



# Novel Contributions to the Taxonomy of the Ciliates Genus *Euplotes* (Ciliophora, Euplotida): Redescription of Two Poorly Known Species, With a Brief Note on the Distributions of This Genus in Coastal Waters of Southern China

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As the typical periphytic ciliate, the genus *Euplotes* Ehrenberg, 1830 is highly diversified and commonly observed in marine water. In this study, the living morphology, infraciliature and silverline system of two poorly known *Euplotes* species, *E. neapolitanus* Wichterman, 1964 and *E. antarcticus* Fenchel and Lee, 1972, isolated from coastal water of southern China, were investigated. The original description of these two species were brief, and thus we provided detailed redescription based on our Chinese population. Their diagnoses were improved by adding some morphology characteristics and their detailed illustrations and photomicrographs were first supplied here. Based on the sufficient justification for identification of our population by morphology, their small subunit ribosomal RNA gene sequences which have been reported were linked to the accurate species name. Phylogenetic analyses showed that these two species cluster with their congeners which shared high morphological similarities with them. In addition, the geographic distribution of the genus *Euplotes* in coast of southern China was revealed, and the mangrove was considered as the ideal habitat for them by possessing the higher species richness.

**Keywords:** *Euplotes neapolitanus* Wichterman, 1964, *Euplotes antarcticus* Fenchel and Lee, 1972, biogeography, morphology, phylogeny

## INTRODUCTION

The species of the ciliate genus *Euplotes* Ehrenberg, 1830 are frequently observed in marine samples (Lynn, 2008; Song et al., 2009). This genus is typical periphytic microorganism which can colonize the submerged substrates and are usually the most primary components of aufwuchs in high abundance and richness. They often feed on bacteria and microalgae, and play a crucial role in the flow of energy in microbial food webs (Lynn, 2008).

The genus *Euplotes* is highly diverse with over 150 species, which were often distinguished using the characteristics including the body size and shape, the dorsal and ventral ridges, the cirral pattern, the adoral zone of membranelles, the macronucleus shape, the dorsal kineties, and the silverline system pattern (Tuffrau, 1960; Borrer, 1972; Carter, 1972; Curds, 1975; Lynn, 2008). Recently many reports on this group integrate aspects of their living morphology, infraciliature and molecular sequences, thus offering a more comprehensive understanding of their taxonomy. Despite of many descriptions of new species and redescrptions of insufficiently known species (Lobban et al., 2005; Fan et al., 2010; Jiang et al., 2010a,b; Pan et al., 2012; Chen et al., 2013; Fotedar et al., 2016; Lian et al., 2018, 2019; Yan et al., 2018; Hu et al., 2019; Gao et al., 2020), some species still require a re-investigation due to lacking detailed morphological description *in vivo* or after protargol preparation, as well as the molecular data (Gao et al., 2017; Liu et al., 2017).

Two poorly known species, namely *E. neapolitanus* Wichterman, 1964 and *E. antarcticus* Fenchel and Lee, 1972, were collected in coastal waters of southern China, which gave us an opportunity to re-recognize them. In previous studies, their SSU rRNA gene sequences have been reported, but no detailed morphological data from specimens are linked with them (Yi et al., 2009; Gao et al., 2017; Zhao et al., 2018). In present paper, the morphology of these two species is thus redescrbed based on live observation and protargol-impregnated material. For the first time, their detailed illustrations and photomicrographs were supplied here. In addition, the study on the diversity of *Euplotes* species has been extensively conducted in the coastal waters of southern China in the past decade, and a large number of species have been collected and reported with taxonomy description (Hu et al., 2019; Lian et al., 2020). Based on a compilation of their faunal data, the biogeographical patterns of the genus *Euplotes* in different sites and habitats of southern China coast were reviewed here.

## MATERIALS AND METHODS

### Collection and Identification

Both species were found near a fishing dock of Daya Bay, Huizhou, China (22°42'N; 114°32'E). *Euplotes neapolitanus* were collected directly using 100 ml bottle from surface water that contained some organic debris on March 31, 2007. The salinity was about 28.6‰, the water temperature 23.1°C and pH 8.2. *Euplotes antarcticus* was collected using 20 µm mesh plankton nets from the upper 0.5 m water on March 31, 2007. The salinity was about 28.7‰, the water temperature 29.7°C and pH 8.4. After isolation, specimens were maintained in Petri dishes in the laboratory at room temperature. Attempts to culture the two species failed; therefore all studies were carried out on freshly isolated specimens.

Cells were observed *in vivo* using a light microscope equipped with differential interference contrast (Zhang et al., 2020). Protargol (Wilbert, 1975), silver nitrate (Foissner, 2014) staining methods were used respectively to reveal the infraciliature and silverline systems. Measurements and counts were made at

1,000 × magnification; drawings of stained cells were performed with the help of a camera and photomicrographs. Terminology is mainly according to Curds (1975).

### Phylogenetic Analyses

The SSU rRNA gene sequences of *E. neapolitanus* and *E. antarcticus* have been published in previous studies with accession number FJ998024 and FJ998023, respectively. The two sequence were aligned with the sequences of 127 other ciliates downloaded from GenBank da ase (see **Figure 5** for accession numbers) using the GUIDANCE2 algorithm<sup>1</sup>. Representative species of Discocephalida, Aspidiscidae, Gastrocirrhidae, Uronychiidae were chosen as outgroup taxa. The final alignment used for phylogenetic analyses included 1,506 sites. Maximum likelihood (ML) analysis with 1,000 bootstrap replicates was performed on the CIPRES Science Gateway applying the GTRGAMMA model (Miller et al., 2010) using RAXML-HPC2 on XSEDE 8.2.9 (Stamatakis, 2014). Bayesian inference (BI) analysis was carried out with MrBayes 3.2.6 on XSEDE (Ronquist et al., 2012) on the CIPRES Science Gateway using the model GTR + I + G selected by AIC in MrModeltest 2.2 (Nylander, 2004). Markov chain Monte Carlo (MCMC) simulations were run for 1,000,000 generations with sampling every 100 generations and a burn-in of 1,000 trees (Dong et al., 2020). MEGA 6 (Tamura et al., 2013) was used to visualize the tree topologies.

