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Morphological redescription of two endocommensal ciliates, *Entorhipidium fukuii* Uyemura, 1934 and *Madsenia indomita* (Madsen, 1931) Kahl, 1934 from digestive tracts of sea urchins of the Yellow Sea, China (Ciliophora; Scuticociliatida)

Hongan Long[a], Weibo Song[a,b,*], Yangang Wang[a], Jiqiu Li[b]

[a]*Laboratory of Protozoology, KLM, Ocean University of China, 266003, Qingdao, P.R. China*
[b]*Laboratory of Protozoology, College of Life Science, South China Normal University, Guangzhou 510631, China*

Abstract

Definitions of the genera *Entorhipidium* and *Madsenia* have been updated on the basis of the results of studies on the living morphology and infraciliature of the endocommensal ciliates, *Entorhipidium fukuii* Uyemura, 1934 and *Madsenia indomita* (Madsen, 1931) Kahl, 1934, isolated from digestive tracts of the sea urchin, *Hemicentrotus pulcherrimus*. *Entorhipidium* are flattened marine endocommensal scuticociliates with a sigmoid body shape and conspicuous tail; the buccal cavity is in the anterior half of the cell and the buccal ciliature has a *Uronema*-like pattern; the somatic kineties form both apical and post-oral sutures. Because of this new definition, a new combination is suggested: *Entorhipidium caudatum* (Poljansky, 1951) nov. comb. (basionym: *Cryptochilidium caudatum* Poljansky, 1951). *Madsenia* are flattened, slender-bodied endocommensal scuticociliates, having a buccal cavity in the anterior half of the cell with M1 and M2 fused into a single structure and both M3 and the paroral membrane short; the somatic kineties form an anterior suture; no caudal bristle was observed.
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Keywords: *Entorhipidium fukuii*; *Madsenia indomita*; Sea urchins; Endocommensal ciliates; *Entorhipidium caudatum* nov. comb.; Morphology and infraciliature
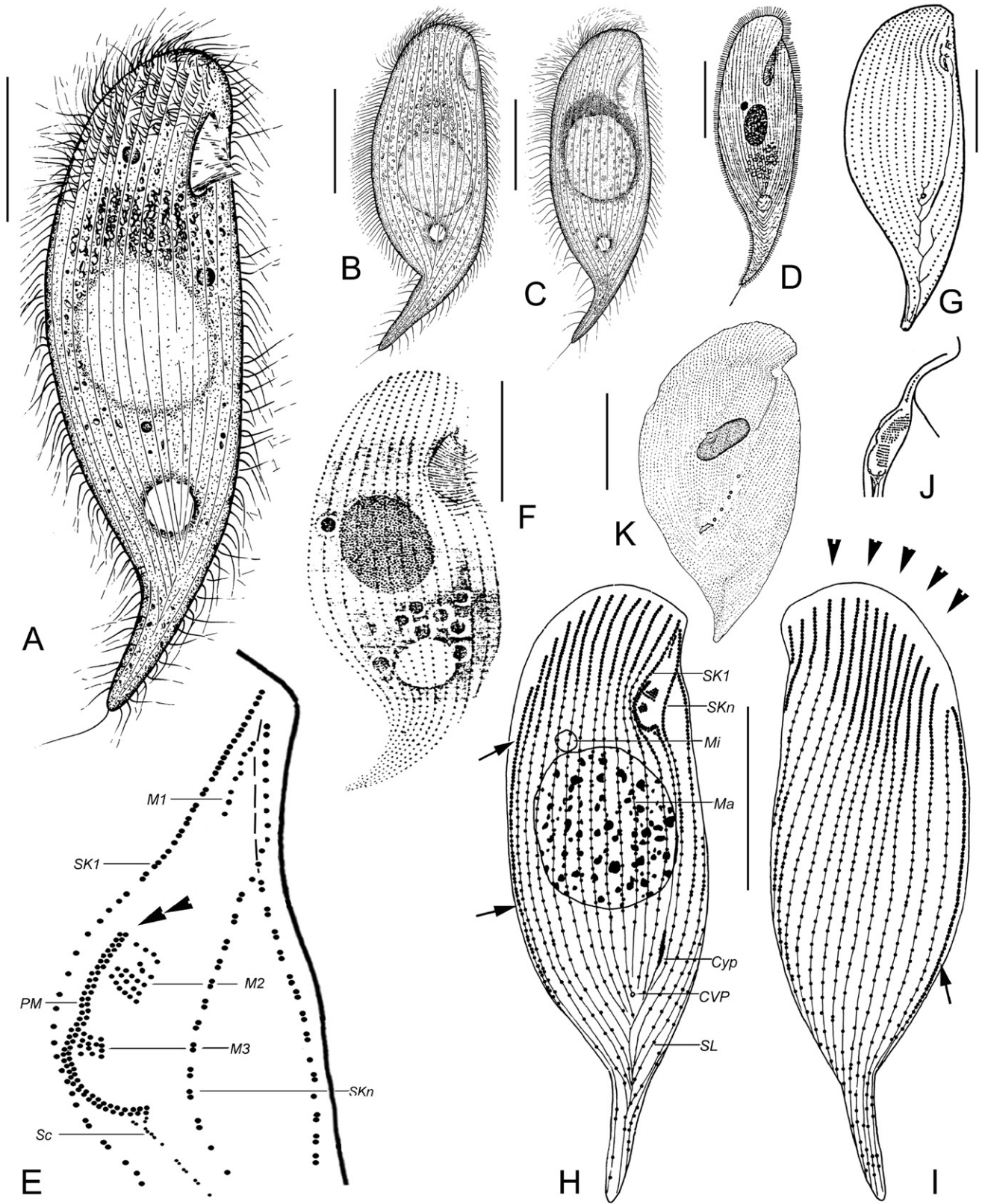
Introduction

Endocommensal ciliates of echinoderms, especially those highly flattened scuticociliates inhabiting the intestines of sea urchins, have attracted the attention of many researchers (Berger 1960, 1961, 1964, dissertation; Hentschel 1924; Jankowski 1973, 1974, 1980; Kahl 1934; Lynch 1929; Madsen 1931; Maupas 1883; Poljansky 1951; Powers 1933a, b, 1935; Profant 1965, dissertation; Russo 1914; Uyemura 1934; Yagiu 1934). However, only a few of these studies were carried out using modern methods, and hence species identification in most groups remains difficult (Berger 1960, 1961, 1964, dissertation, 1965; Foissner 1985; Long et al. 2006; Lynn and Berger 1972, 1973; Lynn and Frombach 1987; Profant 1965, dissertation; Song et al. 1999).

In the summer of 2005, two poorly known endocommensal ciliates were isolated from digestive tracts of the sea urchin *Hemicentrotus pulcherrimus* and then

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*Corresponding author.

E-mail address: wsong@ouc.edu.cn (W. Song).

morphologically studied using in vivo and silver impregnation methods. These species have been re-described from the results of these studies and improved definitions are provided.

Materials and methods

The sea urchin, *Hemicentrotus pulcherrimus* was collected during the summer of 2005 from coastal regions near Qingdao, north China (36°08′N; 120°43′E). The salinity was 30‰ and the water temperature was about 20 °C. To obtain ciliates, the hosts were dissected, and the digestive tracts were transferred to Petri dishes filled with filtered seawater. The released contents were examined and ciliates were picked out with a fine pipette.

After isolation of the ciliates, observations on living cells were carried out with a microscope equipped with Nomarski differential interference optics. Protargol (Wilbert 1975), silver nitrate (Song and Wilbert 1995) and silver carbonate (Ma et al. 2003a) staining methods were used to reveal the infraciliature and silverline systems. Drawings of impregnated specimens were made with the help of a camera lucida; measurements were performed at 100–1250 magnification. Systematics and terminology are mainly according to Corliss (1979).

