J. Mar. Biol. Ass. U.K. (2006), **86**, 823–828
Printed in the United Kingdom

Studies on an endoparasitic ciliate *Boveria labialis* (Protozoa: Ciliophora) from the sea cucumber, *Apostichopus japonicus*

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The morphology and infraciliature of an endoparasitic ciliate, *Boveria labialis*, isolated from respiratory trees of the sea cucumber, *Apostichopus japonicus*, were investigated using living observation and silver impregnation methods. Based on the present and previous studies, an improved diagnosis is supplied: marine *Boveria*, size *in vivo* about 30–100×15–30 μm, body slender and flask-shaped, with a protruding lobe measuring 5–15 μm in length; one ovoid macronucleus and one micronucleus; single contractile vacuole positioned in posterior ¼ of body length; 17–26 somatic kineties; paroral membrane and membranelle 2 forming a double anticlockwise spiral of nearly two turns.

INTRODUCTION

Ciliates assigned to the scuticociliate genus *Boveria* are parasites of some marine animals, e.g. infecting the respiratory trees of Holothuroidea (sea cucumber) and gills of Bivalvia (molluscs) by attaching to inner or outer walls of these organs (Stevens, 1901; Issel, 1903; Kahl, 1931; Chatton & Lwoff, 1949; Fenchel, 1965; Santhakumari & Nair, 1973; Table 1). These parasites sometimes also encyst and sink in connective tissues (Ikeda & Ozaki, 1918). Heavy infection may strongly weaken the respiratory function of the hosts and lead to secondary bacterial diseases (Ikeda & Ozaki, 1918; Xu & Song, 2000).

Taxonomically, four nominal forms of *Boveria* have been reported to date: *B. subcylindrica* Stevens, 1901, *B. subcylindrica* var. *concharum* Issel, 1903, *B. labialis* Ikeda & Ozaki, 1918 and *B. teredinidi* Nelson, 1923. Among these, however, only one species namely *B. subcylindrica* was

Table 1. Boveria *species and their hosts.*

Hosts	*Boveria labialis*[a]	*B. labialis*[b]	*B. subcylindrica*[b]	*B. subcylindrica* var. *concharum*[b]	*B. teredinidi*[b]
Apostichopus japonicus[c]	+				
Capsa fragilis[d]				+	
Cardita sulcata[d]				+	
Cucumaria sp.[c]		+			
Donax politus[d]				+	
Loripes lacteus[d]				+	
Parastichopus californicus[c]			+		
Pinna nobilis[d]				+	
Pinna pectinata[d]			+		
Stichopus japonicus[c]		+	+		
Tapes decussata[d]				+	
Tellina exigua[d]				+	
Tellina nitida[d]				+	
Tellina planata[d]				+	
Tellina sp.[d]		+			
Teredo navalis[d]					+
Venus gallina[d]				+	

a, Based on the Dalian population; b, from Raabe, 1970; c, species belong to sea cucumber Holothuroidea; and d, species belong to mollusc Bivalvia.

