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Morphological description of two new ciliates (Ciliophora, Karyorelictea, Cryptopharyngidae): *Apocryptopharynx discoidalis* spec. nov. and *Cryptopharynx minutus* spec. nov.

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Abstract

The karyorelictids, a highly specialized group of ciliates, mostly inhabit marine biotopes, and there is very little information about their diversity, taxonomy, and systematics. The present paper investigates two new cryptopharyngid ciliates, *Apocryptopharynx discoidalis* spec. nov. and *Cryptopharynx minutus* spec. nov., which were isolated from the intertidal zone of Diaosuyuan Beach in Qingdao, China. *Apocryptopharynx discoidalis* spec. nov. can be distinguished from related species by its oval discoid body shape and highly structured epipellicular scales covering the left lateral surface. *Cryptopharynx minutus* spec. nov. can be recognized by its small body length (30–50 μm), seven to 15 dikinetids in intrabuccal kinety, and two to five macronuclei. Based on our comparison, we believe that *Cryptopharynx* sp. 1 described by Foissner in 1996 represents a population of *C. minutus* spec. nov.

Keywords: Cryptopharyngidae; Epipellicular scales; Karyorelictea; New species; Taxonomy

Introduction

Karyorelictea is widely considered as a key group for studying the phylogeny of ciliated protistans, on account of their characteristic primitive, non-dividing macronuclei (Corliss 1974, 1979; Foissner 1998; Lynn 2008). However, as most species of this class are unculturable and too fragile to be fixed, their somatic and oral ciliature had been poorly explored until the 1990s, when a series of pioneering works was done on karyorelictean ciliates (Al-Rasheid 1996, 1997, 1998; Foissner 1995, 1996a,b, 1997; Foissner and Al-Rasheid 1999; Foissner and Dragesco 1996a,b). In the following ten years, few taxonomic studies on this group had been published (Alekperov et al. 2007; Al-Rasheid 2001). This situation has changed recently, with dozens of new taxa, including one new family and three new genera, being reported, and several little known species being re-described in detail (Campello-Nunes et al. 2015; Mazei et al. 2009; Xu et al. 2011a,b,c, 2012, 2013a,b, 2014, 2015; Yan et al. 2013,

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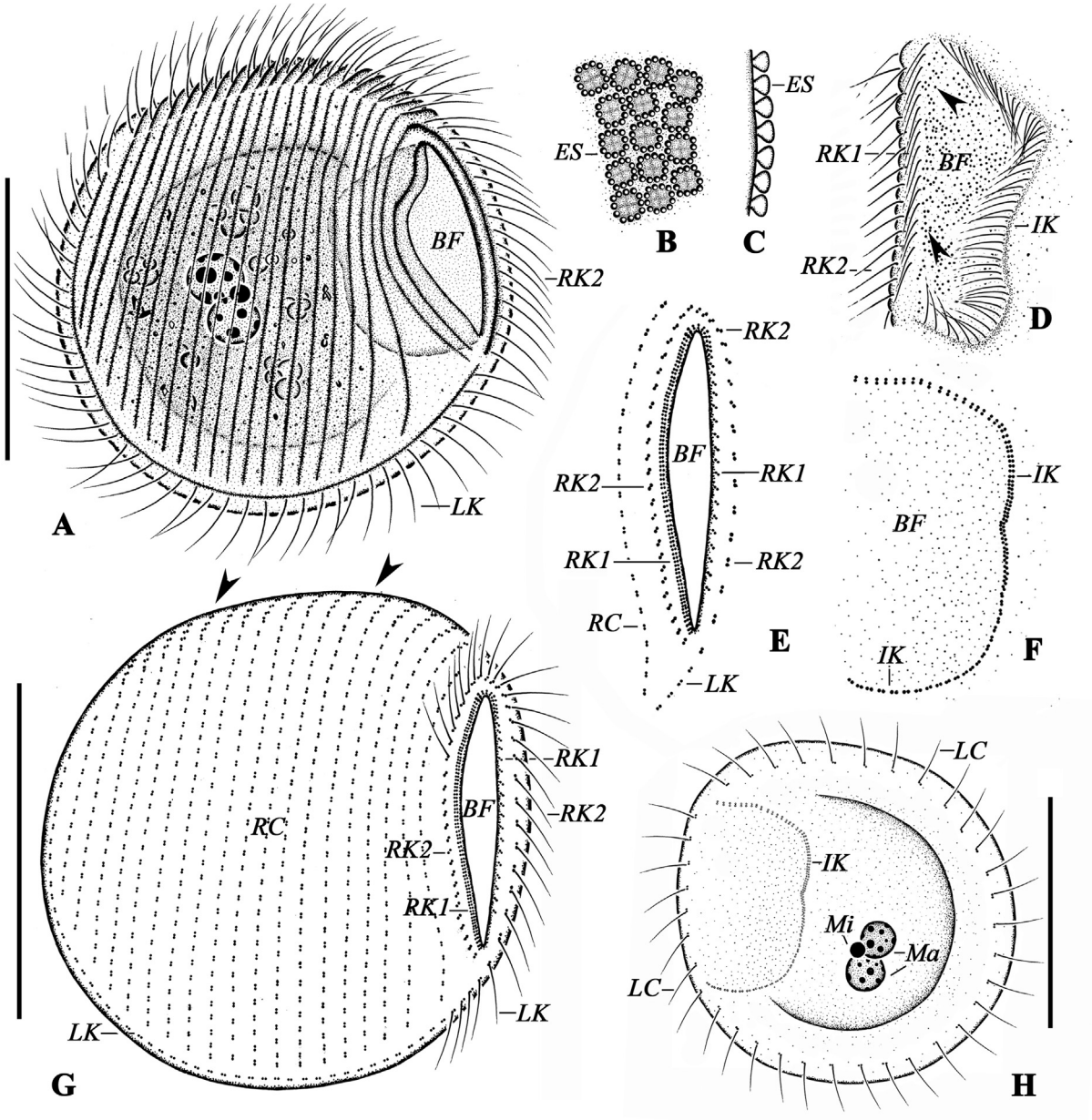