Voucher slides of both species have been deposited in the Laboratory of Protozoology, Ocean University of China, with the following registration numbers: *Entorhipidium fukuii*, 2005050701-1 (with protargol) and 2005050701-2 (with silver nitrate impregnation); *Madsenia indomita*, 2005072301-1 (with protargol) and 2005072301-2 (with silver nitrate impregnation).

Results

Order Scuticociliatida Small, 1967
Family Entorhipidiidae Madsen, 1931
Genus *Entorhipidium* Lynch, 1929

To the authors' knowledge, the most recent definitions of this genus at silver staining level date from the 1960s (Berger 1961, 1964, dissertation; Profant, 1965, dissertation). Based on the data obtained in both previous and the present studies, we here suggest an improved diagnosis.

Improved diagnosis for the genus: Marine endocommensal scuticociliates with a sigmoid body shape and conspicuously narrow posterior end, strongly bilaterally flattened; cytostome located in a concavity anterior to equatorial level; buccal apparatus of *Uronema*-pattern, i.e. M1 single-rowed while M2 and M3 are clearly separate and with multi-row-structure, paroral membrane terminating at anterior level of M2; somatic kineties form both apical and post-oral sutures, and because these kineties are often in a twisted or spiral mode, the post-oral suture is formed on the right side of the cell; found in sea urchin digestive tracts.

Remarks: Considering the buccal apparatus, this genus is extremely similar to the well-known *Entodiscus* Madsen, 1931, whose species are also endocommensal forms in sea urchins and characterized by an oval, bilaterally flattened body shape and *Uronema*-like buccal apparatus (Foissner 1985; Song et al. 1999). *Entorhipidium* can be distinguished from *Entodiscus*, however, by the following features: (1) the sigmoid body shape and slightly twisted ciliary rows, (2) the conspicuous tail (see Foissner 1985; Song et al. 1999).

Another genus, the poorly known *Cryptochilum* was recently redefined by Song et al. (1999); this differs from *Entorhipidium* in possession of a rounded body shape with rounded posterior end (versus S-shaped with a definite tail) and different structure of the buccal apparatus, i.e. M1 long and highly developed with several rows (versus single-rowed M1 with 6–8 kinetosomes in *Entorhipidium*).
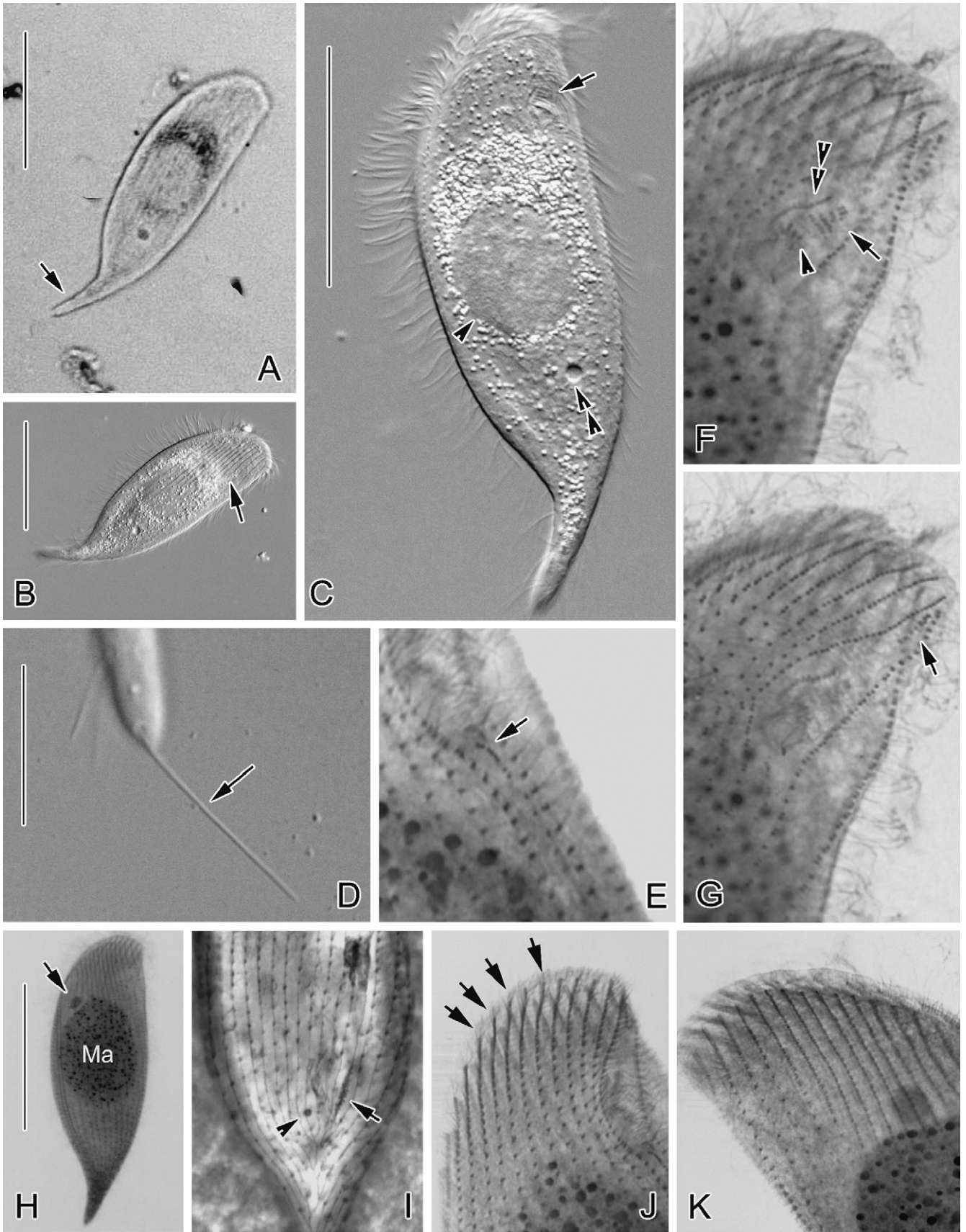
Entorhipidium fukuii Uyemura, 1934, with a description of the Chinese population (Figs. 1–3; Tables 1, 2)

Synonyms:
Cryptochilidium sigmoides Yagiu, 1934
Cryptochilum fukuii (Uyemura, 1934) (Berger, dissertation, 1964)
Cryptochilum sigmoides (Yagiu, 1934) (Berger, dissertation, 1964)
Biggariella fukuii (Uyemura, 1934) (Profant, dissertation, 1965)
Oreoctoma sigmoides (Yagiu, 1934) Jankowski, 1980

Little data concerning the structure of the buccal apparatus of this species was previously available at

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Fig. 1. *Entorhipidium fukuii* (**A–I**) and *E. enchi* (**J**, **K**) from life (**A–D**, **F**), after protargol (**E**) and silver nitrate impregnations (**G**, **H–K**). **A**. Right side of a typical cell. **B**, **C**. Two slightly deformed individuals. **D**. Right side view (after Uyemura, 1934). **E**. Detail of the buccal apparatus, double-arrowheads indicate the anterior end of the paroral membrane. **F**. Right side (called *Cryptochilidium sigmoides*, after Yagiu, 1934). **G**. Right side (called *Biggariella fukuii*, after Profant, 1965, dissertation). **H**, **I**. The infraciliature, arrows mark the dikinetid kineties adjacent to the dorsal margin, arrowheads indicate the apical suture. **J**, **K**. Buccal apparatus and right side of *Entorhipidium enchi* (after Profant, 1965, dissertation). CVP, contractile vacuole pore; Cyp, cytopyge; Ma, macronucleus; M1–M3, membranelles; Mi, micronucleus; PM, paroral membrane; Sc, scutica; SL, silverline; SK, somatic kinety. Scale bars in **A** = 30 μm, in **B**, **C**, **F**, **G**, **H** = 40 μm, in **D** = 35 μm, in **K** = 100 μm.

infraciliature level though the species was repeatedly reported/described (under different generic affiliations, see Berger 1964, dissertation; Profant 1965, dissertation). Hence, an improved diagnosis is suggested here based mainly on the Japanese population of Uyemura (1934) and our newly isolated Chinese specimens.

