

Two New Ciliates from Hong Kong Coastal Water: *Orthodonella sinica* n. sp. and *Apokeronopsis wrighti* n. sp. (Protozoa: Ciliophora)

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ABSTRACT. The morphology and infraciliature of *Orthodonella sinica* n. sp. and *Apokeronopsis wrighti* n. sp., isolated from the coastal water off Hong Kong, were investigated in living and stained specimens. *Orthodonella sinica* n. sp. is diagnosed as: a marine *Orthodonella* 150–310 × 40–80 μm in vivo; with a dominant beak-like projection at the anterior end; about 70 somatic kineties; 84–126 dikinetids in the synhymenium; one contractile vacuole in the posterior one-fourth of cell, near the left margin; one conspicuous dorsal suture. *Apokeronopsis wrighti* n. sp. is diagnosed as: an *Apokeronopsis* about 150–230 × 35–55 μm in vivo; dark-reddish blood-cell-shaped cortical granules grouped in three rows on the ventral side and two rows on the dorsal side; 23–35 cirri in the right mid-ventral rows (MVR) and 23–32 cirri in the left MVR; six to eight buccal, two frontoterminal, 30–42 left marginal, and 32–43 right marginal cirri; 21–30 transverse cirri extending anteriorly beyond the level of mid-body; consistently three dorsal kineties. The separation of *A. wrighti* n. sp. and its highly similar congeners *Apokeronopsis crassa* and *Apokeronopsis bergeri* was supported by comparison of their SSrRNA gene sequences.

Key Words. Marine ciliates, morphology, SSrDNA.

SPECIES identification and separation for the ciliate genus *Orthodonella* Bhatia, 1936 have been difficult, mainly due to insufficient description, misinterpretation of important features, and the absence of data on the infraciliature as revealed by silver impregnation methods (Kahl 1931; Lin, Gong, and Song 2004; Song and Wilbert 2002; Wilbert 1986; Wilbert and Song 2005). To date, only three species have been investigated using silver staining methods (Lin et al. 2004; Song and Wilbert 2002).

Shao et al. (2007) established a new genus *Apokeronopsis* and fixed *Apokeronopsis crassa* (Claparède & Lachmann, 1858) as the type, based on morphological and ontogenetic features of populations isolated from the Yellow Sea, northern China. Species in this genus clearly differ from other genera of the Pseudokeronopsidae based on its unique morphological and morphogenetic features as reported by Shao et al. (2007).

We isolated recently two ciliate species during investigation on ciliates diversity in Clear Water Bay, Hong Kong. Detailed observations have demonstrated that both are hitherto unknown species, belonging to the genera *Orthodonella* and *Apokeronopsis*. Morphological description of the two species and sequence information of small subunit rRNA (SSrRNA) gene of the new *Apokeronopsis* species are here documented.

MATERIALS AND METHODS

Ciliates were collected from the coastal area near the pier of the Hong Kong University of Science and Technology, Clear Water Bay, Hong Kong (22°20'N; 114°17'E), where the water was mesotrophic. Glass slides as artificial substrates were used to enrich microbes by immersing in water for 1 to 2 wk (Gong, Song, and Warren 2005).

Apokeronopsis wrighti n. sp. was isolated once and *Orthodonella sinica* n. sp. was sampled twice in the autumn of 2007. The local ecological features were salinity ~ 33.5‰, temperature ~ 24 °C, and pH ~ 8.1.

Cells were observed in vivo first using a stereomicroscope, and then with high magnification under a compound microscope (100–1,000X). The infraciliature was revealed by the protargol impregnation method (Wilbert 1975). Drawings of living cells were based on free-hand sketches and photomicrographs, and those of impregnated ones were made with the help of a camera

lucida. Terminology is mainly according to Corliss (1979), Lynn and Small (2002), and Shao et al. (2007).

Isolation of cells of *A. wrighti* n. sp. and DNA extraction were according to Gong et al. (2007). In brief, one or more cells of the population were isolated and transferred to a drop of autoclaved seawater. Cells were then repeatedly washed in sterile seawater to remove other potential minute protists. The cells were subsequently transferred to a 1.5-ml microtube with a minimum volume of sterile seawater. Genomic DNA was extracted with REDExtract-N-AmpTM Tissue PCR Kit (Sigma, St. Louis) according to a modified protocol provided by the manufacturer. The modification consisted of the reduction of the recommended volumes for the kit's reagents to 1/10. The concentration and quality of the extracted genomic DNA was checked by NanoDrop-ND1000 spectrophotometer (Wilmington, Delaware). The universal eukaryotic primers (forward 5'-AACCTGGTTGATCCTGC CAGT-3'; reverse 5'-TGATCCTTCTGCAGTTTCACCTAC-3') (Medlin et al. 1988) were used to amplify the SSrRNA gene sequence of *A. wrighti* n. sp. The typical amplification profile consisted of 30 cycles of 1 min at 94 °C, 2 min at 60 °C, and 2 min at 72 °C, followed by 10 min at 72 °C for final extension. The purified PCR product with proper size was inserted into the pUCm-T vector (Sangon, Toronto, ON, Canada) and sequenced by the Sangon sequencing facility in Guangzhou, China.

The SSrDNA sequence of *A. wrighti* n. sp. has been deposited in GenBank (Accession number: EU417963) to compare with those of *Apokeronopsis bergeri* (DQ777742) and *A. crassa* (synonym *Pseudokeronopsis qingdaoensis*, DQ359728).

RESULTS

***Orthodonella sinica* n. sp. (Fig. 1–5, 11, 12, 14–17, 20, 24–31; Table 1)**

Description. The cell size is highly variable, mostly about 220 × 50 μm in vivo; the body is flexible, but non-contractile, sometimes the posterior portion slightly curving to right. The body is lanceolate in outline, with its posterior part fairly narrowed, widest mostly at about mid-body, and with a conspicuous beak-like protrusion at anterior end (Fig. 1, 2, 11, 12, 24). The pharyngeal basket is ca 60-μm long and composed of on average 12 nematodesmal rods surrounding a central tube (Fig. 4, 5, 15, arrows; 25, arrowhead; 28, 29, arrowheads, arrows). The pellicle is thin, with many tiny granules distributed between the ciliary rows (Fig. 3, 16). The numerous food vacuoles contain ingested diatoms, some of which are about two-third of cell length (Fig. 11;

