

Morphology and Ontogenesis of a Marine Ciliate, *Euplotes balteatus* (Dujardin, 1841) Kahl, 1932 (Ciliophora, Euplotida) and Definition of *Euplotes wilberti* nov. spec.

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Summary. *Euplotes balteatus* (Dujardin, 1841) Kahl 1932, collected from coastal waters near Qingdao, northern China, was investigated using live observation and silver staining methods. An improved diagnosis and morphometric data are provided. *Euplotes balteatus* can be identified by the following combination of characters: 10 frontoventral cirri, 2 widely separated marginal cirri, 2 fine caudal cirri, 8 dorsal kineties and a double-*eurystomus* type silverline system. Its morphogenesis, which is similar to that of several congeners, can be summarized as follows: (1) the opisthe's oral primordium appears *de novo* under the pellicle whereas the old oral apparatus is retained by the proter; (2) two groups of frontoventral transverse cirral anlagen, each with five streaks, occur *de novo* and then develop into the frontoventral and transverse cirri separately according to the formula of "3:3:3:3:2" from left to right; (3) the anlagen for the marginal cirri occur *de novo* near the parental oral apparatus; (4) migratory cirri of both dividers derive from the anlagen near the paroral membrane; (5) the dorsal kinety anlagen come from dedifferentiation of the parental structures in the mid-body region; (6) caudal cirri are formed at the posterior ends of the two rightmost dorsal kineties. In the light of the present findings, it was concluded that the Antarctic population of *E. balteatus* reported by Song and Wilbert (2002) was misidentified. A new species, *Euplotes wilberti* nov. spec., is established for this population.

Key words: *Euplotes balteatus*, *Euplotes wilberti* nov. spec., marine ciliate, morphology, ontogenesis.

INTRODUCTION

Species of *Euplotes* have been the subject of numerous morphological, ecological, biochemical and toxico-

logical studies since the genus was established in 1830 by Ehrenberg (e.g. Curds 1975, Wang and Song 1995, Valbonesi *et al.* 1997, Ma *et al.* 2008, Wilbert and Song 2008, Xu *et al.* 2010, Jiang *et al.* 2010a). To date, over 80 species and varieties of this genus have been established based mainly on morphological and morphogenetic characters (Curds 1975; Song and Wilbert 2002; Alekperov *et al.* 2006; Wilbert and Song 2008; Jiang *et al.* 2010a, b; Shao *et al.* 2010).

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Euplotes balteatus was first reported by Dujardin in 1841 with a simple description under the name *Plasconia balteata*. Kahl (1932) supplied an improved description of its morphology *in vivo*. Its silverline system was first described by Tuffrau (1964) following an investigation of silver-impregnated specimens. An Antarctic population was reported by Song and Wilbert (2002), although its identity as *E. balteatus* is doubtful. Details of morphogenesis in *E. balteatus* remain unknown.

During a faunistic survey of ciliates in coastal waters of northern China, a population of *E. balteatus* was isolated giving the opportunity to study its morphology in detail and to document its morphogenetic processes.

MATERIALS AND METHODS

Euplotes balteatus was collected on 18 September 2007 from a seawater pond once used for cultivating abalone near Qingdao (Tsingtao, 36°18'N, 120°43'E), northern China, when the water temperature was about 20°C and the salinity 31‰. Cells were observed *in vivo* using bright field and Nomarski differential interference contrast microscopy (Song *et al.* 2009). The infraciliature and silverline system were revealed with the protargol (Wilbert 1975) and the Chatton-Lwoff (Jiang *et al.* 2010a, b) methods respectively. Counts and measurements were performed at magnifications of $\times 100$ to $\times 1250$.

Uniprotistan cultures were established in filtered seawater with a few rice grains to enrich bacteria as a food resource. After two days, when the cultures were growing fast, cells at different divisional stages were picked out for preparation. Drawings of impregnated specimens were made with the help of a camera lucida under $\times 1250$ magnification. To display the changes during morphogene-

sis, parental cirri are shown in outline whereas new ones are shaded black. Systematics and terminology are mainly according to Curds (1975) and Shao *et al.* (2010).

RESULTS

Eupotes balteatus (Dujardin, 1841) Kahl, 1932 (Figs 1A–P, 2A–F; Table 1)

Improved diagnosis: Small to medium-sized marine *Euplotes*, 30–80 $\mu\text{m} \times 20$ –60 μm *in vivo*; buccal field about 2/3 of cell length, with about 35 adoral membranelles; constantly 10 frontoventral, five transverse, two caudal and two widely separated left marginal cirri; eight dorsal kineties with about 15 dikinetids in mid-dorsal rows. Dorsal silverline system double-*eurystomus* type.