### Biogeographic Distribution

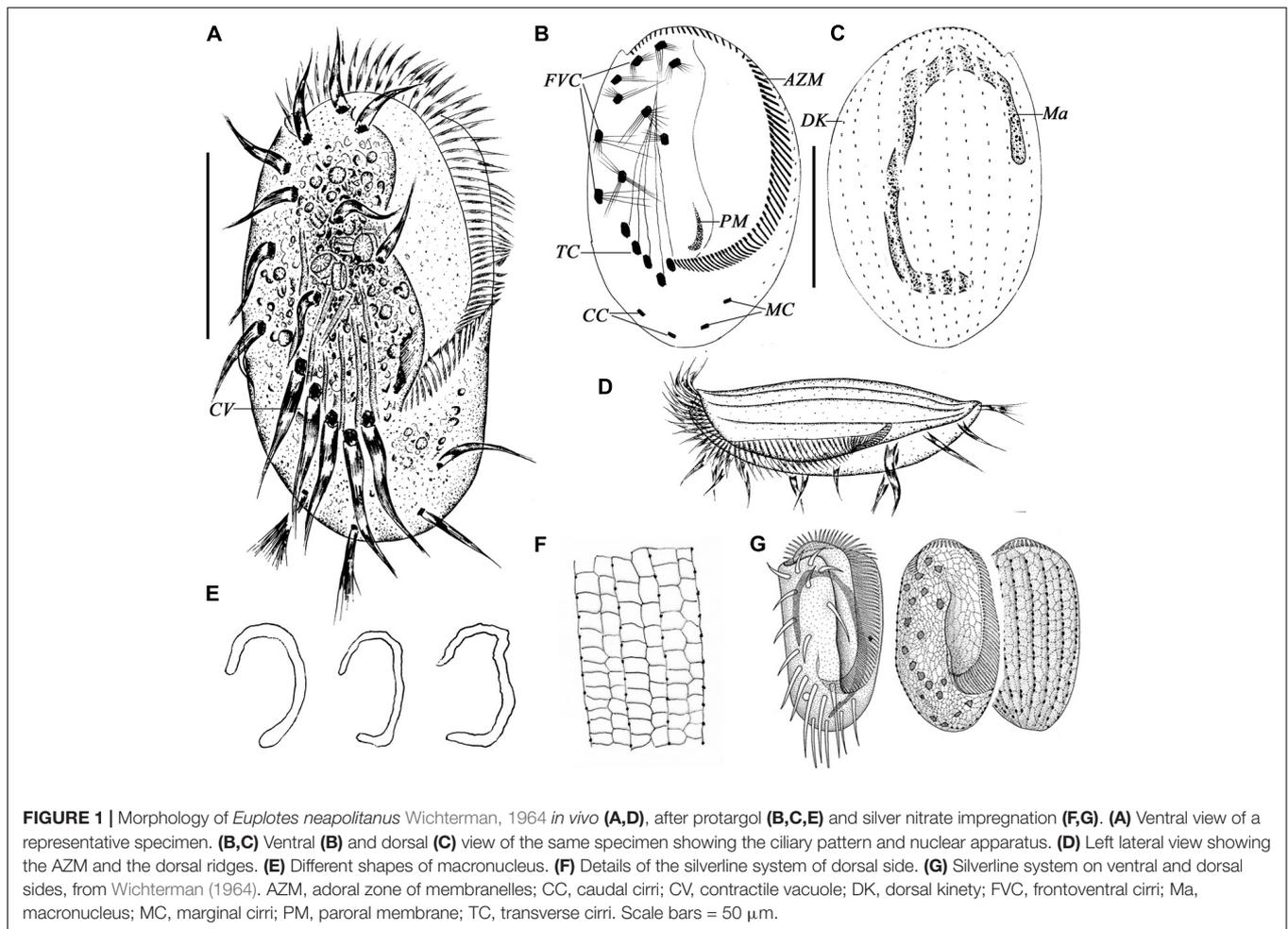
The regional biogeographic patterns of *Euplotes* species are inferred based on a compilation of faunal data from different sources include one monographs (Hu et al., 2019) and all papers on the taxonomy and biodiversity of *Euplotes* in southern China coast. Totally 15 species from six habitats of seven coastal sites were collected as the data set (see **Table 2** for a complete list). Considering that the results in these sources come from the extensive and high-frequency samplings in all sites of southern China coast, the compiled faunal data can generally reflect their distribution characters. Therefore, the presences of each species in these sampling sites and habitats were summarized, based on which the distribution variations of these species among different sites were analyzed and the species richness among habitats were compared.

## RESULTS

### *Euplotes neapolitanus* Wichterman, 1964 Improved Diagnosis

Large marine *Euplotes* with eight or nine low dorsal ridges, 120–150 × 70–80 µm *in vivo*, ellipsoidal in outline, anterior more truncated than posterior; adoral zone of membranelles about 3/4 of cell length, abruptly curved to anterior end of cell, composed of 62–73 membranelles; always 10 frontoventral, five transversal, two caudal and two left marginal cirri; mostly 11 dorsal kineties

<sup>1</sup><http://guidance.tau.ac.il/ver2/>



**FIGURE 1** | Morphology of *Euplotes neapolitanus* Wichterman, 1964 *in vivo* (A,D), after protargol (B,C,E) and silver nitrate impregnation (F,G). (A) Ventral view of a representative specimen. (B,C) Ventral (B) and dorsal (C) view of the same specimen showing the ciliary pattern and nuclear apparatus. (D) Left lateral view showing the AZM and the dorsal ridges. (E) Different shapes of macronucleus. (F) Details of the silverline system on dorsal side. (G) Silverline system on ventral and dorsal sides, from Wichterman (1964). AZM, adoral zone of membranelles; CC, caudal cirri; CV, contractile vacuole; DK, dorsal kinety; FVC, frontoventral cirri; Ma, macronucleus; MC, marginal cirri; PM, paroral membrane; TC, transverse cirri. Scale bars = 50  $\mu\text{m}$ .

with 17–23 dikinetids in mid-kinety rows; Macronucleus 3- or C-shaped; silverline system double-eurystomus type.

