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Morphological studies of six free-living spirotrichean ciliates (Protozoa: Ciliophora) with three new records from the coastal South China Sea

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Abstract

The living morphology and infraciliature of six spirotrichous ciliates collected from the coastal South China Sea were investigated using live observation and protargol impregnation. These are *Euplotes woodruffi* Gaw, 1939, *Hemigastrostyla enigmatica* (Dragesco and Dragesco-Kernéis, 1986) Song and Wilbert, 1997, *Neourostylopsis flavicana* (Wang et al., 2011) Chen et al., 2013, *Protogastrostyla pulchra* (Perejaslawzewa, 1886) Gong et al., 2007, *Pseudoamphisiella alveolata* (Kahl, 1932) Song and Warren, 2000, and *Pseudokahliella marina* (Foissner et al., 1982) Berger et al., 1985. Among these, *Protogastrostyla pulchra*, *Pseudoamphisiella alveolata* and *Pseudokahliella marina* are reported from the South China Sea for the first time, which manifest obvious differences apart from other Chinese populations. As new contribution, the detailed description of isolates from mangrove habitat of *E. woodruffi* and *H. enigmatica*, and aquaculture pond isolate of *N. flavicana*, are present.

Key words: South China Sea, new record, ciliates, Spirotrichea, taxonomy

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1 Introduction

Ciliates are a large group with probably the greatest diversity among all eukaryotic microorganisms (Song et al., 2009). Thousands of free-living ciliates have been reported from marine biotopes, but only a small portion of them are detailedly described with both living morphology and infraciliature informations. Over the last two decades, a series of investigations have been conducted on the ciliate diversity in the coastal waters of the Bohai Sea and Yellow Sea (Song et al., 2009).

Currently, we document six spirotrichous ciliates collected from the South China Sea, i.e., *Euplotes woodruffi* Gaw, 1939, *Hemigastrostyla enigmatica* (Dragesco and Dragesco-Kernéis, 1986) Song and Wilbert, 1997, *Neourostylopsis flavicana* (Wang et al., 2011) Chen et al., 2013, *Protogastrostyla pulchra* (Perejaslawzewa, 1886) Gong et al., 2007, *Pseudoamphisiella alveolata* (Kahl, 1932) Song and Warren, 2000, and *Pseudokahliella marina* (Foissner et al., 1982) Berger et al., 1985, using modern methods. Among them, *Protogastrostyla pulchra*, *Pseudoamphisiella alveolata* and *Pseudokahliella marina* are reported from the South China Sea area for the first time, which manifest obvious differences apart from other Chinese populations. Our brackish isolate of *E. woodruffi*, provides evidence that supports the conclusion, that *E. parawoodruffi* is a junior synonym of *E. woodruffi*. Compaed with previous studies, the mangrove isolate of *H. enigmatica* and the estuary isolate of *Protogastrostyla pulchra* indicate that, the population isolated from habitat with lower salinity has larger body size and more membranelles. Our population from the South China Sea of *Pseudokahliella marina*, is much more similiar with the original description by Foissner et al. (1982) rather than the two populations' colleceted from the Yellow Sea.

2 Materials and methods

The six spirotrichous ciliates were collected from the South China Sea and the detailed information was provided in Table 1 and Fig. 1. The isolated specimens were maintained in Petri dishes at room temperature with rice grains added to enrich bacterial food for ciliates. The isolated cells were observed both *in vivo* using bright field and differential interferences contrast microscopy and then stained using protargol to reveal the infraciliature (Wilbert, 1975). The counts and measurements of the stained specimens were performed at a magnification of 1 000×. The terminology is according to Berger (2006, 2008) and Song and Bradbury (1997).

3 Results and discussion

Euplotes woodruffi Gaw, 1939 (Figs 2a–c and 3a, b)

Morphological description. Cell *in vivo* (140–200 μm) × (90–100 μm), body shape somewhat rectangular with anterior end wider than posterior; both left and right sides almost straight.