Morphological description of Qingdao population: Body size highly variable, usually 30–80 $\mu\text{m} \times 20$ –60 μm *in vivo* (Table 1; Fig. 1A–D, L). Body generally broadly oval in outline, sometimes with a narrow anterior end. Both margins smooth except for a small projection at the right anterior end. Dorsoventrally highly flattened, ventral side somewhat concave, dorsal side convex, without distinct ridges or grooves (Fig. 1N). Each dorsal cilium surrounded by 7 to 9 ellipsoid granules forming a rosette beneath the pellicle (Fig. 1F, M). Cytoplasm colorless, usually with numerous crystals and food vacuoles of different sizes making cell appear opaque or dark gray *in vivo* (Fig. 1A–C, L). Macronucleus generally C-shaped, somewhat variable

Table 1. Morphological characterization of *Euplotes balteatus*. Data are based on protargol impregnated specimens. All measurements in μm . Abbreviations: CV – coefficient of variation in %, Max – maximum, Mean – arithmetic mean, Median – median value, Min – minimum, SD – standard deviation, SE – standard error.

Character	Min	Max	Mean	Median	SD	CV	SE	n
Length of body	52	80	64.2	64	13.3	13.7	2.8	25
Width of body	37	64	49.0	48	6.9	14.0	1.4	25
Length of adoral zone	36	50	42.9	42	3.6	8.3	0.7	25
Number of adoral membranelles	28	43	34.8	34	4.5	12.8	0.9	25
Number of frontoventral cirri	10	10	10.0	10.0	0.0	0.0	0.0	25
Number of left marginal cirri	2	2	2.0	2.0	0.0	0.0	0.0	25
Number of transverse cirri	5	5	5.0	5.0	0.0	0.0	0.0	25
Number of caudal cirri	2	2	2.0	2.0	0.0	0.0	0.0	25
Number of dorsal kineties	8	8	8.0	8.0	0.0	0.0	0.0	25
Number of dikinetids in mid-dorsal rows	12	16	15.0	14.4	12.0	8.1	0.2	25