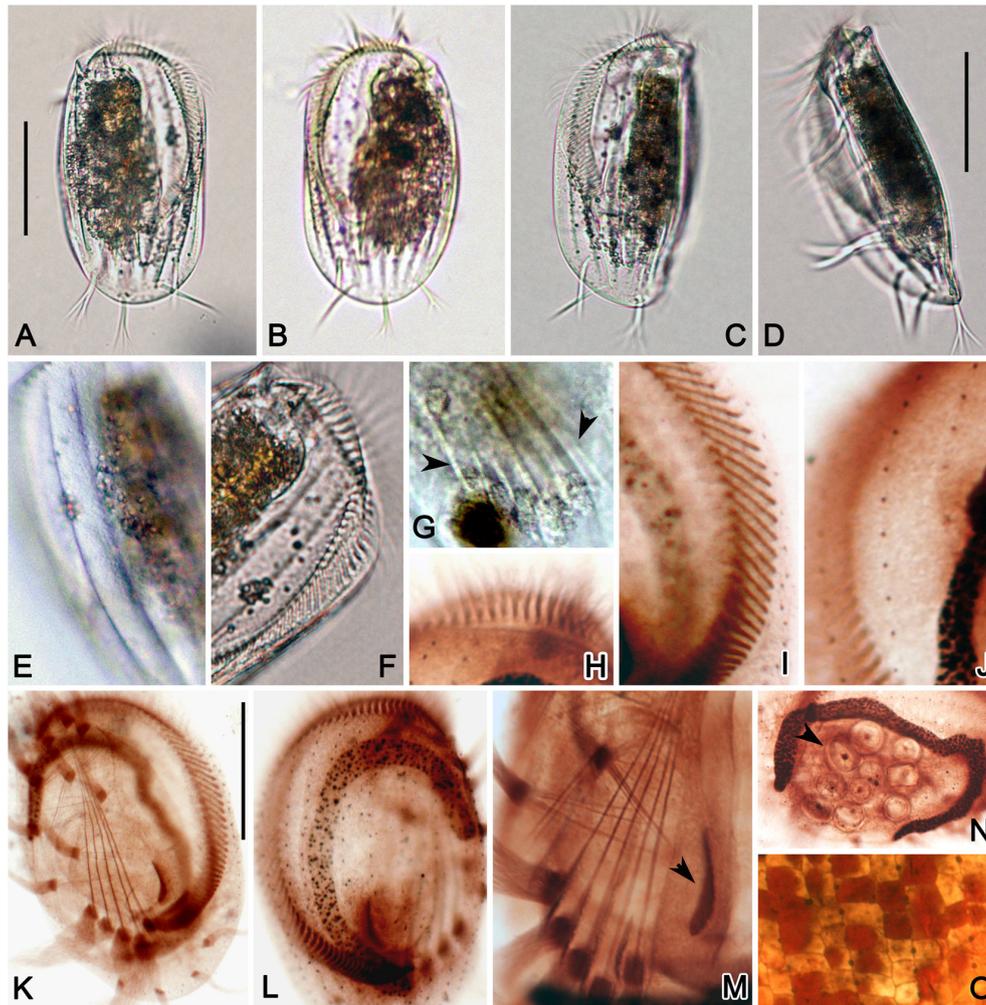
### Description

Body shape generally ellipsoidal with truncated anterior end and bluntly round posterior end, left margin slightly curved while right margin straight (Figures 1A, 2A,B). Size range 120–150  $\times$  70–80  $\mu\text{m}$  *in vivo*, dorsoventrally flattened about 3:2 (Figures 1D, 2C), dorsal surface slightly convex with eight or nine low longitudinal ridges extending over entire length of body (Figures 1D, 2D,E); ventral side more or less concave so transverse section slightly arciform (Figure 2D), five or six short ventral ridges among transverse cirri (Figures 1A, 2G). At anterior end of ventral side, a hyaline collar-like plasmatic protrusion formed at base of adoral zone (Figures 1A, 2F). Buccal field prominent, extending about 3/4 of cell length, right border located almost in middle axis of body, obviously indented in middle portion, forming a broad buccal open area (Figures 1A, 2A–C).

Cytoplasm colorless, highly transparent at marginal area, but dark brownish in right central part due to many lipid globules (3–5  $\mu\text{m}$ ) and food vacuoles (10–12  $\mu\text{m}$ ) containing yellow alga (*Coscinodiscus* sp.) (Figures 1A,

2A,B,F). Contractile vacuole posterior to the rightmost transverse cirrus, about 15  $\mu\text{m}$  in diameter (Figure 1A, arrow). Macronucleus usually untypical 3-shaped with inconspicuous concave notch which even disappeared in some individuals (Figures 1E, 2L,N). Locomotion typically by fast crawling on substrate, occasionally remaining stationary for short periods.

Infraciliature as shown in Figures 1B,C. Paroral membrane easily observed *in vivo* with cilia about 20  $\mu\text{m}$  long, forming an L-shaped kinetosomes area about 20  $\mu\text{m}$  long after staining, with anterior portion thin, and posterior portion thick and slightly curved (Figures 1B, 2M). Adoral zone composed of 62–73 membranelles, which straightly arranged along the left cell margin and abruptly curved to anterior end of cell especially in living cells (Figures 1A,B, 2F,H,I,K). The bases of membranelles up to 10–20  $\mu\text{m}$  long and cilia of membranelles about 15–25  $\mu\text{m}$  long. Consistently 10 frontoventral cirri arranged in normal pattern about 25–30  $\mu\text{m}$  long, and five strong transverse cirri about 40  $\mu\text{m}$  long (Figures 1B, 2K). Usually two, rarely three caudal cirri brush-like, about 30  $\mu\text{m}$  long (Figures 1B, 2K). Two left marginal cirri, positioned below the buccal cavity and close to caudal cirri, the length equal to caudal cirri (Figures 1B, 2K). About 11 or 12 (mostly 11) dorsal kineties almost extending over



**FIGURE 2** | Photomicrographs of *E. neapolitanus* *in vivo* (A–G), and after protargol (H–N) and silver nitrate (O) impregnation. (A,B) Ventral (A) and dorsal (B) views of typical specimens. (C,D) Left-dorsal (C) and lateral (D) views. (E) Detail of dorsal side to show the ridges. (F) Anterior part of ventral side showing the abruptly curved AZM. (G) Detail of ventral side, arrowheads show the short ridge between transverse cirri. (H,I) Anterior (H) and middle (I) portions of AZM. (J) Detail of dorsal kinety. (K,L) Ventral (K) and dorsal (L) views of infraciliature. (M) Center portion of ventral side to show the transverse cirri, paroral membrane (arrowhead) and developed fiber system around the cirri. (N) Macronucleus and food (arrowhead) in cell center. (O) Detailed dorsal view of silverline system. Scale bars = 50  $\mu\text{m}$ .

entire length of cell with the leftmost one usually positioned near body margin on ventral side; middle row with about 17–23 dikinetids (Figures 1C, 2J). Silverline system on dorsal side double-eurystomus type (Figures 1F, 2O).

## *Euplotes antarcticus* Fenchel and Lee, 1972

### Improved Diagnosis

Slender marine *Euplotes*, 50–70  $\times$  25–35  $\mu\text{m}$  *in vivo*, body elongated ellipsoidal shape with anterior end obliquely truncated and right anterior end protruded; left and right margins bended to ventral side; six longitudinal ridges forming three furrows in neighboring pair on dorsal side; buccal filed about 3/4 of cell length with 26–30 membranelles; ventral cirri fine, including 10 frontoventral, five transversal, two caudal and two left

marginal cirri; eight dorsal kineties with 13–17 dikinetids in mid-kinety rows and five or six dikinetids in leftmost one; macronucleus C-shaped or inverted J-shaped; silverline system double-patella type.

### Description

Cells measuring 50–70  $\times$  25–35  $\mu\text{m}$  *in vivo*. Body slender ellipsoidal shaped, anterior end obliquely truncated with right part conspicuously protruded, and posterior end narrowly round (Figures 3A,B, 4A–C,J). Dorsoventrally flattened about 3:2, with left margin strongly and right margin slightly bended to ventral side, which lead to the left margin significantly higher than right one in transverse section of cell (Figures 3E,F, 4D,E). Dorsal surface slightly convex in posterior 2/5 area with about six longitudinal ridges extending over entire length of body (Figures 3E,H, 4D). These ridges separated into three pairs, in

**TABLE 1** | Morphometric characteristics of *Euplotes neapolitanus* (upper rows) and *E. antarcticus* (lower rows) from protargol impregnated specimens.

Characters	Min	Max	Mean	Med	SD	SE	CV	n
Body length	101	142	115.3	115.0	9.06	1.55	7.9	34
	44	56	50.4	51	3.05	0.52	6.1	35
Body width	69	105	81.7	80.0	8.11	1.39	9.9	34
	23	31	26.6	27	2.10	0.36	7.9	35
Length of buccal field	75	96	86.1	85.0	4.57	0.78	5.3	34
	34	44	39.9	40	2.26	0.38	5.7	35
Number of adoral membranelles	62	73	66.6	67	2.49	0.45	3.7	31
	26	30	28.7	29	1.10	0.19	3.8	33
Number of frontoventral cirri	10	10	10.0	10.0	0.00	0.00	0.0	32
	10	11	10.0	10.0	0.18	0.03	1.8	32
Number of transverse cirri	5	5	5.0	5.0	0.00	0.00	0.0	32
	5	5	5.0	5.0	0.00	0.00	0.0	32
Number of left marginal cirri	2	2	2.0	2.0	0.00	0.00	0.0	32
	2	2	2.0	2.0	0.00	0.00	0.0	32
Number of caudal cirri	2	3	2.0	2.0	0.18	0.03	9.0	32
	2	3	2.1	2.0	0.25	0.04	11.9	32
Number of dorsal kineties	11	12	11.0	11.0	0.25	0.04	2.3	32
	8	8	8.0	8.0	0.00	0.00	0.0	32
Number of dikinetids in middle dorsal kinety row	17	23	20.3	21	1.62	0.29	8.0	31
	13	17	14.8	15.0	1.27	0.25	8.6	26

All measurements in  $\mu\text{m}$ .