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Table 1. Sampling information of the six spirotrichid ciliates (Fig. 1)

	Species	Sampling site	Water temperature	Salinity
1	*Euplotes woodruffi*	mangrove, Shenzhen	18°C	18
2	*Hemigastrostyla enigmatica*	mangrove, Shenzhen	20°C	20
3	*Neourostylopsis flavicana*	aquaculture pond, Zhuhai	19°C	13
4	*Protogastrostyla pulchra*	estuary, Guangzhou	24°C	14
5	*Pseudoamphisiella alveolata*	mangrove, Zhanjiang	20°C	30
6	*Pseudokahliella marina*	beach, Huizhou	17°C	30

Body dorsoventrally thin and flattened. Buccal field spacious and long, occupying 75%–90% of body length; midway along right border, buccal lip slightly indented, forming a triangular and large buccal cavity, which ending to left of leftmost transverse cirrus. Evident ridges between transverse cirri. Cytoplasm colorless with numerous fine granules (ca. 1 μm across) beneath pellicle and several large food vacuoles (10–20 μm across) rendering cytoplasm dark grey at low magnification. Contractile vacuole located right of transverse cirri. Locomotion by crawling on substrate and rotating along cell axis occasionally.

Adoral zone of membranelles, comprising of 58–78 and on average 67 membranelles, markedly wide with distal end curved ventrally, near anterior frontal cirri and posterior end terminating in buccal cavity beneath rightmost transverse cirrus. Paroral membrane medium long with anterior portion twisted and like a comb. Invariably nine frontoventral, five transverse, two marginal and two caudal cirri; eight to ten and on average nine dorsal kineties. Macronucleus roughly T-shaped or Y-shaped with two arms; micronucleus not observed.

Comparison. *Euplotes woodruffi* is a species originally isolated from a freshwater pond in Wuhan, China and subsequently found in brackish habitats (Fokin et al., 2008; Gaw, 1939; Pierson, 1943; Shen et al., 2008; Vannini et al., 2012). Song and Bradbury (1997) neotypified *E. woodruffi* base on a freshwater isolate sampled from a pond in Qingdao, China and established *E. parawoodruffi* to separate from the freshwater *E. woodruffi* by several morphological differences and in particular the brackish habibtat. However, by using the morphology, molecular phylogeny and adaptation cultivation, Dai et al. (2013) suggested that *E. parawoodruffi* is a junior synonym of *E. woodruffi*.

Our brackish isolate corresponds well with the previous description of the species (Dai et al., 2013; Shen et al., 2008; Song and Bradbury, 1997). Compared with the four populations reported with detailed morphological data (Dai et al., 2013; Shen et al., 2008; Song and Bradbury, 1997), our specimens have slightly larger body size *in vivo* ((140–200 μm) × (90–100 μm)) than the Putian population ((110–160 μm) × (60–80 μm), Dai et al., 2013) and the Daya Bay population ((90–140 μm) × (60–90 μm), Shen et al., 2008), which is similar with the North American population ((110–200 μm) × (70–130 μm), Song and Bradbury, 1997) and Qingdao populations ((130–160 μm) × (90–110 μm), Song and Bradbury, 1997); the number of adoral membranelles (58–78 and on average 67) is within the range of the four populations (56–85), and the number of mid-dorsal kineties (19–27 and on average 24) is close to the freshwater specimens from Qingdao, China.

Our brackish isolate was collected from a mangrove habitat, with different salinity (18) from the previous sampling environments (freshwater, Qingdao population; salinity 5, Putian and North American populations; and salinity 29, Daya Bay population). The morphological characters of this isolate also fall within the range of the four populations. The South China Sea population provides evidence that supports Dai et al. (2013)'s conclusion, that is, *Euplotes parawoodruffi* is a junior synonym of *E. woodruffi*.