Improved diagnosis: Large *Entorhipidium* about $70–175 \times 25–60\,\mu m$ in vivo; body sigmoid with a conspicuously long tail; about 28–33 bipolar somatic kineties, which form a considerable suture along the apical margin; post-oral suture prominent; M1 composed of a single long row with ca. 9 kinetosomes; one large oval macronucleus and one micronucleus; contractile vacuole pore positioned near the posterior end of somatic kinety Nos. 3 or 4.

Description: The body size and shape in the Chinese population are rather consistent; in vivo they usually measure about $120–160 \times 40–50\,\mu m$ and are compressed bilaterally about 1:3. Their outline is sigmoid when viewed from the side, with the posterior end forming a conspicuous tail about $30\,\mu m$ long, which always shows a distinct curvature towards the dorsal side (Figs 1A–C and 2A–C). The dorsal margin is convex and the ventral margin generally straight with the buccal area slightly concave (Figs 1A–C and 2A–C). The buccal field is narrow and located in the anterior fifth of the body (Figs 1A–C and 2C). A noticeable striation of the cortex, especially in anterior part of cell, corresponds to the ciliary rows (Fig. 2B). The pellicle is thin and no extrusomes were observed. The somatic cilia are ca. $7–10\,\mu m$ long, reaching their maximal density near the anterior end (Figs 1A–C and 2C). The caudal bristle is prominent and stiff, about $15–20\,\mu m$ long (Figs 1A–C and 2D). The endoplasm is colourless, hyaline, often containing abundant tiny granules, especially around the macronucleus (Figs 1A–C, 2B and C). A single large rounded macronucleus is located centrally (Fig. 2C), with one spherical micronucleus attached to it (Figs 1H and 2H). The contractile vacuole is often rather small, positioned in the posterior 1/3 of the body (Figs 1A–C and 2C).

After isolation, most ciliates were seen creeping on the substrate and sometimes swimming with rotation around the longitudinal axis of the cell. The number of individuals decreased greatly within 2 h, when kept in Petri dishes at room temperature.

The buccal ciliature is typical of the genus. Within the deep buccal cavity are three well-developed membranelles (M1–3); M1 is comprised of a single row of about 6–9 kinetosomes (Figs 1E and 2G), while M2 and M3, each composed of 4–5 rows of kinetosomes, are located relatively far away from M1 (Figs 1E and 2F). The paroral membrane is double-rowed, reaching the level of the anterior margin of M2 (Figs 1E and 2F).

The somatic infraciliature consists of about 30 kineties formed almost entirely of monokinetids, which in central parts are conspicuously sparsely ciliated except the two rows on the dorsal margin (Fig. 1H and I). The basal bodies in anterior parts of all kineties are very closely arranged (Figs 1H, I, 2J and K). Kineties of the left and right sides form a slit-like apical suture along the anterior margin (Figs 1I and 2J) and a postoral suture on the ventral side (Fig. 1H), The contractile vacuole pore is located at the posterior end of kinety No. 3 or 4, to the right of the cytopyge (Figs 1H and 2I). The scutica is Y-shaped, consisting of about 15 kinetosomes (Figs 1E and 2E).

The silverline system consists of longitudinal lines connecting the kinetosomes within each somatic kinety (Figs 1H, I and 2I), which do not form a caudal circle (Fig. 1H and I).

Discussion and comparison: With its characteristic body shape, size, prominent tail, habitat, nuclear apparatus and general arrangement of the cilia, the isolate from China corresponds perfectly with the previous reports (Berger, dissertation, 1964; Profant, dissertation, 1965; Uyemura 1934; Yagiu 1934); hence its identification is certain.

Yagiu (1934) described *Cryptochilidium sigmoides*, found also in the intestine of sea urchins, in the same year as Uyemura (1934) described *Entorhipidium fukuii* (Fig. 1D and F). According to the living morphology and the reinvestigation by Profant (1965, dissertation) (Fig. 1G; Table 2), *C. sigmoides* is clearly conspecific with this form, and hence is regarded as a junior synonym of *Entorhipidium fukuii*. For the same reason, *Oreoctoma sigmoides* (Yagiu, 1934) Jankowski, 1980 should also be synonymized with *Entorhipidium fukuii*.

The redefinition by Song et al. (1999) of the genus *Cryptochilum*, with a very different buccal infraciliature (see above), means that the new combinations for this organism, *Cryptochilum fukuii* (Uyemura, 1934)