CV, coefficient of variation in%; Max: maximum, Mean: arithmetic mean; Med, median; Min, minimum; N, number of individuals examined; SD, standard deviation; SE, standard error of mean.

each pair two ridges slightly inclined to each other, which thus producing a 2  $\mu\text{m}$  wide groove between the ridge pair on dorsal surface (Figures 3F, 4H). Three conspicuous ridges in ventral center among transverse cirri. The left and right margins of ventral side uplift, forming the ventral bending (Figures 3A, 4G).

Cytoplasm colorless, several lipid droplets (2–4  $\mu\text{m}$  across) and food vacuoles (3–6  $\mu\text{m}$  across) scattered in cell, rendering cells brown in color at low magnification (Figures 3A, 4B,C). Contractile vacuole located right posterior 1/4 and below the last two ventral cirri, about 8  $\mu\text{m}$  in diameter (Figures 3A, 4B,K). Macronucleus usually open C-shaped and the posterior curve not conspicuous in some individuals which presents an inverted J-shape of macronucleus (Figures 3G, 4L,Q,R). Locomotion untypical, cells usually keep swimming slowly by rotation around the main axis, and occasionally crawls on the substrate.

Buccal field extending to 3/4 of body length, right border slightly indented in lower half, forming a buccal open area accounting for left 40% of body width (Figures 3A, 4A,F). Adoral zone composed of 26–30 membranelles, proximal portion slightly curved and anterior portion obliquely extend along the left cell shoulder to the dorsal anterior end of cell (Figures 3A,C, 4A,L). The bases of membranelles up to 5–9  $\mu\text{m}$  long and cilia of membranelles about 8–12  $\mu\text{m}$  long. Paroral membrane composed of many irregularly arranged kinetosomes which forming a clavate shape about 7  $\mu\text{m}$  long (Figures 3C, 4N).

The ventral cirri generally fine. Invariably 10 frontoventral cirri, about 12  $\mu\text{m}$  long; five transverse cirri about 21  $\mu\text{m}$  long, and two caudal cirri about 15  $\mu\text{m}$  long (Figures 3A,C, 4F,G,L). Two marginal cirri obliquely arranged below the proximal end of adoral membranelles, bearing thicker cilia and larger basal plaque than caudal cirri but equal to the latter in length

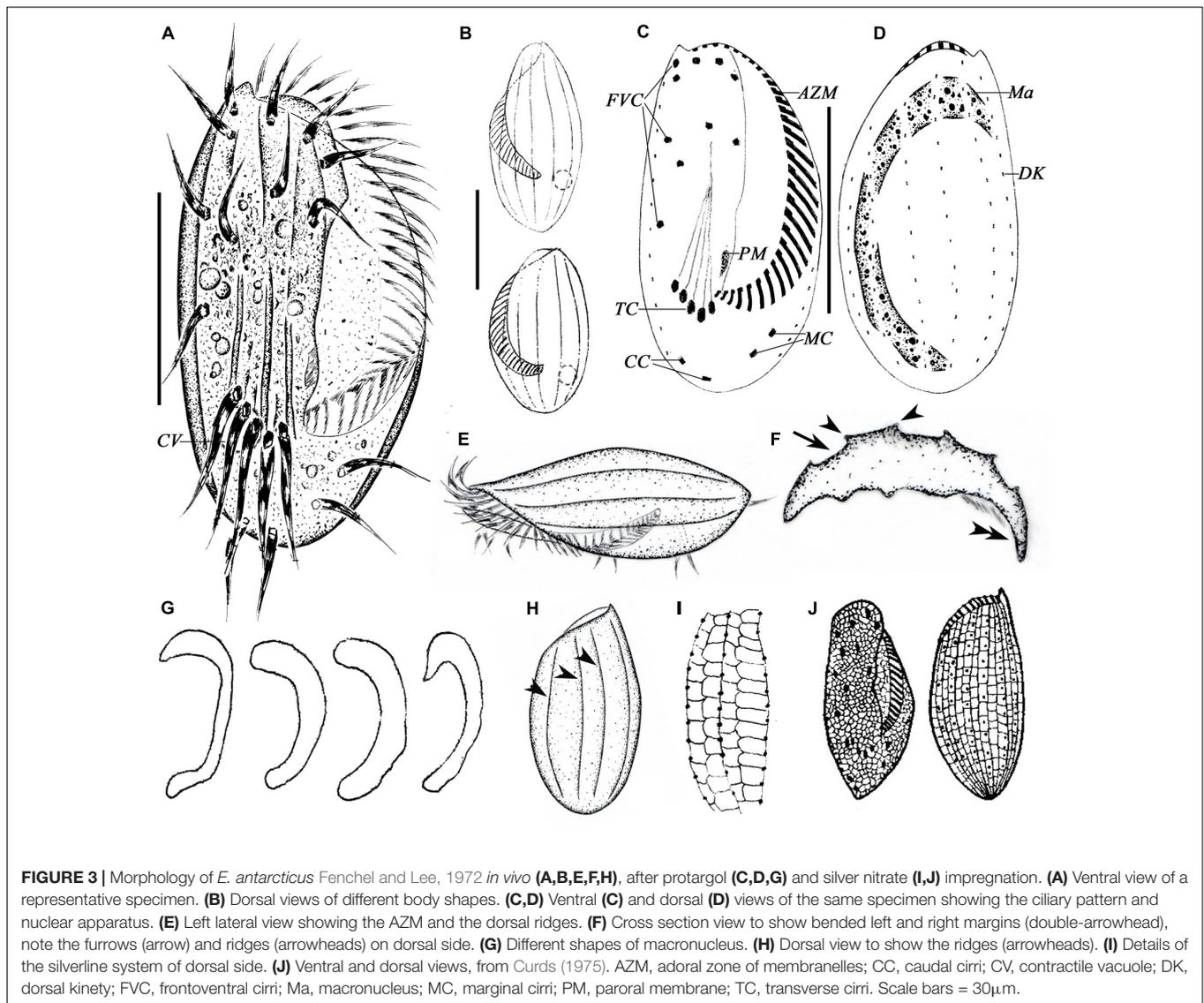
(Figures 3C, 4P). Eight dorsal kineties with the leftmost and rightmost one usually positioned on ventral side. Most dorsal kineties extending over the entire length of the cell, except for the leftmost one which is shortest and positioned below the proximal end of adoral membranelles. The mid-kinety kinety with 13–17 dikinetids, while the rightmost one with only five or six dikinetids (Figures 3D, 4M). The dorsal silverline system double-patella type while in groove area the polygons slightly shrunk due to the inclination of ridges and seem to be double-eurystoma type (Figures 3I, 4O).

### Phylogenetic Position of *E. neapolitanus* and *E. antarcticus* Based on SSU rRNA Gene Sequence Data

In phylogeny trees, *E. neapolitanus* clusters with another population from Korea, which, however, was weakly supported (53ML/0.76BI), and then forms a clade with *E. harpa* and *E. platystoma* (Figure 5). *E. antarcticus* groups together with *E. trisulcatus* with high support value (1.00BI/100ML), which then form a sister branch with the clade consisting of *E. euryhalinus*, *E. sp. 6* and *E. magnicirratu*s (Figure 5).