Fig. 1. A–H. *Apocryptopharynx discoidalis* spec. nov. from life (A–D) and after protargol staining (E–H). **A**. Right side of a representative individual; showing the buccal field, part of second right buccal kinety and dorsolateral kinety. **B, C**. Top (B) and lateral (C) view of epipellicular scales on left side of cell, showing each scale is composed of ca. 12 short rods forming square with cruciform content in the middle in top view while triangular in lateral view. **D**. Lower focal plane of buccal field to indicate intrabuccal kinety, first and second right buccal kineties; arrowheads mark cortical granules distributed in buccal field. **E**. Oral ciliature showing first and second right buccal kineties, and dorsolateral kinety. **F**. Detailed view of buccal field, indicating the distribution of dikinetids of intrabuccal kinety. **G, H**. Ciliature of right (G) and left (H) side and nuclear apparatus of holotype specimen, noting first and second right buccal kineties, right and left ciliary rows, dorsolateral kinety, macronuclei and micronucleus. BF, buccal field; ES, epipellicular scales; IK, intrabuccal kinety; LC, left ciliary row; LK, dorsolateral kinety; Ma, macronuclei; Mi, micronucleus; RC, right ciliary rows; RK1, first right buccal kinety; RK2, second right buccal kinety. Scale bars = 40 μm (A, G, H).

2015, 2016). So far, six families have been reported in this class: Cryptopharyngidae, Geleiidae, Kentrophoridae, Loxodidae, Trachelocercidea and Wilbertomorphidae. Among these families, cryptopharyngids are the least studied.

The family Cryptopharyngidae Jankowski 1980 is described as a "marine small- to medium-sized Loxodida with dorsolateral kinety extending onto the ventral side; with the left body surface covered with distinct, ornamented scales embedded in a mucous layer; with the buccal kineties continuous, and the intrabuccal kinety short and curved or long and clip-shaped; with the buccal overture roundish to ellipsoid, and indistinct because it is not pigmented and narrow" (Lynn

Table 1. Morphometric data from *Apocryptopharynx discoidalis* spec. nov. (first line) and *Cryptopharynx minutus* spec. nov. (second line).

Characteristics[a]	Min	Max	M	Mean	SD	CV	n
Body, length (μm)	47	82	60	60.3	8.8	14.6	17
	23	43	32	32.9	5.1	15.4	15
Body, width (μm)	42	73	54	53.6	7.8	14.5	17
	18	30	22	22.3	3.2	14.4	15
Right ciliary rows, number	15	21	19	18.5	1.6	8.8	17
	10	14	13	12.3	1.1	8.5	15
Dikinetids in first right buccal kinety, number	74	110	95	94.4	11.0	11.6	17
	47	55	50	50.3	2.3	4.5	15
Dikinetids in second right buccal kinety, number	33	58	45	45.6	7.5	16.4	17
	37	45	41	40.9	2.3	5.7	15
Dikinetids in intrabuccal kinety, number	40	80	63	61.4	12.5	20.3	17
	7	15	11	11.1	2.2	19.8	15
Macronuclei, number	2	4	3	2.6	0.7	26.5	17
	2	5	2	2.3	0.8	35.2	15
Micronuclei, number	1	1	1	1.0	0	0	17
	1	2	1	1.1	0.3	24.2	15

Abbreviations: CV, coefficient of variation in %; M, median; Max, maximum; Mean, arithmetic mean; Min, minimum; n, number of specimens investigated; SD, standard deviation of arithmetic mean.

[a] All data are based on protargol-stained specimens.

2008). According to Lynn (2008), this family includes two genera: *Apocryptopharynx* Foissner, 1996 and *Cryptopharynx* Kahl, 1928. Since its establishment, six *Cryptopharynx* species have been reported: *C. setigerus* Kahl, 1928; *C. enigmaticus* Dragesco, 1954; *C. kahli* Dragesco, 1954; *C. mauritanicus* Dragesco, 1965; *C. multinucleatum* Dragesco, 1954; and *C. wardi* Small and Lynn, 1985. Foissner (1996b) transferred *C. wardi* to the genus *Apocryptopharynx*; further, based on the protargol preparation, he described two unidentified forms (*Cryptopharynx* sp. 1 and *Cryptopharynx* sp. 2). So far, *Apocryptopharynx* includes only two species: *A. hippocampoides* Foissner, 1996 and *A. wardi* (Small and Lynn, 1985) Foissner, 1996. Although Jankowski (2007) erected a new genus *Pilopharynx* and designated *A. wardi* as its type species, we think that this genus was established prematurely without further studies on this species (see discussion below). Among all the species mentioned above, data from detailed live observations and other examinations of protargol-stained specimens is available only for *C. setigerus* and *A. hippocampoides* (Foissner 1996b). All the other species have simple descriptions based on either live observation or the protargol-staining method.

