

**Three marine haptorid ciliates from northern China: *Paraspathidium apofuscum* n. sp., *Trachelotractus entzi* (Kahl, 1927) Foissner, 1997 and *Apotrachelotractus variabialis* Long, Song and Warren, 2009 (Protozoa, Ciliophora)**

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Three haptorid ciliates, *Paraspathidium apofuscum* n. sp., *Trachelotractus entzi* (Kahl, 1927) Foissner, 1997 and *Apotrachelotractus variabialis* Long, Song and Warren, 2009, isolated from sandy beaches in Qingdao, China, were morphologically investigated using both *in vivo* observations and silver impregnation methods. The new species, *P. apofuscum*, is highly contractile and has a terminally-located contractile vacuole, approximately 40 somatic kineties, and a dikinetid perioral kinety that does not form a closed circle. Redescriptions of *A. variabialis* and *T. entzi* are also provided based on the Qingdao populations.

**Keywords:** benthos; Haptorida; morphology and infraciliature; new species; taxonomy

## Introduction

In terms of species identification and separation, silver impregnation techniques have contributed greatly to the taxonomy of ciliates. This is particularly true with respect to various poorly-known marine forms, such as the haptorids. Many of these ciliates have never been re-identified since Kahl's review (Kahl 1928, 1930). This is perhaps due to the fact that most haptorids are large and fragile, and hence difficult to fix, although in recent years this problem has been resolved for many species (Dragesco and Dragesco-Kernéis 1986; Song 1990; Foissner 1996a, 1996b, 1996c, 1997a, 1997b, 1998; Dragesco 2002; Foissner and Xu 2007). More recently, we have made some progress in revealing infraciliature of some haptorids found from China Seas (Long et al. 2009).

In the present paper we describe one new haptorid species, *Paraspathidium apofuscum* n. sp., and redescribe two poorly known species, *Trachelotractus entzi* (Kahl, 1927) Foissner, 1997 and *Apotrachelotractus variabialis* Long, Song and Warren, 2009, which we isolated from sandy beaches of the Yellow Sea coast at Qingdao, China.

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## Materials and methods

### Sample collection

Sample collection and isolation of sand-dwelling ciliates followed the protocol of Long et al. (2005); the upper 15 cm layer of sand was collected with sea water from the site, and cells were then picked out using a fine pipette under a stereomicroscope.

The type population of *Paraspathidium apofuscum* n. sp. was collected from the sandy beach of the Second Bathing Bay of Qingdao (36°08'N; 120°43'E) on 22 April 2005. The water temperature was about 15°C, salinity about 30‰.

*Trachelotractus entzi* (Kahl, 1927) Foissner, 1997 was collected on 15 November 2004, from the same location as *P. apofuscum* (water temperature 10°C, salinity approx. 26‰.)

*Apotrachelotractus variabialis* Long, Song and Warren, 2009 was sampled on 15 November, 2004, from a sandy beach at Langya Harbour (39°10'N; 117°06'E) (salinity approx. 29‰, temperature 10°C).

### Morphology

Cells were observed *in vivo* using bright field and differential interference contrast microscopy. Protargol (Wilbert 1975), silver nitrate (Song and Wilbert 1995) and silver carbonate (Ma et al. 2003) impregnations were used to reveal the infraciliature and silverline system. Drawings of live cells were based on free-hand sketches and photomicrographs, and silver impregnated cells were sketched with the aid of a camera lucida; measurements were performed at 100–1250 × magnification (Lin et al. 2008). Systematics and terminology are according to Corliss (1979).

One holotype (LH2005042202-1) and one paratype (LH2005042202-2) slide of *Paraspathidium apofuscum* n. sp. and voucher slides of *Trachelotractus entzi* (LH2004111503-1, LH200411503-2) and *Apotrachelotractus variabialis* (LH2004112401-1, LH2004112401-2) have been deposited in the Laboratory of Protozoology, Ocean University of China.

## Results and discussion

Class **KINETOFRAGMINOPHORA** de Puytorac et al., 1974

Order **HAPTORIDA** Corliss, 1974

Family **PARASPATHIDIIDAE** Foissner, 1997

Genus *Paraspathidium* Noland, 1937

*Paraspathidium apofuscum* n. sp.

(Figures 1, 2 and 8; Table 1)

### Diagnosis

Highly contractile *Paraspathidium* 130–250 µm long *in vivo*; single contractile vacuole terminally located, with no distinct collecting canals; 34–43 somatic kineties; dikinetid perioral kinety not forming a closed circle.

