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Morphology and Small Subunit rDNA Gene Sequence of *Pseudoamphisiella quadrinucleata* n. sp. (Ciliophora, Urostylida) from the South China Sea

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ABSTRACT. The urosylid genus *Pseudoamphisiella* was established by Song (1996) with hitherto only two known congeners. In the present work, the morphology and infraciliature of a new member, *Pseudoamphisiella quadrinucleata* n. sp., a form with conspicuous alveolar layer and four macronuclear nodules isolated from the coastal waters both near Hong Kong and near Guangzhou, South China were investigated using living observation and protargol silver impregnation methods. *Pseudoamphisiella quadrinucleata* differs from other two known forms mainly by the number of macronuclear nodules: constantly four vs. two in *Pseudoamphisiella alveolata* and 24–57 in *Pseudoamphisiella lacazei*. To support this, the sequence of the small subunit rDNA of *P. quadrinucleata* n. sp. showed 14 and 74 nucleotides in comparison with that of the two known congeners, respectively, which hence firmly supports the validity of the new species.

Key Words. Hypotrich, marine ciliate, new species, Pseudoamphisiellidae, taxonomy.

THE ciliate genus *Pseudoamphisiella* Song, 1996 was separated from the closely related *Holosticha* by the following morphological features: (1) there are no frontoterminal cirri vs. frontoterminal cirri always present in *Holosticha*; (2) the midventral rows are well separated and hence these cirri do not form a ''zig-zag'' pattern vs. a closely spaced zig-zag pattern in *Holosticha*; and (3) transverse cirri are highly developed vs. generally inconspicuous in *Holosticha* (Song 1996; Song and Warren 2000). The morphology, morphogenesis, and phylogenetic position of *Pseudoamphisiella* have been repeatedly studied recently (Berger 2006; Hu and Suzuki 2006; Shao et al. 2006; Song 1996; Song and Warren 2000; Song, Warren, and Hu 1997; Yi et al. 2008).

During investigation of the ciliate fauna in the South China Sea, an unknown hypotrich with the ciliary pattern of the genus *Pseudoamphisiella* was isolated at two sampling sites. Here, we present the morphology and small subunit (SSU) rDNA gene sequence of this new species, which we name *Pseudoamphisiella quadrinucleata* n. sp.

MATERIALS AND METHODS

Two populations of *P. quadrinucleata* were collected: one from the coastal waters in Clear Water Bay, Hong Kong (22°20′N, 114°17′E), China on October 15, 2007 with the water temperature ca 24 °C, pH 7.9, salinity 33.5%, and the other from mariculturing waters of Daya Bay near Guangzhou (22°43′N, 114°32′E), China on November 8, 2007 with the water temperature ca 23 °C, pH 8.0, salinity ca 19‰.

Isolated specimens were cultured in Petri dishes for several days at room temperature with rice for breeding bacteria. Observations on living cells were carried out with bright field and differential interference contrast microscopy (Nikon 80i, Tokyo, Japan) at 100–1,000X magnifications. The protargol silver-staining method according to Wilbert (1975) was used to reveal the infraciliature and nuclear apparatus. Measurements of stained specimens were performed at a magnification of 1,250X. Drawings of impregnated specimens were conducted with the help of a camera lucida. Terminology and systematics are mainly according to Corliss (1979).

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Genomic DNA extraction, PCR amplification, and SSU rDNA cloning and sequencing of Guangzhou population were performed

Table **1.** Morphometrical characterization of *Pseudoamphisiella quadrinucleata*, the Daya Bay population (upper line) and the Hong Kong population (lower line).