One remarkable and unique characteristic of this family are the epipellicular scales embedded in the mucous layer. Unfortunately, studies on these structures are scarce (Dragesco 1960; Foissner 1996b). Previous studies (Dragesco 1960; Foissner 1996b) and our observations indicate that these structures are specific to these species and thus can be used as an identifying feature. However, the fine structures and their functions remain unknown, which await further studies including scanning and transmission electron microscopy studies.

In the present work, two novel species of cryptopharyngids have been characterized based on in vivo observation and protargol staining method.

Material and Methods

Samples were collected from sandy sediments in the intertidal zone of Diaosuyuan Beach in Qingdao, China (36°05′03″N, 120°27′37″E) on May 7, 2011. A 15-cm-deep hole was dug in the sand into which seawater gradually seeped in, and then a mixture of seawater (water temperature, 20 °C; salinity, 17‰) and sand from the bottom of the hole was collected as sample. Both *Apocryptopharynx discoidalis* spec. nov. and *Cryptopharynx minutus* spec. nov. occurred at very low abundance in the sample. The cells were isolated and observed in vivo using bright field and differential interference contrast microscopy (100× to 1000× magnification).

The infraciliature was examined using the protargol staining method (Wilbert 1975). Counts and measurements of stained specimens were performed at a magnification of 1000×. Drawings were made with the help of a camera lucida. The terminology used is according to Foissner (1996b).

DNA extraction for both species was unsuccessful, as the cells were too sticky and fragile to be transferred into Eppendorf tubes. Thus, the DNA sequences of these species cannot be reported in the current study.

