Aspidisca beringiana sp. nov. and *Simbiodisca* subgen. nov. (Ciliophora, Euplotida), a symbiont of terebellid polychaetes in the Bering Sea

A.W. Jankowski

A.W. Jankowski, Zoological Institute, Russian Academy of Sciences, Universitetskaya Emb. 1, St. Petersburg 199034, Russia. E-mail: ciliofora@rambler.ru

Terebellids in tidal zone of the Bering Island bear three new symbionts – rhabdophryid suctorians, peritrichs with small rosette-like colonies and aspidiscid hypotrich with a long peristome parallel to left body margin. This is the main feature of a new subgenus of the genus *Aspidisca*, named *Simbiodisca*. It may deserve the full generic rank if the use of protargol silvering method will not reveal any upper left rudiment of the peristomal membranelles.

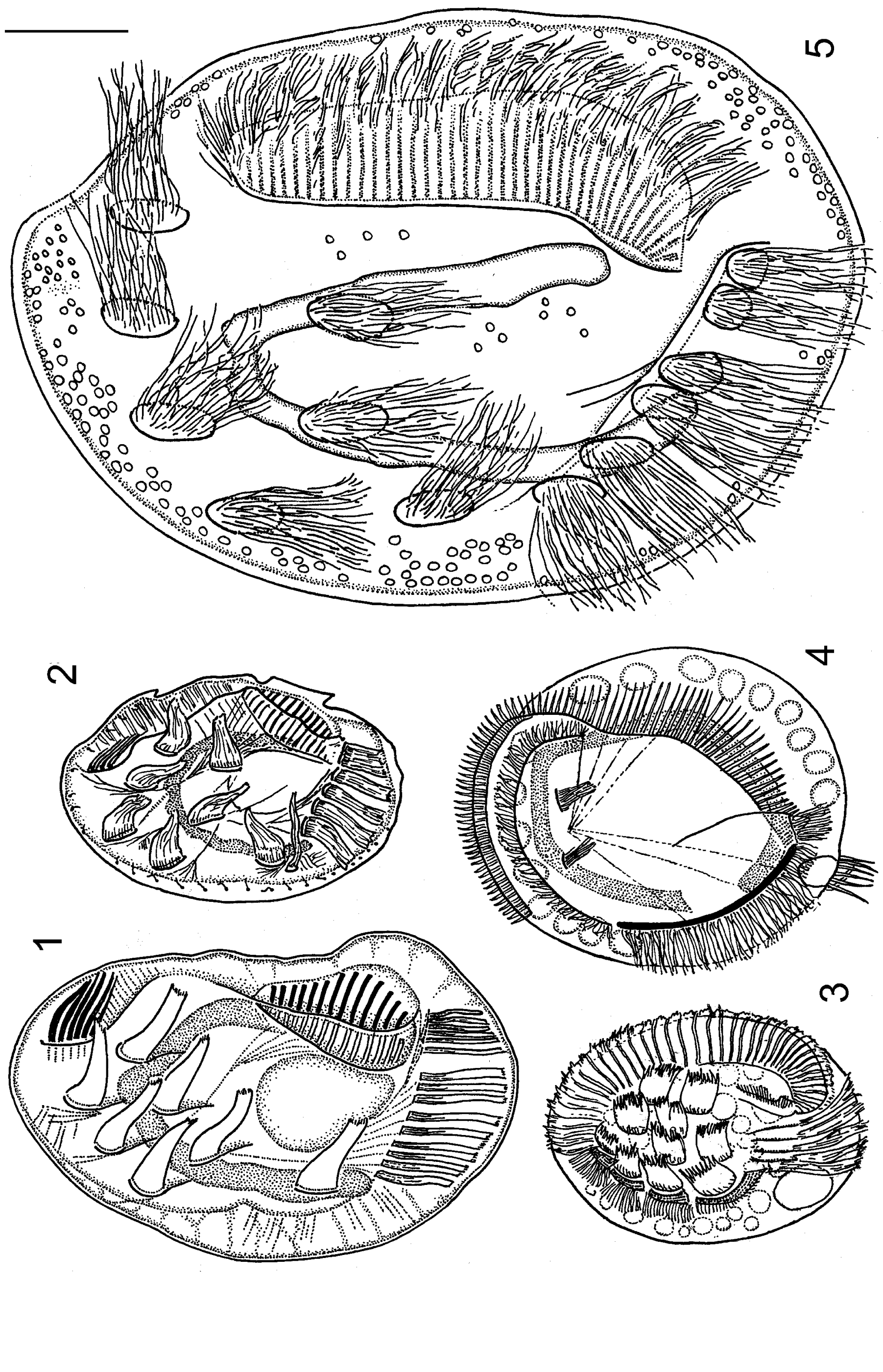
Key words: ciliate symbionts, terebellid polychaetes, Bering Sea, Ciliophora, Euplotida, *Aspidisca*, *Simbiodisca*, new species, new subgenus