Characters	Min	Max	Mean	SD	*n*
Body length	137	175	156.7	12.1	19
	155	253	187.6	22.1	25
Body width	68	133	108.8	21.9	19
	62	171	100.3	20.5	23
Length of buccal field	50	68	58.1	5.4	19
	54	76	64.5	6.9	15
No. of macronuclear nodules	4	4	4	0	21
	4	4	4	0	22
No. of micronuclei	2	5	3.6	1.00	21
	3	6	4.3	0.7	15
No. of adoral membranelles	52	69	61.4	5.0	18
	49	75	61.8	6.5	19
No. of buccal cirri	2	2	2	0	19
	2	2	2	0	21
No. of frontal cirri	3	3	3	0	19
	3	3	3	0	22
No. of cirri in the left midventral row	11	19	15.2	2.1	17
	16	22	18.3	1.6	18
No. of cirri in the right midventral row	14	20	17.4	1.5	17
	14	20	16.6	1.6	20
No. of left marginal cirri	15	29	22.9	3.1	18
	21	31	25.9	2.9	21
No. of right marginal cirri	14	18	16.1	1.1	16
	17	20	18.5	0.9	17
No. of transverse cirri	17	23	19.7	1.4	20
	17	22	19.3	1.3	20
No. of caudal cirri	13	24	17.9	2.8	19
	15	19	16.6	1.1	21
No. of dorsal kineties	12	17	14.8	1.2	16
	13	15	13.3	0.7	9
Length of macronucleus[a]	18	25	20.8	1.8	20
	13	30	22.0	3.9	29
Width of macronucleus[a]	12	18	15.2	1.7	20
	9	23	14.3	3.8	28

All data based on protargol-impregnated specimens. Measurements in micrometers.
[a]The size of posterior most macronucleus.
Max., maximum; Mean, arithmetic mean; Min., minimum; *n*, number of specimens investigated; SD, standard deviation.