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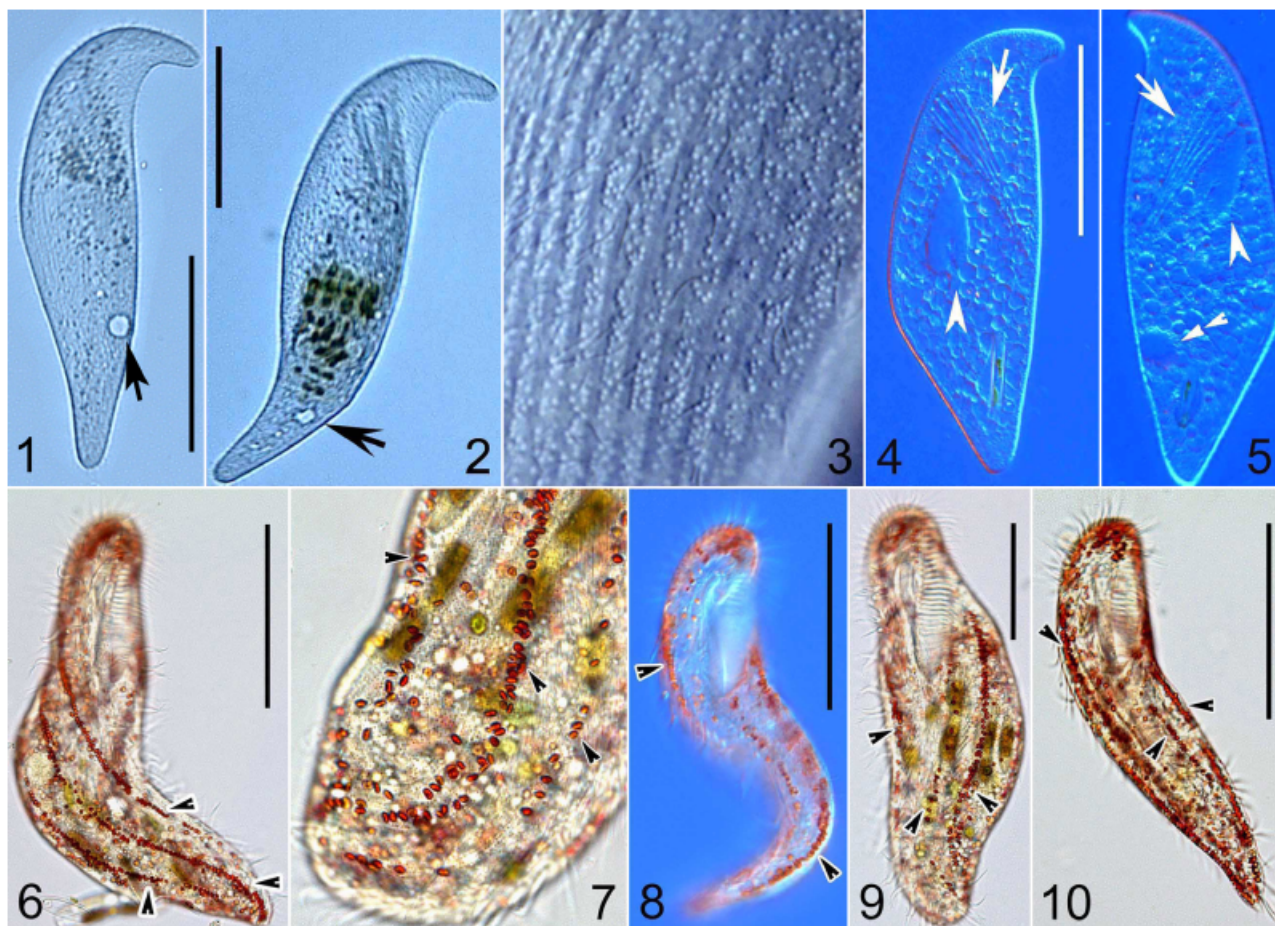


Fig. 1–10. *Orthodonella sinica* n. sp. (1–5) and *Apokeronopsis wrightii* n. sp. (6–10) from life. 1, 2. Ventral view, to show the typical cells and contractile vacuole (CV) (arrows). 3. To show the cortical granules. 4, 5. Ventral and dorsal views, to show the pharyngeal basket (arrows), the macronucleus (arrowheads) and the CV (double arrowheads). 6, 7, 9, 10. To show the arrangement of the dark-reddish granules (arrowheads). 8. A curved individual (arrowheads mark the grouped granules). Scale bars in Fig. 1, 2, 4 = 80 μ m, in Fig. 6, 8, 10 = 60 μ m, in Fig. 9 = 40 μ m.

15, 27, arrowheads). A single contractile vacuole (CV) is located in the posterior one-fourth of cell, consistently near the left margin (Fig. 1, 11, 12, 15, 24, arrows; 5, double-arrowheads). The macronucleus is ellipsoidal and centrally positioned (Fig. 4, 5, arrowheads; 11, 12, 15, 17, 27, 31; 20, arrowheads). Movement is mainly by slowly gliding on substrate.

The synhymenium is composed of ca 100 dikinetids, which are arranged postorally and obliquely on the ventral side, running from the anterior end to about middle portion of right cell margin (Fig. 14, 20, 25, 26, arrows). There are 63–83 longitudinal somatic kineties in mid-body in total. The kineties anterior to synhymenium are parallel to anterior cell margin and mostly continuous, except for some short kineties which seem to be bisected by the oral area (Fig. 14). A suture, which is composed of shortened kineties, is always present on the dorsal side of the posterior portion (Fig. 17, 30, 31, arrows).

***Apokeronopsis wrightii* n. sp. (Fig. 6–10, 32–35, 38, 41–48; Table 2)**

Description. Cells in vivo are mostly $200 \times 45 \mu$ m in size; the body is highly flexible and contractile (Fig. 6, 8, 9). Body is generally elongate in shape and widest at mid-body. The anterior end is broadly rounded, while the posterior end is more or less tapering in typical individuals (Fig. 10, 32). The buccal field is about one-

third of body length (Fig. 10, 32). Cortical granules are rather conspicuous, circular, blood-cell-shaped (BCS), around 2- μ m-sized each, dark-reddish in color, and arranged roughly in three rows on ventral and two rows on dorsal (Fig. 6–10, 33–35, arrowheads). Apart from these large granules, numerous tiny (0.2- μ m across) ones, colorless to grayish, are evenly distributed beneath pellicle as well (Fig. 35, arrows). The CV is not observed. Macronuclear nodules are numerous, each ovoid to ellipsoid and about 3- μ m long, and distributed throughout the body (Fig. 42; 43, arrows). Locomotion, slightly thigmotactic, is by slowly crawling without pause on debris or on bottom of Petri dish.

Infraciliature is as shown in Fig. 41, 42, 48. The distal end of adoral zone of membranelles (AZM) curves strongly posteriorly along the right margin and extends to about level of cytostome (Fig. 32, 41). The bases of membranelles are about 6–10 μ m long (Fig. 38, 41, 46). Paroral and endoral membranes (PM, EM) are ca equal in length and intersected at the anterior one-third in space (Fig. 41; 44, arrow and arrowhead). Somatic cirri are relatively fine and each ca 15 μ m long. The bicorona comprises about 30 slightly enlarged frontal cirri, which are sometimes separated from the mid-ventral complex by an inconspicuous gap (Fig. 41). There are usually two frontoterminal cirri, which are located near the distal end of adoral zone and hence often difficult to