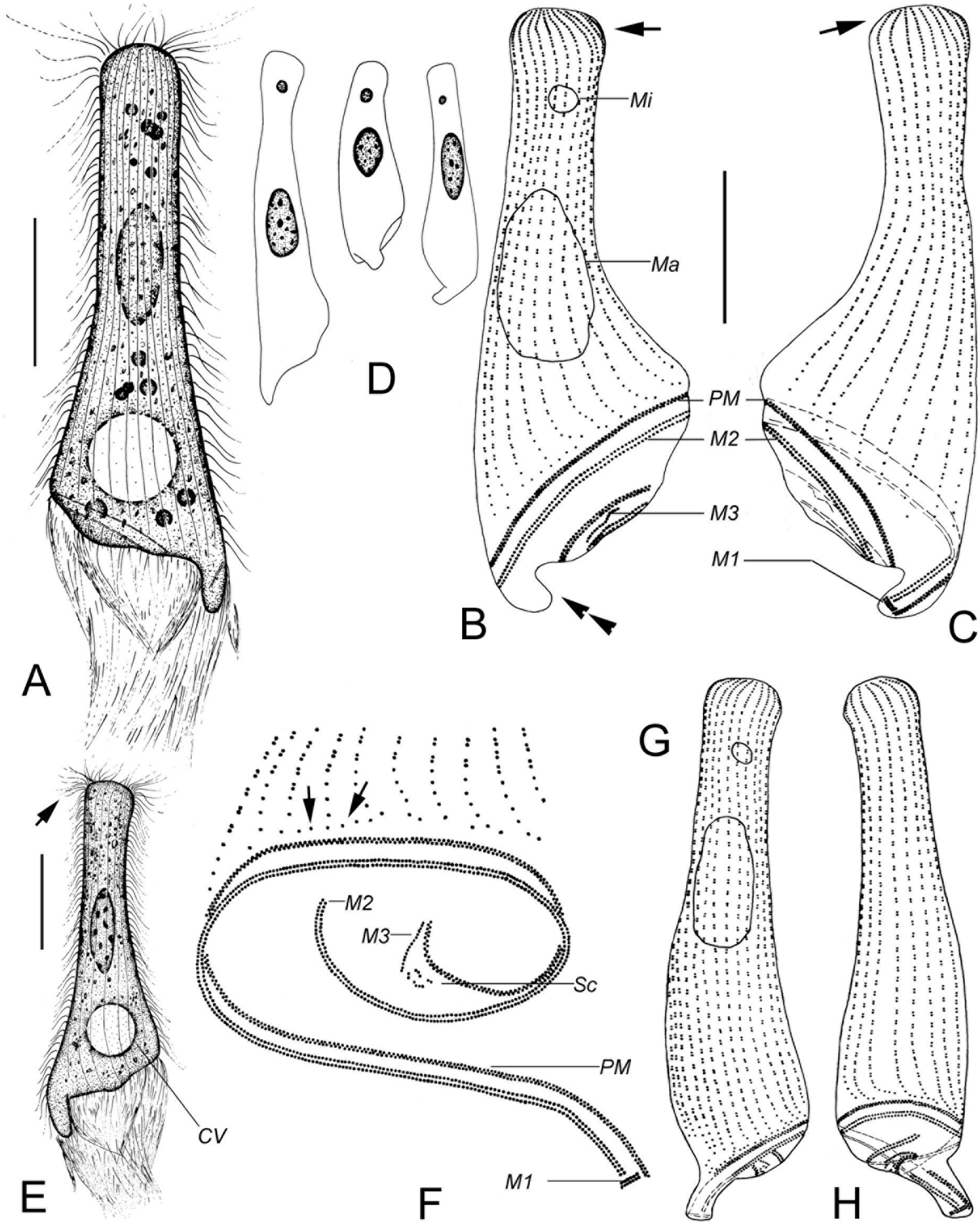

Figure 1. (A & E), *Boveria labialis* Ikeda & Ozaki, 1918 from life; and (B–D,F–H) after protargol impregnation. (A & E) General view of typical cells, arrow shows long cilia of the anterior end of the body; (B,C,G&H) infraciliature, arrows mark densely arranged basal bodies, double-arrowheads indicate the protruding lobe; (D) to show different body shape and the nuclear apparatus; and (F) oral apparatus. CV, contractile vacuole; M1–3, membranelles 1–3; Ma, macronucleus; Mi, micronucleus; PM, paroral membrane; Sc, scutica. Scale bars: 20 μm.

investigated using the protargol impregnation method and all the rest remain unclear at the infraciliature level (Raabe, 1970; Xu & Song, 2000). This renders difficulties in accurate identification and species separation of these taxa.

As a new contribution, the present paper gives a redescription of *Boveria labialis*, based on both living observation and silver staining methods. This is the first study to reveal the infraciliature of this known species.