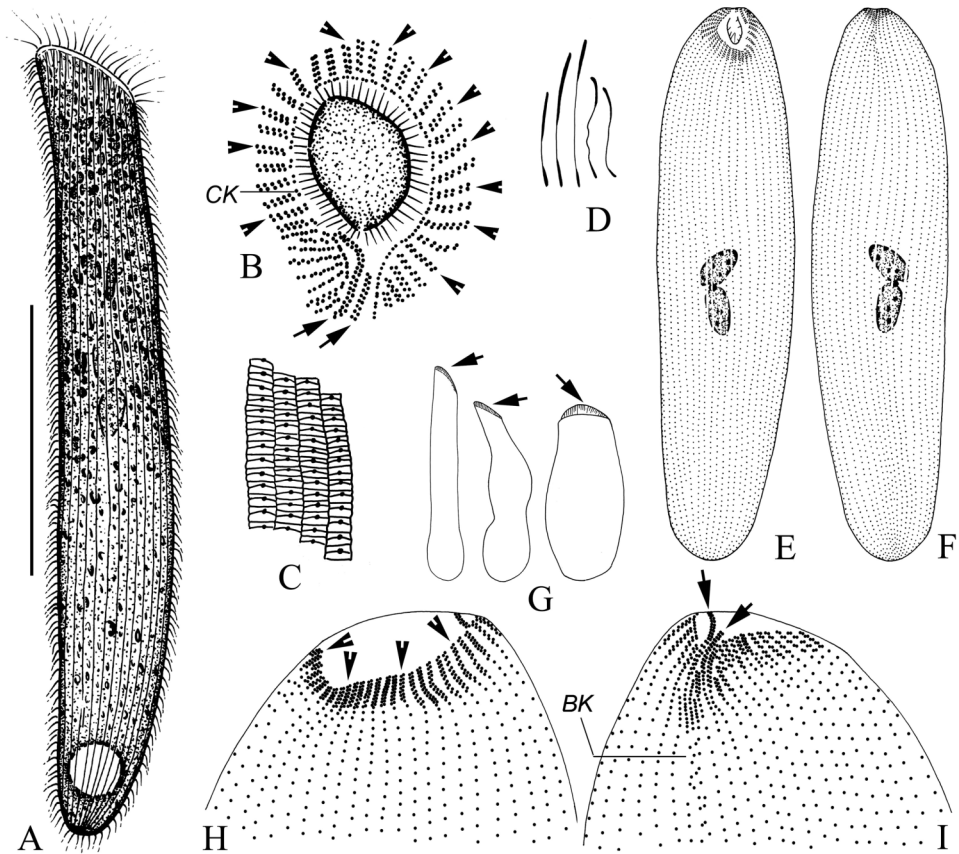


Figure 1. *Paraspithidium apofuscum* n. sp. from live cells (A, G), after protargol (B, D–F), silver nitrate (C), and silver carbonate stainings (H, I). (A) a typical cell; (B) apical view of oral apparatus, arrowheads show the dikinetids in the anterior parts of somatic kineties, arrows indicate the brush kineties; (C) silverline system; (D) extrusomes with different shapes; (E, F) the infraciliature, from the same specimen; (G) half extended cells, arrows indicate extrusomes in the oral area; (H, I) lateral views of the buccal apparatus, arrowheads indicate the circumoral kinety, arrows mark the brush kineties. Notes: BK, brush kineties; CK, circumoral kinety; scale bars: 50  $\mu$ m (A).

#### Type locality

Intertidal zone of a mesotrophic sandy beach near Qingdao (36°08'N; 120°43'E), China.

#### Etymology

Composite of the Greek word *apo-* (unlike) and the known species name *fuscum*, meaning a ciliate different from the congener *P. fuscum*.

#### Description

Size ca. 200  $\times$  40  $\mu$ m *in vivo*, elongate, anterior end shaped like a knife-blade, posterior end rounded (Figures 1A, 2A, D). At rest, cells usually contracted and bucket-shaped