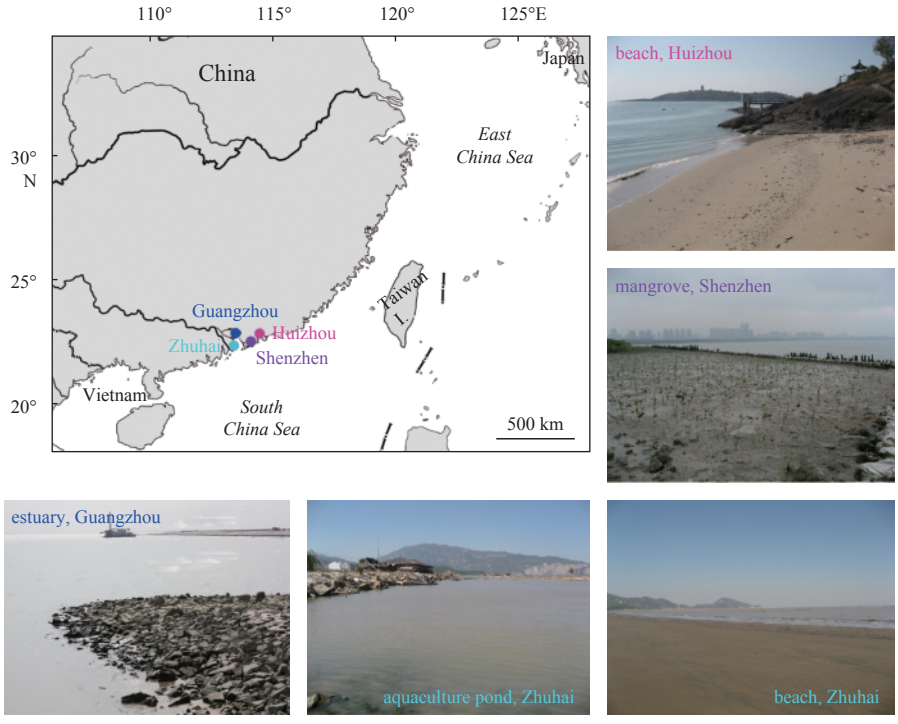

Fig. 1. Sampling sites in Guangdong and Guangxi Provinces of the six spirotrichean ciliates.

***Hemigastrostyla enigmatica* (Dragesco and Dragesco-Kernéis, 1986) Song and Wilbert, 1997 (Figs 2d–e and 3c–e)**

Morphological description. Body size (150–250 μm) × (40–60 μm) *in vivo*, slightly flexible and of variable shape, generally elongate with both ends widely rounded. Left margin straight and right margin slightly convex. Dorsoventrally flattened from 1:2 (dorsal side hunched especially in central portion) to 1:5. Cytoplasm hyaline and colorless with many globular granules and several food vacuoles irregularly distributed; ingested diatoms observed. Colorless and fine cortical granules grouped obliquely and close to dorsal kinetids. Moderately rapid moving on substrate and swimming fast.

Adoral zone 25%–30% of cell length with 59–74 and on average 67 membranelles. One buccal, three frontal, seven frontoventral, two pretransverse, two extra lateroventral and five transverse cirri; 34–40 (on average 36) left and 29–33 (on average 31) right marginal cirri; 4–6 (on average 5) caudal cirri located close to posterior end of left marginal cirral row; usually six (seldom five) dorsal kineties. Two ellipsoidal macronuclear nodules without micronuclei observed.

Comparison. Song and Wilbert (1997) reported a Qingdao population of *Hemigastrostyla enigmatica* from the Yellow Sea (salinity 28–32), with the *in vivo* body size of (120–180 μm) × (30–50 μm), the adoral zone composed of 40–61 membranelles, and 23–30 left and 16–22 right marginal cirri. Gong et al. (2007) presented detailed redescription of it based on two populations collected from a tidal flat (salinity 20–25) on Korea coast of the Yellow Sea, where the species had a larger body size ((110–280 μm) × (40–70 μm) *in vivo*) and more adoral membranelles (42–65) and marginal cirri (24–35 on the left and 23–32 on the right). Cui et al. (2009) isolated a Daya Bay population (salinity 25–32) of *H. enigmatica*, which has smaller body size ((100–150 μm) × (30–50 μm)) and less adoral membranelles (37–48) and marginal cirri (20–28 on the left and 17–24 on the right). Our mangrove population (salinity 20) corresponds well with the Korean population. Compared to the Daya Bay population, our mangrove isolation owns much larger body size *in vivo*, many more adoral membranelles and marginal cirri. Overall, the two populations sampled from the enviroments with low salinity have larger body size, more membranelles and cirri than those from the enviroments with high salinity.

***Neourostylopsis flavicana* (Wang et al., 2011) Chen et al., 2013 (Figs 2g–i and 3f–h)**

Morphological description. Cell *in vivo* (110–150 μm) × (35–50 μm), body slightly contractile and flexible, elongated and somewhat sigmoid with narrowed posterior end, ratio of length to width (3–5):1. Buccal cavity about 25% of body length. Cortical granules bright yellow, some grouped around cirri and dorsal cilia, some arranged in lines between cirral and dorsal ciliary rows,