INTRODUCTION

Euplotid ciliates are diverse and widespread in all biotopes – soil, terrophyton (mosses, lichens, grass), in marshes, lakes, rivers, in brackish estuaries and lagoons, and in the sea, with endemics in Antarctic ice areas. Symbiosis in stichotrichid hypotrichs and in euplotids (Figs 1–4) is an exception – *Kerona* Müller, 1786 on *Hydra* Linnaeus, 1758, two species of *Euplotes* Ehrenberg, 1831 (*E. tuffraui* Berger, 1965 and facultative *E. balteatus* (Dujardin, 1841) in sea urchins, *Euplotaspis* Chatton & Seguela, 1936 (Fig. 3) in tunicates (Chatton & Seguela, 1936; Burreson, 1973; Hill, 1979b), *Paraeuplotes* Wichterman, 1942 (Fig. 4) on corals (Wichterman, 1942) and in actinians (Welch, 1977).

In my samples, *Euplotaspis* occurs in tunicates of the Barents and White seas and on shores of the southern Sakhalin Island. Associations of euplotids *Diophrys* Dujardin, 1841, *Uronychia* Stein, 1859 (*U. bivalvorum* Fenchel, 1965), *Gastrocirrhus* Lepsi, 1928 (*G. stentoreus* Bullington, 1940 in sponges) and of several stichotrichs (like *Holosticha teredorum* Tucolesco, 1962, in teredinid wood borers) with marine invertebrates may be facultative. A large genus *Aspidisca* Ehrenberg, 1830 with world-wide distribution includes only free-living species.

While studying symbionts of marine invertebrates on the Bering Island shores in 1973, I found an *Aspidisca,* which is described here as a new species and a new subgenus, on almost each terebellid polychaete worm collected at low tide near the village of Nikolskoye. Other common symbionts were a new genus of rhabdophryid vermigenean suctorians for a species attached to host tentacles and new peritrichs, probably *Paravorticella* Kahl, 1933, forming small rosettes (one to four specimens on the basodisc). However, the latter identification is not certain for the type species of *Paravorticella* was not described in enough details in the literature. Local terebellids were examined later on the Barents Sea coast at the Dalniye Zelentsy Biostation. *Aspidisca* and suctorians were absent from this area and the examined worms bearid only commensal *Urceolaria* Stein, 1854, peritrichs with another type of the fixon – true *Paravorticella terebellae* (Faure-Fremiet, 1920), and some parasitic intestinal astomes.



Figs 1–5. Free-living and symbiotic euplotids: *Aspidisca polystyla* (from: Tuffrau, 1964) (**1**); *A. lyncaster* (from: Tuffrau, 1964) (**2**); *Euplotaspis cionaecola* (from: Chatton & Seguela, 1936) (**3**); *Paraeuplotes tortugensis* (from: Wichterman, 1942; combination of three drawings; part of symbiotic zooxanthellae omitted) (**4**); *Aspidisca* (*Simbiodisca*) *beringiana* **sp. nov.**, stained by methyl-violet (**5**). Scale bar: 10 µm.