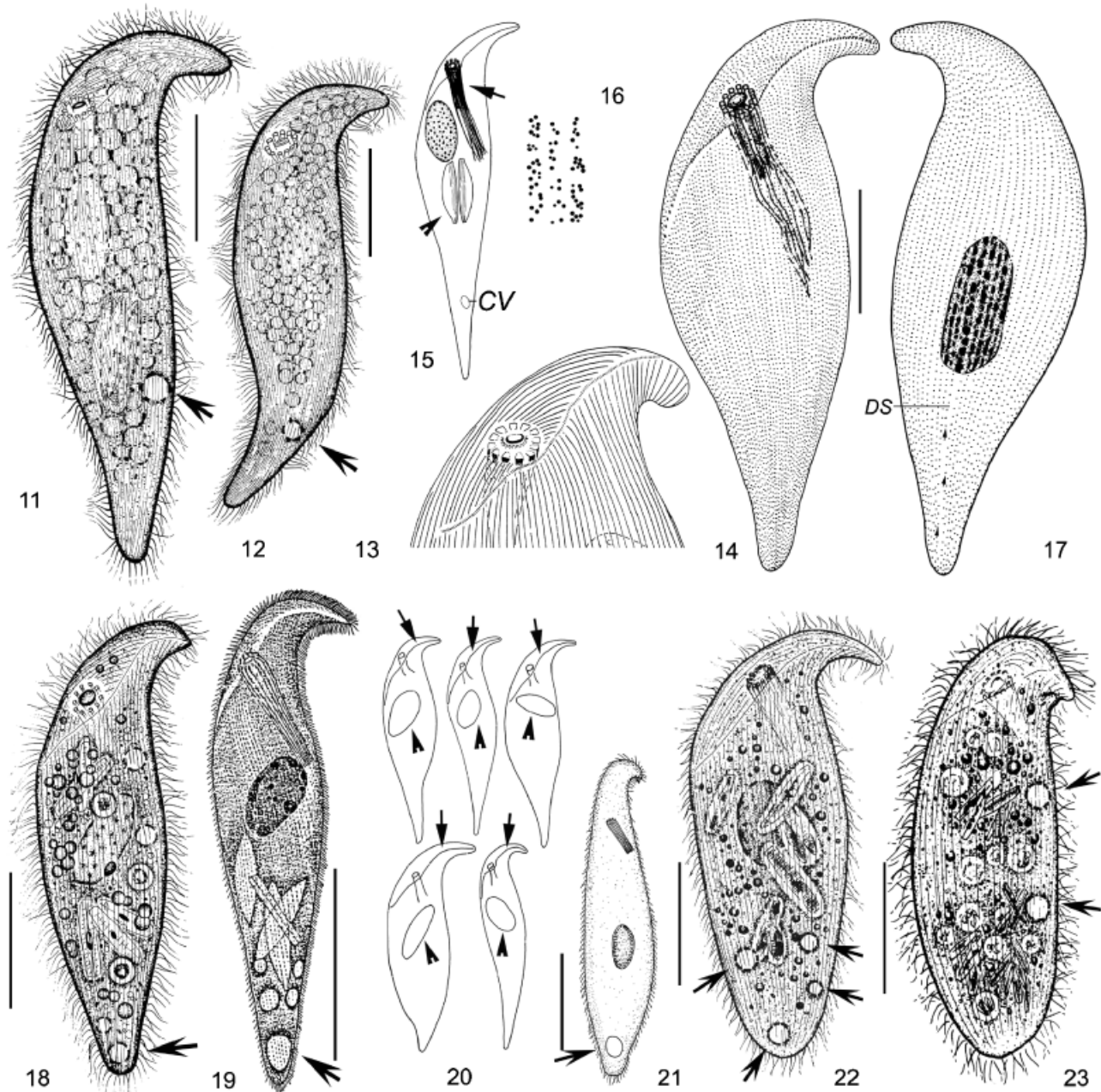


Fig. 11–23. *Orthodonella sinica* n. sp. (11, 12, 16), *Orthodonella apohamatus* (18), *Orthodonella hamatus* (19, 21), *Orthodonella gutta* (22), and *Orthodonella shenae* (23) in vivo and *O. hamatus* (13) and *O. sinica* n. sp. after protargol impregnation (14, 15, 17, 20). 11, 12. Ventral views, to show two typical specimens. Note the contractile vacuole (CV) (arrows). 13. Anterior ventral portion, from Jankowski (1968). 14, 17. Ventral and dorsal views of the same specimen, to show the general infraciliature. Arrows mark the shortened somatic kineties. 15. Ventral view, to show the pharyngeal basket (PB, arrow) and an ingested diatom (arrowhead). 16. Cortical granules. 18, 19, 21–23. Ventral view. Arrows mark CV(s). 18, 21, 22 from Lin et al. (2004), 19 from Ozaki and Yagiu (1941), 23 from Song and Wilbert (2002). 20. To show cells with different morphology of synhymenium (arrows) and macronucleus (arrowheads). CV, contractile vacuole; DS, dorsal suture. Scale bars in Fig. 11, 12, 14 = 50 μ m; in Fig. 18, 19, 21–23 = 40 μ m.

recognize (Fig. 41). Six to eight buccal cirri (BC) are arranged in a row along the PM (Fig. 41; 45, arrowheads). Two mid-ventral rows (MVR) terminate at posterior end of cell, with cirri of each pair conspicuously separated from each other, which are connected with fibrils (Fig. 41; 47, arrows). Twenty-one to 30 transverse cirri (TC) are arranged in a row which extends anteriorly beyond

the level of mid-body (Fig. 41, 48). Cirri in both marginal rows are narrowly spaced, with the right marginal row (RMR) commences near the distal end of AZM; left marginal row (LMR) starts at the cytostome level (Fig. 32, 41). Dorsal kineties are consistently in three rows, in which dikinetids are densely spaced, and each bears a cilium ca 2–3 μ m long (Fig. 42).