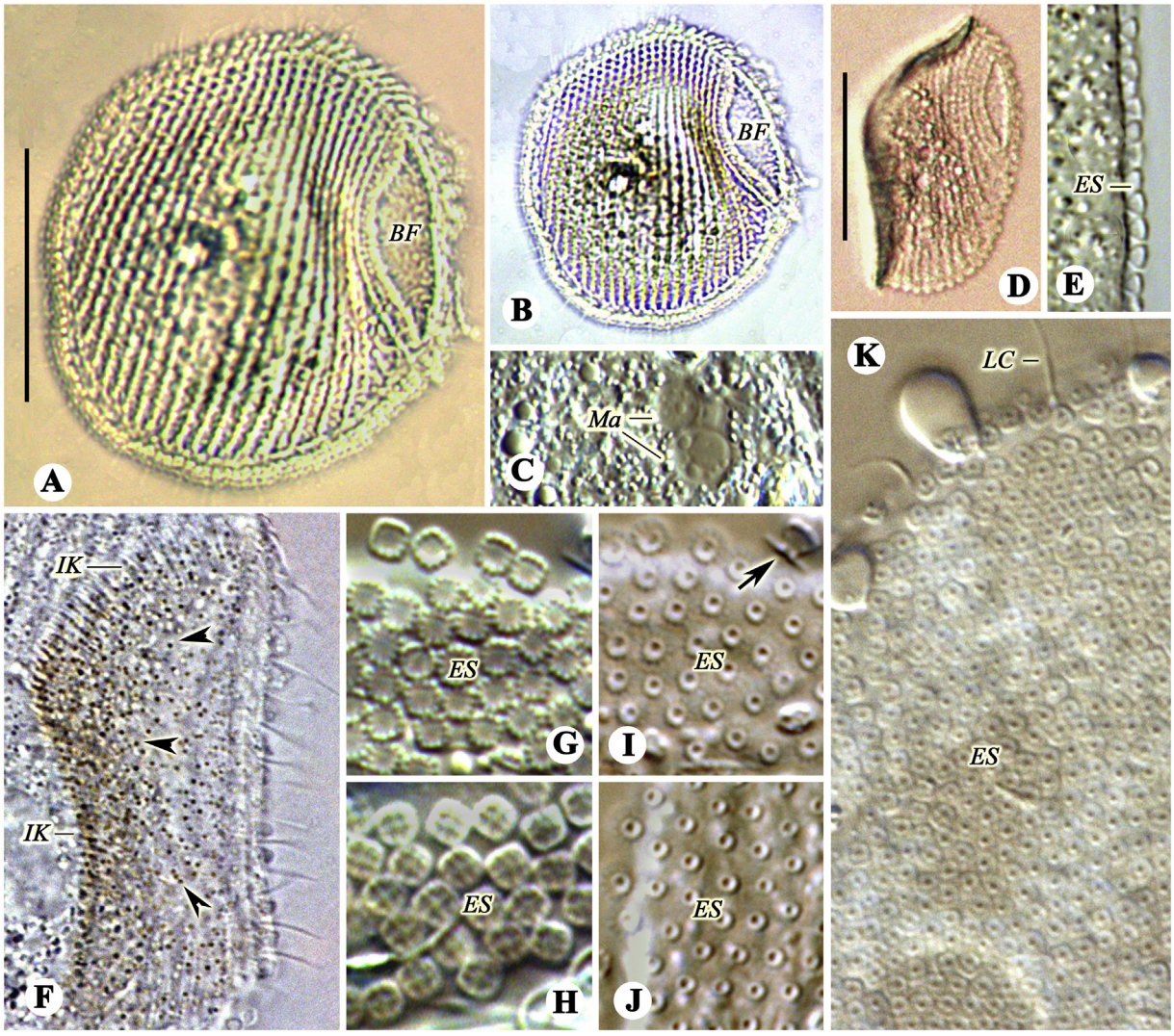

Fig. 2. A–K. *Apocryptopharynx discoidalis* spec. nov. from life. **A, B**. Right side of a representative individual; showing the buccal field. **C**. Macronuclei. **D**. Contorted cell showing the extremely lateral flattening. **E**. Lateral view, showing the triangular shape of epipellicular scales. **F**. Lower focal plane of buccal field indicating intrabuccal kinety; arrowheads mark cortical granules distributed in buccal field. **G–J**. Top view of epipellicular scales from surface (G, H) to lower focal plane (I, J); showing the shape of epipellicular scales changing from square with cruciform content in the middle (G, H) to round fossa (I, J); arrow shows lateral view of one epipellicular scale separated from cell surface. **K**. Left view, marking densely packed epipellicular scales and left ciliary row. BF, buccal field; ES, epipellicular scales; IK, intrabuccal kinety; LC, left ciliary row; Ma, macronuclei. Scale bars = 40 μm (A, D).

Results and Discussion

***Apocryptopharynx discoidalis* spec. nov.** (Figs 1A–H, 2A–K, 3A–H , Table 1)

Diagnosis: Cell in vivo about 70 × 60 μm, oval discoid, with elliptical concave buccal area. Cytoplasm colorless, with brown cortical granules packed in buccal area; left lateral surface covered with mucous layers containing highly specialized epipellicular scales, each composed of ca. 12 short rods arranged in a square, side length 1.5 μm, with cruciform content in the middle from top surface view, and round fossa, 0.8 μm in diameter, from lower focal plane, while triangular, 1.0–1.2 μm long, in lateral view. 15–21 right ciliary rows. First and second right buccal kinety and intrabuccal kinety composed of 74–110, 33–58 and 40–80 dikinetids, respectively. Nuclear apparatus near center of cell consisting of two to four macronuclei and one micronucleus.

Type locality: The intertidal zone of Diaosuyuan Beach at Qingdao (36°05′03″N, 120°27′37″E), China (for details, see Section 'Material and Methods').

Type specimens: The protargol-stained slide containing the holotype specimen (Fig. 1G, H) marked with an inked circle was deposited at the Laboratory of Protozoology, Ocean University of China, China (No. XY11050704). No other slides are available.

Etymology: The species group name *discoidalis* reflects the body shape of this species, which is oval discoid.