MATERIAL AND METHODS

Terebellid worms were collected in tidal zone of Bering Island and neighbouring Mednyy Island (Commandor Islands) in Summer 1973; each worm was individually fixed in jars with 5–8% formalin or in half-diluted Bouin's fluid; both fixatives were diluted by sea water. Isolated symbionts (peritrichs, suctorians and a new species of *Aspidisca* described in this article) were accumulated in drops of diluted sugar on object glass, that was slowly drying (during 2–3 weeks).

In spite of crystallization, dry sugar perfectly retains ciliates and this method is commonly used by me for peritrichs and suctorians, for isolated cells or those intact on their small hosts (hydroids, nematodes, copepods, ostracods, mites). Stored cells may be liberated after adding a drop of water to dry sugar. Before drying, sugar remains long time in dense colloid-like state and fixed material is gradually saturated with sugar of raised concentration and thus persists crystallization. But now I prefer accumulation and storage of isolated fixed cells in glycerin drops in Petri dishes, to prevent from dust, or on object slides, in pure glycerin or in glycerin with hardener – smallest drop of diluted polyethylene-glycol 4000.

Only fixed material was studied in 2001, near 30 years after fixation of samples. At any fixation (less in diluted glutaraldehyde, strongly in formalin) proteins are coagulated, transparency is lost, and study of smallest ciliates becomes difficult. Protargol staining was not used; to reveal kinetome, I have used my method (Jankowski, 2007: 467) with few modifications. Although it was proposed for large hypotrichs only (like *Oxytricha* Bory de Saint Vincent, 1824, *Stylonychia* Ehrenberg, 1830 and *Urostyla* Ehrenberg, 1830), I obtained good staining of cirri in a species under study, except area of peristomal rudiments, too small and heavily granulated. Formol-fixed cells were isolated, washed in water and refixed in mixture of phosphotungstic acid with few glycerol, to prevent cell swelling; after new washing cells were stained in violet ink, differentiated by acetic acid and enclosed in drop of glycerin or sugar solution for study under cover glass. Violet ink, available at sale, is made of 2 different stains, acid or basic; this may be determined by absence or presence of clear water halo surrounding drop of ink on filter paper. Only basic stain (methyl violet) may be used, because acid ink does not permit differentiation of overstained cells. The figures 5–7 are made from such cells stained with basic ink.

TAXONOMIC PART

Order EUPLOTIDA Jankowski, 1980

This order is sometimes attributed to Small and Lynn (1985) but the author and date as given here are correct for the order was described (Jankowski, 1980) in a valid way (for comments see Jankowski, 2007: 480).