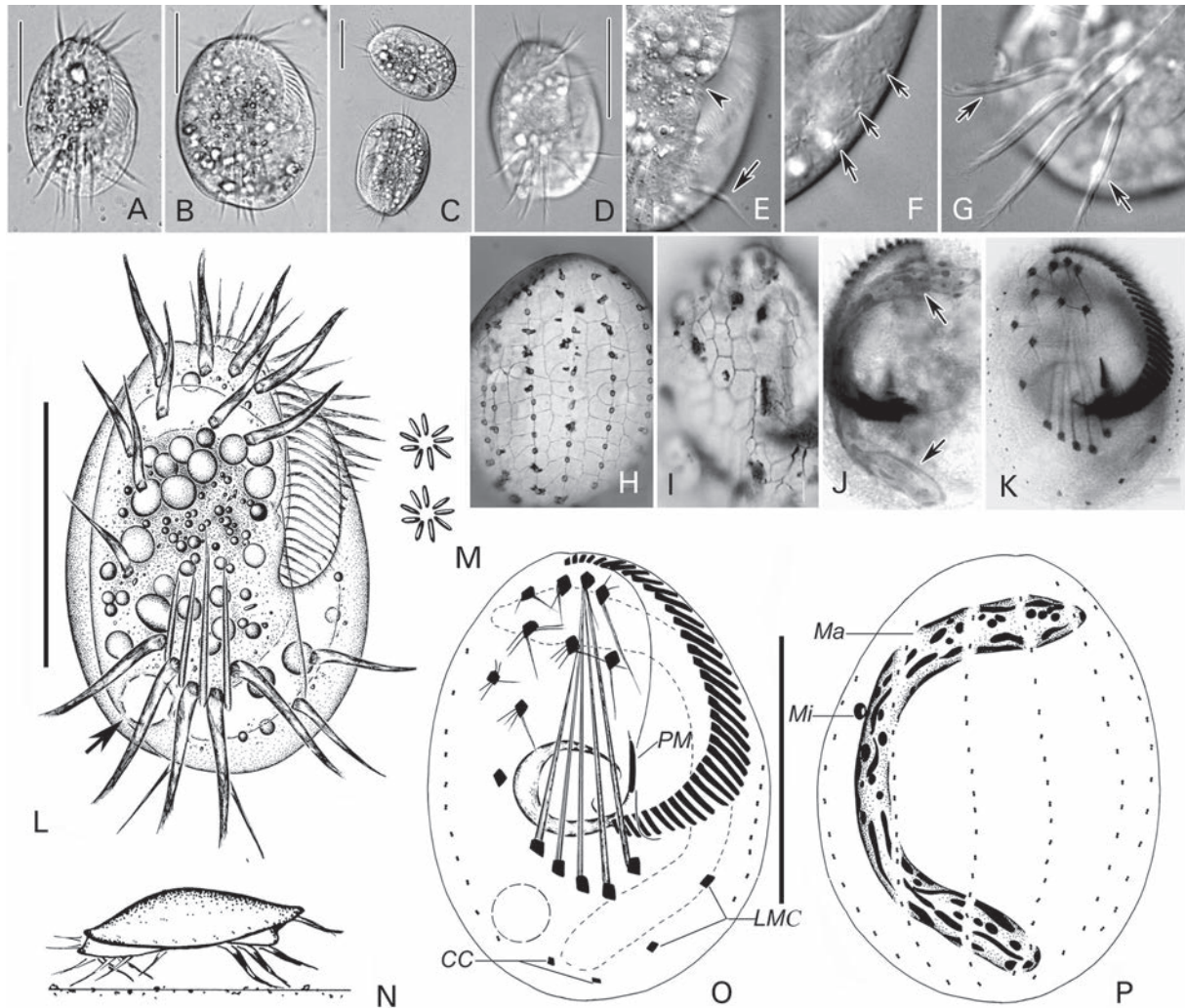


Fig. 1A–P. *Euplotes balteatus* from life (A–G, L–N), after protargol (J, K, O, P) and silver nitrate (H, I) impregnation. **A** – ventral view of a typical individual; **B** – ventral view of a well-fed specimen; **C** – dorsal views showing general body shape; **D** – ventral view to demonstrate the arrangement of cirri; **E, F** – details of cell (ventral view), arrow and arrowhead in **E** indicate the left marginal cirri and paroral membrane respectively; arrows in **F** mark the dorsal cilia; **G** – arrows denote the transverse cirri; **H, I** – silverline system on dorsal and ventral sides respectively; **J** – macronucleus (arrows); **K** – infraciliature on ventral side; **L** – ventral view of a representative individual, arrow indicates the contractile vacuole; **M** – sub-pellicular rod-like structures around dorsal cilia; **N** – lateral view, to show the flattened body shape; **O, P** – ventral and dorsal views, to show the infraciliature and nuclear apparatus. CC – caudal cirri, LMC – left marginal cirri, Ma – macronucleus, Mi – micronucleus, PM – paroral membrane. Scale bar: 40 μ m.

among individuals (Fig. 1J, P). Micronucleus oval, usually attached to mid-region of macronucleus (Fig. 1P).

Adoral zone approximately 55–85% of the body length, composed of 28–43 membranelles, the bases of which are up to 8–10 μ m in length. Paroral membrane thin and long, lying parallel to main body axis and to the right of the posterior portion of the adoral zone of membranelles (AZM). Contractile vacuole about 8 μ m

in diameter, located posteriorly near the right body margin (Fig. 1C, L).

Locomotion typically by moderately fast crawling or incessant jerking on substrate. Most active when near dense growths of bacteria.

Cirri on the ventral side relatively fine with cilia about 14 μ m long *in vivo* (Fig. 1A, D, L). Constantly ten frontoventral cirri (FVC), among which cirrus VI/2