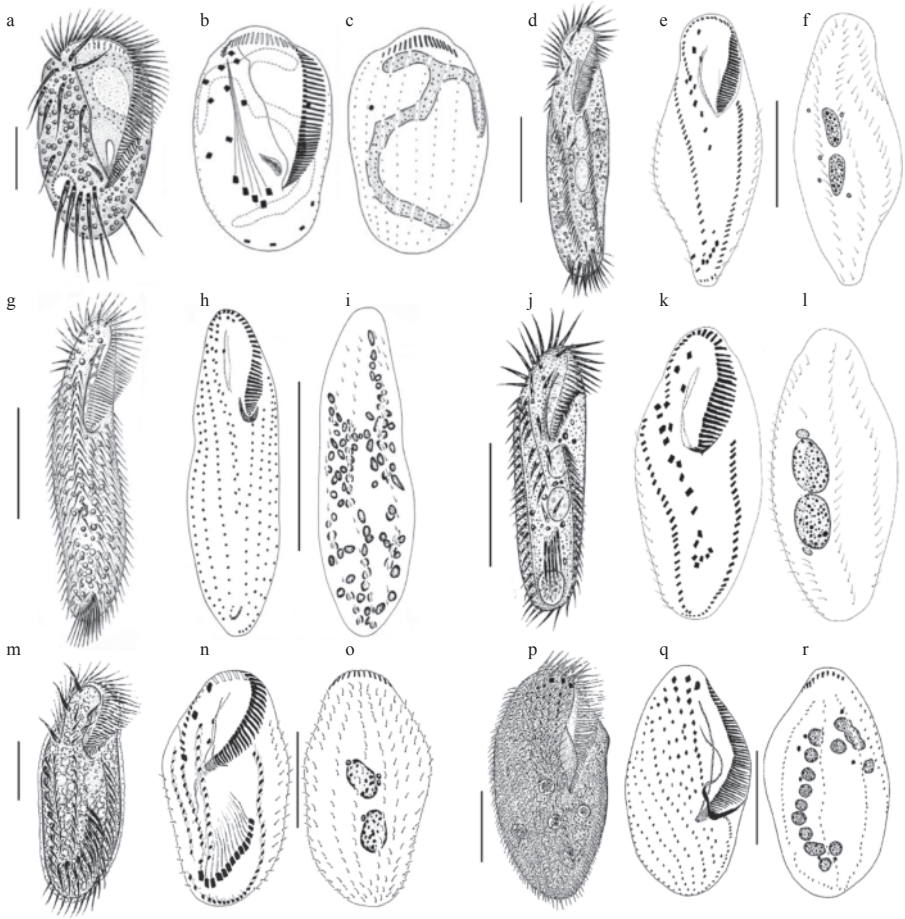

Fig. 2. Living morphology and infraciliature of six spirotrichid ciliates. a–c. *Euplotes woodruffi* from Dai et al. (2013), d–f. *Hemigastrostyla enigmatica* from Gong et al. (2007), g–i. *Neourostylopsis flavicana* from Wang et al. (2011), j–l. *Protogastrostyla pulchra* from Gong et al. (2007), m–o. *Pseudoamphisiella alveolata* from Song and Warren (2000), and p–r. *Pseudokahliella marina* from Hu and Song (2003).

