993; Tong, 1997a; Tong et al., 1997).

Distribution. Marine habitats in Australia, Belize, England, France and USA (Kent, 1880; Boucaud-Camou, 1967; Vørs, 1993; Tong, 1997a; Tong et al., 1997).

ACKNOWLEDGEMENTS

This work was supported by the Kyungnam University Research fund. I would like to thank Drs. Jock Keens (University of Sydney) and M. Wallace (University of Melbourne) for sampling, and thank three reviewers for critical comments.

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Received January 9, 2007
Accepted April 20, 2007