Taxonomic descriptions of three marine colepid ciliates, *Nolandia sinica* spec. nov., *Apocoleps caoi* spec. nov. and *Tiarina fusa* (Claparède & Lachmann, 1858) Bergh, 1881 (Ciliophora, Prorodontida)

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The morphology of three marine colepid ciliates, *Nolandia sinica* spec. nov., *Apocoleps caoi* spec. nov. and *Tiarina fusa* (Claparède & Lachmann, 1858) Bergh, 1881, isolated from Chinese coastal waters, was investigated. *N. sinica* spec. nov. may be separated from its congeners by the structure of its armour plates, each of which may have up to five reniform windows. *A. caoi* spec. nov. is characterized by its large body with broad anterior end and by having 10–12 long, sharp posterior spines. New data and an improved diagnosis are supplied for *Tiarina fusa* (Claparède & Lachmann, 1858) Bergh, 1881, which has a spindle-shaped body, about 16 ciliary rows and a single adoral organelle. Sequence similarities with other available colepid species were determined.

INTRODUCTION

Colepid ciliates have been studied for more than two centuries since *Coleps hirtus* (O. F. Müller, 1786) Nitzsch, 1827 was first reported (Müller, 1786). To date, over 40 nominal species have been recorded from a range of habitats worldwide. The most conspicuous apomorphy of the colepids is their armoured pellicle comprising longitudinal rows of calcium carbonate plates with small lateral teeth. Individual taxa may be characterized by a variety of morphological features including the number of armour tiers, the structure of the plates, the presence or absence of spines, and the number of adoral organelles. The majority of colepids have been isolated from freshwater (Dragesco & Dragesco-Kernéis, 1991; Foissner *et al.*, 1999, 2008; Huttenlauch, 1987; Huttenlauch & Bardele, 1987; Kahl, 1930, 1933; Noland, 1925, 1937; Obolkina, 1995). By contrast, marine habitats are poorly studied and it is likely that many marine colepids await discovery (Chen *et al.*, 2009, 2010).

The present paper provides morphological descriptions of two novel species, namely *Nolandia sinica* spec. nov. and *Apocoleps caoi* spec. nov., from coastal waters of northern

China and new data for a well-known species, *Tiarina fusa* (Claparède & Lachmann, 1858) Bergh, 1881, a population of which was isolated from coastal waters of the South China Sea. Molecular information about the two novel species and the Chinese population of *Tiarina fusa*, including gene sequence data and phylogenetic analyses, have been described by Yi *et al.* (2010), although the novel species were neither named nor described in this article. In the present paper we compare sequence similarities in order to determine inter- and intra-generic relationships among the colepids.

METHODS

Nolandia sinica spec. nov. was collected in May 2007 and *Apocoleps caoi* spec. nov. in June 2007, from the intertidal regions of sandy beaches near Jiaozhou Bay, Qingdao (36° 06' N 120° 29' E), China. The upper 15 cm layer of sand was collected together with seawater from the site. Raw cultures were maintained for one or two weeks at room temperature (Wang *et al.*, 2008a, b). *Tiarina fusa* (Claparède & Lachmann, 1858) Bergh, 1881 was collected in October 2007 using 20 µm plankton nets from coastal waters of Daya Bay, near Shenzhen (22° 36' 3" N 114° 32' 46" E), China.

Ciliates were observed *in vivo* using differential interference contrast microscopy (Nikon D80). The infraciliature was revealed by the silver carbonate and protargol impregnation methods (Ma *et al.*, 2003; Song & Wilbert, 1995). Drawings of stained specimens were made with the help of a camera lucida. Measurements were performed under $\times 100$ – 1250 magnification. Systematics and terminology are mainly according to Corliss (1979), Foissner *et al.* (2008) and Chen *et al.* (2010).

The small subunit (SSU) rRNA gene sequences of *N. sinica* spec. nov., *A. caoi* spec. nov. and *Tiarina fusa* were submitted to the GenBank database by Yi *et al.* (2010) with the accession numbers FJ858215, HM747137 and FJ858217, respectively. Other sequences used in the present study for comparative purposes were obtained from the GenBank database as follows: *Nolandia nolandii* (AM292313), *Apocoleps magnus* (FJ858213), *Coleps hirtus hirtus* (AM292311), *Coleps spetati* (AM292312) and *Levicoleps biwae* (AB354737).