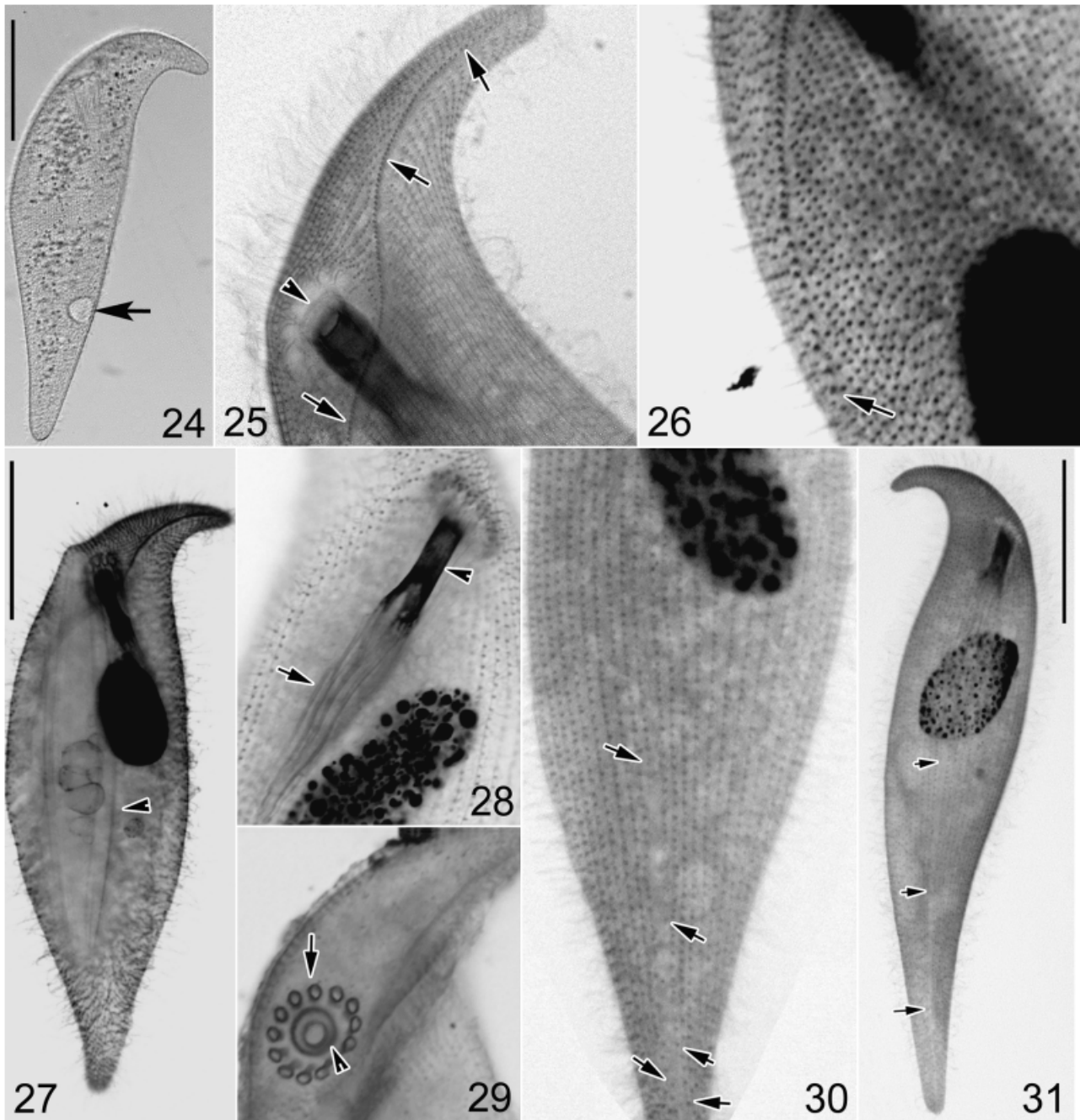


Fig. 24–31. Photomicrographs of *Orthodonella sinica* n. sp. in vivo (24) and after protargol impregnation (25–31). 24. Ventral view, note the contractile vacuole (arrow). 25. Anterior ventral portion, to show the pharyngeal basket (arrowhead) and the synhymenium (arrows). 26. To mark the posterior end of the synhymenium. 27. To show an ingested diatom (arrowhead). 28, 29. Lateral and apical views of the pharyngeal basket, to show the pharyngeal basket tube (arrowhead) and the nematodesmal rods (arrow). 30, 31. Dorsal views, to mark the shortened kineties (arrows). Scale bars = 60 μ m.

DISCUSSION

Comparison of *Orthodonella* species. To date, about five species are included in the genus *Orthodonella* Bhatia, 1936: *Orthodonella apohamatus* Lin et al., 2004, *Orthodonella gutta* (Cohn, 1866) Bhatia, 1936, *Orthodonella hamatus* (Gruber, 1884) Bhatia, 1936, *Orthodonella parvirostrum* (Schewiakoff, 1893) Bhatia, 1936, and *Orthodonella shenae* Song & Wilbert, 2002. Infraciliature, morphometric data, and living morphology of *O. apo-*

hamatus, *O. gutta*, and *O. shenae* have been completely investigated (Lin et al. 2004; Song and Wilbert 2002; Wilbert and Song 2005). There is still no detailed information for the type species *O. hamatus* (i.e. infraciliature and morphological variability), although several re-descriptions have been made, and Jankowski (1968) and Wilbert (1986) provided some illustrations of silver-impregnated specimens (Fig. 13, 19; Table 3; Lin et al. 2004; Ozaki and Yagiu 1941).

Orthodonella sinica highly resembles *O. hamatus* in the cell shape and size, while clearly differs from the latter in the location

Table 1. Morphometrical characterization of *Orthodonella sinica* n. sp. (upper line, Sep. population; lower line, Nov. population).

Character	Mean	Min	Max	SD	CV	<i>n</i>
Body length	255.4	160	318	39.27	15.38	22
	183.1	151	254	23.89	13.05	43
Body width	62.4	43	79	9.68	15.51	22
	53.6	40	68	7.81	14.57	43
Length of pharyngeal tube	24.8	20	30	2.58	10.40	21
	22.3	19	26	1.85	8.30	48
Length of nematodesmal rods	64.0	36	79	12.39	19.36	17
	58.2	40	75	7.38	12.68	30
Length of macronucleus	49.6	37	79	8.51	17.16	22
	45.5	32	66	7.66	16.84	43
Width of macronucleus	25.3	13	40	6.36	25.14	23
	22.3	15	32	4.11	18.43	43
Diameter of cytostome	19.8	17	24	2.21	11.16	23
	15.3	12	19	1.64	10.72	42
Number of dikinetids in synhymenium	108.8	96	126	6.84	6.29	17
	97.0	84	110	5.99	6.18	32
Number of pharyngeal rods	12.3	11	13	0.67	5.45	18
	11.9	10	13	0.79	6.64	41
Number of somatic kineties	74.5	65	81	4.75	6.38	20
	73.4	63	83	4.22	5.75	40

Data based on protargol-impregnated specimens. All measurements in μm . CV, coefficient of variation in %; Max., maximum; Mean, arithmetic mean; Min., minimum; *n*, number of specimens investigated; SD, standard deviation.

of CV (i.e. posterior one-fourth near left cell margin vs. terminally located in *O. hamatus*) (Fig. 21; Table 3; Gruber 1884).

Orthodonella sinica is also similar to *O. apohamatus*, with respect to cell shape and number of pharyngeal rods. However, these two organisms can be distinguished by the numbers of somatic kineties (63–83 vs. 42–60 in *O. apohamatus*) and dikinetids in the synhymenium (84–126 vs. 54–62 in *O. apohamatus*). In addition, the new species has a conspicuous dorsal suture after protargol impregnation (vs. absence in *O. apohamatus*) (Fig. 18; Table 3; Lin et al. 2004).

Orthodonella sinica differs from another two congeners, *O. gutta* and *O. shenae*, by its lanceolate shape (vs. ellipsoidal in *O. gutta* and *O. shenae*), the presence of a dorsal suture (vs. absence in *O. gutta* and *O. shenae*), and more dikinetids in the synhymenium (84–126 vs. 43–70 in *O. gutta* and 80 in *O. shenae*). Additionally, *O. sinica* has more somatic kineties than *O. gutta* (63–83 vs. 50–57 in *O. gutta*) (Fig. 22, 23; Table 3; Lin et al. 2004; Song and Wilbert 2002).

Orthodonella parvirostrum, the only freshwater species, whose infraciliature still remains unknown, can be easily distinguished from *O. sinica* by its smaller body size (60 vs. 150–310 μm), different cell shape (i.e. elliptical, with inconspicuous anterior projection vs. lanceolate, with conspicuous beak-like anterior projection in *O. sinica*), position of CV (i.e. mid-body vs. posterior one-fourth located in *O. sinica*), and shape of macronucleus (i.e. reniform vs. ellipsoidal in *O. sinica*) (Kahl 1931).

Therefore, we recognize this isolate as the new species and provide the following taxonomic information.