Fig. 2. Photomicrographs of *Entorhipidium fukuii* in vivo (**A–D**), after protargol (**E–H**, **J**, **K**) and silver nitrate impregnations (**I**). **A**. Right side view, arrow marks the tail. **B**. Arrow shows cortical striations. **C**. Right side of a typical cell. Arrowhead indicates the macronucleus, arrow marks the oral area and double arrowheads point to the contractile vacuole. Note tiny particles around the Ma. **D**. Arrow indicates the caudal bristle. **E**. Arrow reveals the scutica. **F**. Buccal area showing M2 (arrow), M3 (arrowhead) and paroral membrane (double arrowheads). **G**. Same buccal area focused to show M1 (arrow). **H**. General view to show the macronucleus and micronucleus (arrow). **I**. Posterior part of right side to show the contractile vacuole pore (arrowhead) and cytopyge (arrow). **J**, **K**. Views of the left and right sides showing the arrangement of the anterior parts of somatic kineties, including the densely ciliated parts of the kineties marked by arrows in **J**. Ma, macronucleus. Scale bars in **A**, **B**, **H** = $80\,\mu m$, in **C** = $50\,\mu m$, in **D** = $15\,\mu m$.
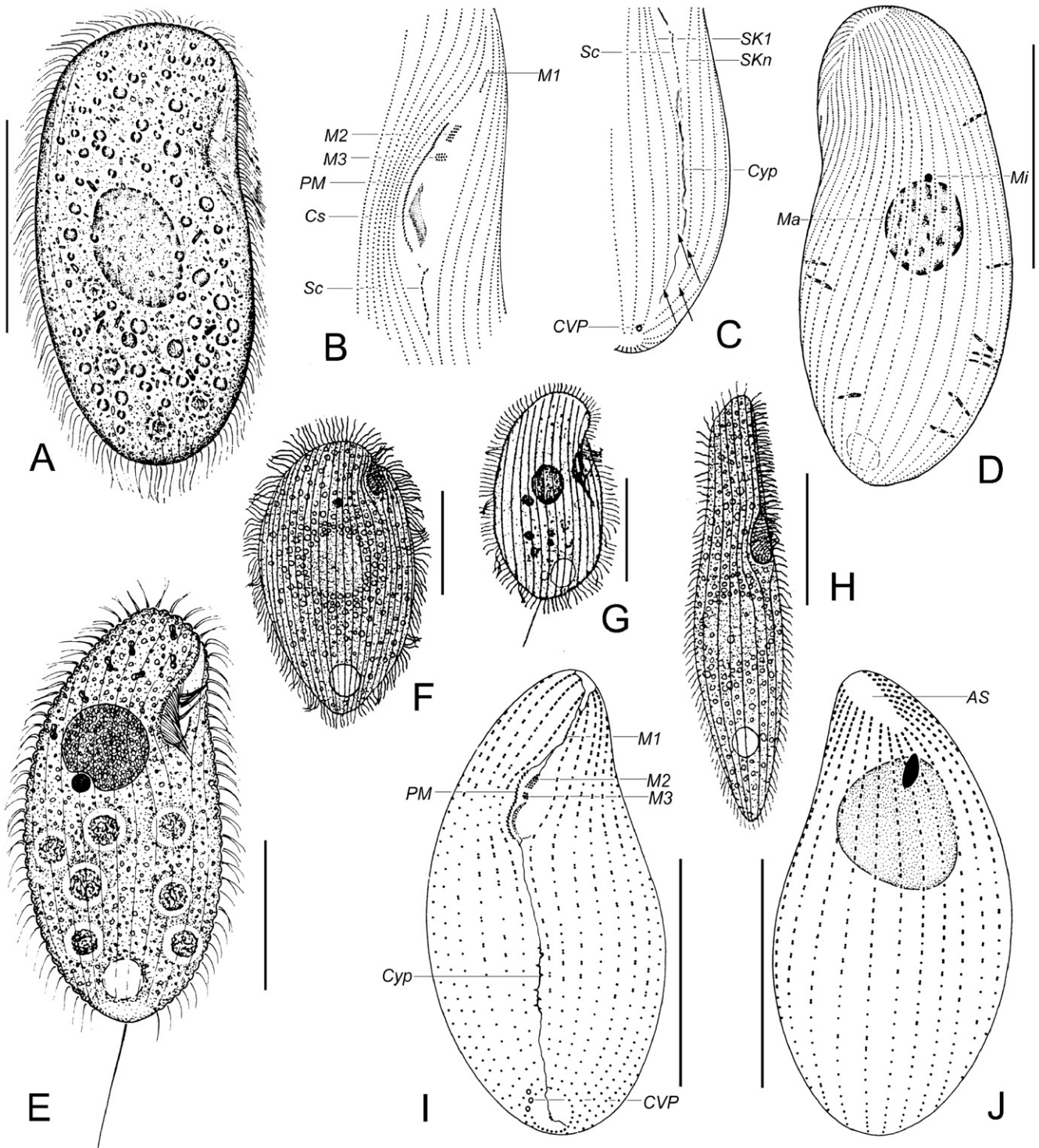
Fig. 3. *Entodiscus pseudoenchi* (**A–D**, after Song et al., 1999), *E. enchi* (**E, I, J**, after Foissner, 1985), *E. borealis* (**F**, after Madsen, 1931), *E. sabulonis* (**G**, after Powers, 1935), *E. indomitus* (**H**, after Madsen, 1931) from life. **A**. Right side of *E. pseudoenchi*. **B**. Part of right side showing the buccal apparatus. **C**. Caudal part of right side, indicating the "shortened" somatic kineties at the posterior end of the cell (arrows). **D**. Left side; note the suture-like apical area. **E**. Right side of *E. enchi* (Russo, 1914) Foissner, 1985. **F**. Right side of *E. borealis*. **G**. Right side of *E. sabulonis*. **H**. Right side of *E. indomitus*. **I, J**. Ventral and dorsal view of *E. enchi* (Russo, 1914) Foissner, 1985. As, apical suture; Cs, cytostome; CVP, contractile vacuole pore; Cyp, cytopyge; Ma, macronucleus; M1–M3, membranelles; Mi, micronucleus; PM, paroral membrane; Sc, scutica; SK, somatic kinety. Scale bars in **A, D** = 50 μm, in **E, I, J** = 25 μm, in **F** = 40 μm, in **G, H** = 30 μm.

Table 1. Morphometric data of the Qingdao population of *Entorhipidium fukuii* Uyemura, 1934 (upper line) and *Madsenia indomita* (Madsen, 1931) Kahl, 1934 (lower line)

Characters	Min	Max	Mean	SD	CV	*n*
Body length in µm	120	165	144.5	13.24	9.2	18
	68	108	89.2	9.44	10.6	30
Body width in µm	36	52	44.5	4.93	11.2	18
	20	34	25.9	3.31	12.8	28
Number of somatic kineties	28	33	30.1	1.18	3.9	18
	18	20	19.2	0.65	3.4	30
Number of macronucleus	1	1	1	0	0	18
	1	1	1	0	0	30
Number of micronucleus	1	1	1	0	0	11
	1	1	1	0	0	30
Length of macronucleus in µm	38	62	46.8	7.22	15.4	18
	14	22	18.8	1.96	10.4	29
Width of macronucleus in µm	28	44	35.2	5.00	14.3	18
	10	14	12.3	1.40	11.4	28
Length of micronucleus in µm	4	8	5.6	1.17	20.9	10
	3	6	4.0	0.74	18.7	29
Width of micronucleus in µm	3	6	4.7	0.82	17.5	10
	2	5	3.3	0.65	19.5	29
Length of buccal field in µm	21	27	24.1	1.85	7.7	15
	—	—	—	—	—	—
Length of paroral membrane in µm	9	17	12.7	2.22	17.1	15
	—	—	—	—	—	—
Length of M1-M2-complex in µm	—	—	—	—	—	—
	9	18	13.2	1.82	13.8	25
Distance from anterior end of paroral membrane to the anterior pole in µm	15	31	23.2	5.69	24.7	18
	—	—	—	—	—	—
Number of kinetosomes in M1	6	9	7.2	0.83	11.9	16
	—	—	—	—	—	—
Kinetosomes in the scutica	10	26	15.6	4.94	30.9	14
	—	—	—	—	—	—

Data are based on protargol impregnated specimens. CV = coefficient of variation in %; max = maximum; mean = arithmetic mean; min = minimum; *n* = number of cells measured; SD = standard deviation.

suggested by Berger (1964, dissertation) and *Cryptochilum sigmoides* (Yagiu, 1934) by Berger (1964, dissertation) are rendered invalid.

Cryptochilum sigmoides sensu Berger, 1961 is clearly not the same species, since it has a significantly different buccal apparatus: M1 is patch-like and multi-rowed. In addition, the body shape is also rather different: slender and sigmoid with relatively long tail (Fig. 6C and D; Berger 1961). This organism clearly requires re-examination.

Profant combined *Entorhipidium fukuii* into his new genus, *Biggariella* in his unpublished dissertation (1965). However, the genus *Biggariella* is a *nomen nudum*

because of the absence of any formal description or definition (Aescht 2001), and in any case his new combination *Biggariella fukuii* (Uyemura, 1934) is a junior synonym.

The congener *Entorhipidium enchi* Lynch, 1929 is much larger (> 200 µm), has conspicuously more somatic kineties, several contractile vacuole pores and a different structure of the buccal apparatus (Fig. 1J and K) (Lynch 1929; Profant, dissertation, 1965); hence it is easily separated from *E. fukuii*.