RESULTS

Nolandia Small & Lynn, 1985

Nolandia sinica spec. nov. (Figs 1 and 2; Tables 1–3)

Diagnosis. Size about 55 – 65×30 – $40 \mu\text{m}$ *in vivo*, body cylindrical in shape; mean 15 transverse and 16 longitudinal ciliary rows; anterior and posterior main plates with five and four reniform windows, respectively; dominant ridge distinct from the left plate margin; one terminal contractile vacuole; marine habitat.

Type locality. Sandy beach near Jiaozhou Bay, Qingdao, China ($36^{\circ} 06' \text{ N } 120^{\circ} 29' \text{ E}$).

Fig. 1. Morphology and infraciliature of *Nolandia sinica* spec. nov. (a–e, g, h), *Nolandia orientalis* Chen *et al.*, 2010 (f, i), *Pinacocoleps similis* (Kahl, 1933) Chen *et al.*, 2010 (j, k), *Nolandia nolandii* (Kahl, 1930) Small & Lynn, 1985 (l, m), *Coleps hirtus hirtus* (Müller, 1786) Nitzsch, 1827 (n, o). (a) Lateral view of a typical individual. (b) Detail of anterior main plate. (c) Detail of posterior main plate. (d, e) Showing variations in body shape. (f) Posterior main plate of *N. orientalis*. (g) Ciliary pattern at apical end of body. (h) Lateral view of a protargol-impregnated specimen, showing infraciliature and nuclear apparatus. (i) Lateral view of *N. orientalis* (from Chen *et al.*, 2010). (j) Lateral view of *Pinacocoleps similis* (from Chen *et al.*, 2010). (k) Detail of armour plates of *Pinacocoleps similis*. (l) Lateral view of *N. nolandii* (from Wilbert & Schmall, 1976). (m) Detail of main plate of *N. nolandii*. (n) *C. hirtus hirtus* (from Foissner, 1984). (o) Detail of main plate of *C. hirtus hirtus*. AO, adoral organelle; AS, anterior spine; CC, caudal cilium; CK, circumoral kinety; CO, ciliary outlet; CV, contractile vacuole; IB, inner oral basket; Ma, macronucleus; Mi, micronucleus; OB, outer oral basket; PC, perioral ciliary; PS, posterior spine; R, ridge; SK, somatic kinety; W, window. Bars, $20 \mu\text{m}$.

Fig. 2. *Nolandia sinica* spec. nov. from life (a–f), after silver carbonate impregnation (g, h). (a, c, d) Lateral views, showing the body shape, posterior spines (arrowheads), anterior spine (arrow) and caudal cilium (double arrowheads in d). (b, e) Lateral views to show the surface detail; note the large, interconnected windows. (f) Detail of a squashed specimen showing the individual plates. (g, h) Lateral views, showing the ciliary pattern. Bars, 20 μ m.

Table 1. Morphometric characteristics of *Nolandia sinica* spec. nov., *Apocollops caoi* spec. nov. and *Tiarina fusa*

Data are based on protargol-impregnated specimens. *n*, Number.

Character	Species	Min	Max	Mean	SD	<i>n</i>
Body length (μ m)	<i>N. sinica</i>	54	60	57.6	± 2.0	25
	<i>A. caoi</i>	96	120	106.7	± 8.1	15
	<i>T. fusa</i>	56	75	64.0	± 5.0	25
Body width (μ m)	<i>N. sinica</i>	28	36	32.2	± 2.7	25
	<i>A. caoi</i>	34	57	49.2	± 5.9	15
	<i>T. fusa</i>	18	25	20.8	± 1.8	25
Somatic kineties (<i>n</i>)	<i>N. sinica</i>	15	17	16.0	± 0.7	25
	<i>A. caoi</i>	19	24	21.1	± 1.5	15
	<i>T. fusa</i>	15	16	15.7	± 0.4	25
Kinetosomes in each kinety (<i>n</i>)	<i>N. sinica</i>	15	15	15.0	± 0	25
	<i>A. caoi</i>	22	24	23.0	± 0.8	15
	<i>T. fusa</i>	32	41	34.5	± 2.3	25
Macronucleus length (μ m)	<i>N. sinica</i>	12	20	13.3	± 1.7	25
	<i>A. caoi</i>	11	16	14.0	± 1.4	15
	<i>T. fusa</i>	12	20	14.6	± 2.1	25

Type material. A protargol slide containing the holotype specimen encircled on the coverslip is deposited in the Natural History Museum, London, UK, with registration number NHMUK 2010.2.2.1. One paratype slide has been deposited in the collection of the Laboratory of Protozoology, OUC, China, with registration number WYG-2007050702.

