

# Studies on a new endocommensal ciliate, *Strombidium foissneri* nov. sp. (Ciliophora, Oligotrichida), from the intestine of the sea urchin *Hemicentrotus pulcherrimus* (Camarodonta, Echinoida)\*

Dapeng XU, Ping SUN, Weibo SONG & Alan WARREN

**Abstract:** The new oligotrich ciliate, *Strombidium foissneri* nov. sp., was isolated from the intestine of the sea urchin *Hemicentrotus pulcherrimus*. Its morphology in vivo and infraciliature were investigated based on observations of specimens from living and fixed protargol-impregnated cells. This new species is characterized as follows: in vivo approximately  $40\text{--}50 \times 30\text{--}45 \mu\text{m}$ ; cell asymmetric heart-shaped, tapering posteriorly and terminating in an elongated tail; apical protrusion prominent; one horseshoe-shaped macronucleus horizontally oriented; girdle kinety discontinuous with a small gap in the left ventral portion and composed of approximately 58 dikinetids; ventral kinety short, with ca. four dikinetids, positioned caudally on left margin; on average 15 anterior and seven ventral membranelles.

**Key words:** Marine ciliate, taxonomy, morphology, Strombidiida.

## Introduction

Taxonomic studies on the free-living oligotrichs have been carried out for more than a century (KENT 1881–1882; LEEGAARD 1915; KAHL 1932; MAEDA & CAREY 1985, MAEDA 1986). In recent years many new taxa have been reported and numerous known species have been reinvestigated using modern methods (SONG & BRADBURY 1998; AGATHA et al. 2005; SONG 2005, XU & SONG 2006). Compared with the free-living groups, little attention has been paid to the endocommensal oligotrichs, especially in sea urchins from which biotope only three species have been described to date: *Spirostrombidium echini*, *Strombidium rapulum* and *S. symbioticum* (YAGIU 1933; POLJANSKY 1951; JANKOWSKI 1974; SONG et al. 1999; XU et al. 2006).

During a survey of the endocommensal ciliates of sea urchins from the coastal waters of Qingdao, a previously unknown oligotrich ciliate was isolated from the intestine of *Hemicentrotus pulcherrimus* (Echinoida, Strongylocentrotidae). In this paper the morphology in vivo and infraciliature of the new isolate are documented and compared with those of similar taxa.

## Materials and methods

The host sea urchin *Hemicentrotus pulcherrimus* was collected from the coastal area off Qingdao (Tsingtao,  $36^{\circ}08'N$ ;  $120^{\circ}43'E$ ), China on November 16, 2005. The water temperature during sampling was about  $14^{\circ}C$ , with pH ca. 8.0 and salinity 29 ‰. In order to obtain ciliates, the intestine was removed from the host, placed in a Petri dish and washed with sterilized sea water.