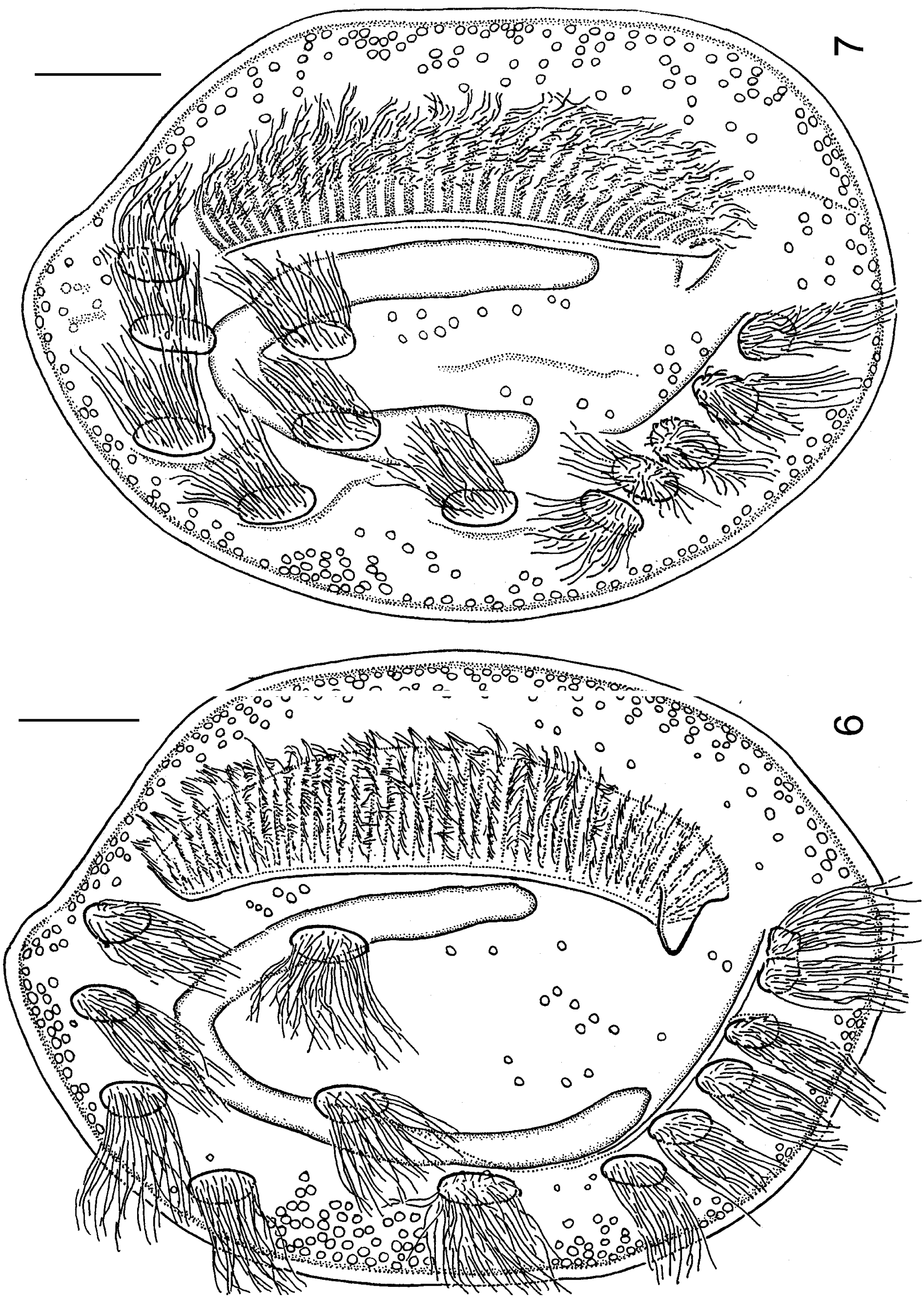
Family ASPIDISCIDAE Ehrenberg, 1838

Genus *Aspidisca* Ehrenberg, 1830

***Aspidisca beringiana* sp. nov.**
(Figs 5–7)

Hapantotype. A slide, 2001-14 (30 formol-fixed unstained specimens enclosed to drop of diluted sugar and then dried), deposited in the Laboratory of Protozoology, Zoological Institute, Academy of Sciences, Saint Petersburg, Russia. The Bering Island shores at Nikolskoye, 1973, coll. A.W. Jankowski.

Diagnosis. Body clear, flat, elongate-ovoid, with narrow, somewhat pointed anterior part (anteron or apex); general body shape resembles that of symbiotic hypostome *Allosphaerium* Kidder & Summers, 1935, inhabiting talitrid amphipods in the same region. Narrow hyaline margin or kiel surrounds entire body; body surface with few folds; cytoplasm densely granulated (layer of mitochondria); food vacuoles without refractile inclusions, such as



Figs 6, 7. *Aspidisca* (*Simbiodisca*) *beringiana* **sp. nov.**, stained by methyl-violet. Scale bar: 10 µm. The upper fragment of the peristome is not revealed using this staining method or it is absent.

diatoms. Macronucleus inverse V-shaped, usually with slightly unequal branches; micronucleus, possibly pale or small, was not detected after staining with acidified methyl-green.