Etymology. The specific epithet *sinica* refers to the fact that this organism was first isolated from China.

Description. Shape of body somewhat variable but generally cylindrical or barrel-shaped; usually slightly narrowed at mid-body where the main plates of armour abut; laterally flattened in a ratio of 5:4. Anterior end flat, truncated; posterior end of body tapering moderately to rounded tip (Figs 1a, d, e and 2a). Endoplasm colourless to brownish and opaque; typically packed with granules 2–5 μ m in diameter and food vacuoles approximately 10 μ m in diameter (Figs 1a and 2a). Macronucleus spherical, measuring approximately 8–10 μ m in diameter; micronucleus approximately 2 μ m in diameter, adherent to macronucleus (Fig. 1h). Contractile vacuole caudal (Fig. 1a, e). Armour composed of *Nolandia*-type plates arranged in six tiers, (1) circumoral, (2) anterior secondary, (3) anterior main, (4) posterior main, (5)

Table 2. Morphological comparison of related colepids

Taxa: 1, *Nolandia nolandii* (data from Foissner *et al.*, 1999); 2, *Nolandia orientalis* (Chen *et al.*, 2010); 3, *Nolandia sinica* spec. nov. (data from this study); 4, *Apocoleps magnus* (Chen *et al.*, 2009); 5, *Apocoleps caoi* spec. nov. (this study); 6, *Tiarina fusa* (this study); 7, *Tiarina meunieri* (Kahl, 1930); 8, *Tiarinella gracilis* (Obolkin, 1995). ?, Data not available; AMP, Anterior main plate; PMP, posterior main plate.

Species	1	2	3	4	5	6	7	8
Body shape	Cylindrical	Cylindrical	Cylindrical	Fusiform	Cylindrical	Twisted fusiform	Fusiform	Fusiform, slightly flattened
Body length (µm)	40–65	70–100	55–65	100–120	100–120	85–135	Approx. 80	Approx. 130
Tiers (<i>n</i>)	6	6	6	8	8	6	6	?
Main plate windows (<i>n</i>)	3 complete	AMP: 6; PMP: 5	AMP: 5; PMP: 4	AMP: 4; PMP: 4	AMP: 4; PMP: 4	Absent	Absent	Absent
Window shape	Uni-window	Uni-window	Uni-window	Uni-window	Uni-window	Absent	Absent	Absent
Ciliary rows (<i>n</i>)	12–14	Approx. 20	Approx. 16	Approx. 22	Approx. 23	15–17	?	10–12

posterior secondary and (6) caudal, each of which comprises approximately 16 rectangular plates. Circumoral tier poorly visible *in vivo*. Anterior secondary plates with sharply pointed anterior ends; some with inconspicuous anterior spines (Figs 1a and 2a). Anterior main plates usually with five windows (Fig. 1a, b), posterior main plates with four (Fig. 1a, c), and posterior secondary plates with two. Caudal tier visible only in oblique or posterior polar views, with posterior spines up to 5 µm long (Figs 1a, d, e and 2a, c, d). Main features of plates include reniform uni-windows near right margin, large interconnected windows, and inconspicuous borders between adjacent windows that combine to form a mean of 16 longitudinal canals across surface of cell (Figs 1a–c and 2b, e, f). Teeth on right margin of each plate approximately 5 µm long, alternating with windows. Longitudinal ridge of plates smooth, thickened and lying slightly nearer left margin than right; left margin very slightly serrated and never wing-like.

Oral opening in centre of anterior pole. Circumoral kinety consisting of ring of dikinetids located next to anterior ends of somatic kineties, interrupted by adoral organelles (Fig. 1g). Adoral organelles 1 and 2 consisting of three dikinetids, organelle 3 consisting of four dikinetids. Oral basket approximately 15 µm in length, located in centre of cytostome.