MATERIALS AND METHODS

The host sea cucumbers (*Apostichopus japonicus*), collected from an offshore mariculture pond near Dalian (39°01′N 121°44′E), China (temperature 20°C, salinity 27), were anatomized and the respiratory trees were checked. After isolation of the ciliates, observations on living cells were carried out with a microscope equipped with Normarski

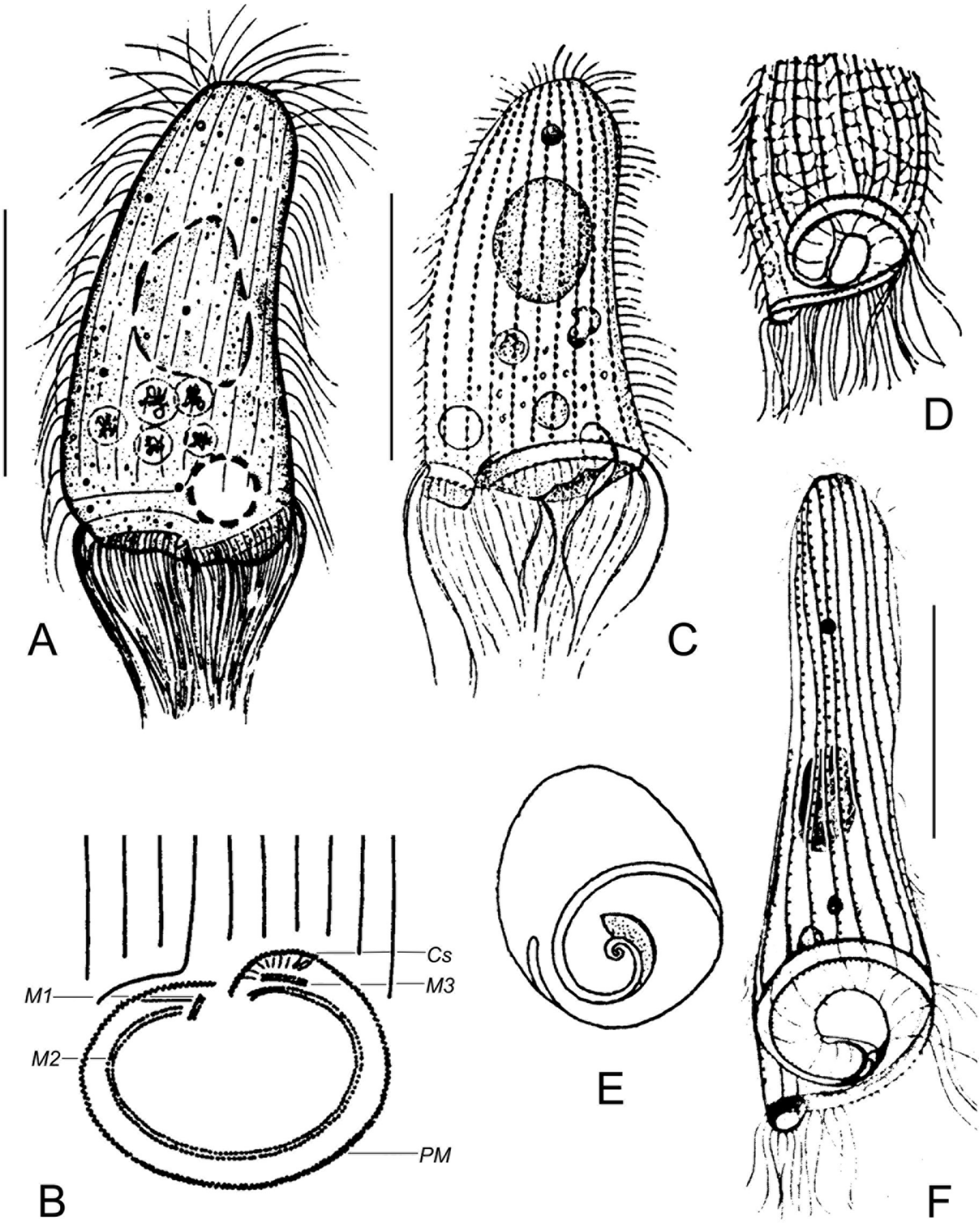

Figure 2. Views and oral field of *Boveria subcylindrica* Stevens, 1901 (A & B from Xu & Song, 2000), *B. subcylindrica* var. *concharum* Issel, 1903 (C & E from Raabe, 1970) and *B. teredinidi* Nelson, 1923 (D & F from Pickard, 1927). Cs, cytostome. Scale bars: 30 μm.

differential interference optics. Protargol (Wilbert, 1975) and silver carbonate (Ma et al., 2003) methods were used for revealing the infraciliature.