In its general morphology, *Entorhipidium fukuii* also resembles the morphotype *Cryptochilidium caudatum* Poljansky, 1951 [called *Cryptochilum caudatum*

Table 2. Morphometric data of different isolates of *Entorhipidium fukuii* Uyemura, 1934 (a) and two populations of the congener *E. caudatum* (Poljansky, 1951) (b)

Synonyms	Size in vivo	Ciliary rows, no.	Host (sea urchins)	Data sources
Entorhipidium fukuii[a]	120–170 × 35–60	28–33	*Hemicentrotus pulcherrimus*	Present work
Entorhipidium fukuii[a]	73–175 × 22–47	ca. 32–40	*Anthocidaris crassispina* *Pseudocentrotus depressus* *Strongylocentrotus pulcherrimus* *Strongylocentrotus intermedius* *Glyptocidaris crenularis*	Uyemura (1934)
Cryptochilidium sigmoides[a]	69–107 × 21–41	ca. 26	*Anthocidaris crassispina*	Yagiu (1934)
Cryptochilum sigmoides[a]	94–153 × 40–55	24–38	*Allocentrotus fragilis* *Strongylocentrotus droebachiensis* *Strongylocentrotus echinoides* *Strongylocentrotus franciscanus* *Strongylocentrotus purpuratus*	Berger (1964)[c]
Biggariella fukuii[a]	76–160 × 33–60	29–33	Various echinoids in the north Pacific Ocean	Profant (1965)[c]
Cryptochilidium caudatum[b]	80–150 × 28–58	ca. 40–50	*Strongylocentrotus nudus*	Poljansky (1951) Berger (1964)[c]
Cryptochilum caudatum[b]	183–306 × 57–114	46–63	*Allocentrotus fragilis*	Berger (1964)[c]

Measurements in μm.
[a] Synonyms of *Entorhipidium fukuii* Uyemura, 1934.
[b] Synonyms of *Entorhipidium caudatum* (Poljansky, 1951) nov. comb.
[c] Dissertation, unpublished.

(Poljansky, 1951) in Berger, 1964, dissertation], which very probably belongs to the genus *Entorhipidium* as the position of its buccal area differs greatly from that of *Cryptochilidium* (in anterior 1/3 versus below the cell equator in *Cryptochilidium*) (Poljansky 1951; Profant, dissertation, 1965). Thus a new combination is suggested: *Entorhipidium caudatum* (Poljansky, 1951) nov. comb. (basionym: *Cryptochilidium caudatum* Poljansky, 1951). *Entorhipidium fukuii* differs from *E. caudatum* in having considerably fewer ciliary rows (24–40 versus ca. 40–63 in the latter) and is much smaller (70–175 × 20–60 μm versus 183–306 × 57–114 μm in the latter) (Fig. 6A; Table 2). Hence, they are unlikely to be conspecific.

The particular form of the S-shaped body and the presence of the conspicuous tail make *Entorhipidium fukuii* easily distinguishable from species of the genus *Entodiscus* from the same biotope: *E. pseudoenchi* Song et al., 1999, *E. borealis* (Hentschel, 1924) Madsen, 1931, *E. enchi* (Russo, 1914) Foissner, 1985 and *E. sabulonis*
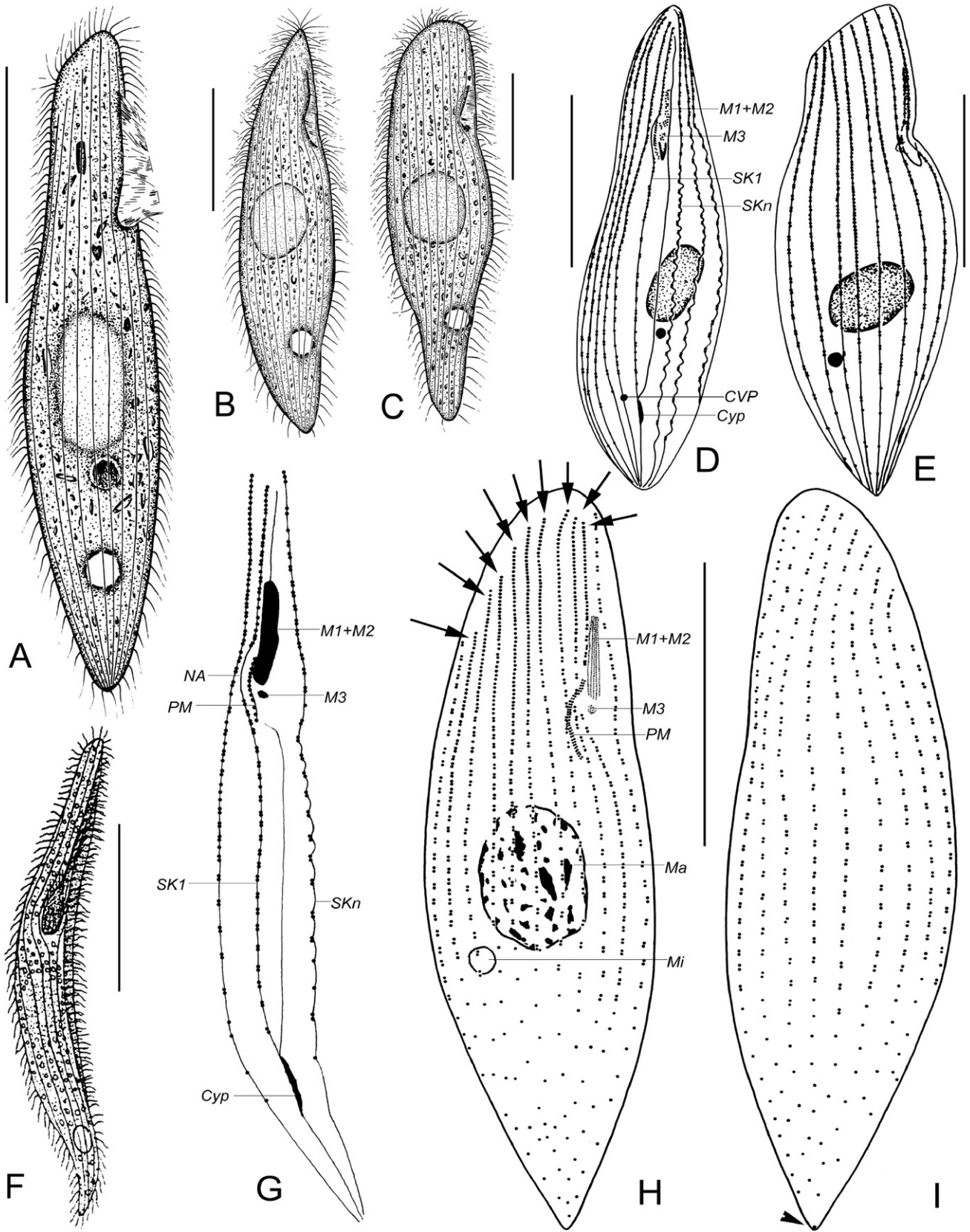
Powers, 1935 (now regarded as synonymous with *E. enchi*) (Fig. 3A–G, I and J; Foissner 1985).
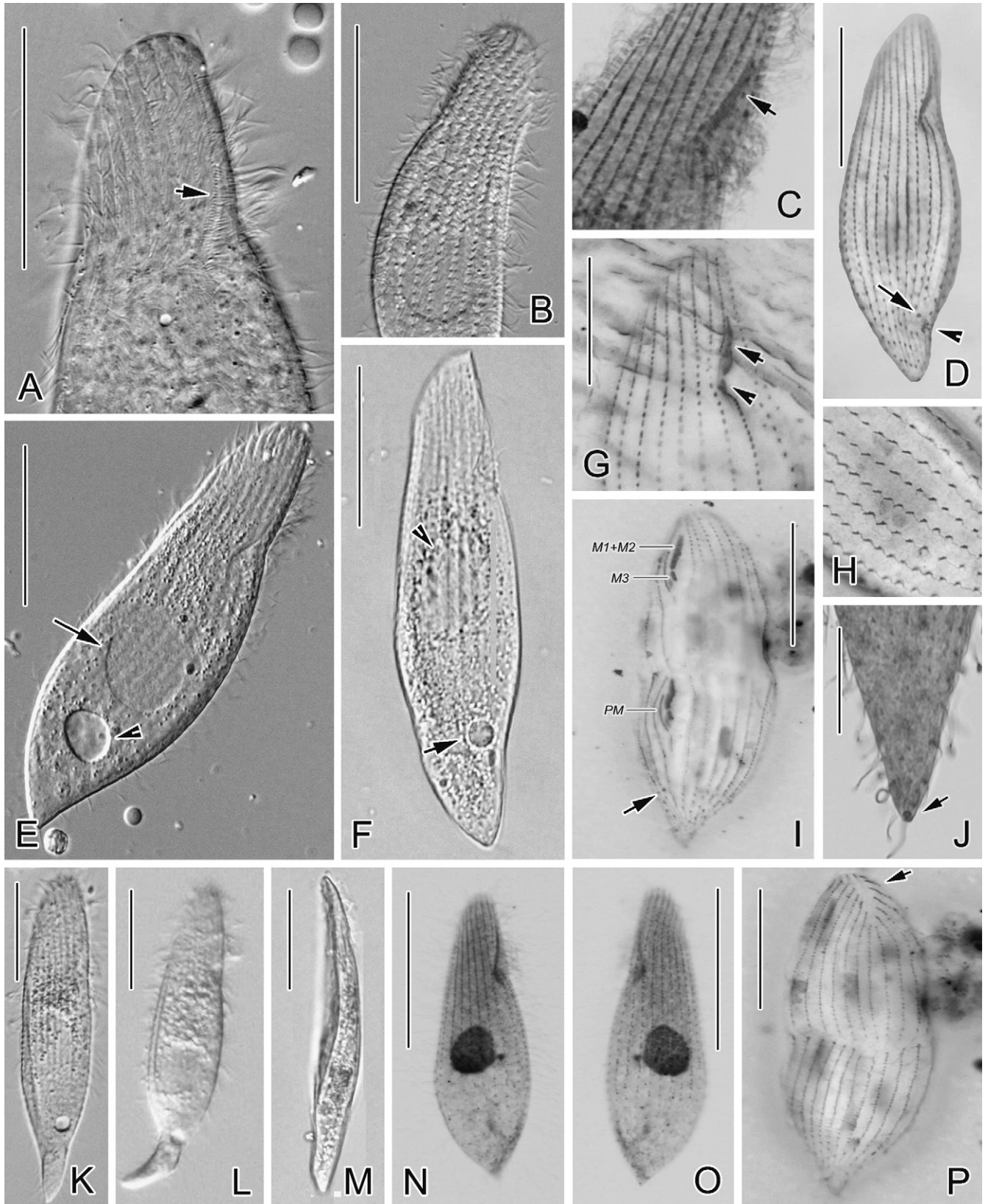
Genus *Madsenia* Kahl, 1934