### Geographic Distribution of the *Euplotes* Species in Coast of Southern China

Totally 15 *Euplotes* species have been found and reported with taxonomy description in seven sites of coast of southern China (Figure 6 and Table 2). Among these species, *E. charon* has a most extensive distribution and was detected in three sites, followed by *E. balteatus*, *E. encysticus*, *E. parawoodruffi*, *E. platystoma*, *E. rarisseta*, and *E. vannus* which were detected in two sites



**FIGURE 3** | Morphology of *E. antarcticus* Fenchel and Lee, 1972 *in vivo* (A,B,E,F,H), after protargol (C,D,G) and silver nitrate (I,J) impregnation. (A) Ventral view of a representative specimen. (B) Dorsal views of different body shapes. (C,D) Ventral (C) and dorsal (D) views of the same specimen showing the ciliary pattern and nuclear apparatus. (E) Left lateral view showing the AZM and the dorsal ridges. (F) Cross section view to show bended left and right margins (double-arrowhead), note the furrows (arrow) and ridges (arrowheads) on dorsal side. (G) Different shapes of macronucleus. (H) Dorsal view to show the ridges (arrowheads). (I) Details of the silverline system of dorsal side. (J) Ventral and dorsal views, from Curds (1975). AZM, adoral zone of membranelles; CC, caudal cirri; CV, contractile vacuole; DK, dorsal kinety; FVC, frontoventral cirri; Ma, macronucleus; MC, marginal cirri; PM, paroral membrane; TC, transverse cirri. Scale bars = 30  $\mu\text{m}$ .

(Figure 7). The rest eight species were only found in one site. The species richness of *Euplotes* is variable among these sites. The highest species richness (13 species) was occurred in Huizhou and the lowest one was occurred in Donghai Island, Zhuhai and Shenzhen with only one species collected there (Figure 7). In addition, regarding the distributions among the six habitat types, the *Euplotes* has highest species number in both mangrove and offshore waters and lowest one in estuary habitat (Figure 7).

## DISCUSSION

### Comparison of *Euplotes neapolitanus* With Related Congeners

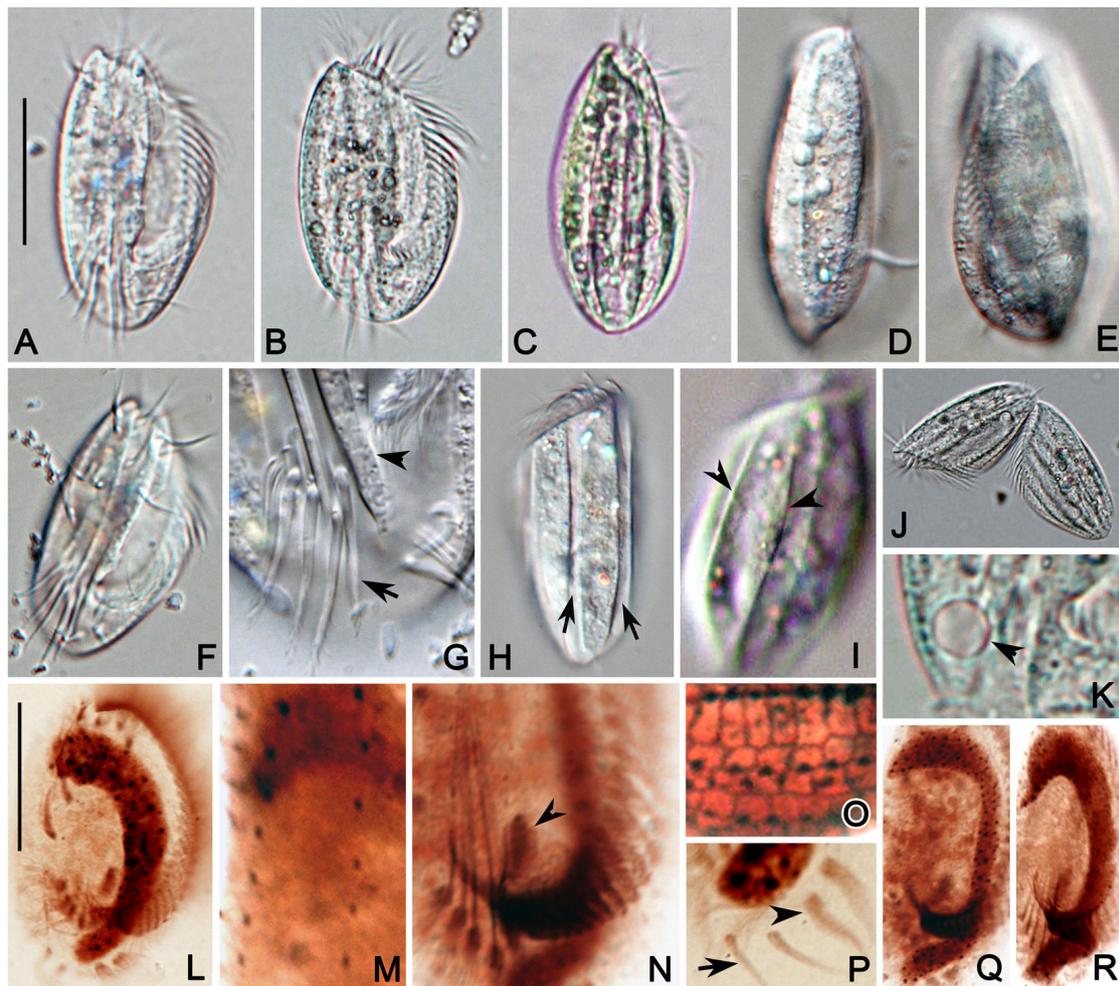
*Euplotes neapolitanus* was first found in Bay of Naples in Mediterranean and described briefly by Wichterman (1964). There was no other record or redescription of this species until present work. The organism we collected corresponds

well with the original description referring to the large cell size, ellipsoidal body shape with wider and truncated anterior end, conspicuous buccal field with adoral zone of membranelles abruptly curved in anterior end, as well as the basic infraciliature.

In terms of the large size (more than 100  $\mu\text{m}$ ), the double-eurystomus type silverline system, 10 frontoventral, two caudal and two marginal cirri, *E. neapolitanus* is similar with five congeners: *E. platystoma* Dragesco and Dragesco-Kernéis, 1986, *E. harpa* Stein, 1859, *E. shanghaiensis* Song et al., 1998, *E. focardii* Valbonesi and Luporini, 1990.

Besides with above aspects, *E. harpa* is similar to *E. neapolitanus* in having dorsal ridges. However, *E. harpa* differs from *E. neapolitanus* by the larger body size (150–160 vs. 130–150  $\mu\text{m}$ ), more dorsal kineties (13 vs. 11), and more dikinetids in mid-kinety rows (40–45 vs. 17–23) (Kahl, 1932).

Although most of the morphometric data of *E. platystoma* overlap with *E. neapolitanus*, *E. neapolitanus* can be separated



**FIGURE 4** | Photomicrographs of *E. antarcticus* *in vivo* (A–K), and after protargol (L–N, P–R) and silver nitrate (O) impregnation. (A) A representative specimen. (B, C) Cells of different shapes. (D, E) Right (D) and left (E) views showing the slightly convex dorsal side. (F) Ventral view showing the cirri. (G) Detail of posterior portion of ventral side to show the transverse cirri (arrow) and ridges (arrowhead). (H, I) Dorsal views to show the grooves (arrow) formed by inclined ridges (arrowhead). (J) A late divider. (K) Detail of contractile vacuole (arrowhead). (L) Ventral view of infraciliature. (M) Detail of dorsal kineties. (N) Center portion of ventral side to show the transverse cirri, paroral membrane (arrowhead) and posterior end of AZM. (O) Detail of dorsal view of silverline system. (P) Posterior portion of ventral side showing the marginal (arrowhead) and caudal (arrow) cirri. (Q, R) Macronucleus of different shapes. Scale bars = 30  $\mu\text{m}$ .

from former by the truncated anterior end of cell (vs. rounded), the anterior adoral zone abruptly curved to dorsal side (vs. evenly curved), and the present of dorsal ridges (vs. absent) (Yan et al., 2018).