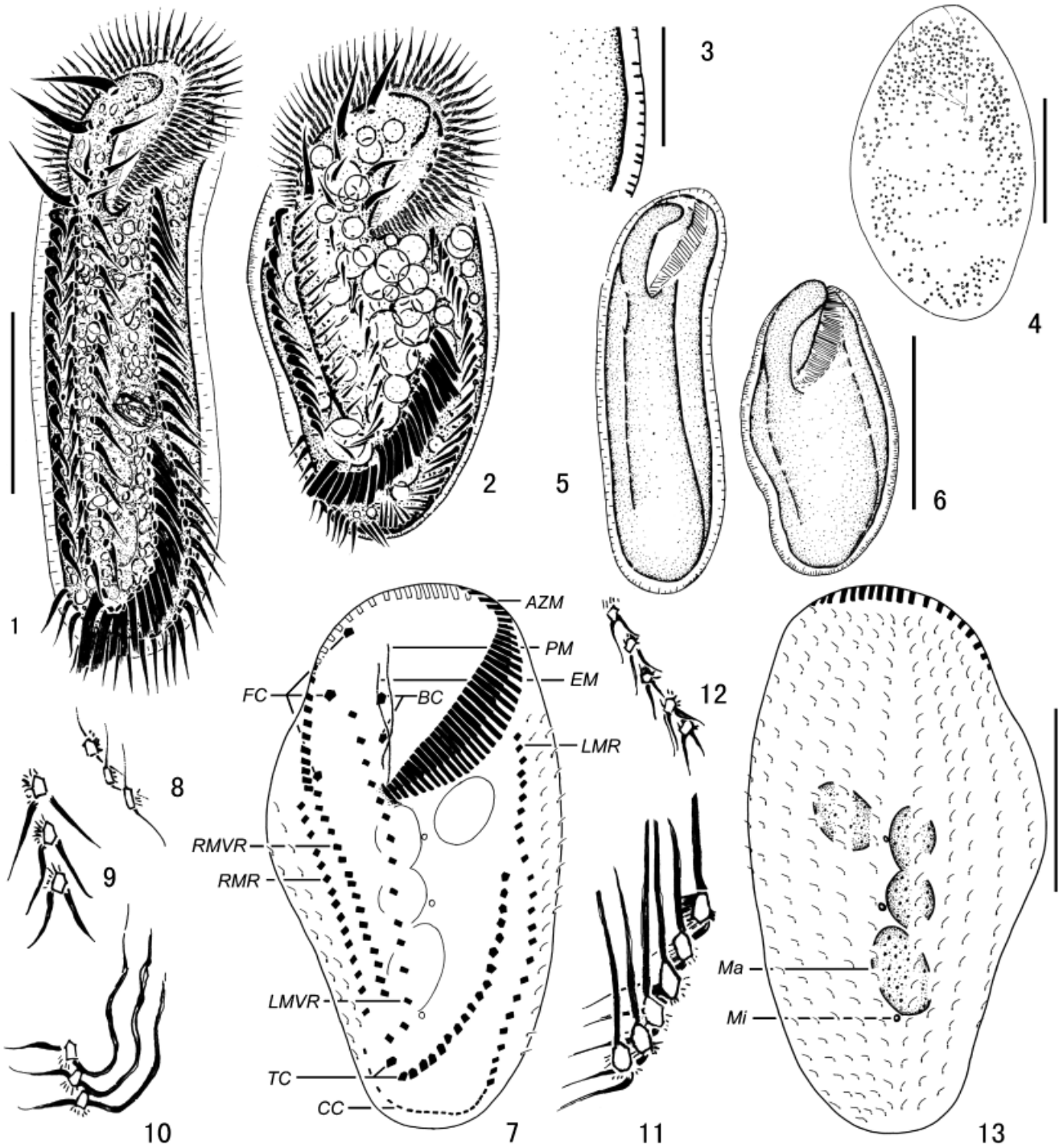

Fig. **1–13.** Morphology of *Pseudoamphisiella quadrinucleata* n. sp. from live observations (1–6) and after protargol impregnation (7–13). **1, 2.** Ventral views, to show the normal (1) and contracted (2) individuals. **3.** Portion of cortex (side view), to show the alveoli and the extrusomes. **4.** Dorsal side, to show the distribution of extrusomes. **5, 6.** Shape variation of the same individual. **7.** Infraciliature of ventral side. **8–12.** Showing the fibrils associated with the right marginal row (RMR) (8), left midventral row (LMVR) (9), right midventral rows (RMVR) (10), transverse cirri (TC) (11), and left marginal row (LMR) (12), respectively. **13.** Dorsal side to show the dorsal kineties and nuclear apparatus. AZM, adoral zone of membranelles; BC, buccal cirri; CC, caudal cirri; EM, endoral membrane; FC, frontal cirri; Ma, macronucleus; Mi, micronucleus; PM, paroral membrane. Scale bars in Fig. 1, 2 = 60 μm, Fig. 3 = 25 μm, Fig. 4–6 = 80 μm, Fig. 7, 13 = 50 μm.

according to Miao et al. (2007). The homologous sequences of *Pseudoamphisiella lacazei* and *Pseudoamphisiella alveolata* are available as GenBank/EMBL DQ777743 and DQ503583, respectively.

RESULTS

The morphology and infraciliature of *P. quadrinucleata* are described based on both Hong Kong and Guangzhou populations.