Class KINETOFRAGMINOPHORA de Puytorac et al. 1974

Order Synhymeniida de Puytorac et al., 1974

Family Orthodonellidae Jankowski, 1968

Genus *Orthodonella* Bhatia, 1936

Synonym *Orthodon* Gruber, 1884

***Orthodonella sinica* n. sp.**

Diagnosis. A marine *Orthodonella* 150–310 \times 40–80 μm in vivo; with a dominant beak-like projection at the anterior end;

about 70 somatic kineties; 84–126 dikinetids in the synhymenium; one CV in the posterior one-fourth of cell, near the left margin; one conspicuous dorsal suture.

Type slides. One holotype and one paratype slides (registration number: LH-2007091204, LWW-2007112303P) with protargol-impregnated specimens have been deposited in the Laboratory of Protozoology, South China Normal University, China.

Type locality. Coastal water of Clear Water Bay, Hong Kong (22°20'N; 114°17'E).

Etymology. The Latin word “*sinica*” (Chinese) indicates that this new species was first discovered in China’s territory.

An updated key to species within the genus *Orthodonella*, based on both living morphology and/or infraciliature, is here supplied:

Key to the genus *Orthodonella*:

- | | |
|---|------------------------|
| 1 Freshwater habitat, with reniform macronucleus | <i>O. parvirostrum</i> |
| 1' Marine habitat, with ellipsoidal macronucleus | 2 |
| 2 Dorsal suture present | <i>O. sinica</i> |
| 2' Dorsal suture absent | 3 |
| 3 Anterior projection beak-like | 4 |
| 3' Anterior projection snout-like | <i>O. shenae</i> |
| 4 Single contractile vacuole, terminally located | <i>O. hamatus</i> |
| 4' More than one contractile vacuole | 5 |
| 5 42–60 somatic kineties; posterior portion fairly thin | <i>O. apohamatus</i> |
| 5' 62–74 somatic kineties; posterior portion blunted | <i>O. gutta</i> |

Comparison of *Apokeronopsis* species. Three species are now included in the genus *Apokeronopsis*: *Apokeronopsis antarctica* (Petz, 1995) Shao et al., 2007, *A. bergeri* Li et al., 2008 and the type species *A. crassa* (Claparède & Lachmann, 1858) Shao et al., 2007. *Apokeronopsis wrighti* n. sp. differs from the type species by having fewer cirri in the right MVR (i.e. 23–35 vs. 33–56), fewer left MVR (i.e. 23–32 vs. 33–53), fewer LMR (i.e. 30–42 vs. 38–70), and fewer RMR (i.e. 32–43 vs. 43–74) (Fig. 32, 40; Table 4). In addition, although these two species have the same two types of cortical granules, their arrangement patterns of BCS granules differ greatly: three rows on ventral and two rows on

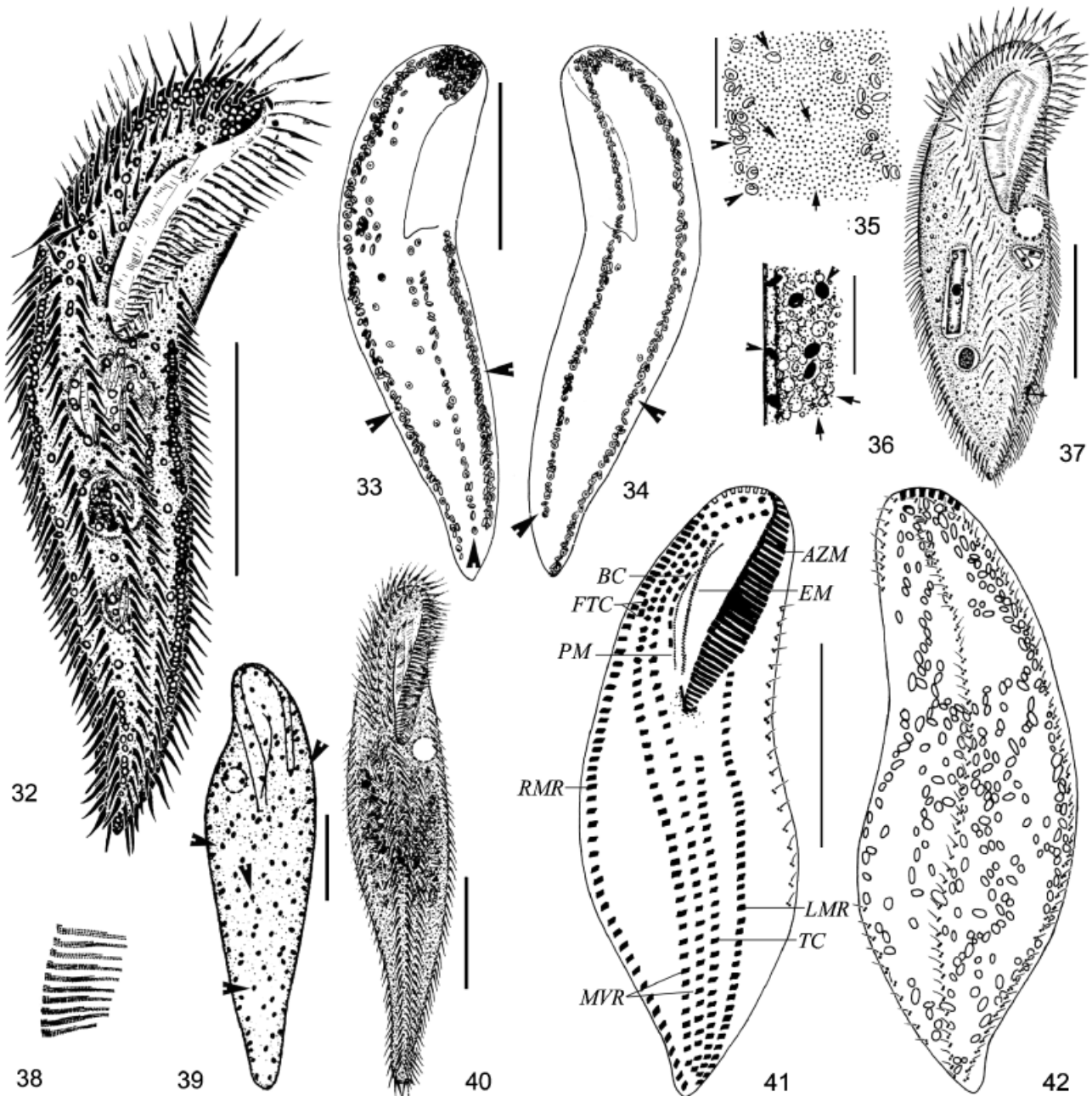


Fig. 32–42. *Apokeronopsis wrighti* n. sp. (32–35), *Apokeronopsis crassa* (36, 39, 40, from Song et al. 2004), *Apokeronopsis antarctica* (37, from Petz 1995) in vivo and *A. wrighti* n. sp. after protargol impregnation (38, 41, 42). 32. Ventral view of a typical cell. 33, 34. To show arrangement of ventral and dorsal blood-cell-shaped cortical granules in groups (arrowheads). 35. Details of part of the pellicle, arrows indicate the small granules, arrowheads mark the big granules. 36. Small (arrows) and big (arrowheads) cortical granules. 37. Ventral view. 38. Structure of the adoral membranelles. 39. To show sparsely distributed blood cell-shaped pigment granules on dorsal side (arrowheads). 40. Ventral view. 41, 42. Ventral and dorsal infraciliature. AZM, adoral zone of membranelles; BC, buccal cirri; EM, endoral membrane; FTC, frontoterminal cirri; LMR, left marginal row; MVR, mid-ventral rows; PM, paroral membrane; RMR, right marginal row; TC, transverse cirri. Scale bars in Fig. 32, 33, 37, 39, 40, 41 = 60 μ m, in Fig. 35, 36 = 10 μ m.

dorsal in *A. wrighti* vs. sparsely distributed in *A. crassa* (Fig. 33–36, 39, arrowheads). As indicated by one comparative study on four marine colored *Pseudokeronopsis* species by Song et al. (2006), even the shape of the BCS granules is a reliable criterion for species separation (i.e. circular vs. oval). With reference to the close systematic relationship of these two pseudokeronopsids, *Apokeronopsis* and *Pseudokeronopsis*, the arrangement of

BCS granules should also be species specific. In addition, comparison of the SSrRNA gene sequences of *A. wrighti* and *A. crassa* shows 50 nucleotides difference (2.8%) (Fig. 49), which, in our opinion, further supports the separation of these two species.