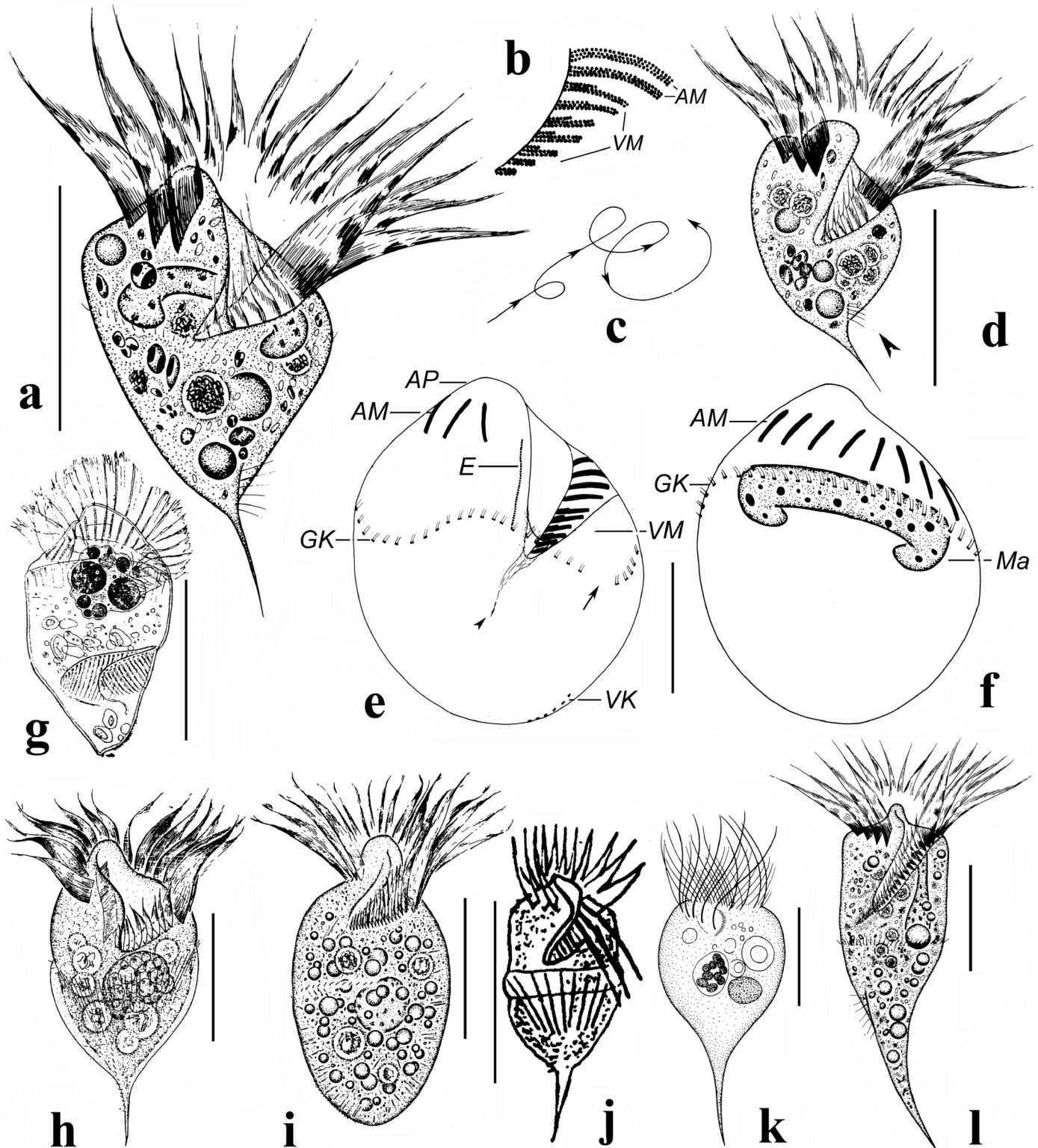
The behaviour of the ciliate in the Petri dish was examined under a dissecting microscope while its morphology was investigated under a compound microscope equipped with a high-power oil immersion objective and differential interference contrast optics. Protargol impregnation followed the protocol of WILBERT (1975). Counts, drawings (with the help of a camera lucida) and measurements were performed at a magnification of  $\times 1250$ .

Terminology is mainly according to AGATHA et al. (2005).