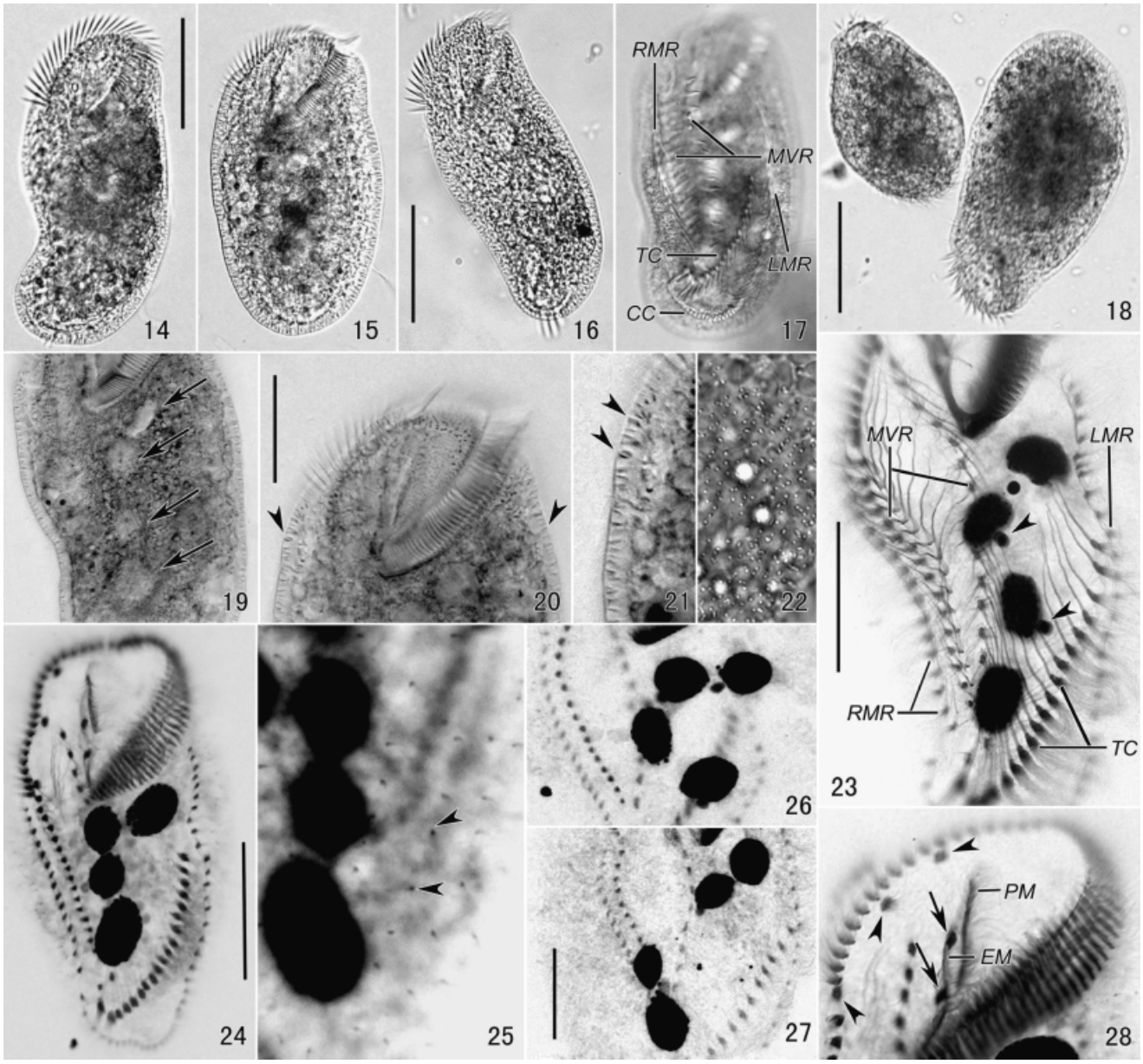

Fig. **14–28.** Morphology of *Pseudoamphisiella quadrinucleata* n. sp. from observations in vivo (14–22) and after protargol impregnation (23–28). **14.** Ventral view of a typical individual. **15, 16.** Ventral views showing different outlines. **17.** Ventral view showing the cirri. **18.** The shape variants of individuals after full feeding. **19.** Ventral view, arrows mark the macronuclear nodules. **20.** Ventral view of anterior portion, arrowheads indicate the alveoli. **21.** Portion of cortex (side view), arrowheads point to the extrusomes. **22.** Dorsal side to show the distribution of extrusomes (as shining dots). **23.** The fibrils associated with cirri, arrowheads refer to the micronuclei. **24.** Ventral view showing the infraciliature and macronuclei. **25.** Dorsal side to show the dorsal kinety (arrowheads). **26, 27.** To exhibit the macronuclei. **28.** Ventral view of anterior portion, arrows mark the buccal cirri, arrowheads indicate the frontal cirri. CC, caudal cirri; EM, endoral membrane; PM, paroral membrane; LMR, left marginal row; MVR, midventral row; RMR, right marginal row; TC, transverse cirri. Scale bars in Fig. 14, 16, 18 = 70 μm, Fig. 20 = 30 μm, Fig. 23, 24 = 50 μm, Fig. 27 = 40 μm.

Morphology. Cell size is typically about 180 × 70 μm with a length to width ratio about 2–3:1 (Table 1). The body shape is conspicuously different among individuals: elongated forms are up to 220 μm in length while oval cells are ca 120 μm wide (Fig. 1, 2, 5, 6, 14–16). The body is flexible, and slightly or conspicuously contractile when disturbed. The body outline is elongated to ellipsoidal, with both ends bluntly rounded; the anterior left region is slightly projected (Fig. 1, 2, 5, 6, 14–18). The body is dorsoventrally flattened about 2:1.