This genus has been placed in the family Entodiscidae Jankowski, 1973 on account of its bilaterally flattened body shape, the pre-equatorial position of the cytostome and its habitat in the sea urchin intestine (Corliss 1979). However, no clear definition of this genus is available, although Profant (1965, dissertation) has roughly described its oral infraciliature. A formal diagnosis is therefore provided here, based on previous data and the present work.

Diagnosis: Endocommensal scuticociliates with slender, bilaterally flattened body; M1 and M2 incorporated into a single structure, i.e. forming an M1–M2 complex; M3 short; paroral membrane short, extending anteriorly to posterior level of M1–M2 complex; apical suture present, cytostome located anterior to equatorial level;

Fig. 4. *Madsenia indomita* (Madsen, 1931) Kahl, 1934 from life (A–C, F), after silver nitrate (D, E, G) and protargol impregnations (H, I). **A**. Right side of a typical individual. **B, C**. To show non-typical body shapes and sizes. **D, E**. Ventral and right views of infraciliature (after Profant, 1965, dissertation). **F**. Ventral view, after Madsen (1931). **G**. Buccal apparatus, note that M1 and M2 are connected. **H, I**. Infraciliature of right and left sides, respectively, arrowhead marks the two basal bodies at the end of the tail, arrows indicate the anterior part of somatic kineties on the right side. Note the structure of the M1–M2 complex. CVP, contractile vacuole pore; Cyp, cytopyge; Ma, macronucleus; M1 + M2, M1–M2 complex; M3, membranelle 3; Mi, micronucleus; NA, naked area of SK1; PM, paroral membrane; SK, somatic kinety. Scale bars in A, I = 30 μm, in B, C = 25 μm, in D–F = 40 μm.

no prominent caudal bristle; marine forms, within sea urchin digestive tracts.

Remarks: One problem remains unsolved concerning the definition of the so-called M1–M2 complex of the oral apparatus. We consider that this complex results from the fusion of membranelles 1 and 2 in the non-division stage, so that it appears as a single ciliary structure. The same appearance could result if the M1 is reduced or missing, so that the apparent M1–M2 complex would then actually consist only of the M2. Study of the morphogenesis might reveal which is the correct explanation.

Fortunately, one specimen in the middle-late stage of stomatogenesis has been observed (Fig. 5I, P). In this specimen no kinetosomes appear anterior to the single structure of the M1–M2 complex, which is about the same shape and size as that in the non-divisional stage. It is the authors' opinion that the structure seen in this specimen represents the primordia of both the M1 and M2 rather than only the M2 (Ma et al. 2003b). Thus, it might be most appropriate to use the term of M1–M2 complex until further morphogenetic details are available.

Madsenia indomita (Madsen, 1931) Kahl, 1934 with a description of the Chinese population (Figs 3H, 4, 5; Tables 1, 3)

Synonyms:
Entodiscus indomitus Madsen, 1931
Anophrys elongata Biggar and Wenrich, 1932

This organism has never been clearly described on the basis of the results of silver staining. Following the previous (Kahl 1934; Madsen 1931; Profant, dissertation, 1965) and the current studies, an improved diagnosis is suggested.

Improved diagnosis: Medium sized *Madsenia* with slender and elongated body shape, posterior end pointed; ca. 70–170×15–$40\,\mu m$ in vivo; about 19 somatic kineties, M1–M2 complex prominent with five longitudinal rows of kinetosomes; contractile vacuole subcaudally positioned, with the pore near posterior end of somatic kinety No. 2.

Description: The size in our isolate was rather constant, in vivo mostly about $90 \times 25\,\mu m$. The body was strongly bilaterally flattened and the body shape was somewhat variable, being generally slender and elongate, narrow or truncated dorsally at the anterior end, but always slightly or strongly pointed at the posterior end (Figs 4A–C, F, 5F and K–M). The buccal field was narrow and slightly concave in the posterior portion, occupying (from cytostome to apical end) about 1/4–1/3 of body length (Figs 4A–C and 5F). The pellicle was thin, with striations corresponding to the ciliary rows (Fig. 5B). No extrusomes were observed and the cytoplasm was transparent and colourless, always containing many tiny shining granules (Figs 4A–C, 5, F, K and L). The contractile vacuole was small, located in posterior 1/4 of the body length near the ventral margin of the cell (Figs 4A–C, 5E, F and K). The macronucleus was large, round to oval in shape, with one spherical micronucleus close to it (Figs 4A–C, H, 5E, F, N and O).

The cilia were about $6\,\mu m$ long, densely arranged on the anterior 1/3 of the cell, but sparsely arranged on other body parts; there was no prominent caudal bristle, but several cilia arise near the posterior end and have the same length as other cilia (Figs 4A–C, I and 5J).

The cells mainly swam in the upper water layer after isolation, moderately fast and rotating about the long axis of the body. The number of swimming cells decreased greatly within an hour after being isolated from sea urchins.