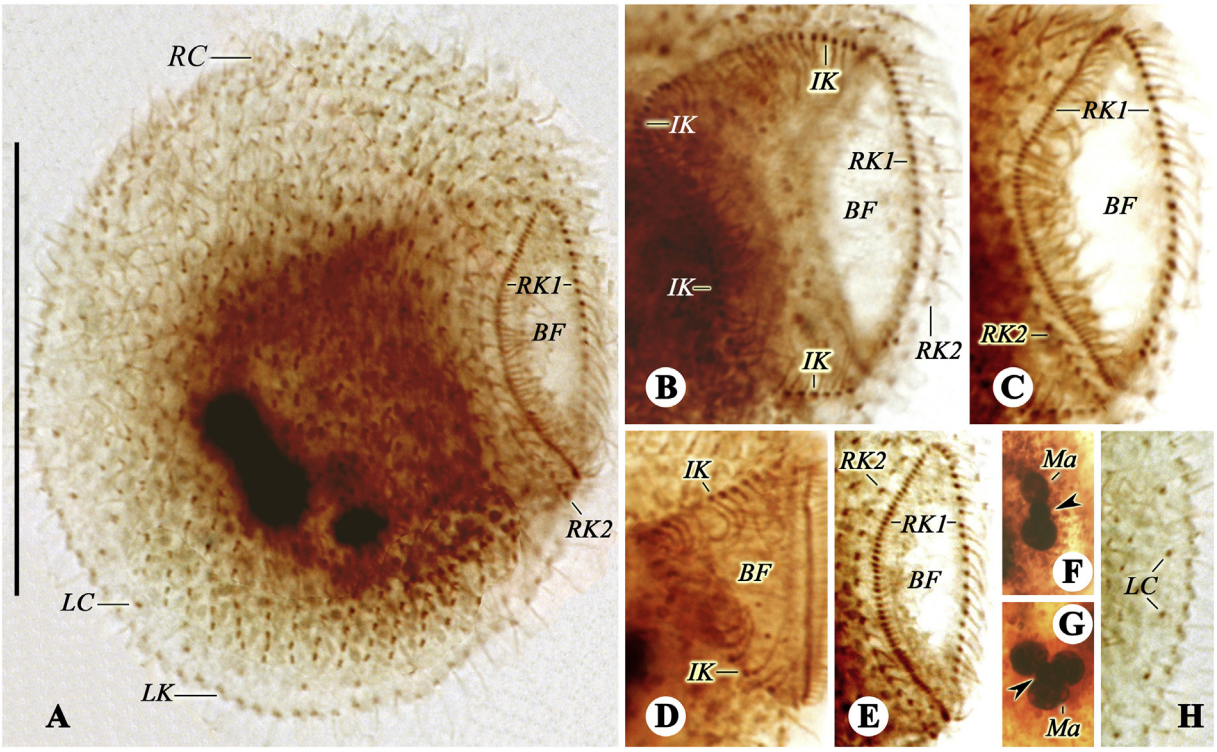

Fig. 3. A–H. *Apocryptopharynx discoidalis* spec. nov. after protargol staining. **A**. Right side of a typical individual; showing the buccal field, first and second right buccal kineties, right ciliary rows, and dorsolateral kinety. **B–E**. Oral ciliature, indicating intrabuccal kinety, first and second right buccal kineties. **F, G**. Macronuclei and micronucleus (arrowhead). **H**. Left side, showing left ciliary row. BF, buccal field; IK, intrabuccal kinety; LC, left ciliary row; LK, dorsolateral kinety; Ma, macronuclei; RC, right ciliary rows; RK1, first right buccal kinety; RK2, second right buccal kinety. Scale bars = 40 μm (A).

Table 2. Comparison among cryptopharyngid species.

Species name	BL (μm)	RC, number	Dikinetids in IK, number	Ma, number	Data source
Apocryptopharynx discoidalis spec. nov.	50–90	15–21	40–80	2–4	Present work
A. hippocampoides	40–90[a]	13–18	16–26	2–4	Foissner (1996b)
A. wardi	ca. 70[b]	18[b]	60[b]	2[b]	Small and Lynn (1985)
Cryptopharynx minutus spec. nov.	30–50	10–14	7–15	2–5	Present work
C. setigerus	25–47[a]	6–11	3 or 4	2 or 3	Foissner (1996b)
C. mauritanicus	110	>40	–	50–70	Dragesco (1965)
C. multinucleatum	90	–	–	10–14	Dragesco (1954)
C. kahli	135	–	–	–	Dragesco (1954)
C. enigmaticus	70–100	17[b]	–	2–5	Dragesco (1954)
*Cryptopharynx*s p. 1	ca. 50[a]	15	ca. 8	2	Foissner (1996b)
Cryptopharynx sp. 2	130[a]	22[b]	ca. 9[b]	6	Foissner (1996b)

Abbreviations: BL, body length; IK, intrabuccal kinety; Ma, macronuclei; RC, right ciliary rows; – data not available.
[a] Data based on protargol-stained specimens.
[b] Data counted from illustrations in reports cited.

Description: Size in vivo about 50–90 × 35–70 μm, mostly 70 × 60 μm. Body oval discoid in outline, elliptical concave oral area, about 35 μm long, located at anterior cell portion (Figs 1A, 2A, B). Extremely lateral flattening, with oral area and hyaline cell margins flexible and often curled; middle region slightly thicker than cell margins (Figs 1A, 2A, B, D). Anterior margin deeply notched as a result of encroaching right ciliary rows (Figs 1A, 2A, B). Right side flat, left vaulted in central region, somewhat protruded, and filled with 1–3 μm sized bright fat globules (Figs 1A, 2A–C). Left surface densely covered with epipellicular scales embedded in thin mucous layer; scales composed of ca. 12 short rods arranged in a square, side length 1.5 μm, with cruciform content in the middle as observed from the top surface view (Figs 1B, 2G, H), and round fossa, 0.8 μm in diameter, from lower focal plane (Fig. 2I–K), while triangular