The pellicle is thin and fragile. Cell surface is covered by a conspicuous, hyaline alveolar layer, which is about 5 μm thick and is visible even under low magnifications (Fig. 15, 16). The extrusomes are bar-like, about 2–3 μm long; they are sparsely distributed within the alveolar layer, one end sticking on the alveolar layer making the cell surface look like it has numerous ''shining dots'' (Fig. 3, 4, 20–22).

The oral field is moderately narrow; the right wall of buccal cavity protrudes strongly to left (Fig. 1). The adoral zone of mem-

Table **2.** Morphological and morphometrical comparison of *Pseudoamphisiella quadrinucleata* n. sp., *Pseudoamphisiella lacazei* (Maupas, 1883) Song, 1996 and *P. alveolata* (Kahl, 1932) Song and Warren, 2000.

Character	*P. quadrinucleata*	*P. lacazei*	*P. alveolata*
Body size in vivo (μm)	160–220 × 60–100	120–240 × 50–80	120–300 × 40–80
No. of macronuclear nodules	4 ($n = 43$)	24–57 ($n = 20$)	2 ($n = 16$)
No. of micronuclei	2–6 ($n = 36$)	7–10 ($n = 6$)	2–5 ($n = 11$)
No. of adoral membranelles	49–75 ($n = 37$)	39–49 ($n = 25$)	47–59 ($n = 9$)
No. of buccal cirri	2–3 ($n = 40$)	2 ($n = 25$)	2 ($n = 18$)
No. of frontal cirri	3 ($n = 41$)	3 ($n = 25$)	3 ($n = 18$)
No. of left midventral cirri	11–22 ($n = 35$)	16–23 ($n = 26$)	10–14 ($n = 11$)
No. of right midventral cirri	14–20 ($n = 37$)	11–16 ($n = 26$)	11–15 ($n = 11$)
No. of left marginal cirri	15–31 ($n = 39$)	21–34 ($n = 16$)	14–20 ($n = 11$)
No. of right marginal cirri	14–20 ($n = 33$)	20–31 ($n = 16$)	12–14 ($n = 11$)
No. of transverse cirri	14–23 ($n = 40$)	16–23 ($n = 26$)	12–16 ($n = 16$)
No. of caudal cirri	13–24 ($n = 40$)	9–11 ($n = 5$)	11–16 ($n = 9$)
No. of dorsal kineties	12–17 ($n = 25$)	8–11 ($n = 25$)	10–12 ($n = 16$)
Alveolar layer	Present	Absent	Present
Data source	Present work	Song (1996)	Song and Warren (2000)

n, number of specimens investigated.

branelles (AZM) is conspicuous, and about one-fourth to one-third of cell length. Cilia in anterior membranelles are ca 30 μm long. The frontal and transverse cirri are strong, about 25 μm in length. The midventral, marginal, and caudal cirri are ca 15 μm long. The caudal cirri are densely arranged; as in other known congeners, they are closely connected with the left marginal cirri (Fig. 7, 17, 24). Dorsal cilia are about 5 μm long.

Cytoplasm is usually dark gray, and typically contains numerous granular inclusions, which are 5–10 μm across and give the cell a dark, opaque appearance, especially at low magnifications (Fig. 14–16). Food vacuoles are difficult to recognize. Contractile vacuole is not observed.

The cell constantly has four spherical or ellipsoidal macronuclear nodules (Ma), and the anteriormost one is usually distinctly displaced leftwards; each one is about 13–30 × 9–23 μm in size; they usually appear as four transparent areas in vivo at central portion (Fig. 13, 19, 24). The cell has two to five micronuclei, about 3 μm across, which are often within the indentations of macronuclear nodules and undetectable in vivo (Fig. 13; 23, arrowheads).

Locomotion is by continuous crawling on substrate, relatively fast in speed. The body reacts quickly when disturbed by contracting and remaining motionless for a short while.