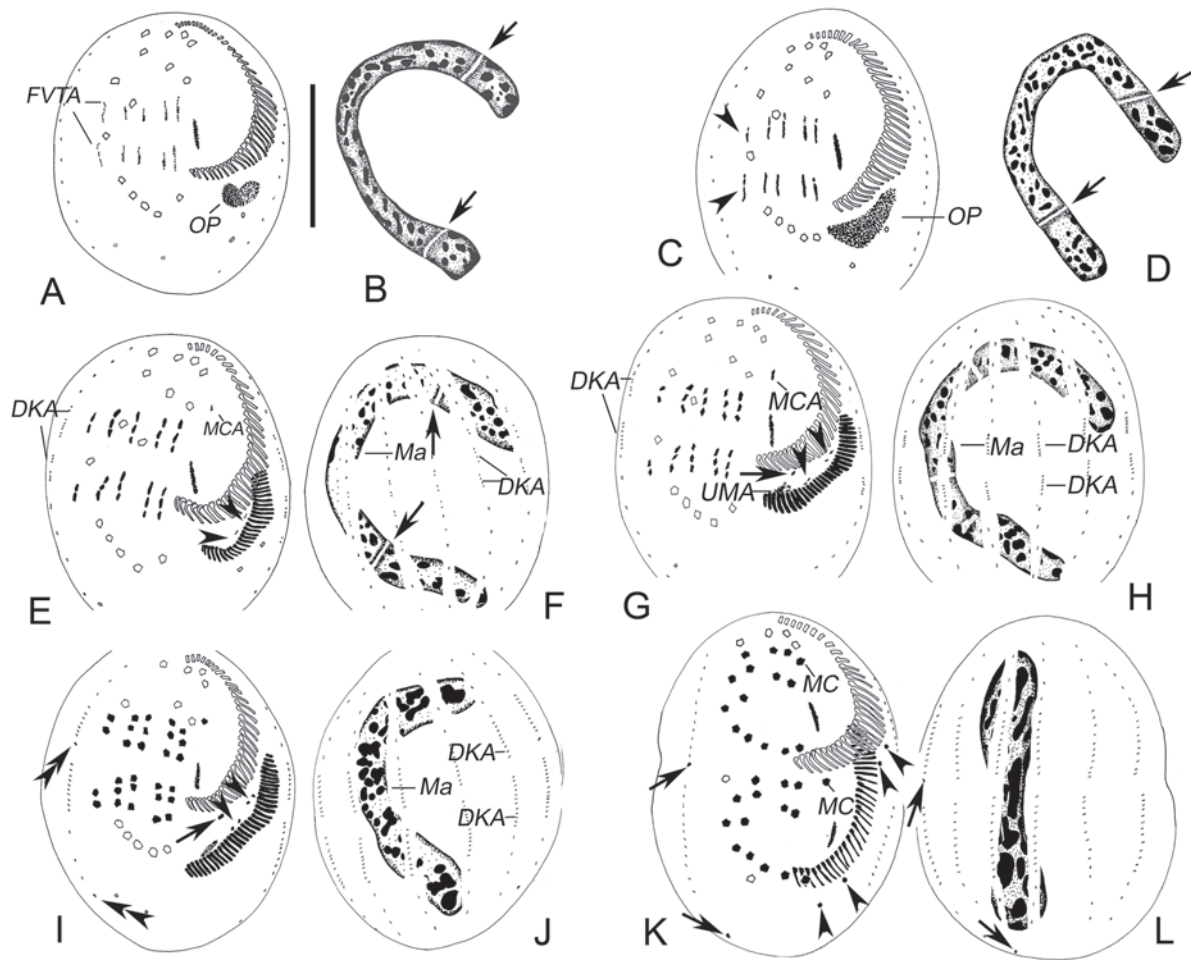


Fig. 2A–L. Morphogenesis of *Euplotes balteatus* after protargol impregnation. **A, B** – ventral view and macronucleus of the same specimen at an early stage to show the appearance of frontoventral transverse cirral anlagen, the developing oral primordium and macronuclear replication bands (arrows); **C, D** – slightly later divider, ventral view and macronucleus of the same specimen, indicating 5 FVT-cirral streaks (arrowheads) and the enlarging oral primordium; arrows point to the replication bands; **E, F** – ventral and dorsal views of the same specimen to show the marginal cirral anlagen (arrowheads), cirrus I/1 anlage and the newly formed dorsal kinety anlagen (DKA, macronuclear replication bands (arrows)); **G, H** – ventral and dorsal views of the same specimen, showing the fragmentation of the cirral anlagen, the formation of cirrus I/1 anlage for the opisthe (arrow), the UM-anlage for the opisthe and the development of the anlagen both of the marginal cirri (arrowheads) and of the dorsal kineties; **I, J** – ventral and dorsal views of the same middle-stage divider to show the completion of the cirral development, the differentiation of the caudal cirri (double arrowheads), the development of cirrus I/1 for the opisthe (arrow) and the marginal cirri (arrowheads); **K, L** – ventral and dorsal views of a late divider, showing the migration of the cirri, the development of the dorsal kineties and the dividing macronucleus. Arrowheads indicate the marginal cirri and arrows point to the caudal cirri. FVTA – frontoventral-transverse anlagen, Ma – macronucleus, DKA – dorsal kinety anlagen, MCA – migratory cirral anlagen, UMA – undulating membrane anlagen, MC – migratory cirri, OP – oral primordium. Scale bar: 30 μ m.

is nearer to cirrus V/2 than to cirrus V/3, the other seven frontoventral cirri located in frontal area of body (Fig. 1K, O). Constantly five strong transverse cirri, cilia of which are about 30 μ m long; cirrus VI/1 is located slightly higher than cirrus II/1 (Fig. 1K, O). Two fine, widely separated marginal cirri. Anterior marginal cir-

rus and leftmost transverse cirrus located at about the same level (Fig. 1K, O). Two fine caudal cirri (Fig. 1O). Invariably eight dorsal kineties that extend entire length of body. First and last dorsal kineties usually positioned near the edge on the ventral side, the middle ones consist of 12–16 dikinetids (Fig. 1O, P).

Dorsal silverline system irregular double-*eurys-tomus* type, i.e. distances from the silverline to both neighboring ciliary rows about equal (Figs 1H, 4F) with moderately dense silverline grids on ventral side (Figs 1I, 4E).

Morphogenesis

Stomatogenesis: Stomatogenesis begins with the emergence of the opisthe's oral primordium which develops independently as a group of anarchic kinetosomes formed beneath the cortex behind the parental oral zone (Figs 2A, 3A). The oral primordium then enlarges and the anterior part starts aggregating to form the membranelles. As the new membranelles are progressively formed in a posterior direction, the whole

structure elongates, eventually becoming the AZM for the opisthe (Figs 2K, 3O). At the same time a second anlage forms to the right of the oral primordium. This anlage enlarges, extends to form a streak, and subsequently develops into the paroral membrane for the opisthe (Fig. 2G, I, K). The old oral apparatus remains intact for the proter.

Development of the frontoventral transverse cirral anlagen: At about the same time of the appearance of the oral primordium in the opisthe, five short thread-like frontoventral transverse cirral anlagen with loosely arranged basal bodies occur *de novo* among the parental cirri near the cell surface in each divider (Figs 2A, 3A). Later, these streaks gradually elongate and broaden, then split into several fragments in the mid-