Somatic cilia 8–10 µm long, regularly arranged, forming a mean of 15 transverse circumferential rows and 16 longitudinal rows (Figs 1g, h and 2g, h). Two dikinetids at anterior end of each ciliary row collectively form perioral ciliature. One caudal cilium approximately 20 µm long (Figs 1a and 2d).

Remarks and comparisons

The genus *Nolandia* was established by Small & Lynn (1985) although neither a diagnosis nor a detailed description was provided. The genus *Nolandia* is characterized by the structure of its armour plates, these being arranged in six tiers. Two species have previously been described: *N. nolandii* (Kahl, 1930) Small & Lynn, 1985 and *Nolandia orientalis* Chen *et al.*, 2010.

N. sinica differs from *N. nolandii* in the following plate features: (1) teeth of right plate margin alternately arranged with windows (vs at same level as windows); (2) teeth very long and finger-shaped (vs teeth short and thorn-like) (Fig. 1b, c, 1 and Table 2; Wilbert & Schmall 1976). The divergence of these two forms is also supported by SSU rRNA gene sequence data which show a difference of 65 nt (Table 3).

N. sinica can likewise be distinguished from *N. orientalis* by the structure of the armour plates: (1) windows large, border between adjacent windows inconspicuous (vs windows with wide and conspicuous border between adjacent windows); (2) ridge conspicuous and thickened, slightly nearer the left margin (vs ridge inconspicuous and slightly nearer the right margin); (3) absence of granules near left plate margin (vs

Table 3. SSU rRNA gene sequence dissimilarities (lower triangle) and number of different sites (upper triangle) among eight colepids. Species described in the present study are marked in bold.

Species	SSU rRNA gene sequence dissimilarities/number of different sites with:							
	1	2	3	4	5	6	7	8
1 <i>Tiarina fusa</i>	*	47	71	53	49	109	114	107
2 <i>Nolandia sinica</i>	2.79	*	65	37	33	102	101	110
3 <i>Nolandia nolandii</i>	4.16	3.88	*	71	67	70	74	76
4 <i>Apocoleps magnus</i>	3.15	2.20	4.24	*	4	106	111	106
5 <i>Apocoleps caoi</i>	2.91	1.96	4.00	0.24	*	102	107	102
6 <i>Coleps hirtus hirtus</i>	6.39	6.09	4.11	6.32	6.09	*	20	105
7 <i>Coleps spetai</i>	6.69	6.03	4.34	6.62	6.38	1.17	*	107
8 <i>Levicoleps biwae</i>	6.12	6.54	4.46	6.30	6.06	6.16	6.28	*

granules present) (Fig. 1b, c, f, i and Table 2; Chen *et al.*, 2010).

In terms of body shape and size, position of contractile vacuole, and the caudal cilium, two other colepid species should be compared with *N. sinica*, namely *Pinacocoleps similis* (Kahl, 1933) Chen *et al.*, 2009 and *Coleps hirtus* (Müller, 1786) Nitzsch, 1827. Both can be easily distinguished from *N. sinica* by the plate windows, these being absent in *Pinacocoleps similis* and pretzel-shaped bi-windows in *C. hirtus*, whereas *N. sinica* has reniform uni-windows (Fig. 1a, b, j, k, n, o and Table 2; Chen *et al.*, 2010; Foissner *et al.*, 1999).

***Apocoleps* Chen *et al.*, 2009**

***Apocoleps caoi* spec. nov. (Figs 3 and 4; Tables 1–3)**

Diagnosis. Large *Apocoleps* with elongated body, about $100\text{--}120 \times 35\text{--}60\text{ }\mu\text{m}$ *in vivo*; anterior tertiary, anterior secondary and anterior main tier plates all with four uni-windows, posterior main and secondary tier plates each with three or four uni-windows, posterior tertiary tier plate with two uni-windows; left plate margin slightly serrated; a mean of about 23 transverse and 21 longitudinal ciliary rows; one terminal contractile vacuole; marine habitat.

Type locality. Sandy beach near Jiaozhou Bay, Qingdao, China ($36^{\circ} 06' \text{ N } 120^{\circ} 29' \text{ E}$).

Type material. A protargol slide containing the holotype specimen encircled on the coverslip is deposited in the Natural History Museum, London, UK, with registration number NHMUK 2010.2.2.2. One paratype slide has been deposited in the collection of the Laboratory of Protozoology, OUC, China, with registration number WYG-2007060702.