Drawings of impregnated specimens were conducted with the help of a camera lucida; measurement was performed under ×1250 magnification. The systematic theme and terminology are according to Corliss (1979). Voucher slides with protargol impregnated specimens are deposited in the Laboratory of Protozoology, Ocean University of China, China, registration numbers: 2004122001-1, 200412201-2, 2004122001-3.

RESULTS

Systematic position of Boveria labialis

The ciliate we described systematically belongs to the order Scuticociliatida and family Ancistridae according to Corliss (1979).

Improved diagnosis for Boveria labialis *Ikeda & Ozaki, 1918*

Flask-shaped *Boveria* with a protruding lobe in adoral region, size *in vivo* about 30–100×15–30 μm; one ovoid macronucleus and one micronucleus; conspicuous

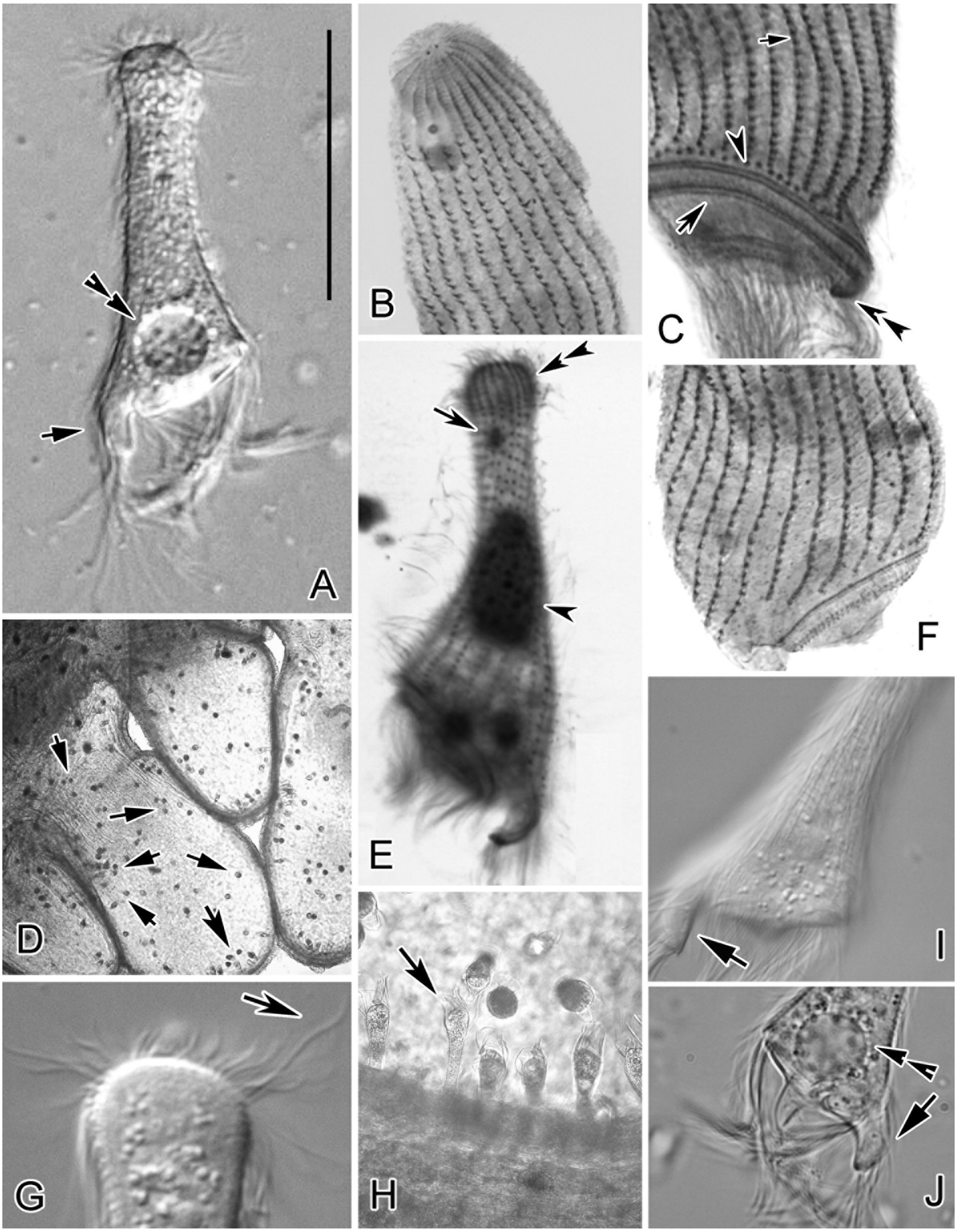

Figure 3. (A,D,G,H,I & J) *Boveria labialis* Ikeda & Ozaki, 1918 from life, (E) after protargol staining; and (B,C &F) silver carbonate impregnation. (A) A typical cell, arrow shows the protruding lobe, double arrowheads indicate the contractile vacuole; (B) apical view; (C & F) posterior portion, to note paroral membrane (arrowhead), membranelle 2 (arrow), membranelle 1 (double-arrowheads) and myonemes (small arrow); (D) respiratory trees of a sea cucumber, arrows mark cells attaching to the inner wall of respiratory trees; (E) infraciliature, showing the macronucleus (arrowhead), micronucleus (arrow) and densely arranged basal bodies (double-arrowheads) at the aboral end; (G) anterior portion, arrow marks the long cilia of aboral end; (H) arrow indicates an individual adhering to the epithelium of host with its aboral end; and (I & J) arrows note the protruding lobe; double arrowheads refer to the contractile vacuole. Scale bar: 50 μm.

Table 2. *Morphometric data of the Dalian population of* Boveria labialis. *Data are based on protargol impregnated specimens.*

Character	Minimum	Maximum	Mean	SD	CV	N