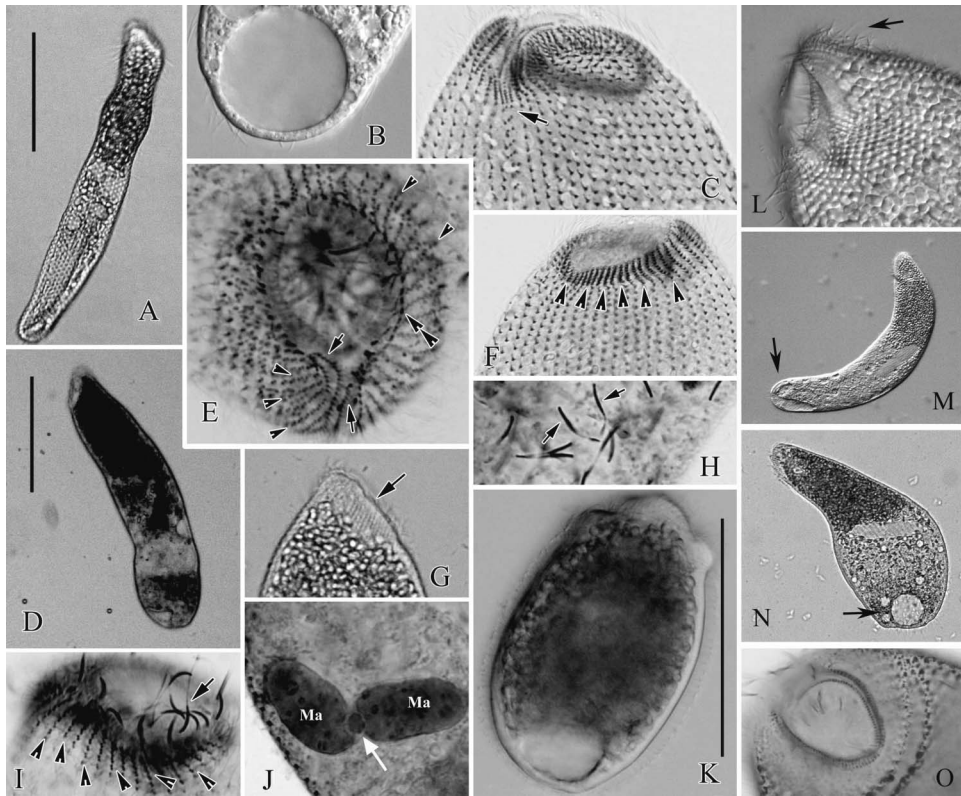


Figure 2. *Paraspathidium apofuscum* n. sp. from live cells (A, B, D, G, K–N) and after protargol (E, H–J), silver carbonate (C, F) and silver nitrate (O) impregnations. (A, D) lateral views of cells; (B, M, N) showing the contractile vacuole (arrows in M, N); (C, F) lateral view of the buccal apparatus, arrowheads indicate the dikinetids in the anterior part of somatic kineties, arrow marks the brush kineties; (E) apical view of buccal apparatus; (G) the anterior portion of the cell, arrow marks the extrusomes; (H) arrows mark extrusomes; (I) to show anterior part of somatic kineties (arrowheads) and extrusomes (arrow); (J) to show macronuclear nodule (Ma) and micronucleus (arrow); (K) a highly contracted cell; (L) arrow indicates the dorsal brush; (O) apical view of the oral apparatus. Notes: Ma, macronuclear nodule; scale bars: 50  $\mu$ m (A, D, K).

(Figure 2K). Anterior half of body full of dark granules, giving cell “half black, half transparent” appearance under low magnifications (Figures 1A, 2A, D). Extrusomes thread-like, 8  $\mu$ m long, thinner in middle portion than at either end, widely distributed in cytoplasm especially around the slit-like, apically located cytostome (Figures 1A, D, G, 2G, H, I). Two ellipsoidal macronuclei, with one micronucleus between them (Figures 1E, F, 2J). One contractile vacuole, terminally located (Figures 1A, 2B, M, N). Somatic cilia ca. 7  $\mu$ m long *in vivo*; and oral cilia ca. 12  $\mu$ m (Figure 1A). Generally inactive, often float in the water, occasionally crawling slowly among sand grains.

Infraciliature as shown in Figures 1E, 1F, 1H, 1I, 2C, 2E, 2F, 2I. On average, 37 somatic kineties present, each composed of monokinetids plus five to seven dikinetids in anterior portion (Figures 1B, 1H, 1I, 2C, 2E, 2F, 2I). Brush kineties composed of

Table 1. Morphometric data of *Paraspathidium apofuscum* n. sp. after protargol impregnation.

Characters	Min.	Max.	Mean	SD	CV	n
Body length ( $\mu\text{m}$ )	152	240	190	27.66	14.56	10
Body width ( $\mu\text{m}$ )	44	72	53.2	8.01	15.06	10
Number of somatic kineties	34	43	37.4	2.81	7.51	12
Length of macronuclear nodule ( $\mu\text{m}$ )	16	33	24.1	5.53	22.95	16
Width of macronuclear nodule ( $\mu\text{m}$ )	8	16	11.9	2.5	21.01	15
Number of micronuclei	1	1	1	0	0	16
Number of macronuclear nodules	2	2	2	0	0	16

Note: CV, coefficient of variation in %.

three parts: (1) two short dikinetid kineties; (2) four or five short monokinetid kineties; (3) ca. 20 irregularly distributed kinetosomes (Figures 1B, 1I, 2E). Buccal apparatus located at anterior end of cell. Oral opening apical and irregularly elliptical (Figure 2O). Perioral kinety, consists of ca. 50 pairs of kinetosomes, does not form a closed circle. Numerous fine fibres associated with the buccal margin (Figures 1B, 2E). Reticular silverline system consists of quadrangular parts, with one cross line going through the kinetosome in each part (Figure 1C).

#### Remarks and comparison

Hitherto, *Paraspathidium* was a monotypic genus, the only species being *P. fuscum* (Kahl, 1928) Fjeld, 1955, which was redescribed by Foissner (1997b). Our new species differs clearly from *P. fuscum* in the following combination of characters: (1) fewer somatic kineties (34–43 vs. 50–60); (2) the absence of conspicuous dorsal brush (vs. the presence of conspicuous, highly differentiated dorsal brush; see Figures 2L, 8D, 8E); (3) perioral kinety open (vs. closed in *P. fuscum*) (Figures 8D, 8E); (4) contractile vacuole without detectable collecting canals (vs. with several collecting canals extending to mid-body in *P. fuscum*) (Foissner 1997b).

Family **HELICOPRORODONTIDAE** Small and Lynn, 1985

Genus *Trachelotractus* Foissner, 1997

*Trachelotractus entzi* (Kahl, 1927) Foissner, 1997

(Figures 3, 4, 8; Table 2)

#### Remarks

The morphology and infraciliature of the Qingdao population of *Trachelotractus entzi*, which is the first report of this ciliate from China, corresponds well with former studies (Kahl 1927; Dragesco 1960; Foissner 1997a; Figure 8A–C). Here, we provide a brief redescription and some photomicrographs for this species.