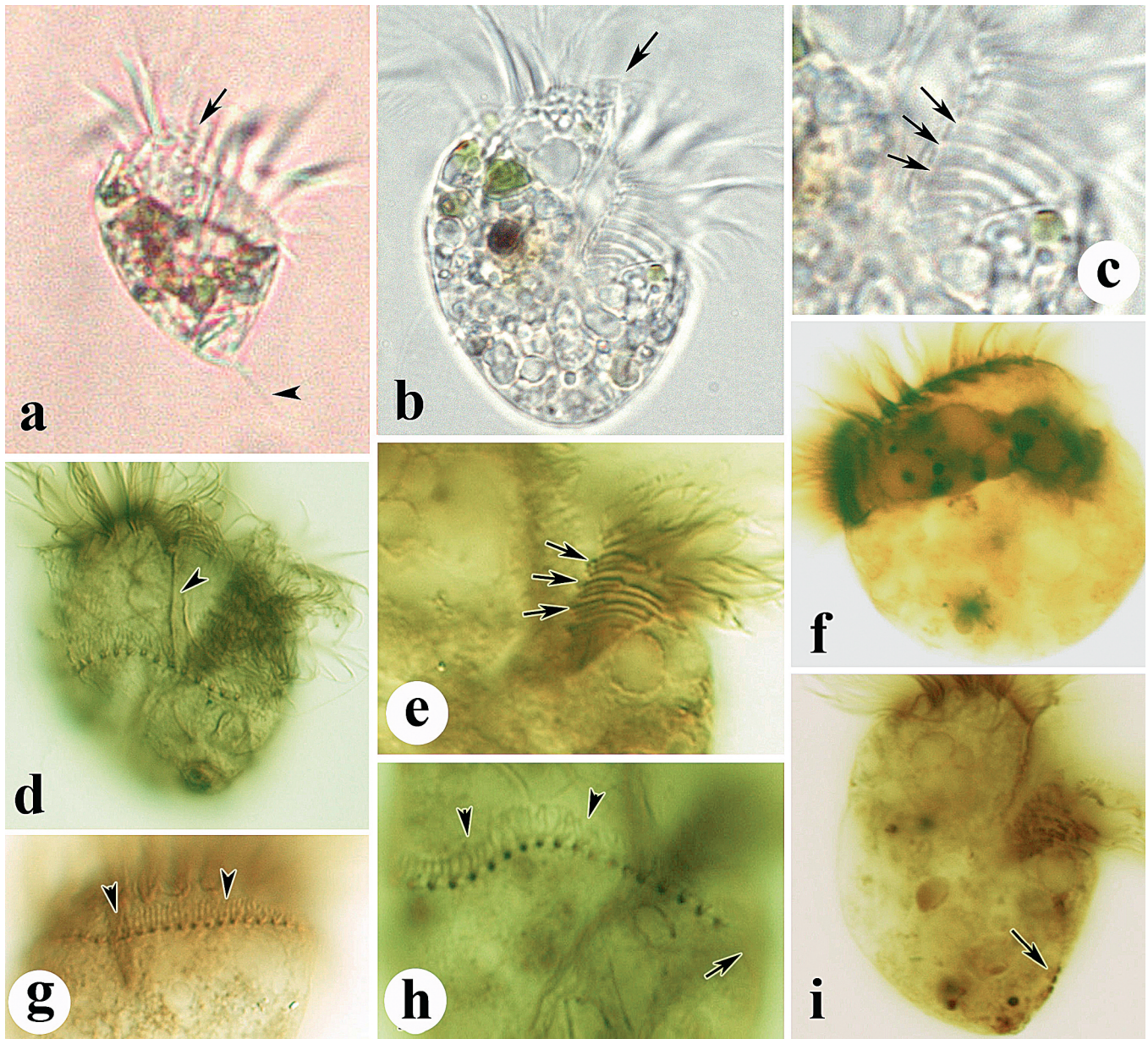
## Results and discussion

**Diagnosis:** Medium-sized, sea urchin-inhabiting *Strombidium*, in vivo approximately  $40\text{--}50 \times 30\text{--}45 \mu\text{m}$ ; cell asymmetric heart-shaped, tapering posteriorly with a pointed tail; apical protrusion prominent; one

\* The authors dedicate this article to Prof. Dr. Wilhelm FOISSNER, on the occasion of his 60<sup>th</sup> birthday.