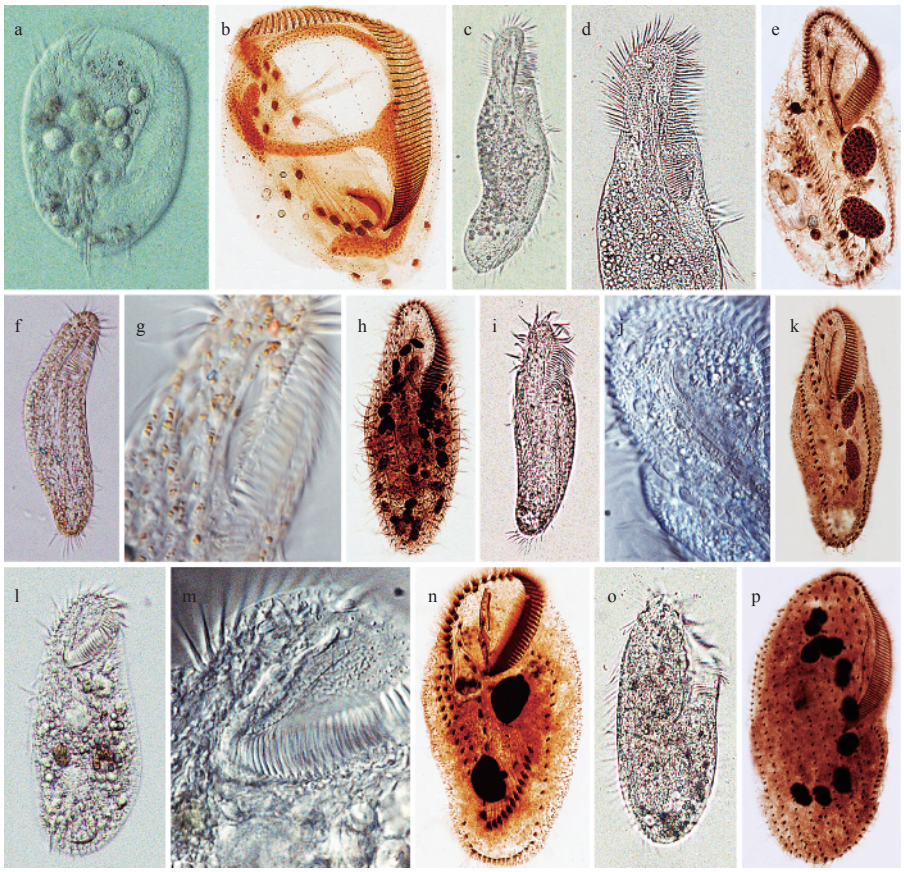

Fig. 3. Photomicrographs of the six spirotrichean ciliates from life (a, c, d, f, g, i, j, l, m and o) and after protargol impregnation (b, e, h, k, n and p). a and b. Ventral views of *Euplotes woodruffi*; c–e. ventral views of *Hemigastrostyla enigmatica*, to indicate the buccal field (d); f–h. ventral views of *Neourostylopsis flavicana*, to show the cortical granules (g); i–k. ventral views of *Protogastrostyla pulchra*, to show the cortical granules (j) and the infraciliature (k); l–n. ventral views of *Pseudoamphisiella alveolata*, to indicate the buccal field (m) and the infraciliature (n); and o and p. *Pseudokahliella marina*.

rendering cell reddish. Cytoplasm colorless with tiny lipid droplets and food vacuoles. Contractile vacuole located anterior of mid-body and near left margin. Crawling fast on debris and sometimes swimming along axis of body.

Adoral zone composed of 28–41 (on average 34) membranelles. Pharyngeal fibers conspicuous after protargol impregnation. One buccal, four frontal, two frontoterminal, two pretransverse and 7–10 (on average 8) transverse cirri. Midventral complex of 11–18 (on average 14) pairs of cirri in a zigzag pattern. Invariably four left and three right marginal cirral rows. Three complete kineties plus one short row anterior of rightmost marginal row and 56–92 (on average 72) macronuclear nodules.

Comparison. *Neourostylopsis flavicana* was first discovered from a mangrove habitat (salinity 20) in Shenzhen. Our population which was isolated from an aquaculture pond (salinity 13) corresponds well with the type population. Although both populations were isolated from the South China Sea, our isolates have a slightly smaller body size (*in vivo* (110–150 μm) × (35–50 μm) vs. (130–200 μm) × (30–60 μm)) and a sligthly lower number of adroal membranells (28–41, on average 34 vs. 33–45, on average 37) (Wang et al., 2011).

Protogastrostyla pulchra **(Perejaslawzewa, 1886) Gong et al., 2007 (Figs 2j–l and 3i–k)**

Morphological description. Cell *in vivo* (160–300 μm) × (50–100 μm), flexible and non-contractile, usually slender and elongate. Both ends widely rounded, body margins slightly convex and dorsoventrally flattened about 2:1. Cortical granules (like extrusomes) colorless elliptic in lateral view, irregularly grouped between ventral cirral row and dorsal kineties rows. Endoplasm colorless to grayish. Moving moderately rapid and crawling on substrate.

Adoral zone composed of 40–50 (on average 45) membranelles. Invariably 16 frontoventral and five transverse cirri, 31–37 (on average 34) left and 24–30 (on average 27) right marginal cirri, and five dorsal kineties.

Comparison. Gong et al. (2007) provided a detailed redescription of *Protogastrostyla pulchra* based on two populations collected from a coastal beach (salinity 30) of the Jawol Island and a tidal flat at Ganghwa (salinity 20–25) on Korea coast of the Yellow Sea. Our population was isolated from an estuary habitat (salinity 14) in the South China Sea for the first time. It corresponds well with the previous description, except for the larger size *in vivo* ((160–300 μm) × (50–100 μm) vs. (110–180 μm) × (30–50 μm)) and more adoral membranelles (40–50 vs. 30–42). Our study indicated that under similar environmental conditions the body size and number of adoral membranelles of *P. pulchra* have a tendency to increase with decreasing salinity.