Length of body	46	100	73	12.82	17.5	25
Width of body	16	30	21	3.97	18.9	26
Number of somatic kineties	17	22	20	1.33	6.7	27
Number of macronucleus	1	1	1	0	0	27
Length of macronucleus	11	22	15	2.66	17.2	28
Width of macronucleus	7	14	10	2.32	22.5	28
Number of micronucleus	1	1	1	0	0	27
Length of the protruding lobe	5	9	7	0.98	14.4	21

Measurements in μm. CV, coefficiency of variation in%; N, number of cells measured; SD, standard deviation.

protruding lobe measuring 5–15 μm; contractile vacuole positioned in posterior $^1/_4$ of body length; 17–26 somatic kineties; paroral membrane and membranelle 2 forming a double anticlockwise spiral of nearly two turns. Infection on inner walls of respiratory trees of sea cucumbers.

Morphological description of Boveria labialis *Ikeda & Ozaki, 1918 from respiratory trees of the sea cucumber,* Apostichopus japonicus (Figures 1 & 3; Table 2)

Body usually slender and flask-shaped, size *in vivo* mostly about 70×25 μm. Adoral end obliquely truncated, with a conspicuous protruding lobe measuring 5–9 μm in length (Figures 1B,E & 3A,I,J). Margins of anterior half of the cell straight and in parallel, whereas posterior half gradually widened, reaching the maximum width at oral field. Pellicle thin, no extrusomes distinguishable *in vivo*. Cytoplasm colourless and hyaline, often with several small food vacuoles and shining globules (Figures 1A,E & 3G). One contractile vacuole, about 12 μm in diameter, located at posterior $^1/_4$ of body length (Figures 1A,E & 3A,J). Macronucleus ovoid, single micronucleus spherical in shape, usually positioned anteriorly to macronucleus (Figures 1D & 3E). Oral cilia about 25 μm long *in vivo* (Figures 1A,E & 3A). Somatic cilia about 5–7 μm in length, slowly undulating when isolated; two types of cilia distinguishable with obviously different length (12 vs 6 μm) at aboral end (Figures 1E & 3G), with which cells attach to tissues of hosts (Figure 3D,H).