**Fig. 1:** *Strombidium foissneri* nov. sp. from live cells (**a**, **c**, **d**), after protargol impregnation (**b**, **e**, **f**) and some morphologically similar species (**g**–**l**). **a**: Ventral view of a representative specimen. **b**: Details of ventral membranelles. **c**: Swimming trace. **d**: Shape variant; arrowhead marks the cilia of ventral kinety. **e**, **f**: Ventral and dorsal views of the same specimen, to show the infraciliature. Arrowhead marks pharyngeal fibres, arrow denotes gap in girdle kinety. **g**: *Strombidium symbioticum* JANKOWSKI, 1974 (from JANKOWSKI 1974). **h**: *Strombidium styliferum* LEVANDER, 1894 (from SONG & PACKROFF 1997). **i**: *Spirostrombidium echini* SONG et al., 1999 (from SONG et al. 1999). **j**: *Strombidium minor* (KAHL, 1932) MAEDA & CAREY, 1985 (from KAHL 1932, called "*S. calkinsi*"). **k**: *Strombidium longipes* MEUNIER, 1910 (from MAEDA & CAREY 1985). **l**: *Strombidium rapulum* (YAGIU, 1933) KAHL, 1934 (from XU et al. 2006). AM – anterior membranelles; AP – apical protrusion; E – endoral membrane; GK – girdle kinety; Ma – macronucleus; VK – ventral kinety; VM – ventral membranelles. Scale bars: 30 µm (**a**, **d**, **g**, **j**–**l**); 15 µm (**e**, **f**).



**Fig. 2:** Photomicrographs of *Strombidium foissneri* nov. sp. from live cells (**a–c**) and after protargol impregnation (**d–i**). **a:** A typical individual, arrow indicates the prominent apical protrusion, arrowhead marks the tail. **b:** A slightly compressed specimen, to show the apical protrusion (arrow). **c, e:** Details of ventral membranelles of a living (**c**) and a stained cell (**e**); note that the first kinety in each membranelle is shorter than the other two (arrows). **d:** Ventral view, arrowhead marks the endoral membrane. **f:** Horseshoe-shaped macronucleus. **g:** Dorsal view, to show girdle kinety; note that both basal bodies in each dikinetid bear short cilia (arrowheads). **h:** Ventral view, to show the argentophilic girdle (arrowheads) and the small gap of girdle kinety (arrow) on the left ventral portion. **i:** Arrow marks the ventral kinety. Length of live specimens 40–50  $\mu\text{m}$ , length of prepared specimens 30–42  $\mu\text{m}$ .

horseshoe-shaped macronucleus horizontally oriented; girdle kinety composed of approximately 58 dikinetids and discontinuous with small gap in left ventral portion; ventral kinety short, with ca. four dikinetids, positioned caudally on left margin; on average 15 anterior and seven ventral membranelles.

**Dedication:** This new species is dedicated to Prof. Dr. Wilhelm FOISSNER, in recognition of his outstanding contributions to ciliatology.

**Type location and host:** Type locality: coastal waters off Qingdao (Tsingtao, 36°08'N, 120°43'E), China. Host: the sea urchin *Hemicentrotus pulcherrimus* (Echinoida, Strongylocentrotidae). Host tissue: intestine.

**Type deposition:** One holotype and one paratype slide of protargol-impregnated specimens are deposited in the Laboratory of Protozoology, Ocean University of China (registration numbers 05111601, 05111602). One paratype slide is also deposited at the Natural History Museum, London (registration number 2007:11:1:1).

**Table 1:** Morphometric data for *Strombidium foissneri* nov. sp.

Characteristics <sup>a</sup>	Min	Max	Mean	Median	SD	n
Cell length	30	42	35.2	35.0	3.4	16
Cell width	22	36	29.8	30.0	3.8	16
Cell length: width, ratio	1.1	1.4	1.2	1.2	0.1	16
Apex to buccal vertex, distance	16	23	18.8	19.0	2.4	16
Anterior membranelles, number	14	16	15.1	15.0	0.9	16
Ventral membranelles, number	6	7	6.5	6.5	0.5	16
Girdle kinety, number of dikinetids	53	62	57.3	57.5	2.9	10
Ventral kinety, number of dikinetids	3	5	4.0	4.0	0.6	10

<sup>a</sup> Data based on randomly selected, protargol-impregnated specimens. Measurements in  $\mu\text{m}$ . Max – maximum; Min – minimum; n – number of specimens investigated; SD – standard deviation.