The infraciliature in silver-stained preparations consists of 18–20 sparsely arranged, bipolar somatic kineties, usually composed of dikinetids in the anterior 3/4 of the body, with kinetids in the anterior 1/4 of kineties on the right side closely spaced (Figs 4D, E, H, I, 5D, N and O) and with left and right somatic kineties forming the apical suture along the anterior pole (Fig. 5P). The contractile vacuole pore is located at posterior fourth of the ventral side (Figs 4D, 5D and I), near the short cytopyge (Figs 4D, G and 5D).

In the buccal apparatus, shown in Fig 4G and H, M1 and M2 form a single structure, the M1–M2 complex, which consists of five longitudinal rows of kinetosomes separated from a fairly short M3. The paroral membrane at the right of the shallow buccal cavity is gently curved and extends anteriorly to the level of the posterior 1/5 of the M1–M2 complex (Fig. 4G and H). The silverline system takes the form of longitudinal

Fig. 5. Photomicrographs of *Madsenia indomita* (Madsen, 1931) Kahl, 1934 from life (**A**, **B**, **E**, **F**, **K–M**), after protargol (**C**, **N**, **O**), silver carbonate (**J**) and silver nitrate impregnations (**D**, **G**, **H**, **I**, **P**). **A**, **C**. To show M1–M2 complex (arrows). **B**. Showing the cortical structure. **D**. Contractile vacuole pore (arrow) and cytopyge (arrowhead). **E**. The macronucleus (arrow) and contractile vacuole (arrowhead). **F**, **K**, **L**. Individuals with different body shapes, arrow indicates the contractile vacuole and arrowhead marks the macronucleus. **G**. Arrow shows M1–M2 complex and arrowhead shows M3. **H**. Silverline system. **I**, **P**. Ventral and dorsal views of an individual in morphogenesis, note that M1 + M2 form a single structure (**I**); arrow in **I** indicates the contractile vacuole pore, while the arrow in **P** points to the apical suture. **J**. Arrow points to basal bodies at the end of the tail. **M**. Dorsal view. **N**, **O**. Right and left views of the same specimen. M1 + M2, M1–M2 complex; M3, membranelle 3; PM, paroral membrane. Scale bars in **A** = $20\,\mu m$, in **B**, **D**, **I**, **P** = $40\,\mu m$, in **E**, **F**, **K**, **L**, **M** = $30\,\mu m$, **G** = $15\,\mu m$, in **J** = $10\,\mu m$, in **N**, **O** = $50\,\mu m$.

Table 3. Morphological comparison of populations of *Madsenia indomita* (Madsen, 1931) Kahl, 1934

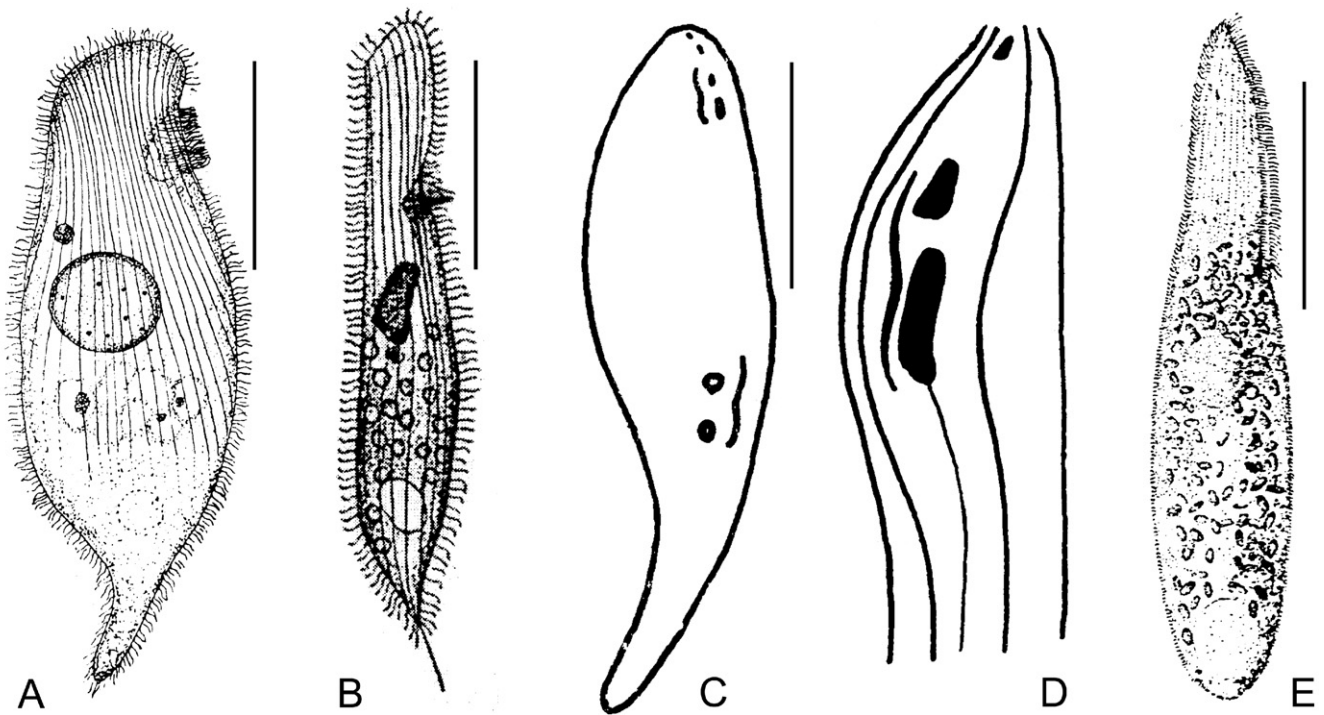| Synonyms	Size in vivo	Ciliary rows, no.	Host sea urchins	Data sources
Madsenia indomita	70–110 × 20–35	18–20	*Hemicentrotus pulcherrimus*	Present work
Entodiscus indomitus Madsen, 1931	80–117 × 20–23	ca. 14	*Strongylocentrotus droebachiensis*	Madsen, 1931
Anophrys elongata Biggar and Wenrich, 1932	166 × 33	16–18	*Toxopneustes variegates Echinometris subangularis*	Biggar and Wenrich, 1932

Measurements are in μm.

Fig. 6. Some related morphotypes. **A.** *Cryptochilidium caudatum* Poljansky, 1951, right side (after Poljansky 1951). **B.** *Entodiscus indomitus* Madsen, 1931, right side (after Biggar & Wenrich 1932). **C, D.** *Cryptochilum sigmoides* sensu Berger, 1961 after silver nitrate impregnation, right side and buccal apparatus (presumed misidentification, after Berger 1961). **E.** *Anophrys elongata* Biggar & Wenrich, 1932, right side (after Biggar & Wenrich 1932). Scale bars in **A, C** = 40 μm, in **B, E** = 50 μm.

lines connecting kinetosomes of each somatic kinety (Figs 4D and 5H).

Discussion: *Madsenia indomita* was originally described by Madsen (1931) under the name, *Entodiscus indomitus* Madsen, 1931. Later, it was transferred into the monotypic genus *Madsenia* by Kahl (1934). Profant (1965, dissertation) gave a detailed re-description based mainly on the infraciliature. The Qingdao isolate corresponds very well with the previous reports (Figs 3H, 4A, D, E and G–I; Table 3). Thus, the identification of this population is quite certain.

Powers (1933a) redescribed *Entodiscus indomitus* Madsen, 1931, but reported that his isolate possessed a 16 μm long caudal bristle (versus caudal cilia as long as somatic ones in Madsen's records; Figs 3H and 6B). Since Powers' description was based only on living cells and the character of a caudal bristle is usually a reliable feature for species identification, we are not convinced that they could be conspecific.