Etymology. This species is named in honour of Professor Wenxuan Cao, Institute of Hydrobiology, Wuhan, Chinese

Academy of Sciences, in recognition of his contributions to zoology.

Description. Body $100\text{--}120 \times 35\text{--}60\text{ }\mu\text{m}$ *in vivo*, elongated and sometimes slightly curved, body widest in anterior quarter, gradually narrowing posteriorly (Figs 3a, c and 4a, b). Cytoplasm slightly greyish, often with numerous oily globules, $4\text{--}5\text{ }\mu\text{m}$ in diameter, that render the main part of the body opaque, and several large, approximately $10\text{ }\mu\text{m}$ diameter, brownish food vacuoles. One spherical macronucleus, approximately $12\text{--}15\text{ }\mu\text{m}$ in diameter, located in mid-body; one micronucleus, approximately $2\text{ }\mu\text{m}$ across, attached to macronucleus (Figs 3d and 4e). One contractile vacuole usually terminally positioned (Figs 3a, c and 4b).

Armour composed of *Nolandia*-type plates arranged in eight tiers, (1) circumoral, (2) anterior tertiary, (3) anterior secondary, (4) anterior main, (5) posterior main, (6) posterior secondary, (7) posterior tertiary and (8) caudal, each tier consisting of a mean of 21 rectangular plates. Circumoral tiers hardly recognizable *in vivo*. Anterior tertiary plates with acutely pointed anterior ends, some with anterior spines (Figs 3a and 4b, g). Anterior secondary and main plates each with four windows, posterior main and secondary plates each with three or four windows, posterior tertiary plates each with two windows. Caudal tier with several long, conspicuous posterior spines (Figs 3a, c and 4a, b). Detailed structure of armour plates as shown in Figs 3(g) and 4(f–h). Main features are as follows: plate approximately $20\text{ }\mu\text{m}$ in length, with teeth about $2\text{ }\mu\text{m}$ long; ridge at left plate margin, slightly serrated; conspicuous bridge connecting teeth and plate ridge; reniform uni-windows near right margin.

Oral opening in centre of anterior pole. Circumoral kinety consisting of ring of dikinetids located next to anterior ends of somatic kineties, interrupted by adoral organelles. Adoral organelles formed by three short, obliquely arranged rows (Figs 3f and 4i). Organelles 1 and 2 each consisting of four dikinetids, organelle 3 of three dikinetids.

Fig. 3. Morphology and infraciliature of *Apocoleps caoi* spec. nov. (a, c, d, f, g) and three related morphotypes. (a) Lateral view of a typical individual. (b) *Planicoleps psammophilus* Dragesco & Dragesco-Kernéis, 1991 (from Dragesco & Dragesco-Kernéis, 1991). (c) Lateral view of a slightly curved individual of *A. caoi*. (d) Lateral view, showing the ciliary pattern of *A. caoi*. (e) *Apocoleps magnus* Chen *et al.*, 2009 (from Chen *et al.*, 2009). (f) Ciliary pattern of anterior end of *A. caoi*. (g) Detail of armour plate of *A. caoi*. (h) *Kotinia heterolobata* (Obolkin, 1995) Obolkin in Aesch, 2001 (from Obolkin, 1995). (i) Detail of armour plate of *A. magnus* (from Chen *et al.*, 2009). AS, anterior spine; CC, caudal cilium; CK, circumoral kinety; CV, contractile vacuole; IB, inner oral basket; Ma, macronucleus; PC, perioral cilia; PS, posterior spine; SK, somatic kinety. Bars, 30 µm.

Oral basket in centre of cytostome, conspicuous in protargol-stained specimens (Figs 3f and 4e, i).

Somatic cilia regularly arranged, forming a mean of 23 circumferential rows and 21 longitudinal rows. Two dikinetids at anterior end of each ciliary row collectively form perioral ciliature. One caudal cilium approximately 30 µm long (Fig. 3a, d).

Remarks and comparisons

The genus *Apocoleps* was established as a monotypic genus with *A. magnus* as the type species by original designation (Chen *et al.*, 2009). It is characterized by the possession of *Nolandia*-type plates arranged in eight tiers.