Pseudoamphisiella alveolata **(Kahl, 1932) Song and Warren, 2000 (Figs 2m–o and 3l–m)**

Morphological description. Cell *in vivo* (90–140 μm) × (40–90 μm), flexible and contractile with length to width ratio 3:2 to 4:1. Outline shape variable, slender or broad oval to elliptical. Dorsovent-

rally flattened about 2:1. Left margin of anterior region ear-shaped rendering appearance of cephalization. Cell surface covered by conspicuous, hyaline alveolar layer (3–5 μm thick) and sparsely arranged, bar-like (2–3 μm long) extrusomes within it. Cytoplasm containing numerous granular inclusions giving cell a dark, opaque appearance. Locomotion typified by continuous crawling on substrate.

Adoral zone composed of 40–47 (on average 43) membranelles. Invariably three frontal and two buccal cirri. 12–14 cirri in left and 12–13 cirri in right midventral row. 19–24 (on average 21) left and 11–14 (on average 13) right marginal cirri. 14–16 (on average 15) transverse, about 10 dorsal kineties with 12–15 (on average 14) caudal cirri. Two macronuclear nodules and about three micronuclei.

Comparison. Kahl (1932) first reported this species, and then Song and Warren (2000) provided a detailed redescription based on a population collected from the Yellow Sea coastal water off Qingdao. Our population which was isolated from a mangrove habitat (salinity 30) in the South China Sea corresponds well with previous description, except for the shorter body length (*in vivo* 90–140 μm vs. 120–240 μm), a lower number of adoral membranelles (40–47, on average 43 vs. 47–59, on average 51), and more cirri in the left marginal row (19–24, on average 21 vs. 14–20, on average 17).

***Pseudokahliella marina* (Foissner et al., 1982) Berger et al., 1985 (Figs 2p–r and 3o, p)**

Morphological description. Cell *in vivo* (130–180 μm) × (50–60 μm), body shape elliptical with both ends rounded, and dorsoventrally flattened 3:2 to 3:1. Oral field large, half of body length and one-third of body width, with a roof-like structure covering posterior part of adoral zone. Thick pellicle slightly flexible with conspicuous and colorless cortical granules. Endoplasm dark greyish with numerous granular inclusions and several large food vacuoles. Moving moderately rapid, crawling on substrate and swimming around long axis of cell.

Adoral zone composed of 60–85 (on average 67) membranelles and invariably three enlarged frontal cirri. About 13 cirral rows, 23–30 (on average 26) left and 34–48 (on average 40) right marginal cirri. Three complete dorsal kineties without caudal cirri. Seven to twelve (on average 9) macronuclear nodules.

Comparison: *Pseudokahliella marina* was first described by Foissner et al. (1982) and then Hu and Song (2003) redescribed it based on two populations collected from mollusc-culturing waters along Qingdao coast (salinity 30). Our population was isolated from the South China Sea (salinity 30), which corresponds well with the previous descriptions. Interestingly, the South China Sea population is morphologically much more similiar with the European population described by Foissner et al. (1982) than the two Yellow Sea populations described by Hu and Song (2003). Likely, the habitats (e.g., the salinity of the microenvironment) rather than the geographic distance played a more important role in shaping the morphological variations among these populations.

References

Berger H. 2006. Monograph of the Urostyloidea (Ciliophora, Hypotricha) (Monographiae Biologicae, vol. 85). Berlin: Springer, 1–1303

Berger H. 2008. Monograph of the Amphisiellidae and Trachelostylidae (Ciliophora, Hypotricha) (Monographiae Biologicae, vol. 88). Berlin: Springer, 1–737

Cui Fulian, Liu Weiwei, Lin Xiaofeng, et al. 2009. Morphological studies on seven hypotrichous ciliates (Ciliophora, Protozoa) from the Daya Bay, South China. Acta Zootaxonomica Sinica (in Chinese), 34(2): 335–339