Swimming only for a very short while (usually several seconds) before attaching: moderately fast, rotating around long axis of body, and then sticking firmly to the bottom or the wall of Petri dish. No culture could be maintained; this species might live in isolation for several hours at room temperature.

Infraciliature as shown in Figure 1B,C,F–H. Somatic ciliature composed of 17–22 (mostly 20) bipolar rows of dikinetids. Several monokinetids positioned beside the end of each somatic row and adjacent to the paroral membrane (PM). Basal bodies on aboral end densely arranged (Figures 1B,C,F & 3B,C,F).

Oral field rounded. Membranelle 1 (M1) composed of two short rows of basal bodies, positioned on the protruding lobe and perpendicular to the PM. Membranelle 2 (M2) two-rowed, whereas basal bodies of PM arranged in zig-zag pattern. Membranelle 2 and PM about equal in length, both starting at the protruding lobe and forming a double anticlockwise spiral of nearly two turns; membranelle 3 (M3) relatively short, consisting of a single row of basal bodies, with some sparsely distributed scutica beside (Figures 1B,C,F & 3C).

DISCUSSION AND COMPARISON

Boveria labialis was first isolated and reported by Ikeda & Ozaki (1918) from the respiratory trees of two sea cucumbers, *Cucumaria* sp. and *Stichopus japonicus*, as well as from gill lamellae of a shellfish, *Tellina* sp., which documented the living features in detail: body trumpet-shaped, aboral end rounded, oral strongly widened equipped with a distinctly protruding lobe, on which the adoral kineties start their course. Hence, it corresponds perfectly with the Dalian population considering the body shape, size, biotope and especially the presence of the protruding lobe (Table 3). We are therefore convinced about the identification of our organism.

To date, four nominal morphotypes in the genus have been reported: *Boveria subcylindrica* Stevens, 1901, *Boveria subcylindrica* var. *concharum* Issel, 1903, *Boveria labialis* Ikeda & Ozaki, 1918 and *Boveria teredinidi* Nelson, 1923. Among them, only *Boveria subcylindrica* Stevens, 1901 was investigated using the protargol impregnation method (Xu & Song, 2000) and all the rest remain unclear at the infraciliature level.

Boveria subcylindrica can be easily separated from *B. labialis* by the absence of the protruding lobe, a

Table 3. *Comparison of* Boveria *species.*

Species	Body shape	Body length	Protruding lobe	Data source
Boveria labialis	flask-shaped	45–100	conspicuous, 5–9 μm	present work
B. labialis	flask-shaped	30–100	conspicuous, 10–15 μm	Ikeda & Ozaki, 1918
B. subcylindrica	truncate	54–81	absent	Xu & Song, 2000
B. subcylindrica var. *concharum*	truncate	27–169	absent	Issel, 1903; Pickard, 1927
B. teredinidi	slender	27–173	absent	Pickard, 1927

Measurements in μm.

conspicuously cylindrical body shape (vs flask-shaped or trumpet-shaped) and a one-turn double anticlockwise spiral of the oral structure which is formed by PM and M2 (vs two turns in *B. labialis*) (Figure 2A,B; Table 3) (Xu & Song, 2000).

Considering the body size and general appearance, *Boveria teredinidi* Nelson, 1923 slightly resembles *B. labialis* as well. The former can be identified as it lacks the protruding lobe at the *in vivo* level. In addition, *B. labialis* is distinctly flask-shaped or trumpet-shaped (vs truncate) (Figure 2D,F; Table 3) (Pickard, 1927; Raabe, 1970).

Another similar morphotype, *Boveria subcylindrica* var. *concharum* Issel, 1903 differs from *B. labialis* in the body shape (elongate vs flask-shaped or trumpet-shaped) and also lacking the protruding lobe (Figure 2C,E; Table 3) (Issel, 1903).

This work was supported by the Natural Science Foundation of China (Project no. 30430090). Help in supplying samples by Mr Xiaojun Rong (Yellow Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences) is gratefully acknowledged.

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Submitted 24 December 2005. Accepted 24 March 2006.