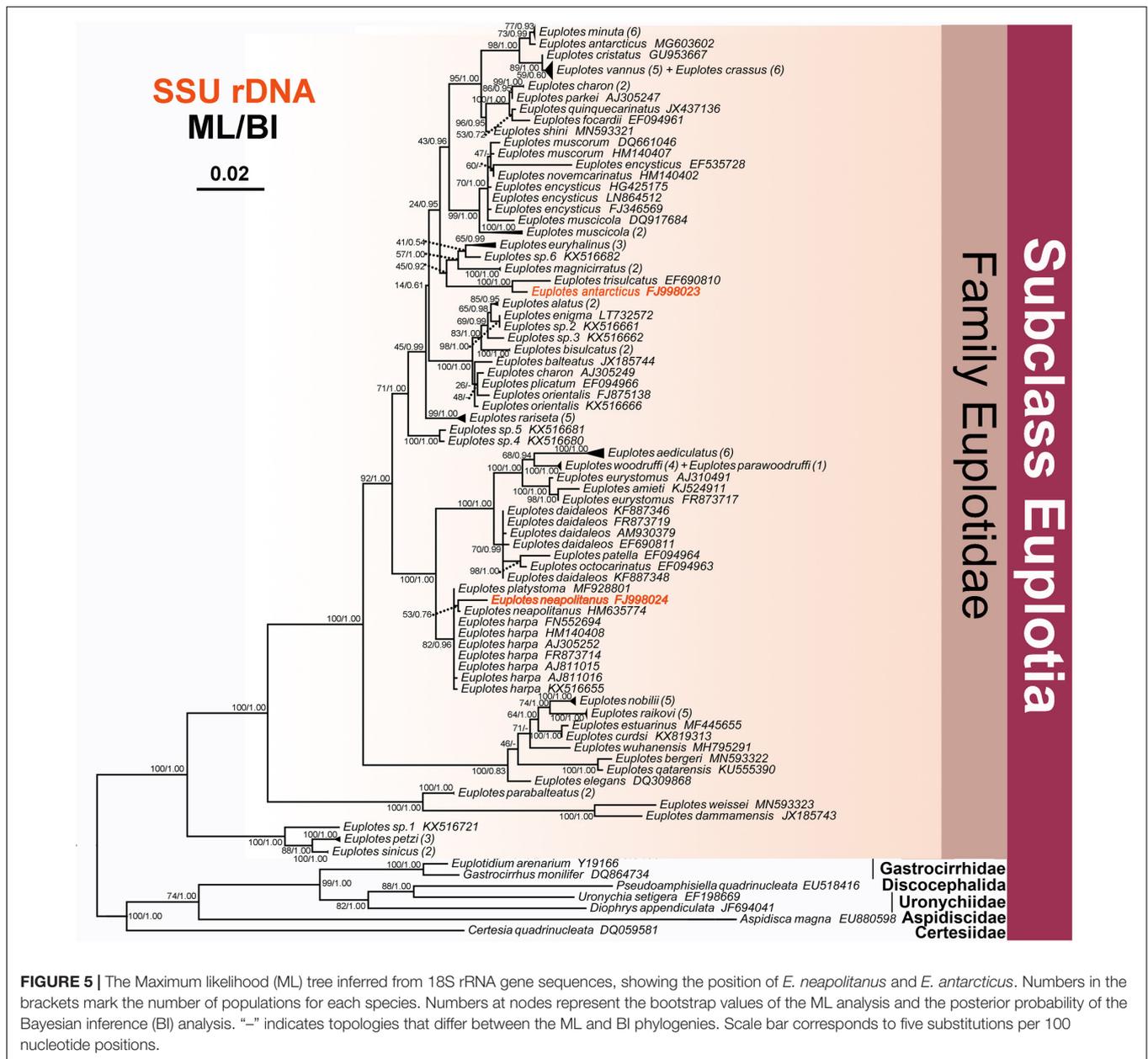
*Euplotes shanghaiensis* can be distinguished from *E. neapolitanus* by the smaller body size (80–120  $\times$  30–35 vs. 130–150  $\times$  70–75  $\mu\text{m}$  *in vivo*), fewer adoral membranelles (53–58 vs. 62–73), more dorsal kineties (12–13 vs. 11), and freshwater habitat (vs. marine) (Song et al., 1998).

*Euplotes focardii* differs from *E. neapolitanus* by the smaller body size (38–110  $\times$  30–92 vs. 130–150  $\times$  70–75  $\mu\text{m}$  *in vivo*), fewer dorsal kineties (10 vs. 11), and the absent of dorsal ridges (vs. present) (Valbonesi and Luporini, 1990).

Beside, *E. damammensis* Chen et al., 2013 is also a large *Euplotes* with 10 frontoventral, two caudal and two marginal cirri

as well as 11 dorsal kineties, although its silverline system was not revealed yet. It can be separated from *E. neapolitanus* by fewer adoral membranelles (44–51 vs. 62–73), and the shape of the macronucleus (sigmoidal vs. C-shaped) (Chen et al., 2013).

In phylogeny trees, *E. neapolitanus* clusters with *E. harpa* and *E. platystoma*, which agrees with their high morphologic similarities. There is another available SSU rRNA gene sequence under the species name *E. neapolitanus* [Korean isolation; GenBank accession number: HM635774; submitted by Khan et al., unpublished] in the GenBank, which, however, display a significant difference with our population in 55 nucleotides and 5% dissimilarity but a high similarity to *E. platystoma* with only a difference of four nucleotides. Considering the sufficient justification for identification of our population by morphology, the SSU rRNA gene sequence under the name *E.*