#### Description of the Qingdao population

Body vermiform, highly contractile and flexible (Figures 3A, 3B, 4A, 4C–E). Contracted cells ca.  $250 \times 40 \mu\text{m}$  and extended ones can reach ca.  $750 \times 30 \mu\text{m}$ . “Head” bulkier than posterior region, and filled with numerous dark granules, body widest at

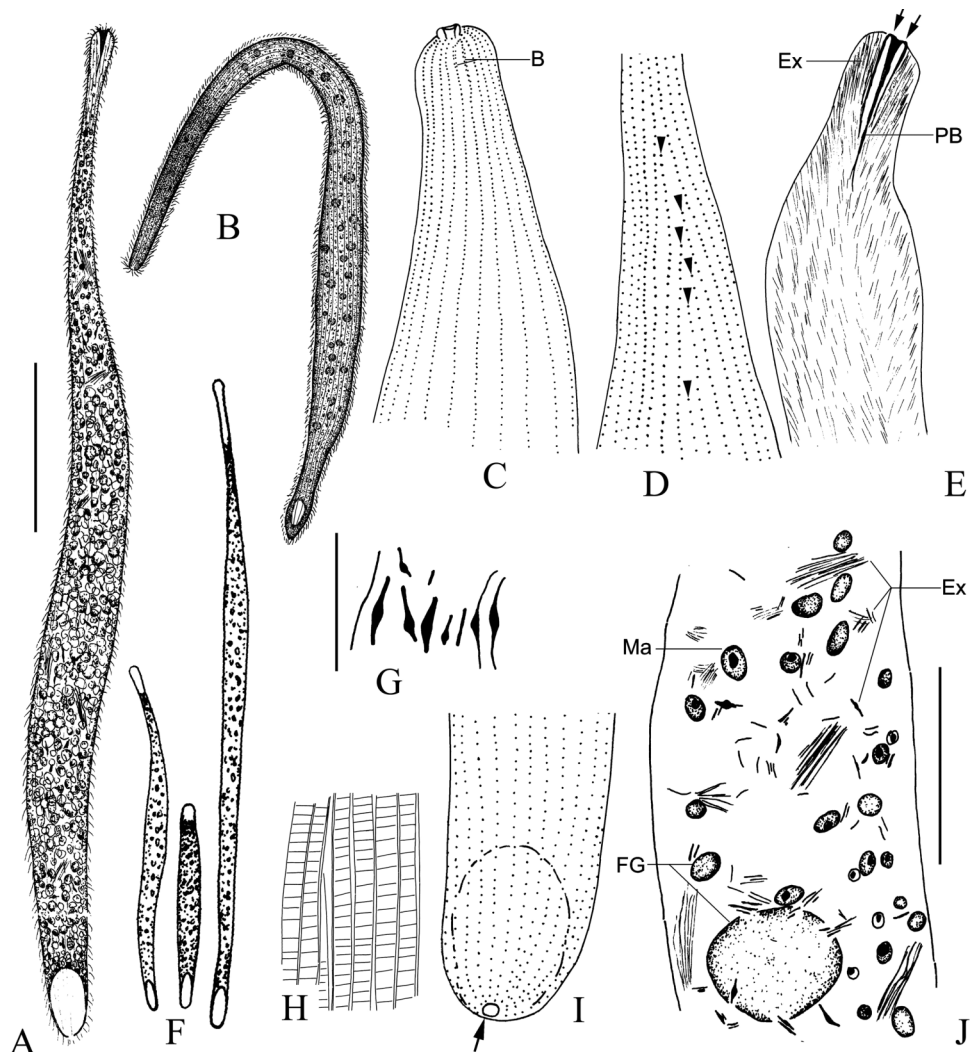


Figure 3. *Trachelotractus entzi* (Kahl, 1927) Foissner, 1997 from live cells (A, B, F) and after silver nitrate (H) and protargol stainings (C–E, G, I, J). (A) a typical cell; (B) a not fully extended cell; (C, I) infraciliature of anterior and posterior body portion, arrow marks the pore of contractile vacuole; (D) showing infraciliature in neck region, arrowheads mark the shortened somatic kineties; (E) showing pharyngeal basket (PB) and nematodesmata in neck region; (F) showing extended, contracted and half-extended cells (from right to left); (G) showing various extrusomes; (H) silverline system; (J) cytoplasmic inclusions in trunk region. Notes: B, brush kineties; Ex, extrusome; FG, fat globules; Ma, macronuclear nodules; PB, pharyngeal basket; scale bars: 70  $\mu\text{m}$  (A); 20  $\mu\text{m}$  (G); 40  $\mu\text{m}$  (J).

trunk (Figures 3B, 4A–E). One contractile vacuole terminally located, pore opening at posterior end of cell (Figures 3A, 3B, 3F, 3I, 4A, 4C, 4D). Somatic cilia 4  $\mu\text{m}$  long *in vivo*. Extrusomes thread-, fibril- or rod-like, randomly distributed in the cytoplasm, 2–16  $\mu\text{m}$  long (Figure 3E, 3G, 3J). Pellicle coarse, composed of quadrangular units (Figure 4F). Locomotion by crawling between sand grains.