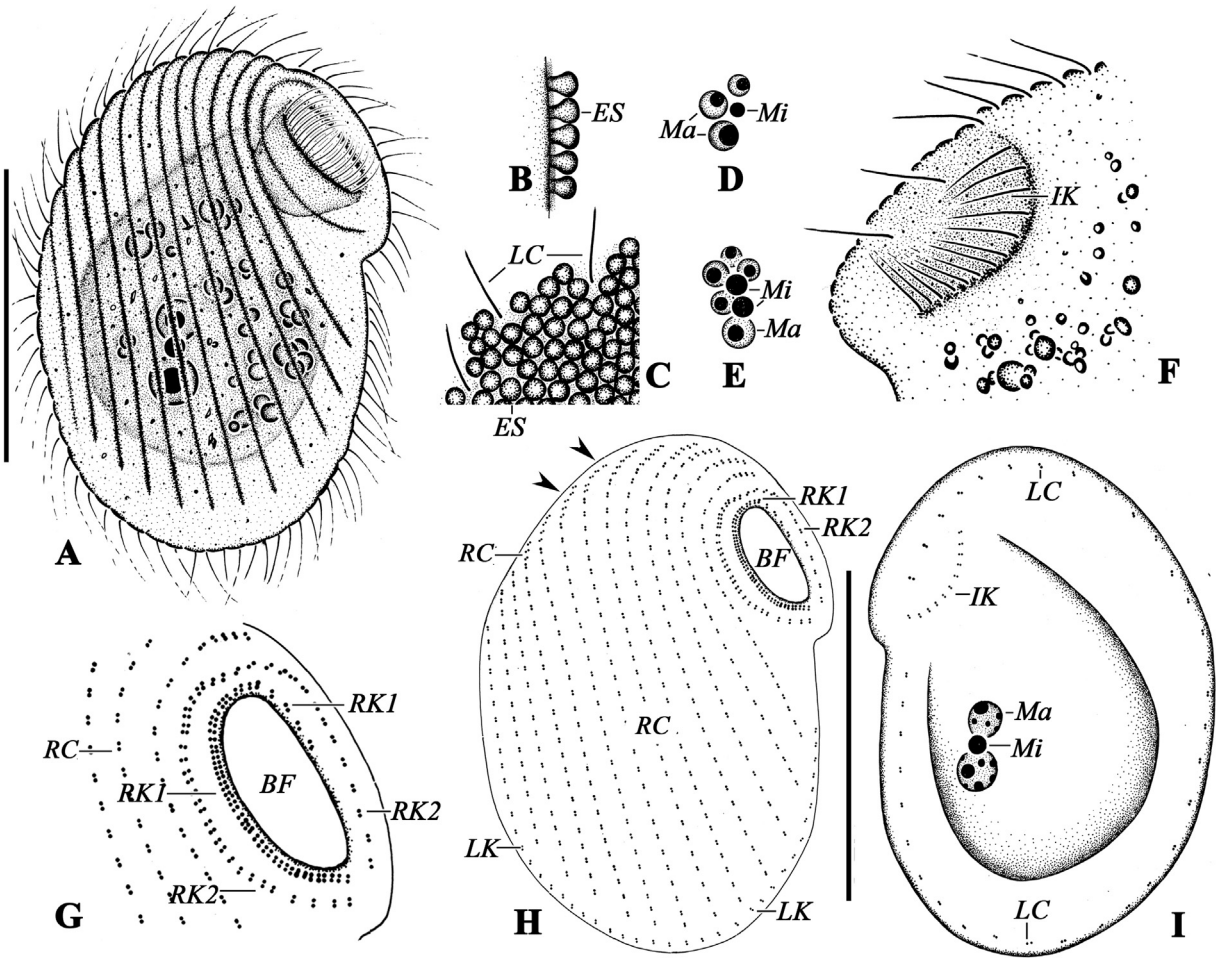

Fig. 4. A–I. *Cryptopharynx minutus* spec. nov. from life (A–F) and after protargol staining (G–I). **A**. Right side of a representative specimen. **B, C**. Lateral (B) and top (C) view of epipellicular scales, showing they are embedded in mucuous material, appears circular in outline when viewed from the top and becomes narrow at the bottom when focused through. **D, E**. Nuclear group with three or five macronuclei and one or two micronuclei. **F**. Lower focal plane of buccal field, indicating intrabuccal kinety. **G**. Oral ciliature, showing first and second right buccal kineties, and right ciliary rows. **H, I**. Infraciliature of right (H) and left (I) side of holotype specimen, noting first and second right buccal kineties, right and left ciliary rows, dorsolateral kinety, macronuclei and micronucleus; arrowheads mark obliquely oriented and more closely spaced dikinetids of anterior end of right ciliary rows. BF, buccal field; ES, epipellicular scales; IK, intrabuccal kinety; LC, left ciliary row; LK, dorsolateral kinety; Ma, macronuclei; Mi, micronucleus; RC, right ciliary rows; RK1, first right buccal kinety; RK2, second right buccal kinety. Scale bars = 20 μm (A, H, I).

(1.0–1.2 μm long), as observed from the lateral view (Figs 1C, 2E). Cytoplasm colorless and transparent. Cortical granules brown, ca. 0.5 μm in diameter, packed in buccal area (Figs 1D, 2F). Movement moderately rapid for some time on substrate, occasionally, still and stuck tightly to the bottom.

Oral and somatic cilia about 5 μm and 7 μm long, respectively. 15–21 right ciliary rows, with dikinetids aligned approximately parallel to kinety axes, except in anterior portion of kineties, where they become almost transversely orientated and tightly spaced (Figs 1A, G, 3A). No kinetids in postoral area (Figs 1G, 3A). Buccal overture roundish to broadly elliptical (Figs 1A, 2A, B). Oral ciliature composed of three different kineties; first and second right buccal kineties extending along right and anterior margin of buccal overture, curving backward along left overture margin and ending almost at bottom of buccal area; first right buccal kinety composed of 74–110 densely packed dikinetids, especially those on right of buccal field, slightly shorter than second right buccal kinety; second right buccal kinety, composed of 33–58 dikinetids with only anterior basal body ciliated, almost connect with dorsolateral kinety; intrabuccal kinety composed of 40–80 dikinetids, and those in anterior part are orientated almost perpendicular to kinety axes, while others in posterior part almost parallel to kinety axes, extending deep into cell (Figs 1D–H, 3B–E). Dorsolateral kinety extending along posterior body margin to bottom of buccal area, connecting with second right buccal kinety (Figs 1A, G, 3A). Left ciliary row extending around cell composed of dikinetids with only one basal body ciliated (Figs 1H, 3H). Nuclear apparatus located near center of cell, com-