Apokeronopsis antarctica has no cortical granules (vs. presence) and no transverse cirri (vs. presence) and hence on both

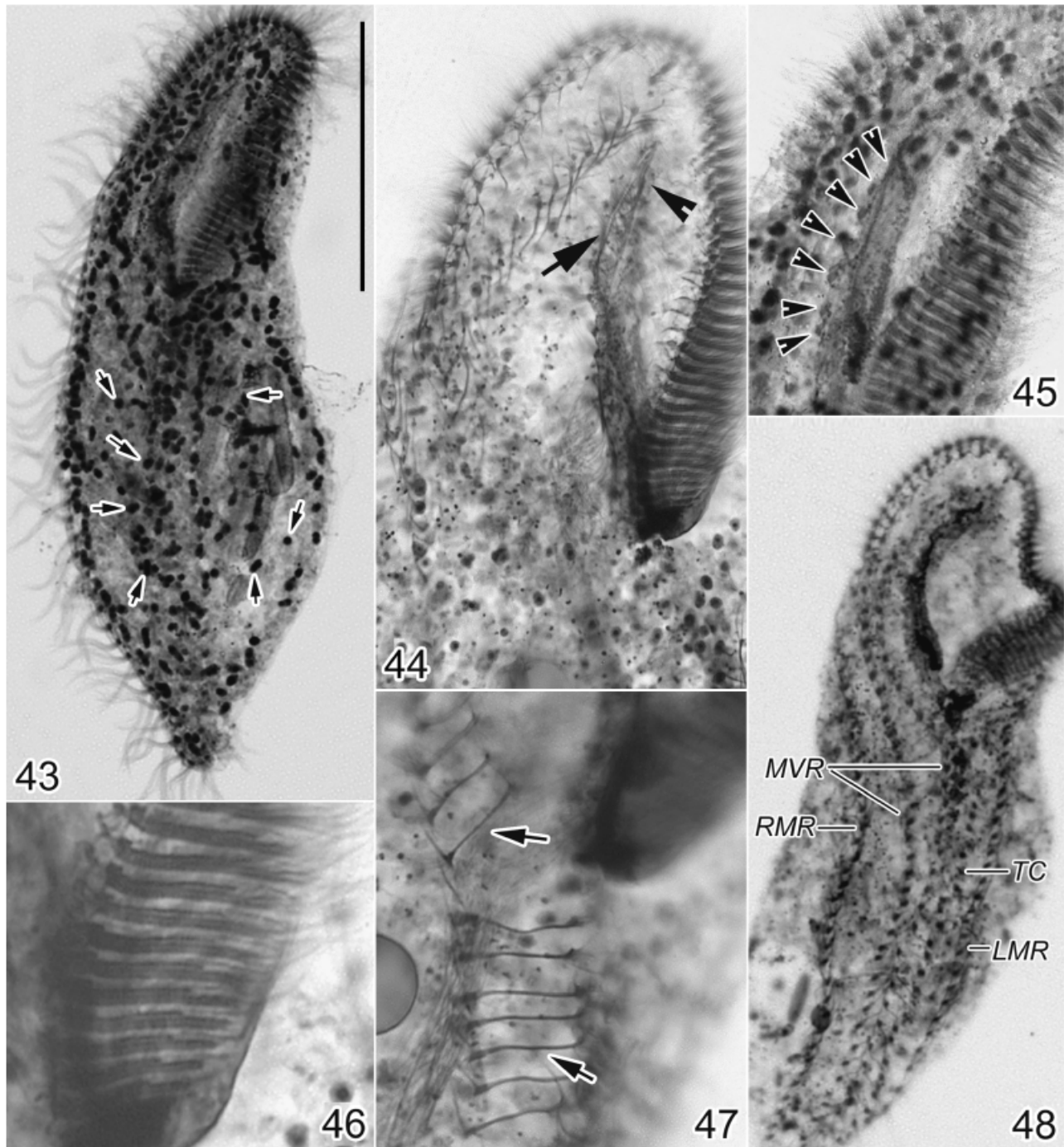


Fig. 43–48. Photomicrographs of *Apokeronopsis wrighti* n. sp. after protargol impregnation. 43. Ventral view, note the macronuclear nodules (arrows). 44. Anterior ventral portion, to show endoral membrane (arrowhead) and paroral membrane (arrow). 45. To mark the buccal cirri (arrowheads). 46. To show fine structure of adoral membranelles. 47. To show fibrils connecting cirri in mid-ventral rows (arrows). 48. Ventral view. LMR, left marginal row; MVR, mid-ventral rows; RMR, right marginal row; TC, transverse cirri. Scale bar = 60 μ m.

characters can be distinguished from our new species (Fig. 32, 37; Table 4; [Petz 1995](#)).

Compared with another marine species, *A. bergeri* (Li et al. 2008, in press), the new species can be clearly separated by possessing fewer adoral membranelles (i.e. ca 50 vs. 80), fewer bicoxonal cirri (i.e. ca 30 vs. 38), and fewer BC (i.e. 6–8 vs. 9–13).

Comparison of the SSrRNA gene sequences of *A. wrighti* and *A. bergeri* also shows 46 nucleotides difference (2.6%), indicating that they are not likely conspecific (Fig. 49).

Therefore, because this new isolate is so distinctly different from other described species, we provide the following diagnosis of this new *Apokeronopsis* species.

Table 2. Morphometrical characterization of *Apokeronopsis wrighti* n. sp.

Character	Mean	Min	Max	SD	CV	<i>n</i>
Body length	192	160	233	21.51	11.20	24
Body width	68.0	46	94	11.61	17.08	23
Buccal length	74.9	61	85	7.19	9.60	24
Number of AM	52.1	44	61	4.40	8.45	21
Number of cirri in LMR	35.3	30	42	2.89	8.19	20
Number of cirri in RMR	38.4	32	43	3.47	9.04	17
Number of cirri in TC	27.1	21	30	2.63	9.70	15
Number of DK	3	3	3	0	0	2
Number of cirri in BC	7.3	6	8	0.71	9.73	9
Number of cirri in LMV	26.9	23	32	2.60	9.67	15
Number of cirri in RMV	28.5	23	35	3.31	11.61	15
Number of cirri in LB	16.3	13	22	2.52	15.46	16
Number of cirri in RB	14.0	11	17	1.69	12.14	16
Number of Ma	207.3	161	286	33.72	16.27	16

Data based on protargol-impregnated specimens. All measurements in μm .