Dai Renhai, Xu Kuidong, He Yingying. 2013. Morphological, physiological, and molecular evidences suggest that *Euplotes parawoodruffi* is a junior synonym of *Euplotes woodruffi* (Ciliophora, Euplotida). Journal of Eukaryotic Microbiology, 60(1): 70–78, doi: 10.1111/jeu.2012.60.issue-1

Foissner W, Adam H, Foissner I. 1982. Morphologie und Infraciliatur von *Bryometopus pseudochilodon* Kahl, 1932, *Balantidioides dragescoi* nov. spec. und *Kahliella marina* nov. spec. und Revision des Genus *Balantidioides* Penard, 1930 (Protozoa, Ciliophora). Protistologica, 18: 211–225

Fokin S I, Di Giuseppe G, Erra F, et al. 2008. *Euplotespora binucleata* n. gen., n. sp. (Protozoa: Microsporidia), a parasite infecting the hypotrichous ciliate *Euplotes woodruffi*, with observations on microsporidian infections in Ciliophora. Journal of Eukaryotic Microbiology, 55(3): 214–228, doi: 10.1111/jeu.2008.55.issue-3

Gaw H Z. 1939. *Euplotes woodruffi*, sp. nov.. Arch Protistenk, 93: 1–5

Gong Jun, Kim S J, Kim S Y, et al. 2007. Taxonomic redescriptions 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. Journal of Eukaryotic Microbiology, 54(6): 468–478

Hu Xiaozhong, Song Weibo. 2003. Redescription of the morphology and divisional morphogenesis of the marine hypotrich *Pseudokahliella marina* (Foissner et al., 1982) from scallop-culture water of North China. Journal of Natural History, 37(17): 2033–2043, doi: 10.1080/00222930210132265

Kahl A. 1932. Urtiere oder Protozoa. I: Wimpertiere oder Ciliata (Infusoria), 3. Spirotricha. Arch Protistenk, Tierwelt Dtl, 25: 399–650

Pierson B F. 1943. A comparative morphological study of several species of *Euplotes* closely related to *Euplotes patella*. Journal of Morphology, 72(1): 125–165, doi: 10.1002/(ISSN)1097-4687

Shen Zhuo, Lin Xiaofeng, Li Jiqiu. 2008. Morphological redescription of a rare marine euplotids, Euplotes parawoodruffi (Protozoa, Ciliophora, Euplotida). Acta Zootaxonomica Sinica (in Chinese), 33(2): 335–339

Song Weibo, Bradbury P C. 1997. Comparative studies on a new brackish water Euplotes, *E. parawoodruffi* n. sp., and a redescription of *Euplotes woodruffi* Gaw, 1939 (Ciliophora; Hypotrichida). Archiv Protistenk, Tierwelt Dtl, 148(4): 399–412, doi: 10.1016/S0003-9365(97)80019-X

Song Weibo, Warren A. 2000. *Pseudoamphisiella alveolata* (Kahl, 1932) nov. comb., a large marine hypotrichous ciliate from China (Protozoa, Ciliophora, Hypotrichida). European Journal of Protistology, 36(4): 451–457, doi: 10.1016/S0932-4739(00)80051-6

Song Weibo, Warren A, Hu Xiaozhong. 2009. Free-living Ciliates in the Bohai and Yellow Seas, China (in Chinese). Beijing: Science Press, 1–518

Song Weibo, Wilbert N. 1997. Morphological investigations on some free living ciliates (Protozoa, Ciliophora) from China Sea with description of a new hypotrichous genus, *Hemigastrostyla* nov. gen. Archiv für Protistenkunde, 148(4): 413–444, doi: 10.1016/S0003-9365(97)80020-6

Vannini C, Ferrantini F, Ristori A, et al. 2012. Betaproteobacterial symbionts of the ciliate *Euplotes*: origin and tangled evolutionary path of an obligate microbial association. Environmental Microbiology, 14(9): 2553–2563, doi: 10.1111/emi.2012.14.issue-9

Wang Yangang, Hu Xiaozhong, Huang Jie, et al. 2011. Characterization of two urostylid ciliates, *Metaurostylopsis flavicana* spec. nov. and *Tunicothrix wilberti* (Lin & Song, 2004) Xu et al., 2006 (Ciliophora, Stichotrichia), from a mangrove nature protection area in China. International Journal of Systematic and Evolutionary Microbiology, 61(Pt 7): 1740–1750

Wilbert N. 1975. Eine verbesserte Technik der Protargolimprägnation für Ciliaten. Mikrokosmos (in German), 64: 171–179