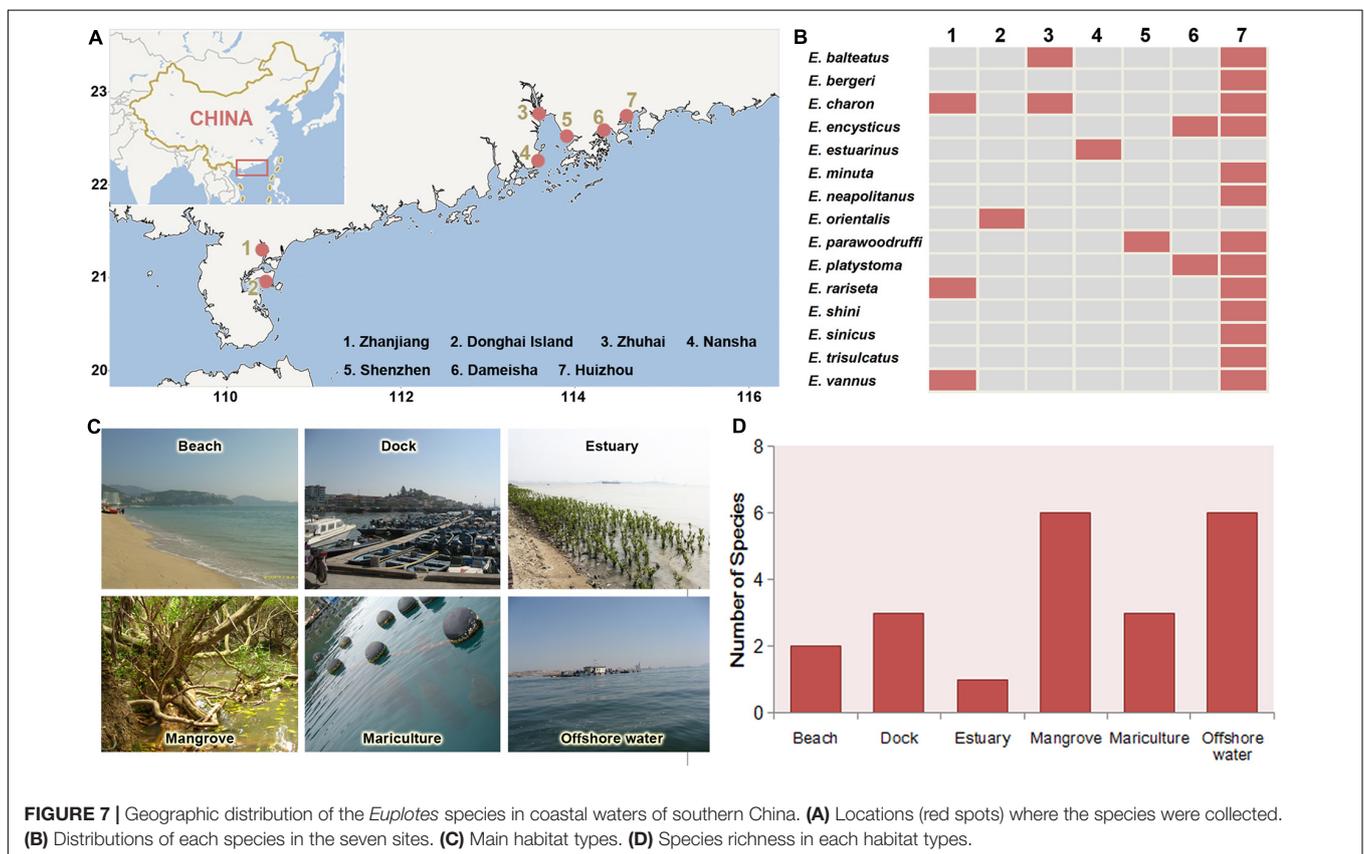
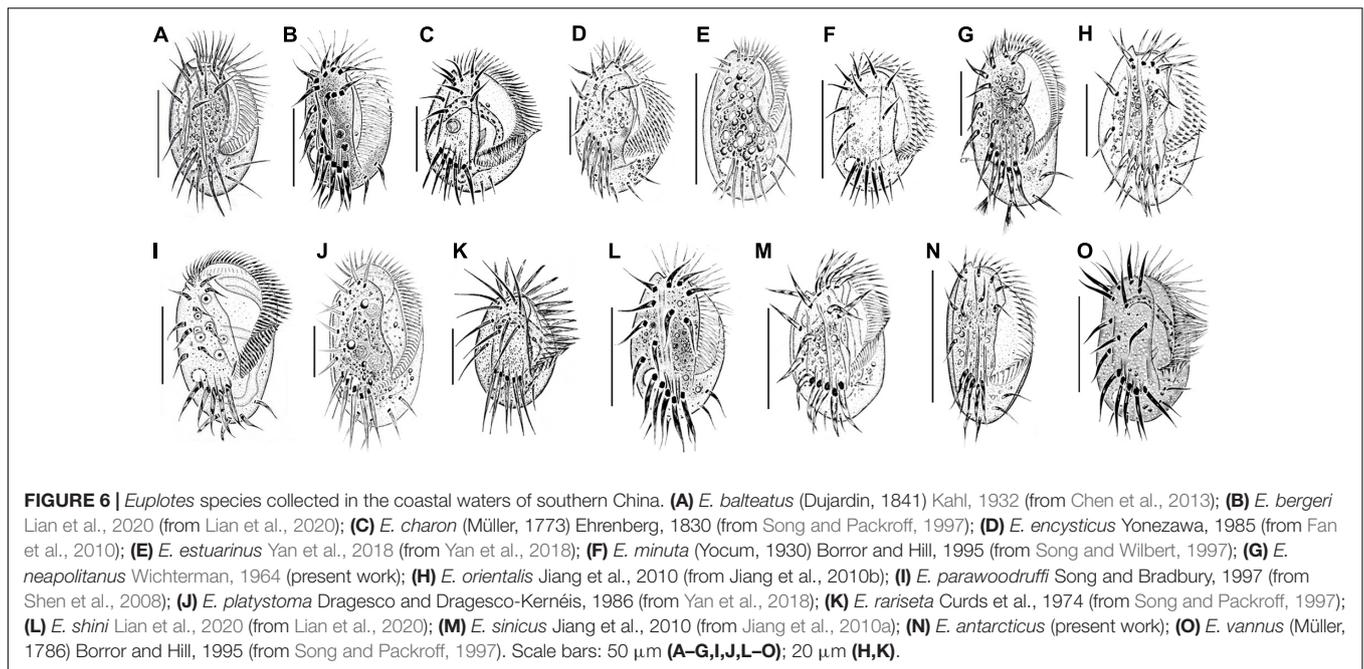


*neapolitanus* should be undoubtedly linked to our population. Any conclusion about the taxonomy identification of the Korean isolations shouldn't be made until its morphological data are available.

### Comparison of *Euplotes antarcticus* With Related Congeners

*Euplotes antarcticus* was initially reported by Fenchel and Lee (1972) with some brief description by emphasizing its oblong body outline. The specimens studied here corresponds well with the original reports regarding its body shape, six dorsal ridges and basic infraciliature such as the numbers of adoral membranelles, ventral cirri, and especially the eight

dorsal kineties of which six were on the dorsal surface. Some slight differences could be observed between these two populations, in terms of the cell size *in vivo* (50–70 × 25–35 μm in our specimens vs. 85–90 × 30–35 μm), and the sampling location (subtropical water in our specimens vs. Antarctica). In addition, the dorsal furrows observed in our specimens were not mentioned in previous descriptions, which was probably because that the dorsal ridges didn't incline to forming the obvious groove in original population and suggest that the furrows are population-dependent characteristics for this species. Curds (1975) reviewed the original study and supplied some detailed characteristics such as rectangular outline with pointed posterior end, and a cleft presented in the right peristomial margin, which were not prominent or



lacked in our specimens. Considering that these supplementary characters were obtained based on the observation on the original figures, those may be over-interpreted and cannot represent the general characters for this species. Moreover, the

dargyrome also displayed dissimilarity between them (double-patella type in our specimens vs. double-eurystomus type in Curds's description). Given that the inclined ridges were observed *in vivo* in our specimens, the size of each argyrome grid close to

TABLE 2 | *Euplotes* species collected in the coastal waters of southern China and their morphological comparison.