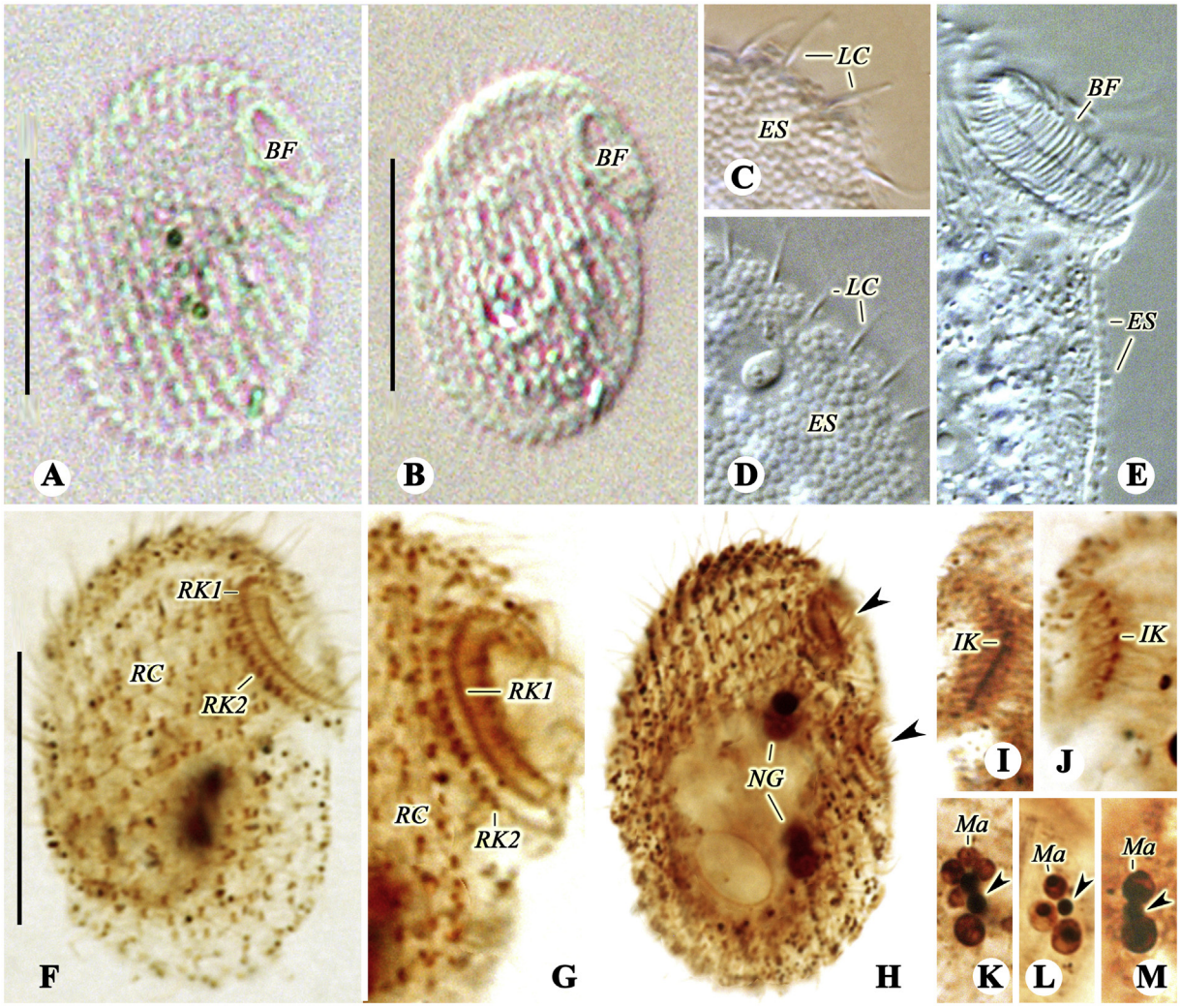

Fig. 5. A–M. *Cryptopharynx minutus* spec. nov. from life (A–E) and after protargol staining (F–M). **A, B**. Right side of a representative individual. **C, D**. Left view, marking densely packed epipellicular scales and left ciliary row. **E**. Noting buccal field and lateral view of epipellicular scales on cell margin. **F**. Infraciliature of right side, marking right ciliary rows, first and second right buccal kineties. **G**. Oral ciliature, showing right ciliary rows, first and second right buccal kineties. **H**. Dividing cell, noting two groups of nuclear apparatus; arrowheads indicate two buccal fields. **I, J**. Intrabuccal kinety. **K–M**. Nuclear apparatus including macronuclei and micronuclei (arrowhead). BF, buccal field; ES, epipellicular scales; IK, intrabuccal kinety; LC, left ciliary row; Ma, macronuclei; NG, nuclear groups; RC, right ciliary rows; RK1, first right buccal kinety; RK2, second right buccal kinety. Scale bars = 20 μm (A, B, F).

posed of two to four macronuclei (4–5 μm across) and one micronucleus, forming cluster (Figs 1A, 2C, 3F, G).

Comparison: Foissner (1996b) established the genus *Apocryptopharynx*, which is characterized by "intrabuccal kinety long and composed of many kinetids forming clip-shaped row extending deeply into the organism." Based on the similarity of the intrabuccal kinety with *Apocryptopharynx* spp., it can be assumed that this new species belongs to the genus *Apocryptopharynx*. So far, two species, i.e., *A. hippocampoides* and *A. wardi*, have been included in this genus. Although Jankowski (2007) transferred the latter to *Pilopharynx* as a type species due to its serrate lateral edges, no further studies have been carried out on it. However, the cell margins of both new species reported in the current study were notched by encroaching right ciliary rows (Figs 2D, 5A, B) which are thought to be not as remarkable as in *A. wardi*, but it is unclear how different these two features are without any micrographs of *A. wardi*. Therefore, we think it was too early to establish the new genus *Pilopharynx* without further details, and therefore, *A. wardi* should be included in *Apocryptopharynx* for now.

The two known species of *Apocryptopharynx* and all species of the genus *Cryptopharynx* are ellipsoid in shape, while the new species differs with regard to this feature as it has a discoidal body. Additionally, *A. hippocampoides* can be distinguished from *A. discoidalis* spec. nov. as it has fewer dikinetids in the intrabuccal kinety (16–26 vs. 40–80) (Table 2; Foissner 1996b).