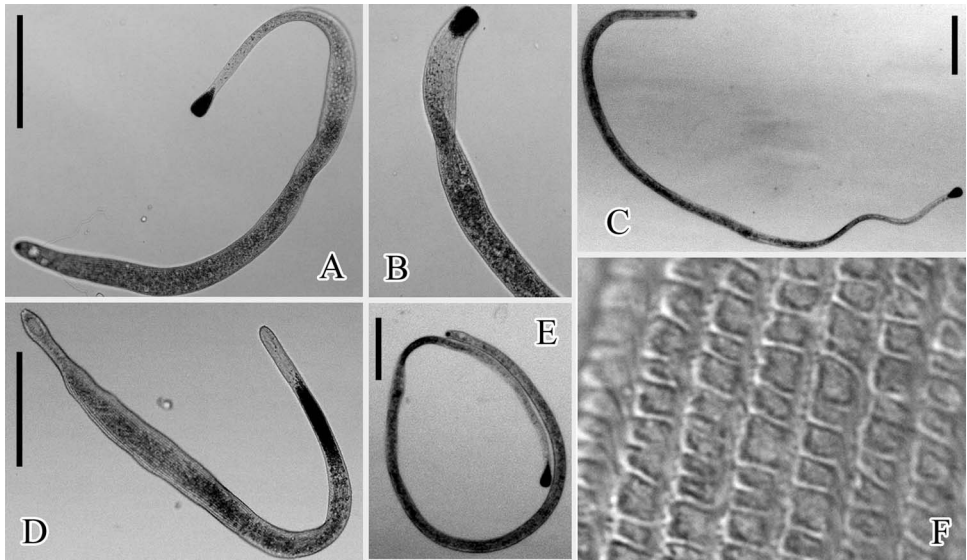


Figure 4. Photomicrographs of *Trachelotractus entzi* (Kahl, 1927) Foissner, 1997 from live cells (A–F). (A, D) half-extended cells; (B) anterior part of a cell; (C) a fully extended cell; (E) a highly winding cell, note the black “head” of the cell; (F) pellicle structure. Note: scale bars: 100  $\mu\text{m}$ .

Table 2. Morphometric characteristics of *Trachelotractus entzi* (Kahl, 1927) Foissner, 1997 (upper line) and *Apotrachelotractus variabilis* Long, Song and Warren, 2009 (lower line) from protargol impregnated specimens.

Characters	Min.	Max.	Mean	SD	CV	n
Body length ( $\mu\text{m}$ )	284	696	398.8	105.53	26.5	20
	240	448	335.7	56.63	5.20	27
Body width ( $\mu\text{m}$ )	29	60	41.0	8.72	21.30	19
	32	56	41.1	5.72	5.10	26
Head width ( $\mu\text{m}$ )	12	19	15.8	1.59	10.06	20
	16	24	19.4	3.41	5.48	30
Pharyngeal basket length ( $\mu\text{m}$ )	16	25	20.9	2.59	12.40	18
	22	40	28.1	4.81	4.58	21
Pharyngeal basket width ( $\mu\text{m}$ )	3	8	4.37	1.16	26.50	19
	4	6	4.7	0.69	4.90	24
Number of somatic kineties on trunk	20	26	22.5	1.26	5.60	19
	23	30	25.8	1.92	4.69	22
Number of kinetosomes in 10 $\mu\text{m}$	8	17	11.0	83.8	23.90	19
	11	15	12.6	1.36	4.00	16
Number of brush kineties	2	2	2	0	0	6
	1	1	1	0	0	7
Number of somatic kineties between neck suture and the brush kinety	–	–	–	–	–	–
	6	8	7.1	0.46	4.36	19

Note: CV, coefficient of variation in %.

Infraciliature shown in Figure 3C, 3D, 3I. Pharyngeal basket long (Figure 3E). Two short dikinetid brush kineties composed of ca. 30 kinetosomes in total. On average, 23 monokinetid somatic kineties on trunk, with several short kineties on the neck, forming a suture (Figure 3d). Kinetosomes in neck region interconnected by dense nematodesmata (Figure 3E). Numerous irregularly distributed, spherical or elliptical macronuclear nodules, number varying with cell size (Figure 3J).

*Apotrachelotractus variabialis* Long, Song and Warren, 2009  
(Figures 5–7; Table 2)

*Remarks*

Long et al. (2009) established the genus *Apotrachelotractus* but provided only a brief description of the type species *A. variabialis*. Here, we supply some supplementary information including morphometric data and detailed descriptions of certain structures.