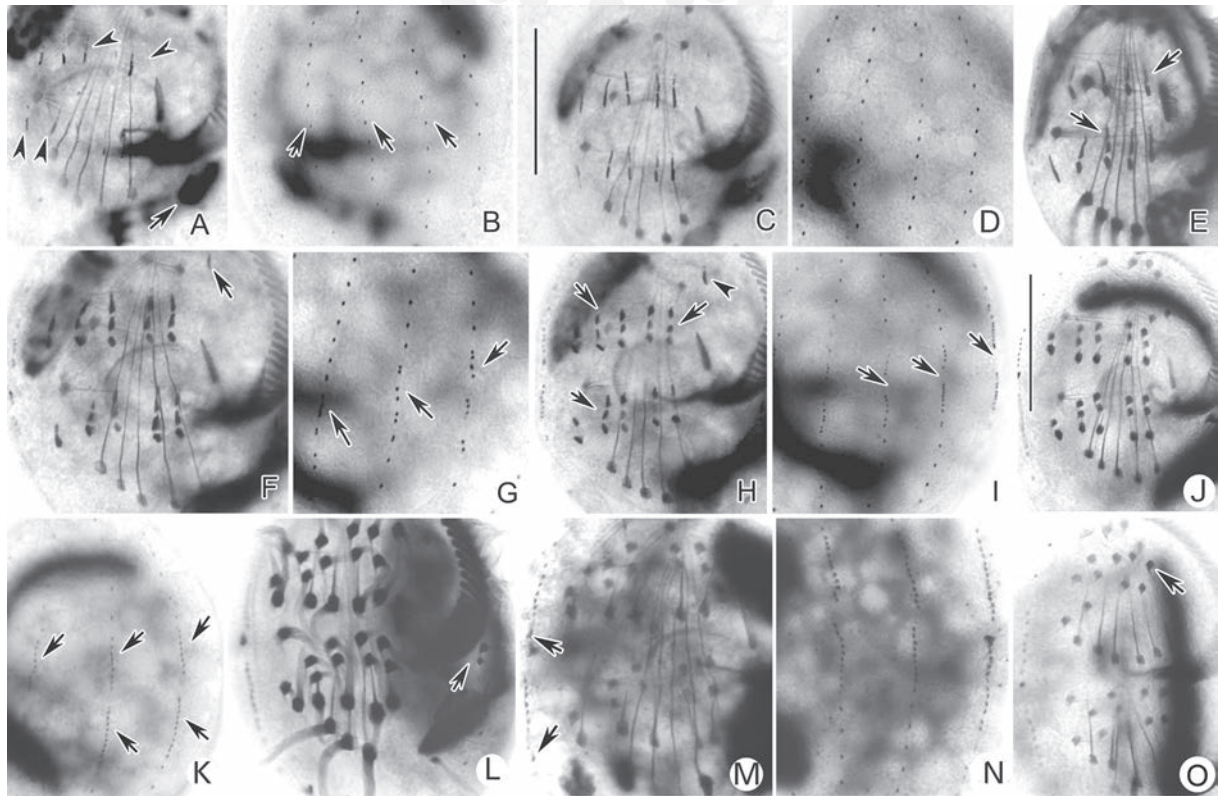


Fig. 3A–O. Photomicrographs of *Euplotes balteatus* during binary division after protargol impregnation. **A** – ventral view of an early divider, arrowheads mark the frontoventral transverse cirral anlagen (FVT-anlagen), arrow indicates the oral primordium of the opisthe; **B** – dorsal view of the same specimen as **A**, arrows denote the parental dorsal kineties; **C**, **D** – ventral and dorsal views of the same early divider; **E** – ventral view, arrows indicate the FVT-anlagen; **F** – ventral view, arrow depicts the migratory cirri anlage in the proter; **G** – dorsal view of the same divider as **F**, arrows mark the dorsal kinety anlagen; **H**, **I** – ventral and dorsal views of the same mid-divider, arrows and arrowhead in **H** indicate the newly formed frontoventral transverse cirri and migratory cirral anlage in the proter respectively, arrows in **I** mark the gaps between the dorsal kinety anlagen in both dividers; **J**, **K** – ventral and dorsal views of the same divider, arrows in **K** mark the dorsal kinety anlagen; **L** – ventral view, to show the marginal cirral anlagen in the proter (arrow); **M**, **N** – ventral and dorsal views of the same late divider, arrows mark the new caudal cirri; **O** – ventral view of a late divider, arrow indicates the new migratory cirri in the proter. Scale bar: 40 μm .

region of body (Figs 2A, C, G, 3C, E, F, H). These fragments migrate and eventually develop into the mature frontoventral and transverse cirri for both dividers following a “3:3:3:3:2” pattern (Figs 2I, K, 3J, L, M, O).

The generation of the leftmost frontoventral cirrus (*viz.* migratory cirrus) occurs in a typical *Euplotes* pattern, *i.e.* the cirral anlage for the proter appears behind the parental migratory cirrus at an early stage (Figs 2E, 3F), then enlarges, differentiates and migrates to its final position (Figs 2G, I, K, 3H, O). For the opisthe, the cirral anlage occurs on the surface of the cell beneath the oral primordium. Eventually this anlage gives rise to the migratory cirrus although at relatively later stage in the process than that for the proter.

Development of marginal cirri: When the streaks of frontoventral transverse cirral anlagen begin to fragment, two small anlagen appear *de novo* on the surface of the cell between the parental and newly formed oral membranelles (Fig. 2E); each of these anlagen will split and eventually develop into two new marginal cirri in each divider (Figs 2G, I, K, 3L).