CV, coefficient of variation in %; Max., maximum; Mean, arithmetic mean; Min., minimum; *n*, number of specimens investigated; SD, standard deviation; AM, Adoral membranelles; BC, buccal cirri; DK, dorsal kineties; LB, left bicorona; LMR, left marginal row; LMV, left mid-ventral row; Ma, macronuclear nodules; RB, right bicorona; RMR, right marginal row; RMV, right mid-ventral row; TC, transverse cirri.

Class SPIROTRICHEA Bütschli, 1889

Order Urostylida Jankowski, 1979

Family Pseudokeronopsidae Borror & Wicklow, 1983

Genus *Apokeronopsis* Shao et al., 2007

***Apokeronopsis wrighti* n. sp.**

Diagnosis. An *Apokeronopsis* about $150\text{--}230 \times 35\text{--}55 \mu\text{m}$ in vivo; dark-reddish BCS cortical granules grouped in three rows on the ventral side and two rows on the dorsal side; 23–35 cirri in the right MVR and 23–32 cirri in the left MVR; six to eight buccal, two frontoterminal, 30–42 left marginal, and 32–43 right marginal

cirri; 21–30 transverse cirri extending anteriorly beyond the level of mid-body; consistently three dorsal kineties.

Type slides. One holotype and one paratype of slides (registration number: LH-2007091202, LH-2007091202P) with protargol-impregnated specimens have been deposited in Laboratory of Protozoology, South China Normal University, China.

Type locality. Coastal water of Clear Water Bay, Hong Kong ($22^{\circ}20'N$; $114^{\circ}17'E$).

Dedication. We dedicate this species to our eminent colleague Dr. Andre-Denis G. Wright, in recognition of his contributions to phylogenetic evolution in ciliates.

Table 3. Morphological comparison of *Orthodonella sinica* n. sp. with its congeners.

Character	<i>O. sinica</i> n. sp.	<i>O. hamatus</i>	<i>O. hamatus</i>	<i>O. apohamatus</i>	<i>O. gutta</i>	<i>O. shenae</i>
Cell size in vivo	150–310	90–125 up to 200	90–150 up to 350	60–160	140–200	120–180
Total number of kineties	63–83	—	—	42–60	62–74	ca 70
Number of dikinetids in synhymenium	84–126	—	ca 90	54–62	43–70	80
Number of pharyngeal rods	10–13	9–12	ca 8	8–13	11–14	13
Number of contractile vacuole(s)	1	4–5	1	4	4	2
Position of contractile vacuole(s)	Posterior portion, near left margin	Posterior portion	Posterior end	Two near left margin and two terminally located	Posterior portion	Near left margin
Shape of anterior projection	Dominant, beak-like	Dominant, beak-like	Dominant, beak-like	Dominant, beak-like	Dominant, beak-like	Small, snout-like
Geographic location	Hong Kong	Japanese Sea	A saline lake, Canada	Yellow Sea, China	Yellow Sea, China	Antarctic
Data sources	Present work	Ozaki and Yagiu (1941)	Wilbert (1986)	Lin et al. (2004)	Lin et al. (2004)	Song and Wilbert (2002)

Measurements are in μm .

Table 4. Comparison of *Apokeronopsis* species.

Character	<i>A. wrighti</i> n. sp.	<i>A. crassa</i>	<i>A. antarctica</i>
Body length in vivo	ca 200	ca 200–250	ca 200
Cortical granules	Two types, big ones brown-reddish, grouped in line	Two types, big ones brown-reddish, sparsely distributed	absent
Buccal length	61–85 (75)	62–79 (ca 73)	68–121 (98)
Number of macronuclear nodules	161–286	195–253	ca 120–170
Number of adoral membranelles	44–61 (52)	46–65 (ca 54)	45–71 (61)
Number of cirri forming the bicorona	32–45 (36)	21–35 (ca 27)	20–42 (33)
Number of frontoterminal cirri	2	2	1–3 (2)
Number of buccal cirri	6–8 (7)	6–13 (9)	2–5 (4)
Number of cirri in right mid-ventral row	23–35 (29)	33–63 (ca 41)	41–64 (ca 47)
Number of cirri in left mid-ventral row	23–32 (27)	33–59 (ca 41)	40–63 (46)
Number of transverse cirri	21–30 (27)	25–45 (ca 32)	0
Number of right marginal cirri	32–43 (38)	43–74 (ca 51)	51–77 (63)
Number of left marginal cirri	30–42 (35)	38–70 (ca 52)	45–80 (63)
Number of dorsal kineties	3	3	3
Data source	Original	Hu & Song (2000), Song, Wilbert, and Hu (2004), Berger (2006).	Petz (1995)

Measurements in µm. Numbers in parentheses refer to the mean value.