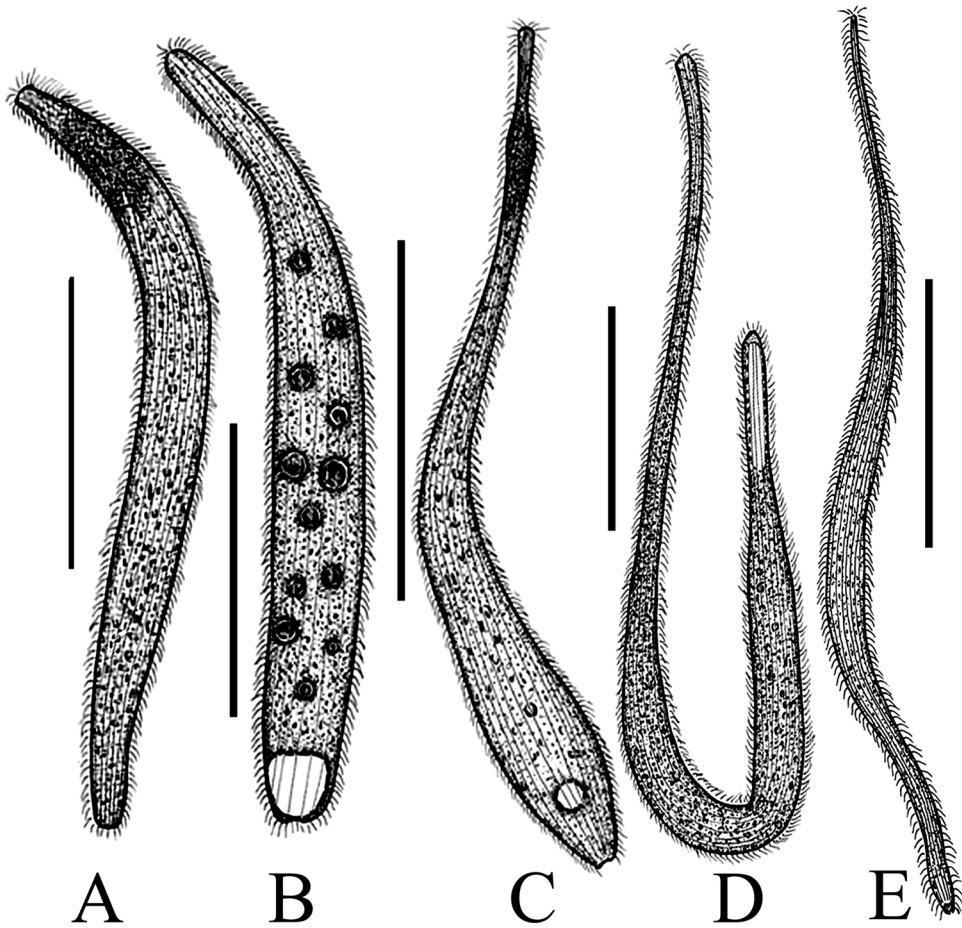


Figure 5. Different shapes of *Apotrachelotractus variabialis* Long, Song and Warren, 2009. (A, B) highly contracted; (C, D) not fully-extended; (E) fully-extended. Note: scale bars: 100  $\mu\text{m}$  (A, B); 200  $\mu\text{m}$  (C, D); 500  $\mu\text{m}$  (E).