**Morphological description** (Fig. 1, 2; Tab. 1): Cell size rather constant, mostly  $45 \times 40 \mu\text{m}$ . Usually asymmetric heart-shaped, not flattened or only slightly flattened dorsoventrally (Fig. 1a, d, 2a, b). Anterior end domed, always obliquely truncated with prominent, hyaline apical protrusion (ca.  $5 \mu\text{m}$  high) at right side of peristome that can be recognized in vivo but may disappear or be undetectable after protargol impregnation (Fig. 1a, d, 2a, b). Cell tapering posteriorly and terminating in an elongated tail (Fig. 1a, d, 2a). Tail thin, fragile but not contractile, about 25–35 % cell length, often curving towards left and difficult to detect when compressed by cover-glass or after staining (Fig. 2b). Buccal cavity deep and prominent, extending towards right side of cell and terminating at about equatorial region (Fig. 1a, 2b). Pellicle thin, no hemitheca observed. Cytoplasm colourless, often filled with numerous granules; several to many food vacuoles (usually containing algae including diatoms), giving cell grey to green-brown appearance (Fig. 2a, b). No extrusomes recognized in vivo although an argentineophilic girdle anterior to the girdle kinety is revealed in protargol-impregnated specimens (arrowheads in Fig. 2h). Single macronucleus horseshoe-shaped, horizontally oriented and containing many small chromatin aggregates (diameter ca.  $2\text{--}4 \mu\text{m}$ ; Fig. 1f, 2f). Micronucleus, contractile vacuole, and cytophyge not observed.

Locomotion with two patterns: rotating moderately fast and irregularly around cell axis when swimming with cilia of anterior membranelles stretching anteriorly (Fig. 1a, c, d), or moving very fast when crawling on debris, using its adoral membranelles for attachment with ventral side facing down.

Oral apparatus typical of genus. Anterior portion of adoral zone with 14–16 membranelles, ventral portion with six or seven membranelles (Fig. 1e, 2e). Bases of anterior membranelles about  $8 \mu\text{m}$  long, each composed of three kinety rows; cilia approximately  $25 \mu\text{m}$  long. Bases of ventral membranelles about  $3\text{--}6 \mu\text{m}$  long, gradually shortening from anterior to posterior. Most ventral

membranelles composed of three kinety rows; first row approximately 35–50 % length of the other two (Fig. 1b, 2c, e, arrows); in proximal one or two ventral membranelles only two kinety rows were observed (Fig. 1b). Endoral membrane on inner wall of buccal lip on right side of buccal cavity, composed of a single row of densely packed basal bodies (Fig. 1e, 2d, arrowhead). Pharyngeal fibres approximately  $10 \mu\text{m}$  in length (Fig. 1e, arrowhead).

Somatic ciliature as shown in Fig. 1e, f, 2g–i, comprising a girdle and a ventral kinety. Girdle kinety horizontally positioned and forming an incomplete circle; with a small gap about  $5 \mu\text{m}$  wide on left ventral portion (Fig. 1e, 2h, arrow), composed of approximately 57 (range 53–62) dikinetids, both basal bodies bearing a short (ca.  $2.5 \mu\text{m}$ ) cilium (Fig. 2g, h, arrowheads). Ventral kinety reduced, positioned at base of tail, extending longitudinally along left margin of cell and composed of approximately four (three to five) dikinetids, cilia about  $6 \mu\text{m}$  long (Fig. 1d, arrowhead, 2i, arrow).