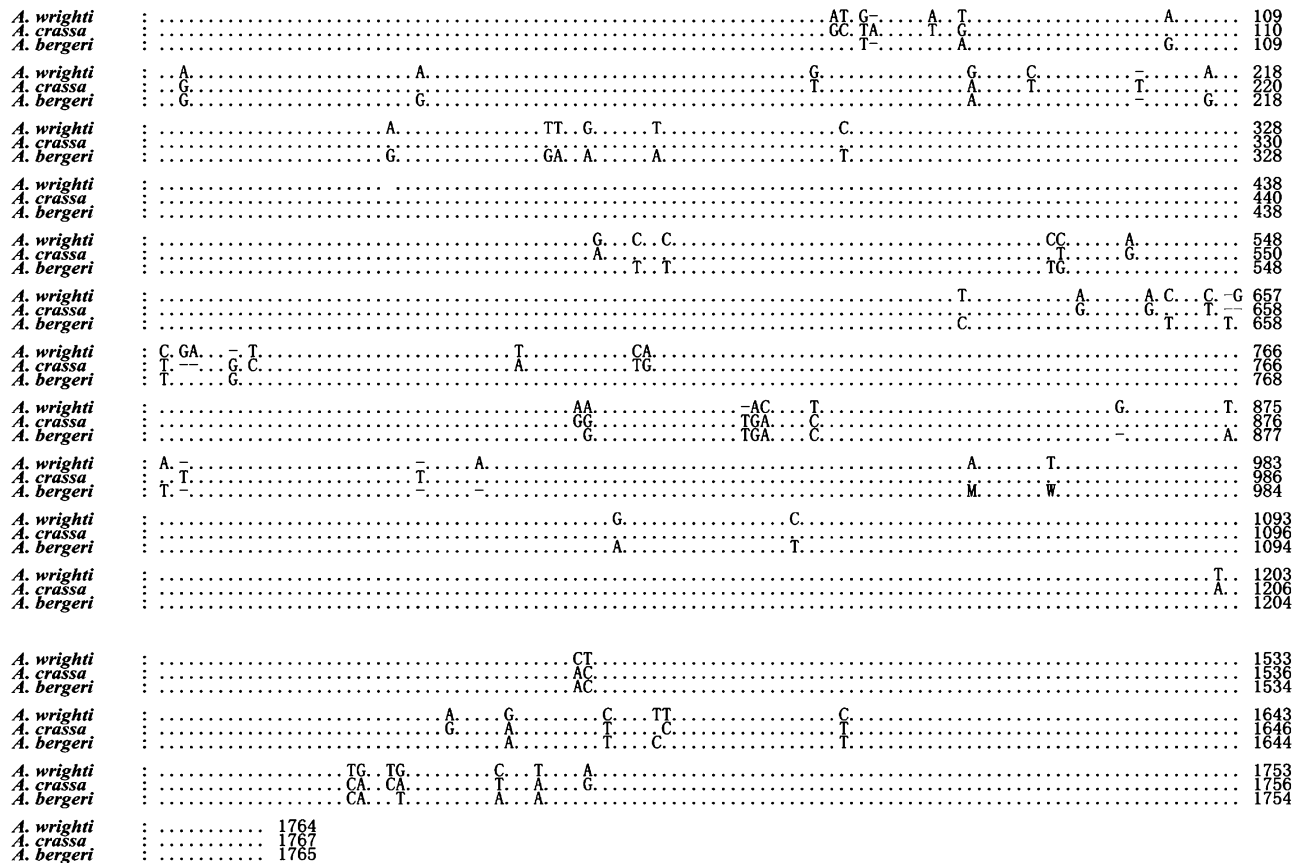


Fig. 49. Small subunit rRNA gene sequence of *Apokeronopsis wrighti* n. sp. aligned with the sequences of *Apokeronopsis crassa* and *Apokeronopsis bergeri*. Numbers at the end of lines indicate the number of nucleotides. The differences in sequence length were compensated for by introducing alignment gaps (–) in the sequences. Matched sites are marked with dots.

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LITERATURE CITED

- Berger, H. 2006. Monograph of the Urostyleloidea (Ciliophora, Hypotricha). *Monogr. Biol.*, **85**:i–xvi, 1–1304.
- Bhatia, B. L. 1936. Protozoa: Ciliophora. The Fauna of British India, Including Ceylon and Burma. Taylor and Francis Press, London, 493 p.
- Corliss, J. O. 1979. The Ciliated Protozoa. Characterization, Classification and Guide to the Literature. 2nd ed. Pergamon Press, Oxford, 455 p.
- Gong, J., Song, W. & Warren, A. 2005. Periphytic ciliate colonization: annual cycle and responses to environmental conditions. *Aquat. Microb. Ecol.*, **39**:159–170.
- Gong, J., Kim, S.-J., Kim, S.-Y., Min, G.-S., Roberts, D. McL., Warren, A. & Choi, J. K. 2007. Taxonomic redescription of two ciliates, *Protogastrostyla pulchra* n. g., n. comb. and *Hemigastrostyla enigmatica* (Ciliophora: Spirotrichea: Stichotrichia), with phylogenetic analyses based on 18S and 28S rRNA gene sequences. *J. Eukaryot. Microbiol.*, **54**:468–478.
- Gruber, A. 1884. Die Protozoen des Hafens von Genua. *Nova Acta Acad. Caes. Leop. Caro. German Nat. Cur.*, **46**:473–539.
- Hu, X. & Song, W. 2000. Infraciliature of *Pseudokeronopsis qingdaoensis* sp. n. from marine biotope (Ciliophora: Hypotrichida). *Acta Zootax. Sin.*, **25**:361–364.
- Jankowski, A. W. 1968. Taxonomy of the suborder Nassulina Jank., 1967 (Ciliophora, Ambihymenida). *Zool. Zh.*, **47**:990–1001. (In Russian with English summary)
- Kahl, A. 1931. Urtiere oder Protozoa I: Wimpertiere oder Ciliata (Infusoria) 2. Holotricha außer den im 1. Teil behandelten Prostomata. *Tierwelt Dtl.*, **21**:181–398.
- Li, L., Song, W., Warren, A., Yi, Z., Roberts, D. & Hu, X. 2008. Morphology and morphogenesis of a new marine hypotrichous ciliate, *Apokeronopsis bergeri* nov. spec. from the Yellow Sea, China (Ciliophora, Hypotrichida). *Eur. J. Protistol.*, (in press)
- Lin, X., Gong, J. & Song, W. 2004. Morphological studies on a new species of *Orthodonella*, with redescription of *O. gutta* (Cohn, 1866) Kahl, 1931 (Protozoa: Ciliophora: Synhymeniida) from coastal water off Qingdao, China. *J. Nat. Hist.*, **38**:2001–2011.
- Lynn, D. H. & Small, E. B. 2002. Phylum Ciliophora. In: Lee, J. J., Bradbury, P. C. & Leedale, G. F. (ed.), An Illustrated Guide to the Protozoa. Society of Protozoologists, Allen Press, Lawrence, KS. p. 371–656.
- Medlin, L., Elwood, H. J., Stickel, S. & Sogin, M. L. 1988. The characterization of enzymatically amplified eukaryotic 16S-like rRNA-coding regions. *Gene*, **71**:491–499.
- Ozaki, Y. & Yagiu, R. 1941. Studies on the marine ciliates of Japan, mainly from the Setonaikai (the inland sea of Japan) II. *J. Sci. Hiroshima Univ. (Ser. B)*, **9**:159–180.
- Petz, W. 1995. Morphology and morphogenesis of *Thigmokeronopsis antarctica* n. sp. and *T. crystallis* n. sp. (Ciliophora, Hypotrichida) from Antarctic sea ice. *Eur. J. Protistol.*, **31**:137–147.
- Shao, C., Hu, X., Warren, A., AL-Rasheid, K. A. S., AL-Quraishy, S. A. & Song, W. 2007. Morphogenesis in the marine spirotrichous ciliate *Apokeronopsis crassa* (Claparède & Lachmann, 1858) n. comb. (Ciliophora: Stichotrichia), with the establishment of a new genus, *Apokeronopsis* n. g. and redefinition of the genus *Thigmokeronopsis*. *J. Eukaryot. Microbiol.*, **54**:392–401.
- Song, W., Warren, A., Roberts, D., Wilbert, N., Li, L., Sun, P., Hu, X. & Ma, H. 2006. Comparison and redefinition of four marine, colored *Pseudokeronopsis* spp. (Ciliophora, Hypotrichida), with emphasis on their living morphology. *Acta Protozool.*, **45**:271–287.
- Song, W. & Wilbert, N. 2002. Faunistic studies on marine ciliates from the Antarctic benthic area, including descriptions of one epizoic form, 6 new species and 2 new genera (Protozoa: Ciliophora). *Acta Protozool.*, **41**:23–61.
- Song, W., Wilbert, N. & Hu, X. 2004. New contributions to the marine hypotrichous ciliate, *Pseudokeronopsis qingdaoensis* Hu & Song, 2000 (Protozoa: Ciliophora: Stichotrichida). *Cah. Biol. Marine*, **45**:335–342.
- Wilbert, N. 1975. Eine verbesserte Technik der Protargolimprägung für Ciliaten. *Mikrokosmos*, **64**:171–179.
- Wilbert, N. 1986. Ciliates from saline lakes in Saskatchewan. *Symp. Biol. Hung.*, **33**:249–256.
- Wilbert, N. & Song, W. 2005. New contributions to the marine benthic ciliates from the Antarctic area, including description of seven new species (Protozoa, Ciliophora). *J. Nat. Hist.*, **39**:935–973.

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