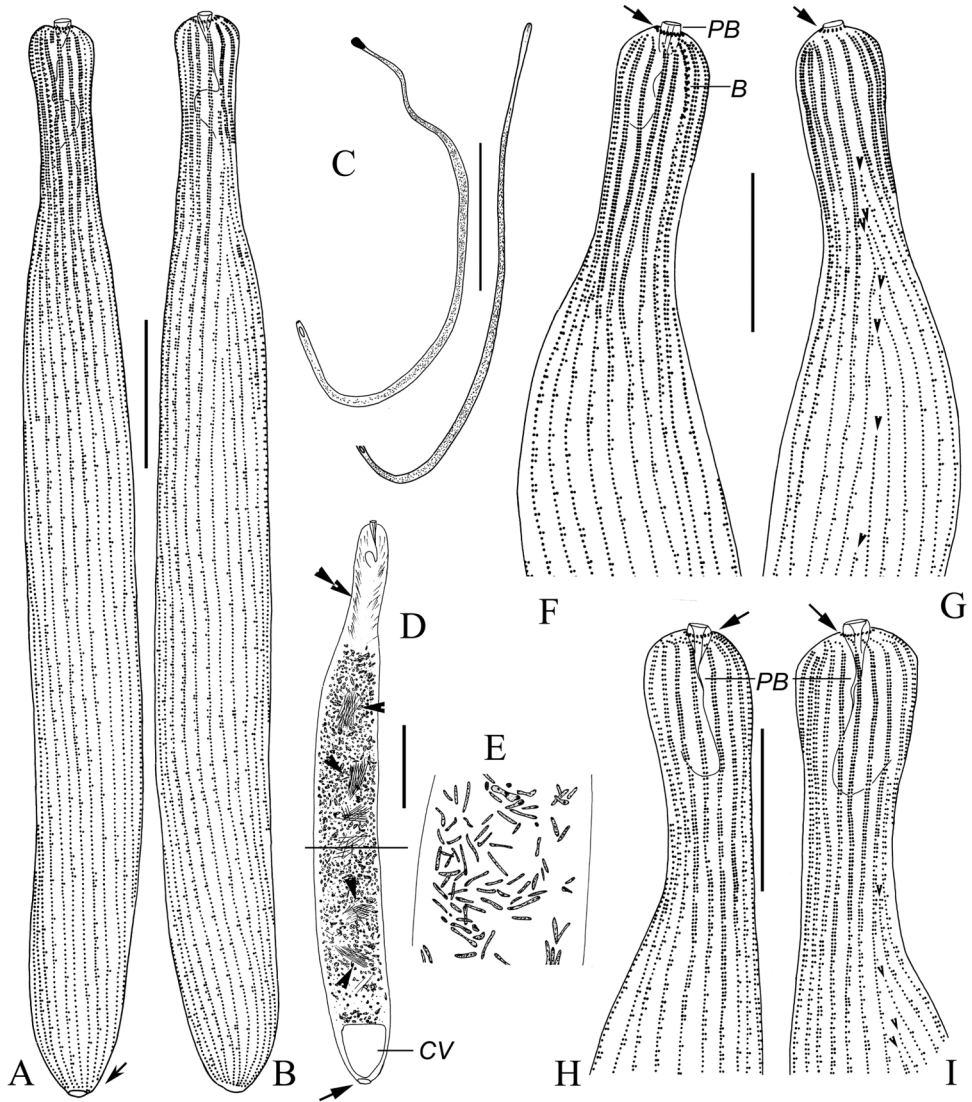


Figure 6. *Apotrachelotractus variabilis* Long, Song and Warren, 2009 after protargol staining (A, B, D–I) and from live cells (C). (A, B) infraciliature, arrow marks the contractile vacuole pore; (C) fully-extended individuals; (D, E) to show extrusomes (arrowheads) and macronuclear nodules, double-arrowheads indicate nematodesmata, arrow marks the contractile vacuole pore; (F–I) structure of anterior parts, arrows indicate the circumoral kinety, arrowheads show the suture on the “neck”. Notes: B, brush kinety; PB, pharyngeal basket; scale bars: 60  $\mu\text{m}$  (A); 45  $\mu\text{m}$  (D); 30  $\mu\text{m}$  (F, H).

### Redescription

Body contractile and flexible, vermiform in shape. Body size variable, ca. 290–500  $\times$  25–35  $\mu\text{m}$  *in vivo* when contracted and about 800–1700  $\times$  15–20  $\mu\text{m}$  *in vivo* when extended. On average, 335  $\times$  40  $\mu\text{m}$  after fixation (Figure 5A–E). Head usually

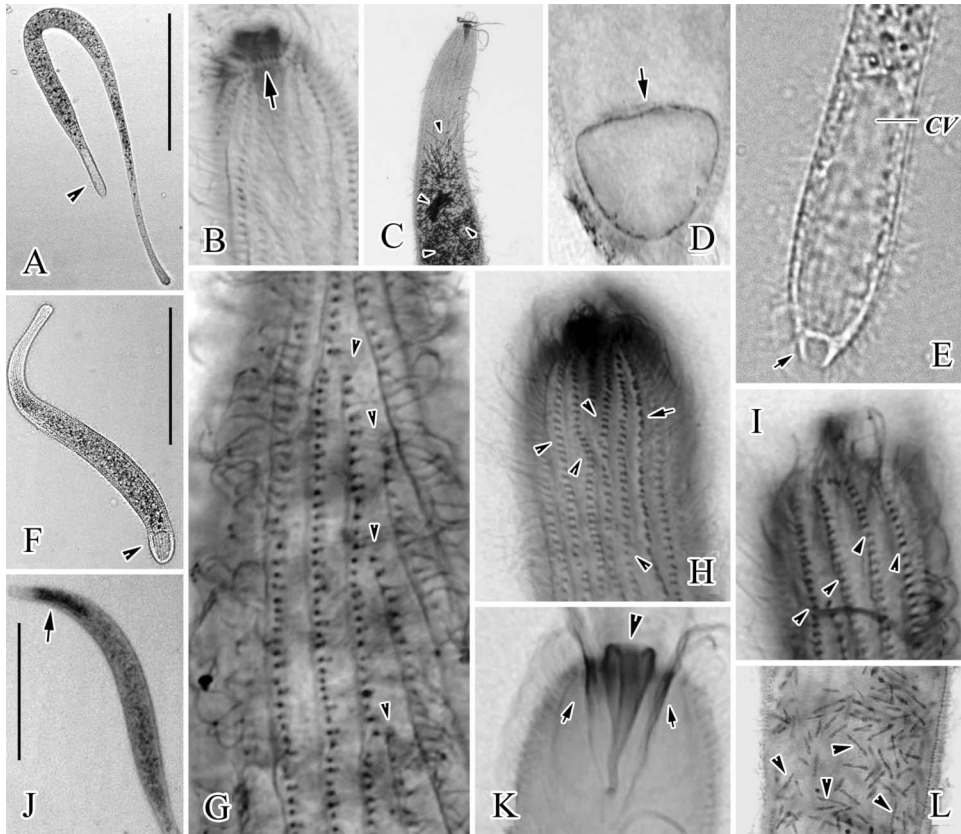


Figure 7. Photomicrographs of *Apotrachelotractus variabilis* Long, Song and Warren, 2009 from live cells (A, E, F, J) and after protargol impregnation (B–D, G–I, K, L). (A) lateral view of a not fully-extended cell, arrowhead indicates the contractile vacuole; (B) to show the circumoral kinety (arrow); (C) arrowheads show the extrusomes in the cell trunk; (D) the contractile vacuole (arrow); (E) posterior end of a cell, arrow marks the contractile vacuole pore; (F) lateral view of a contracted cell; (G) to show the neck suture (arrowheads); (H) anterior end, arrow marks the brush kinety, arrowheads indicate dikinetid kineties; (I) to show the dikinetids in the anterior end of a cell; (J) a highly contracted cell, arrow marks the dark area in the anterior part; (K) pharyngeal basket (arrowhead) and extrusomes beside the cytostome (arrows); (L) the macronuclear nodules (arrowheads). Note: scale bars: 300  $\mu\text{m}$  (A); 200  $\mu\text{m}$  (F, J).

globular, sometimes full of dark granules (Figures 5A, C, 6C, 7J). Macronuclear nodules rod-like, 500–700 in number, distributed throughout cell trunk (Figures 6D, 6E, 7L). Single contractile vacuole terminally located, irregularly rounded, 30  $\mu\text{m}$  in diameter (Figures 6D, 7A, 7D–F). Contractile vacuole pore at posterior end of body (Figures 5C, 5E, 6A, 6D, 7E). Three types of extrusomes exist: (1) perioral extrusomes ca. 22  $\mu\text{m}$  long (Figure 7K); (2) fibril-like extrusomes in neck region, about 10  $\mu\text{m}$  long; (3) needle-like somatic extrusomes, ca. 20  $\mu\text{m}$  long (Figures 6D, 7C). Cytoplasm hyaline, containing numerous small food vacuoles (<12  $\mu\text{m}$ ). Somatic cilia ca. 6  $\mu\text{m}$  long *in vivo*.