**Comparison with related species:** Only three oligotrich ciliates inhabiting the intestine of sea urchins have previously been reported: *Spirostrombidium echini*, *Strombidium rapulum* and *S. symbioticum* (YAGIU 1933; POLJANSKY 1951; JANKOWSKI 1974; SONG et al. 1999; XU et al. 2006).

*Spirostrombidium echini* SONG et al., 1999, which was isolated from unidentified sea urchins collected from the Weddell Sea, Antarctica (SONG et al. 1999), clearly differs from *Strombidium foissneri* in that it lacks a tail (vs. tail present in *S. foissneri*) and in having a different somatic ciliary pattern (*Spirostrombidium*-like vs. *Strombidium*-like in *S. foissneri*) (Fig. 1i; SONG et al. 1999).

*Strombidium rapulum* (YAGIU, 1933) KAHL, 1934 has been reported from various sea urchins but its infraciliature has only recently been revealed (XU et al. 2006; Fig. 1l). It can be separated from the new species

by the following combination of features: (1) body shape (elongated-conical vs. asymmetric heart-shaped); (2) body size in vivo ( $50\text{--}130 \times 25\text{--}65 \mu\text{m}$  vs.  $40\text{--}50 \times 30\text{--}45 \mu\text{m}$ ); (3) shape of macronucleus (elongated ellipsoidal vs. horseshoe-shaped); (4) number of ventral membranelles (28–31 vs. six or seven), and (5) position of ventral kinety (located at the posterior right side of cell vs. located at the posterior left side of cell (XU et al. 2006).

*Strombidium symbioticum* JANKOWSKI, 1974 was found in the stronglylocentrotid sea urchin *Strongylocentrotus intermedius* (JANKOWSKI 1974). Although its infraciliature remains unknown it can easily be separated from *Strombidium foissneri* by the absence of a tail (vs. tail present) and the shape of its macronucleus (irregular globular vs. horseshoe-shaped). Furthermore, *S. symbioticum* might have more adoral membranelles since the illustrations in the original report show c. 30 membranelles in total (vs. 22 in *S. foissneri*) (Fig. 1g; JANKOWSKI 1974).

Three other marine *Strombidium* species (*S. styliferum*, *S. minor* and *S. longipes*) have a pointed tail and should therefore be compared with *S. foissneri* although each is free-living and none has so far been isolated from sea urchins. *Strombidium styliferum* LEVANDER, 1894 differs from *S. foissneri* by the following combination of characters: presence of conspicuous extrusomes (vs. extrusomes absent or not recognizable); shape of macronucleus (globular vs. horseshoe-shaped); number of ventral membranelles (9–11 vs. six or seven) (Fig. 1h; SONG & PACKROFF 1997).

*Strombidium minor* (KAHL, 1932) MAEDA & CAREY, 1985 can be clearly separated from *S. foissneri* by the possession of three prolonged membranelles (vs. absent in *S. foissneri*), and the presence of a contractile vacuole and extrusomes (vs. absent or not recognizable in *S. foissneri*) (Fig. 1j; KAHL 1932; MAEDA & CAREY 1985).

Although *Strombidium longipes* MEUNIER, 1910 is poorly known, it can be separated from *S. foissneri* by the shape of its macronucleus (ovoid vs. horseshoe-shaped), and its larger body (ca.  $80 \mu\text{m}$  vs.  $40\text{--}50 \mu\text{m}$ ) (Fig. 1k; MEUNIER 1910).

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#### Addresses of authors:

Dr. Dapeng XU  
Dr. Ping SUN  
Dr. Weibo SONG (corresponding author)  
Laboratory of Protozoology  
Ocean University of China  
Qingdao 266003  
China  
E-mail: [wsong@ouc.edu.cn](mailto:wsong@ouc.edu.cn)

Dr. Alan WARREN  
Department of Zoology  
The Natural History Museum  
Cromwell Rd, London SW7 5BD  
United Kingdom  
E-mail: [A.Warren@nhm.ac.uk](mailto:A.Warren@nhm.ac.uk)

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