Biggar and Wenrich (1932) reported a new species, *Anophrys elongata*, whose living characters, such as body shape, general appearance of the ciliary structure and nuclear apparatus correspond well with our *Madsenia indomita* population except that the described *Anophrys elongata* is relatively bigger (166 × 33 versus 90 × 25 μm) and its contractile vacuole is caudally

located (versus subcaudally located in *Madsenia indomita*; Fig. 6E). Since these differences might be population-dependent, *Anophrys elongata* could be the junior synonym of *Madsenia indomita* (Madsen, 1931) Kahl, 1934. Detailed study of the buccal ciliature is necessary to resolve this question.

Acknowledgements

References

Aescht, E., 2001. Catalogue of the generic names of ciliates (Protozoa, Ciliophora). Denisia 1, 1–350.

Berger, J., 1960. The entocommensal ciliate fauna of *Strongylocentrotus* spp. from the northeast Pacific (Abstr.). J. Protozool. 7 (suppl.), 17.

Berger, J., 1961. The comparative buccal morphology of certain hymenostome ciliates entocommensal in echinoids. Progress in Protozoology. In: Proceedings of the First International Congress in Protozoology, Prague, pp. 86–88.

Berger, J., 1964. The morphology, systematic and biology of the endocommensal ciliates of echinoids. Doctoral Thesis. University of Illinois, USA.

Berger, J., 1965. The infraciliary morphology of *Euplotes tuffraui* n. sp., a commensal in strongylocentrotid echinoids, with comments on echinophilous populations of *Euplotes balteatus* (Dujardin) (Ciliata: Hypotrichida). Protistologica 1, 17–31.

Biggar, R.B., Wenrich, D.H., 1932. Studies on ciliates from Bermuda sea urchins. J. Parasitol. 18, 252–257.

Corliss, J.O., 1979. The Ciliated Protozoa. Characterization, Classification and Guide to the Literature, second ed. Pergamon Press, Oxford.

Foissner, W., 1985. The morphology and the infraciliature of some ciliates (Protozoa: Ciliophora) inhabiting the gut of the sea urchins (Euechinoidea) *Paracentrotus lividus* and *Arbacia lixula*. Arch. Protistenk. 130, 355–366.

Hentschel, C.C., 1924. On a new ciliate, *Cryptochilum boreale* nov. sp., from the intestine of *Echinus esculentus* Linn., together with some notes on the ciliates of echinoids. Parasitology 16, 321–328.

Jankowski, A.W., 1973. Parasitic Ciliophora I. *Pectenita golikowi* gen. et sp. n. (Entodiscidae fam. n.) from *Mizuhopecten yessoensis*. Parazitologia 7, 214–219.

Jankowski, A.W., 1974. Commensological sketches. 6. Endocommensals of *Strongylocentrotus intermedius* in the Busse Lagoon (Southern Sakhalin). Gidrobiol. Zh. 10, 60–68.

Jankowski, A.W., 1980. Conspectus of a new system of the phylum Ciliophora. Trudy Zool. Inst., Leningr. 107, 80–115 (in Russian with English translation).

Kahl, A., 1934. Ciliata endocommensalia et parasitica. In: Grimpe, G., Wagler, E., Die Tierwelt der Nord und Ostsee. Lief. (Lieferung) 26 (Teil II, c4), Leipzig, pp. 147–183.

Long, H., Song, W., Chen, J., Gong, J., Ji, D., Hu, X., Ma, H., Zhu, M., Wang, M., 2006. Studies on an endoparasitic ciliate *Boveria labialis* (Protozoa: Ciliophora) from the sea cucumber *Apostichopus japonicus*. J. Mar. Biol. Assoc. UK 86, 823–828.

Lynch, J.E., 1929. Studies on the ciliates from the intestine of *Strongylocentrotus* I. *Entorhipidium* gen. nov. Univ. California Publ. Zool. 33, 27–56.

Lynn, D.H., Berger, J., 1972. Morphology, systematics, and demic variation of *Plagiopyliella pacifica* Poljansky, 1951 (Ciliatea: Philasterina), an endocommensal of strongylocentrotid echinoids. Trans. Am. Microsc. Soc. 91, 310–336.

Lynn, D.H., Berger, J., 1973. The Thyrophylacidae, a family of carnivorous philasterine ciliates endocommensal in strongylocentrotid echinoids. Trans. Am. Microsc. Soc. 92, 533–557.

Lynn, D.H., Frombach, S., 1987. The ultrastructure and systematics of *Schizocaryum dogieli*, a ciliate endocommensal in Pacific echinoids. Can. J. Zool. 65, 3133–3143.

Ma, H., Choi, J.K., Song, W., 2003a. An improved silver carbonate impregnation for marine ciliated protozoa. Acta Protozool. 42, 161–164.

Ma, H., Song, W., Hu, X., Warren, A., 2003b. Morphology and stomatogenesis of *Pseudocohnilembus hargisi* (Ciliophora: Scuticociliatida). J. Mar. Biol. Assoc. UK 83, 399–405.

Madsen, H., 1931. Bemerkungen über einige entozoische und freilebende marine Infusorien der Gattungen *Uronema*, *Cyclidium*, *Cristigera*, *Aspidisca* und *Entodiscus* gen. nov. Zool. Anz. 96, 99–112.

Maupas, E., 1883. Contribution à l'étude morphologique et anatomique des infusoires ciliés. Arch. Zool. Exp. Gén. 1, 427–664.

Poljansky, I.G., 1951. Intestinal infusoria of sea urchins. Parasitol. Sb. Akad. Nauk SSSR Zool. Inst. 13, 371–393 (in Russian).

Powers, P.B.A., 1933a. Studies on the ciliates from sea urchins I. General taxonomy. Biol. Bull. 65, 106–121.

Powers, P.B.A., 1933b. Studies on the ciliates from sea urchins II. *Entodiscus borealis* (Hentschel). (Protozoa, Ciliata). Behavior and morphology. Biol. Bull. 65, 122–136.

Powers, P.B.A., 1935. Studies on the ciliates of sea-urchins, a general survey of the infestations occurring in Tortugas echinoids. Pap. Tortug. Lab. Carnegie Inst. Washington 29, 293–327.

Profant, R.J., 1965. The morphology, systematics and distribution of ciliates infaunating three species of echinoids in the Eastern Pacific Ocean. Doctoral Thesis, University of California.

Russo, A., 1914. Species di ciliate viventi nell'intestino dello *Strongylocentrotus lividus* Brandt. Boll. Accad. Gioen. Sci. Nat. Catania 32, 1–10.

Song, W., Wilbert, N., 1995. Benthische Ciliaten des Süßwassers. In: Röttger, R. (Ed.), Praktikum der Protozoologie. Gustav Fischer Verlag, New York, pp. 156–168.

Song, W., Wilbert, N., Warren, A., 1999. Three new endocommensal ciliates from digestive tract of sea urchins of the Weddell Sea, Antarctica (Protozoa, Ciliophora). Polar. Biol. 22, 232–240.

Uyemura, M., 1934. Über einige neue Ciliaten aus dem Darmkanal von japanischen Echinoideen (I). Sc. Rep. Tokyo. Bunr. Daig. 1, 181–191.

Wilbert, N., 1975. Eine verbesserte Technik der Protargolimprägnation für Ciliaten. Mikrokosmos 64, 171–179.

Yagiu, R., 1934. Studies on the ciliates from the intestine of *Anthocidaris crassispina* (A. Agassiz). II. *Cryptochilidium sigmoides* sp. nov. and *Cryptochilidium minor* sp. nov. J. Sci. Hiroshima Univ. Ser. B., Div. 1. 3, 25–31.