***Cryptopharynx minutus* spec. nov.** (Figs 4A–I, 5A–M , Table 1)

Diagnosis: Cell in vivo about 40 × 20 μm, shape oval with elliptical protruding oral area. Cytoplasm colorless; left lateral surface covered by epipellicular scales, circular in outline when viewed from the top, about 0.8 μm in diameter, with bottom part narrowed and embedded in mucous material. 10–14 right ciliary rows; first and second right buccal kinety and intrabuccal kinety composed of 47–55, 37–45 and 7–15 dikinetids, respectively. Nuclear apparatus near center of cell consisting of two to five macronuclei and one or two micronuclei.

Type locality: The intertidal zone of Diaosuyuan Beach located at Qingdao (36°05′03″N, 120°27′37″E), China (for details, see Section 'Material and Methods').

Type specimens: The protargol-stained slide containing the holotype specimen (Fig. 4H, I) marked with an inked circle has been deposited at the Laboratory of Protozoology, OUC, China (No. XY11050702). No other slides are available.

Etymology: The species group name *minutus* reflects the body length of this species, which is small.

Description: Size in vivo about 30–50 × 15–25 μm, mostly 40 × 20 μm. Cell oval-shaped with elliptical protruding oral area, about 15 μm long, located at anterior cell portion (Figs 4A, 5A, B). Distinctly lateral flattening, with middle region slightly thicker than cell margins (Figs 4A, 5A, B). Cell margin notched by encroaching right ciliary rows (Figs 4A, 5A, B). Right side flat, left vaulted in central part, which is filled with 1–3 μm-sized bright fat globules and food vacuoles containing digested algae (Figs 4A, 5A). Left surface densely covered with epipellicular scales embedded in thin, mucous layer; scales circular (about 0.8 μm in diameter) in outline when viewed from the top, while approximately triangular in lateral view, 1 μm long, with bottom part narrowed and embedded in mucous material (Figs 4B, C, 5C–E). Cytoplasm colorless and transparent (Fig. 5A, B). Movement by moderately rapid crawling on substrate, sometimes keeping motionless and sticking tightly to the bottom.

Somatic cilia about 5 μm long. 10–14 right ciliary rows, with dikinetids aligned approximately parallel to kinety axes, except in anterior portion of kineties, where they become almost transversely orientated and more closely spaced (Figs 4H, 5F, G). Buccal overture roundish to broadly elliptical (Figs 4G, 5F, G). Oral ciliature composed of three different kineties; first and second right buccal kineties extending along right and anterior margin of buccal overture, curving backward along left overture margin; first right buccal kinety composed of 47–55 densely packed dikinetids and ending at middle of left side of buccal area; second right buccal kinety, composed of 37–45 dikinetids, ending at bottom of left side of buccal area; intrabuccal kinety composed of 7–15 dikinetids extending into buccal cavity (Figs 4F, I, 5I, J). Dorsolateral kinety extending along posterior body margin to bottom of first right ciliary row (Figs 4H, 5F). Left ciliary row extending around cell (Figs 4I, 5C, D). Nuclear apparatus located near center of cell, composed of 2–5 macronuclei, 4–5 μm in diameter, and one or two micronuclei, forming a cluster (Figs 4D, E, I, 5K–M). One dividing cell observed with two nuclear groups and two oral apparatus (Fig. 5H).

Comparison: Foissner (1996b) reinvestigated *Cryptopharynx setigerus* and improved the genus diagnosis as follows: "intrabuccal kinety short and composed of a few kinetids forming a slightly curved row." Therefore, this new species should be assigned to this genus.

Cryptopharynx sp. 1 described by Foissner (1996b) resembles the new species the most. Although no in vivo information is available, their body size is similar (50 μm vs. 30–50 μm), as is the number of right ciliary rows (15 vs. 10–14) and the number of dikinetids in the intrabuccal kinety (ca. 8 vs. 7–15) (Table 2; Foissner 1996b). Since these morphometric data almost overlap, we believe both are conspecific.

The new species resembles *Cryptopharynx setigerus* with regard to body shape and the number of right ciliary rows and macronuclei. However, it can be distinguished from *C. setigerus* by the number of dikinetids in the intrabuccal kinety, which is much higher in the new species (7–15 vs. 3 or 4) (Table 2; Foissner 1996b).

Cryptopharynx minutus spec. nov. differs from *C. kahli*, *C. enigmaticus* and *Cryptopharynx* sp. 2 by its much smaller body length (30–50 μm vs. 135 μm, 70–100 μm, and 130 μm, respectively) (Table 2; Dragesco 1954; Foissner 1996b). In addition, the new species has fewer right ciliary rows than *C. enigmaticus* and *Cryptopharynx* sp. 2 (10–14 vs. 17, 22 respectively) (Table 2; Dragesco 1954; Foissner 1996b).

Cryptopharynx multinucleatum and *C. mauritanicus* can be distinguished from the new species by their higher number of macronuclei (10–14, 50–70 vs. 2–5) and longer bodies (90 μm, 110 μm vs. 30–50 μm). Moreover, *C. mauritanicus* has more right ciliary rows than the new species (at least 40 vs. 10–14) (Table 2; Dragesco 1954, 1965).

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