Body size “in fixo” (not contracted in balsam; measurements of 10 cells in formalin or sublimate at oil immersion, 10 × 90) 67–76 × 48–54 μm.

As usually in euplotids, cilia composing cirri are separated at fixation and look like brushes or bundles, not as cones unlike those in living ciliates. The ventral kinetome includes 7 somatic cirri in 2 groups (4 + 3) and 5–6 caudal cirri in diagonal row. The peristome is long, extends parallel to the left body margin as a wide slightly curved cross-striated stripe with near 25–28 transversal membranelles; their bases are diminished at size to both ends of a stripe, but not so sharply as in other aspidiscids. The absence of upper rudiment on the drawings does not mean its absence in ciliates; it was not distinctly revealed in fixed cells after long storage in formalin; this species must be restudied later with protargol silvering. The argyrome and the dorsal setae were not revealed by the ink staining used.

Hosts: terebellid polychetes (family Terebellidae Malmgren, 1867) with dense crown of cephalic tentacles *Neoamphitrite figulus* (Dalyell, 1853) and *Thelepus cincinnatus* (Fabricius, 1780); additional host – *Cirratulus cirratus* (Müller, 1776), belonging to another family of polychaetes (Cirratulidae Ryckholt, 1851) with somatic tentacles on somites. The site of sampling – littoral at low tide near Nikolskoye, Bering Island, and on Mednyj Island (Commandor Islands, Bering Sea).

DISCUSSION

Almost all species of *Aspidisca* Ehrenberg, 1830 have small diagonal rhombic peristome with few membranelles (examples: Figs 1, 2). Only *A. orthopogon* Deroux & Tuffrau, 1965 is an exception – it has very long peristome composed of numerous membranelles, but it retains rhomboid shape with sharp diminution of membranellar width to the upper end (Deroux &Tuffrau, 1965; Li et al., 2008).

Except this species, any modern description of *Aspidisca* made by 4 procedures of silvering (methods of Klein, Chatton & Lwoff, Bodian and Fernandez-Galiano) may be used for comparison with a new symbiont, and all free-living species of *Aspidisca* are different from *A. beringiana* in peristomal shape and membranellar pattern. Some examples of this vast literature are publications of Tuffrau (1964), Hill (1979a), Foissner (1982), Dragesco & Dragesco-Kerneis (1986), Foissner et al. (1991), Augustin & Foissner (1992), Pang & Fu (1992), Song (1995, 2003), Wang et al. (1997), Song & Wilbert (2002), Chen & Song (2002), Song & Warren (2009).

Any large genus must include subgroups – groups of species that may be recognized as subgenera by structural features of minute significance. Example in ciliates is *Paramecium* Müller, 1773 with 3 subgenera based on body shape and degree of peristomal shift from cell apex. Attempt to distinguish subgenera in *Aspidisca* was made by me (Jankowski, 1979, 2007: 489) – subgenera *Aspidisca* Ehrenberg, 1830 s. str., *Dimaspidisca* Jankowski, 1979 and *Netaspidisca* Jankowski, 1979, were based, like those in *Euplotes*, on the pattern of dorsal argyrome. This alveolar net is difficult for silvering and is rarely revealed in *Aspidisca*.

Three groups of species distinguished in *Aspidisca* (Wu & Curds, 1979; Borror & Hill, 1995) are based on presence or absence of small additional cirri and, if present, on their location; these groups were not regarded as subgenera and have not received Latin names. At present, subgeneric value of dorsal argyrome pattern is denied in related genus *Euplotes*, and this genus was split into several genera with different cirral pattern (Borror & Hill, 1995). Thus separation of the above mentioned subgenera in *Aspidisca* based on differences in dorsal argyrome also is not acceptable, and instead only two distinct groups of species (subgenera) may be distinguished – *Aspidisca* Ehrenberg, 1830 s. str. with small rhombic oblique peristome carrying few diagonal membranelles and *Simbiodisca* subgen. nov. with a long peristome extending along almost the entire left cell margin, and carrying a large number of transversal membranelles.