A. caoi may be separated from *A. magnus*, its only congener, by the following features: (1) body widest at anterior end, posterior portion gradually narrowed (vs body widest in mid-region, both ends conspicuously narrowed) (Fig. 3a, e); (2) 10–12 long, sharp posterior spines (vs approx. 6–8 short and

thorn-like posterior spines) (Chen *et al.*, 2009). The separation of these two species is also weakly supported by molecular data, their SSU rRNA gene sequences differing by 4 nt (Table 3).

Considering the large size and high number of armour tiers, two other species should also be compared with *A. caoi*, namely *Kotinia heterolobata* (Obolkin, 1995) Obolkin in Aesch, 2001 and *Planicoleps psammophilus* Dragesco & Dragesco-Kernéis, 1991. *A. caoi* differs from *K. heterolobata* (Obolkin, 1995) in the body shape (posterior end moderately tapering vs posterior end truncated), body size (100–120 × 35–60 µm vs 120–200 × 50–63 µm), the number of adoral organelles (three vs five), and habitat (marine vs freshwater) (Fig. 3h; Table 2; Obolkin, 1995). *A. caoi* can be separated from *Planicoleps psammophilus* by the following characters: body shape (body widest in anterior region, circular in transverse section vs body widest in posterior region and conspicuously flattened), the number and shape of main plate windows (four reniform uni-windows vs approx. 10 oval uni-windows), the shape of the left plate margin (slightly serrated vs smooth and

Fig. 4. *Apocoleps caoi* spec. nov. from life (a, b, f–h), after protargol impregnation (e, i) and silver carbonate impregnation (c, d). (a) Lateral view of a typical specimen, showing the body shape and posterior spines (arrowhead). (b) Lateral view of an unusual specimen, showing the curved body and the anterior spine (arrow) and posterior (arrowhead) spines. (c, e) Lateral views showing the ciliary pattern. (d) Detail of posterior region, showing the ciliary pattern. (f) Detail of main armour plate; arrowhead indicates the gap between two plates. (g) Detail of anterior region, showing the anterior tertiary tier plates and anterior spine (arrow). (h) Surface detail showing armour plates. (i) Anterior end of cell, showing the ciliary pattern and adoral organelles (arrowheads). Bars, 30 µm (a–e) and 15 µm (f–i).

straight), and the number of perioral kinetosomes (two pairs vs approx. seven or eight pairs) (Fig. 3b; Table 2; Dragesco & Dragesco-Kernéis, 1991).

***Tiarina* Bergh, 1881**

***Tiarina fusa* (Claparède & Lachmann, 1858) Bergh, 1881 (Fig. 5; Tables 1–3)**

Synonym: *Coleps fusus* Claparède & Lachmann, 1858

Some new data have been obtained from the population studied here; thus we present an updated description and an improved diagnosis.

Improved diagnosis. About $60\text{--}135 \times 20\text{--}30$ µm *in vivo*, body spindle-shaped; a mean of 16 longitudinal ciliary rows, each with approximately 35 kinetosomes; armour plates rectangular, with conspicuous longitudinal ridge and triangular teeth; one macronucleus and one micronucleus; marine habitat.

Slide deposition. Voucher slides of protargol-stained cells have been deposited in the collections of the Natural History Museum, London, UK (registration number NHMUK 2010.2.2.3) and the Laboratory of Protozoology, OUC, China (registration number LWW-20071013).

Description. Body usually about 80×20 µm *in vivo*, ratio of length to width about 4:1, circular in cross-section.

Fig. 5. Morphology and infraciliature of *Tiarina fusa* (Claparède & Lachmann, 1858) Bergh, 1881. (a) Lateral view of a typical specimen, showing the body shape and wing-like structure (arrow). (b) Transverse section, showing the arrangement of armour plates; arrows indicate the cilia. (c) Ciliary pattern of anterior end. (d) Detail of the main armour plate. (e, f) Lateral views of two specimens (from Kahl, 1930). (g) Lateral view, showing the ciliary pattern and three adoral organelles (from Small & Lynn, 1985). (h) Lateral view, showing the ciliary pattern and nuclear apparatus. (i) Lateral view of a typical specimen, showing the