Development of dorsal ciliature: During the middle stage of cell division, several basal bodies in the mid-region of the old dorsal kineties dedifferentiate into anarchic basal bodies (Figs 2F, 3G). These then proliferate as short primary rows of densely packed dikinetids forming the dorsal kinety anlagen (DKA). Subsequent development of the DKA occurs in two parts (Figs 2H, 3I). Each part then extends along the longitudinal axis in both directions and eventually replaces the old structures (Figs 2J, L, 3K, N). One caudal cirrus is formed at the posterior end of each of the two rightmost dorsal kinety anlagen in the proter. In the opisthe one caudal cirrus is formed at the end of the two rightmost parental dorsal kinety rows each (Figs 2I, K, L, 3M).

Macronuclear division: The macronucleus develops in the same way as in other congeners. That is, at the beginning of morphogenesis, two replication bands appear at the both ends of the macronucleus. As the two bands gradually converge towards the center, the C-shaped macronucleus becomes increasingly short and straight (Figs 2L, 3O). At the late stage of cell division, the replicated macronucleus splits in the middle forming two C-shaped macronuclei, one for each daughter cell (Fig. 3M).

DISCUSSION

Euplotes balteatus

Comparison with other populations: In spite of the variability of its body shape and size, the high stability of the dorsal silverline system pattern, ventral infraciliature, and dorsal structure *in vivo* are important characters for the identification of *Euplotes balteatus* (Tuffrau 1960, Curds 1975, Song and Wilbert 2002, Jiang *et al.* 2010c). The China population corresponds closely with the populations studied previously in terms of its body shape, appearance of adoral zone of membranelles, ventral ciliature, double-*eurystomus* type silverline system and marine habitat (Kahl 1932, Tuffrau 1964, Curds 1975). It differs slightly, however, in body size *in vivo* (< 80 μm long vs. > 100 μm long) and the number of dikinetids of the mid-dorsal rows (*ca.* 15 vs. *ca.* 20). These, however, are regarded as population-level differences; hence its identity as *E. balteatus* is not in doubt (Fig. 4A–C).

Comparison with similar congeners from the same location: Four similar (small sized, ovoid body shaped) *Euplotes* species have been reported from the coastal waters of Qingdao in addition to *E. balteatus*, namely *E. minuta* Yocum, 1930, *E. parabalteatus* Jiang *et al.*, 2010, *E. orientalis* Jiang *et al.*, 2010 and *E. raikovi* Agamaliyev, 1966. *E. minuta* and *E. raikovi* can be easily separated from *E. balteatus* by the type of silverline system and the number of frontoventral cirri (Fig. 5A–E, P–R). Disparities between *E. balteatus* and *E. parabalteatus* include: 1) the number of dorsal kineties (8 vs. 6–7); 2) the number of adoral membranelles (28–43 vs. 19–23); 3) the shape of macronucleus (C-shaped vs. slightly curved rod shaped); 4) the arrangement of frontoventral cirri (V/2 close to V/3 vs. V/2 close to VI/2) (Fig. 5F–J). *E. orientalis* is distinguished from *E. balteatus* in having two reduced cirri (IV/2 and V/2) each composed of single dikinetids (vs. two normal-sized cirri in *E. balteatus*) (Fig. 5K–O).

Morphogenesis: Five types of morphogenesis pattern have been recorded in *Euplotes* based on the segmentation pattern of the frontoventral transverse cirral anlagen, the absence/presence of reduced cirri and the migration of frontoventral cirri during the ontogenesis process; these are the *affinis*-type, the *eurystomus*-type, the *charon*-type, the *raikovi*-type, and the *orientalis*-type (Curds 1975, Voss 1989, Wang and Song 1995, Martín-González *et al.* 1998). *E. balteatus* can be as-