Infraciliature. The AZM consists of 49–75 membranelles. The base of AZM is about 10–15 μm long, and those of the distal end are distinctly shorter than the proximal portion (Fig. 7, 24). Paroral membrane (PM) is about 40 μm long, usually across the endoral membrane (EM) (Fig. 7, 28). Two buccal cirri (BC) are close to the right of PM (Fig. 7; 28, arrow). The body has three frontal cirri (FC), of which the right-most one is located near the distal end of AZM (Fig. 7, 28, arrowheads). The right midventral row (RMVR) is relatively short, contains 14–20 cirri and terminates anteriorly at the right-most frontal cirrus. The left midventral row (LMVR) is composed of 11–22 loosely arranged cirri; the anterior end of LMVR extends to about half-way up the frontal field (Fig. 7, 23, 24). Highly developed transverse cirral (TC) row is composed of 17–23 close-set cirri, which are arranged in a J-shaped row; TC anteriorly terminates near the equatorial level, and posteriorly to the end of LMVR (Fig. 1, 7, 17, 23, 24). Marginal cirri are closely spaced; there are 14–20 cirri in the right marginal row (RMR) and 15–31 in the left marginal row (LMR); the posterior end of LMR is almost continuous with the caudal cirri (CC). The CC are distinctly smaller than the remaining cirri, consist of 13–24 close-set cirri (Fig. 7, 17). Fibrils, which are highly developed and group dependent in terms of their length and structure, are characteristically associated with midventral, marginal, and transverse cirri (Fig. 8–11, 23). There are 12–17 dorsal kineties extending over entire length of body (Fig. 7, 13, 25).

Gene sequence. The length of the SSU rDNA for *P. quadrinucleata* n. sp. is 1,771 bp (GenBank Accession number EU518416).

DISCUSSION

To date only two species in the genus *Pseudoamphisiella* have been reported, namely *P. lacazei* (Maupas, 1883) Song, 1996 (formerly *Holosticha lacazei*) and *P. alveolata* (Kahl, 1932) Song and Warren, 2000 (formerly *Holosticha alveolata*) (Song 1996; Song et al. 1997; Song and Warren 2000).

These three species show significant morphological differences (Table 2). The general living morphology and infraciliature of *P. quadrinucleata* n. sp. are most similar to that of *P. alveolata*. However, the new species consistently has four macronuclear nodules (vs. two in *P. alveolata*). Thus, *P. quadrinucleata* can be easily separated from *P. alveolata*; also based mainly on the number of macronuclear nodules, our new species can be clearly differentiated from the multinuclear *P. lacazei* (four vs. 24–57). Furthermore, *P. quadrinucleata* has a higher number of adoral membranelles than *P. lacazei* (49–75 vs. 39–49), and exhibits a conspicuous alveolar layer (vs. absent in *P. lacazei*).

The dissimilarity is also supported by the molecular data. Comparison of the SSU rDNA gene sequence of *P. quadrinucleata* n. sp. with that of *P. alveolata* and *P. lacazei* reveals 14 (0.8%) and 74 (4.2%) nucleotides difference, respectively, which supports, in our opinion, the validity of this new species, *P. quadrinucleata* n. sp.

Family Pseudoamphisiellidae Song et al., 1997

Pseudoamphisiella quadrinucleata n. sp.

Diagnosis. Marine *Pseudoamphisiella* about 160–220 × 60–120 μm in vivo with conspicuous alveolar layer, elongated to ellipsoidal shaped; four macronuclear nodules; 49–75 adoral membranelles; three frontal and two buccal cirri; 17–23 transverse cirri extending about two-fifth of cell length; two genus-typical, widely-separated midventral rows, the left one with about 11–22 and right with 14–20 cirri; 15–31 and 14–20 cirri in left and right marginal rows, respectively; about 17 caudal cirri and 12–17 dorsal kineties.

Type slides. Two permanent slides of protargol-impregnated specimens of Guangzhou population are in the Laboratory of

Protozoology, South China Normal University, one as the holotype (No. SZ07110804-1) and the other as the paratype (No. SZ07110804-2). A voucher slide (No. LH2007101504) of protargol-impregnated specimens of the Hong Kong population is also deposited in the same place.

Etymology. The *quadri* and *nucleata* refer to the four macronuclear nodules of this species.

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