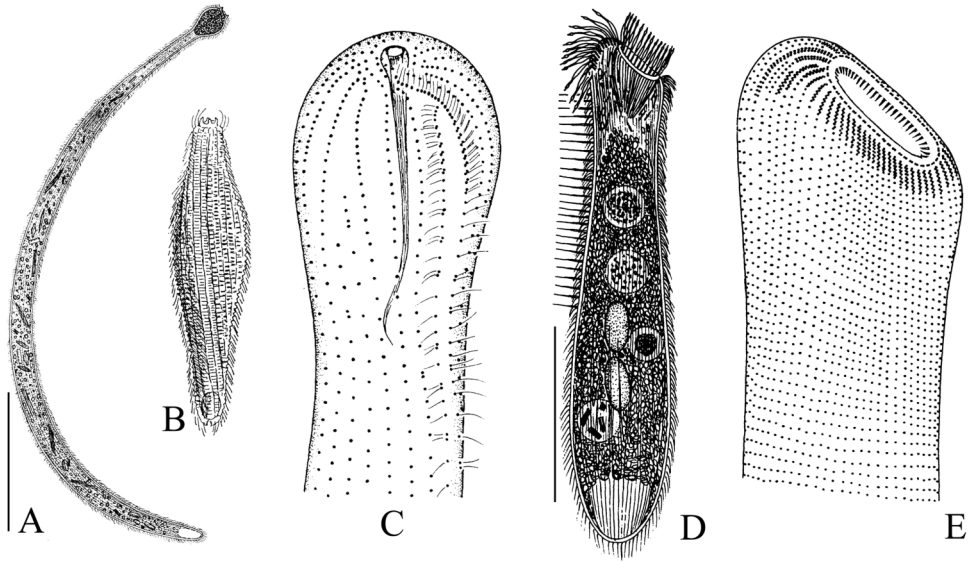


Figure 8. *Trachelotractus entzi* (Kahl, 1927) Foissner, 1997 (A–C) and *Paraspathidium fuscum* (Kahl, 1928) Fjeld, 1955 (D, E), from life (A, B, D) and after protargol impregnation (C, E). (A) lateral view, from Foissner (1997a); (B) a contracted cell, from Kahl (1927); (C) infraciliature of anterior part, from Foissner (1997a); (D, E) lateral view and infraciliature of anterior part, from Foissner (1997b). Note: scale bars: 200  $\mu\text{m}$  (A); 90  $\mu\text{m}$  (D).

Cells usually float in water, rotating around their long axis and continuously transforming their shape (Figure 5A–E); sometimes crawling among sand grains, slightly thigmotactic.

Buccal apparatus funnel-like, apically located, with long posterior nematodesmata (Figures 6F–I, 7K). Cytostome encircled by perioral kinety (Figures 6F, 6H, 7B).

Infraciliature as shown in Figure 6A, 6B, 6F–I. Twenty-three to 30 somatic kineties on cell trunk, each composed of monokinetids below neck and dikinetids above neck (Figures 6A, 6B, 6F–I, 7H, 7I). One brush kinety located in neck region, consisting of 60–100 kinetosomes arranged in about 20–30 groups of three kinetosomes. Brush kinety difficult to discern when located on cell margin (Figures 6F, 6H, 7H). Suture on cell neck conspicuous, ca. seven kineties from brush kinety (Figures 6F, 6G, 6I, 7G; Table 2).

## Discussion

*Apotrachelotractus variabialis* closely resembles *Trachelotractus entzi* (Kahl, 1927) Foissner, 1997 in terms of its live morphology (e.g. elongate body shape, the presence of pharyngeal basket and extrusomes) and marine habitat. However, *A. variabialis* can be distinguished from *T. entzi* by its brush kinety (one highly specialized group of kinetosomes vs. two short rows of dikinetids), the anterior portion of the somatic kineties (dikinetid vs. monokinetid in *T. entzi*) (Figure 8A, 8C; Foissner 1997a), and the peribuccal ridge (inconspicuous vs. conspicuous in *T. entzi*).

At first glance at its infraciliature, *A. variabialis* may seem like an early divider of *T. entzi*, although no dividing cells were observed. Furthermore, protargol impregnations of *A. variabialis* were conducted every day for 10 days following their isolation and no *T. entzi* morphotype was found. In addition, the stable statistical data on the infraciliature also indicates that this form is a different species from *T. entzi* (Table 2).

Unfortunately, the original sampling beach was destroyed to build an abalone-culturing pond and so we are not able to re-isolate *A. variabialis* from the same location. Nevertheless, further studies on other populations of *A. variabialis*, including analyses of gene sequencing data, are needed in order to determine the precise systematic position of this species.

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