body shape, pointed posterior end (arrowhead) and wing-like structure (arrow). (j) Lateral view of cell during binary fission; the posterior body half is brighter because the new armour plates are colourless and transparent. (k, l) Lateral views to show the ciliary pattern and nuclear apparatus. (m–o) Detail of plates, showing the ridge (arrows) and serrated left margin (arrowheads). (p) Detail of armour plates; arrows mark the conspicuous teeth. (q) Transverse view, showing the circular cross-section. (r, s) Detail of anterior end, showing the ciliary pattern and adoral organelle (arrow). AO, adoral organelle; CC, caudal cilium; Ma, macronucleus; PC, perioral ciliary; PS, posterior spine; R, ridge; SK, somatic kinety; T, tooth. Bars, 30 μm (a–l) and 15 μm (m–s).

Body spindle-like, anterior end truncated, posterior region gradually tapering to a sharp point (Fig. 5a, b, i, j). Endoplasm colourless to brownish and opaque, packed with granules 2–3 μm in diameter and food vacuoles 5–8 μm across (Fig. 5a). Macronucleus spherical, approximately 10 μm in diameter, single spherical micronucleus near macronucleus (Fig. 5a, h, l).

Armour composed of *Tiarina*-type plates arranged in six tiers, (1) circumoral, (2) anterior secondary, (3) anterior main, (4) posterior main, (5) posterior secondary and (6) caudal, each tier comprising a mean of 16 rectangular plates. Circumoral tier plates difficult to observe *in vivo*. Anterior secondary plates rectangular, one of which possesses a conspicuous wing-like structure (Fig. 5a, i). Anterior main, posterior main and posterior secondary tier plates each with three sturdy teeth. Caudal tier plates triangular, sharply pointed posteriorly. Usually with a single conspicuous posterior spine. Fine structure of armour plates as shown in Fig. 5(d, m–o). Main features as follows: windows absent but with three thin, transparent rounded areas at levels of teeth of right plate margin; teeth sturdy and triangular; longitudinal ridge thickened and smooth, slightly nearer right margin; left plate margin distinctly crenellated and wing-like.

Oral opening in centre of anterior pole. Only one adoral organelle observed, formed by two densely arranged kinety rows, each comprising about 12–15 kinetosomes (Fig. 5c, r, s). Oral basket approximately 16 μm long.

Somatic cilia 5–6 μm long and regularly arranged, forming about 16 longitudinal rows, each comprising about 35 kinetosomes. Anterior ends of ciliary rows each with 7 or 8 densely packed kinetosomes that collectively form the perioral ciliature (Fig. 5h, s). One caudal cilium 15–20 μm long (Fig. 5a).

Remarks and comparisons

Tiarina fusa was first reported by Claparède & Lachmann (1858) under the name *Coleps fusus*, but Bergh (1881) subsequently transferred it to the genus *Tiarina*. The divergence of *Tiarina fusa* from the genus *Coleps* is supported by the SSU rRNA gene sequence data, which shows that the former differs in 109 and 114 nt from *C. hirtus hirtus* and *C. spetai*, respectively (Table 3).

Although *Tiarina fusa* has previously been investigated several times based on living and fixed specimens, the detailed structure of the armour plates and adoral organelles

has never been reported. Small & Lynn (1985), for example, provided only a brief description and a schematic figure showing three oblique adoral organelles. By contrast, the present study has revealed only one long, oblique adoral organelle in the China population.

Morphologically, *Tiarina fusa* is very similar to *Tiarina meunieri* Kahl, 1930 in terms of its body shape and size and its marine habitat. However, the former can be distinguished from the latter by the wing-like structure at the anterior end of the body (absent in *Tiarina meunieri*) and the presence of a single spherical macronucleus (vs generally moniliform with 5–8 macronuclear segments in *Tiarina meunieri*) (Table 2; Kahl, 1930).

With reference to the body shape and size, *Tiarina fusa* should also be compared with *Tiarinella gracilis* Obolkina, 1995, the former being separated from the latter as follows: (1) body never flattened vs body slightly flattened; (2) anterior end with wing-like structure vs anterior without a wing-like structure; (3) single posterior spine vs three or four posterior spines; (4) approximately 16 longitudinal somatic kineties vs 10–12 longitudinal somatic kineties; (5) single long, oblique adoral organelle vs three short adoral organelles (Table 2; Obolkina, 1995).

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