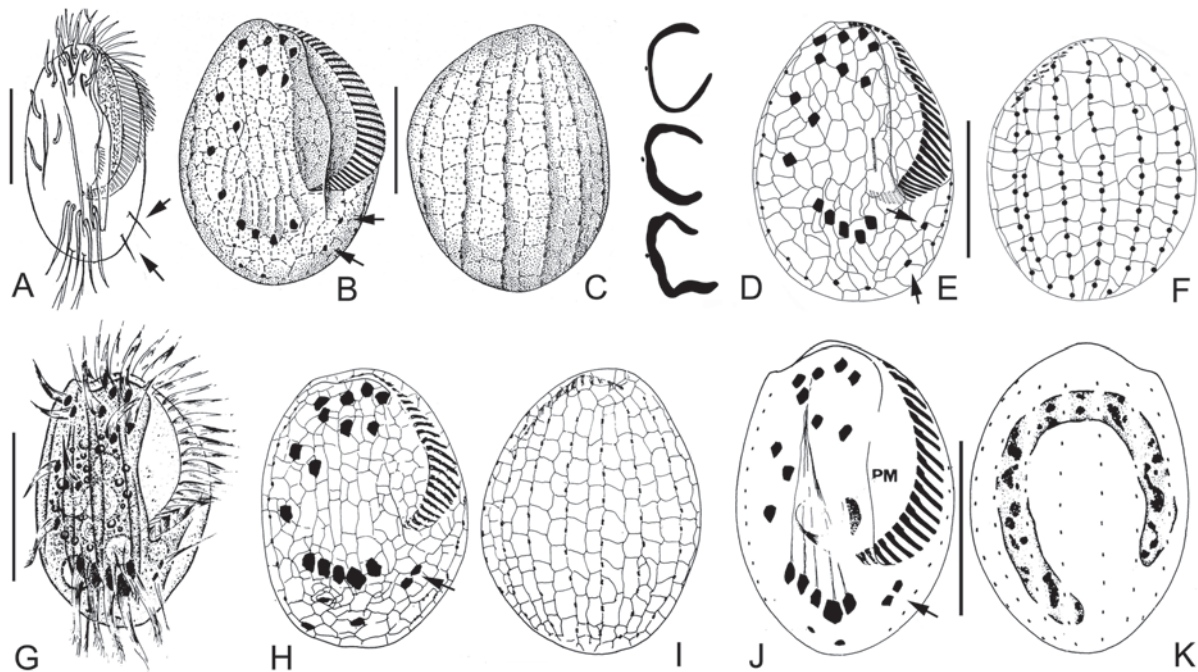


Fig. 4A–K. *Euplotes balteatus* and *Euplotes wilberti* nov. spec. from life (A, G), after protargol (D, J, K) and silver nitrate (B, C, E, F, H, I) impregnation. Arrows point to the marginal cirri. A – ventral view of *Euplotes balteatus*, to show the ventral ciliature (from Kahl 1932); B, C – ventral and dorsal views of *Euplotes balteatus*, showing the silverline system (from Tuffrau 1964); D – *Euplotes balteatus*, to show variations in the shape of the macronucleus; E, F – ventral and dorsal views showing the silverline system; G–K – *Euplotes wilberti* nov. spec., to show the live morphology (G), silverline system (H, I), infraciliature (J, K) and macronucleus (K) (from Song and Wilbert 2002). Scale bar: 30 μ m.

signed to the *charon*-type, that is the frontoventral and transverse cirri are formed following the pattern 3:3:3:3:2. Based on the mode of formation of the caudal cirri, *Euplotes* can be divided into three further types: *focardii*-type, *vannus*-type and *charon*-type (Serrano *et al.* 1992, Ma *et al.* 2008, Jiang *et al.* 2010c). *E. balteatus* belongs to the *focardii*-type in which a caudal cirrus is formed posterior to each of the rightmost two dorsal kineties/DKA in both dividers, hence the number of caudal cirri is constant (Shao *et al.* 2010).

One noteworthy feature in the morphogenesis of *E. balteatus* is that it commences with the dedifferentiation of basal bodies in the mid-region of each dorsal kinety. These basal bodies proliferate to form sparsely distributed elementary anlagen, which then break apart and extend towards the both directions from the fraction spot along the longitudinal axis. These findings are consistent with those of Shao *et al.* (2010) who noted that the dorsal kineties in *E. charon* develop in an atypical primary mode.

Euplotes wilberti nov. spec.

Syn. *Euplotes balteatus* sensu Song & Wilbert, 2002

In 2000, a *Euplotes* species collected from King George Island, Antarctica, was reported as another population of *Euplotes balteatus* (Song and Wilbert 2002). It was diagnosed as follows: 40–70 \times 30–50 μ m *in vivo*, 25–33 adoral membranelles, 10 frontoventral, 5 transverse, 2 caudal and two close-set marginal cirri; 8 to 10 dorsal kineties with ca. 11 dikinetids in mid-rows; silverline system double-*eurystomus* type (Fig. 4G–K). However, the Antarctica population differs from *E. balteatus* in several respects including: 1) the dorsal grooves (distinct vs. inconspicuous in *E. balteatus*); 2) marginal cirri (densely ranged vs. widely separated in *E. balteatus*); 3) the number of dorsal kineties (9 vs. 8 in *E. balteatus*) (Dujardin 1841, Kahl 1932, Tuffrau 1964, Curds 1975). These are significant differences from which we conclude that the Antarctica population is not *E. balteatus*. Indeed, it cannot be identified with