Simbiodisca may be regarded as an intermediate form between ancestral euplotids, which possess a complete peristome typical for the hypotrichs, with anterior and lateral zones, and *Aspidisca* s. str. with two rudiments of the ancestral peristomal stripe.

***Simbiodisca* subgen. nov.**

Gender. Feminine.

Etymology. Subgeneric name is a combination of a word “symbiosis” with a generic name *Aspidisca*.

Type species: *Aspidisca beringiana* **sp. nov.**

Diagnosis. A long peristome; retention of a long membranellar stripe along the left body margin. Other characters as described for the type species.

Included species: type species.

Remark. If any anterior segment (rudiment) of a stripe is not revealed in the future by using silver protein or ammoniacal silver staining methods (the methods of Bodian and Fernandez-Galiano), this subgenus will need to be raised to the generic level.

REFERENCES

Augustin, H. & Foissner, W. 1992. Morphologie und Ökologie einiger Ciliaten (Protozoa: Ciliophora) aus dem Belebtschlamm. *Archiv für Protistenkunde*, **141**: 243–283.

Borror, A.Ch. & Hill, B. F. 1995. The order Euplotida (Ciliophora): taxonomy, with division of *Euplotes* into several genera. *Journal of Eukaryotic Microbiology*, **42**: 457–466.

Burreson, E.M. 1973. Symbiotic ciliates from solitary ascidians in the Pacific Northwest, with a description of *Parahypocoma rhamphisokarya* n. sp. *Transactions of the American Microscopical Society*, **92**: 517–522.

Chatton, E. & Seguela, J. 1936. Un hypotriche de la branchie de *Ciona intestinalis* L., intermèdiaire entre les Euplotidae et les Aspidiscidae: *Euplotaspis cionaecola* n. g., n. sp. *Bulletin de la Socièté Zoologique de France*, **61**: 332–340.

Chen, Z. & Song, W. 2002. Phylogenetic positions of *Aspidisca steini* and *Euplotes vannus* within the order Euplotida (Hypotrichia: Ciliophora) inferred from complete small subunit ribosomal RNA gene sequences. *Acta Protozoologica*, **41**: 1–9.

Deroux, G. & Tuffrau, M. 1965. *Aspidisca orthopogon*, n. sp. Rèvision de certains mècanismes de la morphogenèse a l'aide de une modification de la tèchnique au protargol. *Cahiers de Biologie Marine*, **6**: 293–310.

Dragesco, J. & Dragesco-Kerneis, A. 1986. Ciliès libres de l'Afrique intertropicale. *Faune Tropicale*, **36**: 1–559.

Foissner, W. 1982. Ökologie und Taxonomie der Hypotrichida (Protozoa: Ciliophora) einiger Österreichischer Boden. *Archiv für Protistenkunde*, **126**: 19–143.

Foissner, W., Blatterer, H., Berger, H. & Kohmann, F. 1991. Taxonomische und ökologische Revision der Ciliaten des Saprobiensystems. Band I. Cyrtophorida, Oligotrichida, Hypotrichia, Colpodea. *Informations-Berichte der Bayerischen Landesamtes für Wasserwirtschaft*, München, **1/91**: 1–478.

Hill, B.F. 1979a. Reconsideration of cortical morphogenesis during cell division in *Aspidisca* (Ciliophora, Hypotrichida). *Transactions of the American Microscopical Society*, **98**: 537–542.

Hill, B.F. 1979b. Structure and cortical morphogenesis during cell division in *Euplotaspis cionaecola* Chatton and Seguela, 1936 (Ciliophora, Hypotrichida). *Journal of Protozoology*, **26**, Supplement 3(1): 18.