Species	Body size (μm)	No. adoral membranelles	No. dorsal kinety	Silverline system	Location	Habitat	References
<i>E. balteatus</i>	70–100 × 50–75	31–39	7	Double-eurystomus	Huizhou, Zhuhai	Mangrove, Mariculture	Hu et al., 2019
<i>E. bergeri</i>	70–80 × 45–50	43–57	12–14	Double-eurystomus	Huizhou	Offshore water	Lian et al., 2020
<i>E. charon</i>	70–100 × 65–90	51–60	9–10	Double-eurystomus	Huizhou, Zhanjiang, Zhuhai	Mangrove, Mariculture	Hu et al., 2019
<i>E. encysticus</i>	80–90 × 50–65	50–80	7	Complex	Huizhou, Dameisha	Beach	Fan et al., 2010; Hu et al., 2019
<i>E. estuarius</i>	50–75 × 30–50	25–33	7	Double-eurystomus	Nansha	Estuary	Yan et al., 2018
<i>E. minuta</i>	50–70 × 40–55	33–41	7–9	Single-vannus	Huizhou	Offshore water	Hu et al., 2019
<i>E. neapolitanus</i>	120–150 × 70–80	62–73	11	Double-eurystomus	Huizhou	Dock	Present work
<i>E. orientalis</i>	35–45 × 20–30	18–25	6–7	Double-patella	Donghai Island	Mariculture	Hu et al., 2019
<i>E. parawoodruffi</i>	90–140 × 60–90	60–70	9	Double-eurystomus	Shenzhen, Huizhou	Mangrove, Offshore water	Shen et al., 2008; Hu et al., 2019
<i>E. platystoma</i>	100–150 × 50–80	46–54	10–11	Double-eurystomus	Huizhou, Dameisha	Beach, Mangrove	Lian et al., 2018; Yan et al., 2018
<i>E. rarisseta</i>	30–50 × 20–40	17–22	7	Double-patella	Zhanjiang, Huizhou	Mangrove, Offshore water	Hu et al., 2019
<i>E. sinicus</i>	60–95 × 35–65	38–46	7	Double-patella	Huizhou	Offshore water	Hu et al., 2019
<i>E. shini</i>	65–75 × 35–45	37–46	9	Single-vannus	Huizhou	Offshore water	Lian et al., 2020
<i>E. antarcticus</i>	50–70 × 25–35	26–30	8	Double-patella	Huizhou	Dock	Present work
<i>E. vannus</i>	90–140	60	9–10	Single-vannus	Huizhou, Zhanjiang	Mangrove, Dock	Hu et al., 2019

the ridge would be shrunk in the appearance in this case and thus double-patella might visually shift to double-eurystomus. Similar result was proposed in the study of Foissner et al. (1991).

Petz et al. (1995) described a *Euplotes* species under the name of *E. antarcticus*. However, it is clearly different from the original description *E. antarcticus* in terms of the cell size *in vivo* (90–145 × 30–80 vs. 85–90 × 30–35 μm), the numbers of adoral membranelles (38–70 vs. about 30 in original description), dorsal kineties (9–14 vs. 8 in original description), dorsal ridges (7–10 vs. 6 in original description), and caudal cirri (three or two vs. two in original description). Therefore, it was probably a misidentification.

Several congeners with elongated ellipsoidal body shape and double-type silverline system should be compared with *E. antarcticus*, namely *E. affinis* (Dujardin, 1841) Kahl, 1932, *E. trisulcatus* Kahl, 1932, *E. dogieli* Agamaliyev, 1967, *E. poljanskyi* Agamaliyev, 1966 and *E. zenkewitchi* Burkovsky, 1970.

*Euplotes trisulcatus* is most similar to *E. antarcticus* in the body shape and having dorsal ridges and furrows. However, *E. antarcticus* has a larger body size (50–90 × 25–35 vs. 35–50 × 25–40 μm *in vivo*), more dorsal kineties (eight vs. seven), and more dikinetids in the middle row (13–17 vs. 11) (Tuffrau, 1960; Carter, 1972). Therefore, it can be clearly separated from *E. trisulcatus*.

Both *E. dogieli* and *E. poljanskyi* can be easily distinguished from *E. antarcticus* by the absent of dorsal ridges (vs. present), the number of adoral membranelles (35–38 in *E. dogieli* and 36–40 in *E. poljanskyi* vs. 26–30 in *E. antarcticus*), dorsal kineties (seven in *E. dogieli* and *E. poljanskyi* vs. eight in *E. antarcticus*) frontoventral (nine in *E. dogieli* and eight in *E. poljanskyi* vs. 10 in *E. antarcticus*) and marginal cirri (one in *E. dogieli* and *E. poljanskyi* vs. two in *E. antarcticus*) (Agamaliyev, 1966, 1967).

*Euplotes zenkewitchi* differs from *E. antarcticus* in having more adoral membranelles (50–55 vs. 26–30), more dorsal kineties (ten vs. eight), fewer frontoventral cirri (nine vs. ten), and distinctively curved macronucleus (vs. slightly curved with angular posterior end) (Burkovsky, 1970).

*Euplotes affinis* is a small organism and resembles *E. antarcticus* with prominent dorsal ridge. It differs from the latter, however, by the feature of freshwater habitat (vs. marine), 3-shaped macronucleus (vs. C-shaped), having fewer adoral membranelles (18–20 vs. 26–30), and fewer frontoventral cirri (nine vs. ten) (Kahl, 1932).

Besides, *E. aberrans* Dragesco, 1960 is also similar to *E. antarcticus* in the body shape although its silverline system was not revealed yet. It can be separated from *E. antarcticus* by the feature of macronucleus shape (horseshoe shaped with ends almost meet one another vs. obviously opened C-shape), having more adoral membranelles (about 50 vs. 26–30), fewer dorsal ridges (four vs. six) and fewer frontoventral cirri (eight vs. ten) (Dragesco, 1960).

In previous phylogeny studies, the gene sequences of our population of *E. antarcticus* have been published under the name *E. cf. antarcticus*. There is another available SSU rRNA gene sequence under the species name *E. antarcticus* [Korean isolation; accession number: MG603602; submitted by Park et al. (2019)]

in the GenBank, which, however, display a significant difference with our population in 94 nucleotides and 5.2% dissimilarity. Considering the sufficient justification for identification of our population by morphology, we suggest to link the SSU rRNA gene sequence of *E. antarcticus* with our population. Detailed morphological description of the Korean specimen (EF690810) is necessary to reconsider the identification. In phylogeny trees, our population of *E. antarcticus* clusters with *E. trisulcatus*, which is consistent with their high morphologic similarities.

## Remarks on the Distribution of Genus *Euplotes*

According to the compilation of faunal data, 15 *Euplotes* species have been found in southern China coast, which is significantly more than eight species reported in northern China coast such as Bohai and Yellow seas (Song et al., 2009). Moreover, six species, i.e., *E. vannus*, *E. charon*, *E. minuta*, *E. rarisseta*, *E. sinicus*, *E. parawoodruffi* were found in both southern and northern China coasts (Song et al., 2009), suggesting their widely distribution in China.

Our results revealed that the highest species richness was occurred in Huizhou, which probably attributes to the diverse habitats there being suitable for different species (Table 2). In addition, mangrove wetlands possess the highest species richness of *Euplotes* species among the habitats in coast of southern China. The high diversity in mangrove has also been reported for other ciliate groups such as oligotrichs and stichotrichs (Zhang et al., 2018; Liang et al., 2019; Liu et al., 2019; Song et al., 2019), which suggest mangrove is an ideal habitat for ciliates. There are several reasons supporting the large ciliate species diversity in mangrove (Langenheder et al., 2010). First, the water in mangrove is normally eutrophic with high productivity due

to the litter decomposition of plants, which supplies sufficient food source for ciliates. Second, the intricate root network of mangrove plant can dissipate the waves and conserve water and soil, which produces a stable habitat for ciliates especially for periphytons. Moreover, the dominant plants and terrain features of the mangroves are spatially varied, which leads to extensive environmental difference among the mangroves and thus supplies a wide range of niches for ciliates.

## DATA AVAILABILITY STATEMENT

All data generated or used during the study appear in the article.

## AUTHOR CONTRIBUTIONS

WL and XL conceived the research. WL wrote the manuscript. JJ and YT critically reviewed the findings and improved the manuscript. All authors contributed to the article and approved the submitted version.

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**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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