Jankowski, A.W. 1979. Taxonomy and phylogeny of the order Hypotrichida Stein, 1859 (Protozoa, Ciliophora). *Proceedings of the Zoological Institute, Russian Academy of Sciences*, **85:** 48–85. (In Russian).

Jankowski, A.W. 1980. Conspectus of a new system of the phylum Ciliophora Doflein, 1901. *Proceedings of the Zoological Institute, Russian Academy of Sciences*, **94**: 103–121. (In Russian).

Jankowski, A.W. 2007. Phylum Ciliophora Doflein, 1901. Review of taxa. In: **Alimov, A.F.** (Ed.). *Rukovodstvo po zoologii* [Guide to Zoology]. Protists, **2**. Nauka, Saint Petersburg: 415–993. (In Russian).

Li, L., Shao, Ch., Yi, Zh., Song, W., Warren, A., Al-Rasheid, K.A.S., Al-Farraj, S.A., Al-Quraishy, S. A., Zhang, Q.Q., Hu, X., Zhu, M., Ma, H. 2008. Redescriptions and SSrRNA gene sequence analyses of two marine species of *Aspidisca* (Ciliophora, Euplotida) with notes on morphogenesis in *A. orthopogon*. *Acta Protozoologica*, **47**: 83–94.

Pang, Y. & Fu, Z. 1992. The morphology and morphogenesis of *Aspidisca costata*. *Journal of the East China Normal University*, Supplement 1992: 91–100.

Small, E.B. & Lynn, D.H. 1985. Phylum Ciliophora Doflein, 1901. *In*: **Lee, J.J., Hutner, S.H. & Bovee, E.C.** (Ed.). An illustrated guide to the Protozoa. Society of Protozoologists, Allen Press, Lawrence, Kansas: 393–575.

Song, W. 1995. Preliminary studies on the phylogenetic relationship of genera within the family Euplotidae (Ciliophora, Hypotrichida). *Oceanologica et Limnologica Sinica*, **26**: 527–534.

Song, W. 2003.Reconsideration of the morphogenesis in the marine hypotrichous ciliate, *Aspidisca leptaspis* Fresenius, 1865 (Protozoa, Ciliophora). *European Journal of Protistology*, **39**: 53–61.

Song, W. & Warren, A. 2009. Chapter 12. Protohypotrichs, discocephalids and euplotids. *In*: **Song, W., Warren, A. & Hu, X.** (Ed.). *Free-living ciliates in the Bohai and Yellow seas, China*. Science Press, Beijing: 415–459.

Song, W. & Wilbert, N. 2002. Faunistic studies on marine ciliates from the Antarctic benthic area, including descriptions of one epizoic form, 6 new species, and 2 new genera (Protozoa: Ciliophora). *Acta Protozoologica*, **41**: 23–61.

Tuffrau, M. 1964. La morphogenèse de bipartition et les structures neuromotrices dans le genre *Aspidisca* (ciliès hypotriches). Rèvue de quelques espèces. *Cahiers de Biologie Marine*, **5**: 173–199.

Wang, M., Mu, Zh., Zhang, Sh. & Song, W. 1997. Morphogenesis of *Aspidisca pulcherrima* (Protozoa: Ciliophora). *Zoological Research*, **18**: 185–188.

Welch, M.P. 1977. The apostome *Foettingeria* and other ciliate symbionts from the sea anemone *Bunodosoma cavernata* from Galveston Island, Texas. *Transactions of the American Microscopical Society*, **96**: 489–496.

Wichterman, R. 1942. A new ciliate from a coral of Tortugas and its symbiotic zooxanthellae. *Papers from the Tortugas Laboratory, Carnegie Institution of Washington*, **524**: 105–111.

Wu, I.C.H. & Curds, C.R. 1979. A guide to the species of the genus *Aspidisca*. *Bulletin of the British Museum (Natural History), Zoology Series*, **36**: 1–34.

Received 5 October 2009 / Accepted 20 November 2009