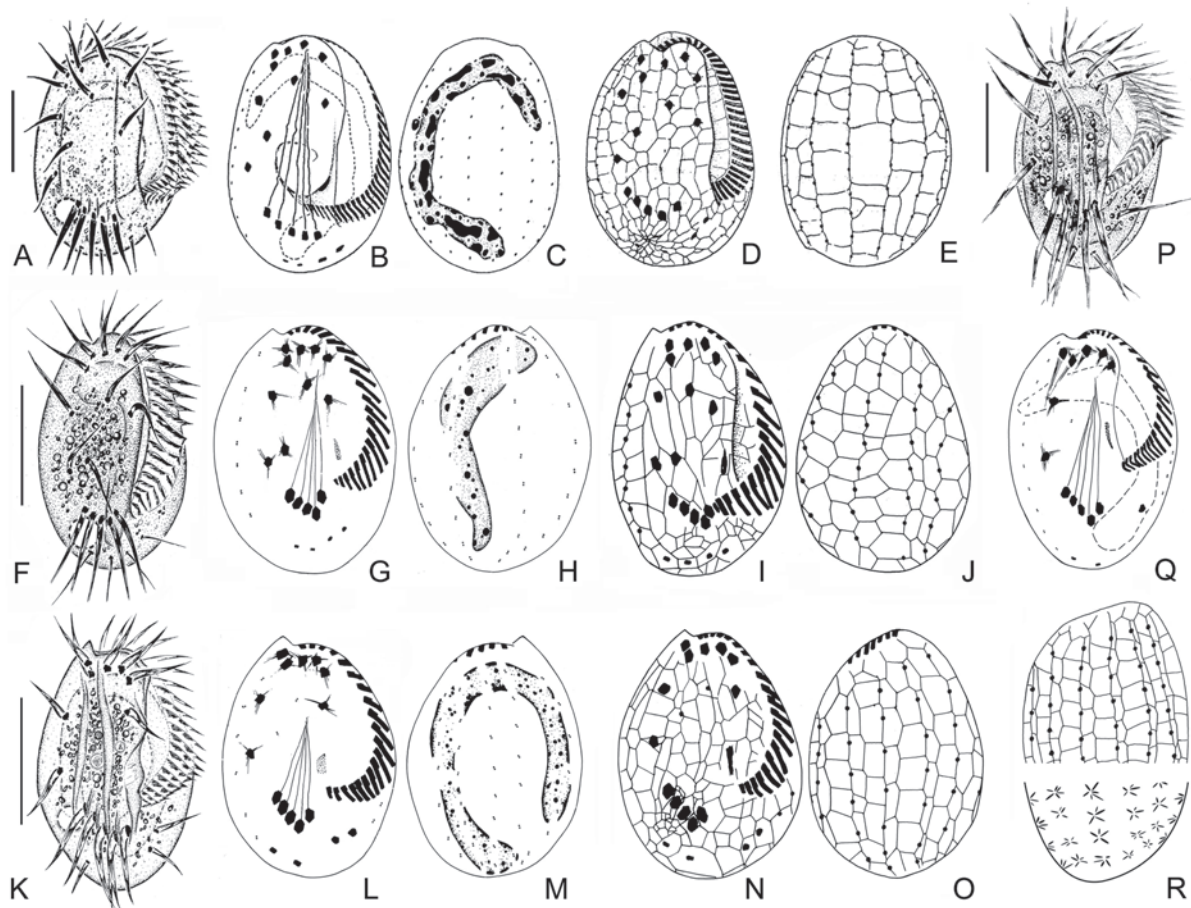


Fig. 5A–R. Morphology from life, infraciliature and silverline system of four morphologically similar *Euplotes* species. **A–E** – *Euplotes minuta* (from Song and Wilbert 1997a); **F–J** – *Euplotes parabalteatus* (from Jiang *et al.* 2010a); **K–O** – *Euplotes orientalis* (from Jiang *et al.* 2010b); **P–R** – *Euplotes raikovi* (from Jiang *et al.* 2010b). Scale bar: 20 μm .

any known species of *Euplotes* so we therefore establish a new species for this population, *Euplotes wilberti* nov. spec.

Diagnosis: Small to medium-sized marine *Euplotes* about $40\text{--}70 \times 30\text{--}45 \mu\text{m}$ *in vivo*, 27–33 membranelles, 10 frontoventral, 5 transverse, 2 caudal and two closely spaced marginal cirri; 8 to 10 dorsal kineties with *ca.* 11 dikinetids in mid-dorsal rows; double-*eurystomus* type silverline system.

Type locality: Rock pool and the littoral of Potter Cove, King George Island ($62^{\circ}14'S$, $58^{\circ}40'W$), Antarctica.

Type specimen: A protargol slide of the Antarctica population was deposited in the Oberösterreichisches Landesmuseum (LI), A-4040 Linz, Austria (Song and

Wilbert 2002). Unfortunately the slide was not registered so it is not currently possible to declare a type specimen.

Dedication: We dedicate this new species to Dr. Norbert Wilbert, Institut für Zoophysologie, Universität Bonn, Germany, in recognition of his contributions to the study of ciliates as well as his excellent descriptions of this organism (Song and Wilbert 2002, Wilbert and Song 2008).

Comparison with other morphologically related forms: To date, there have been reports of 8 small-sized *Euplotes* with a double-*eurystomus* type dorsal silverline system, 10 frontoventral and 2 marginal cirri, namely *E. balteatus* (Dujardin, 1841) Kahl 1932, *E. parabalteatus* Jiang *et al.*, 2010, *E. alatus* Kahl,

Table 2. Comparison of morphologically similar marine *Euplotes* species with 10 frontoventral cirri, 2 marginal cirri and a “double-*eurystomus*” type dorsal silverline system. AM – adoral membranelles, DK – dorsal kineties, Ma – macronucleus, TC – transverse cirri. Measurements in μm .

Character	<i>E. wilberti</i>	<i>E. balteatus</i>	<i>E. parabalteatus</i>	<i>E. alatus</i>	<i>E. quinquecarinatus</i>	<i>E. magnicirratus</i>	<i>E. plicatum</i>	<i>E. trisulcatus</i>
Body size <i>in vivo</i>	40–70	30–80	30–35	36–43	55	54**	42–55	40
No. of AM	27–33	18–25	19–23	ca 30*	25–30*	49–52	22–25	25–36
No. of DK	8–10	8	6–7	8	9	8	10	7
No. of dikinetids in mid-rows	9–14	12–16	8–11	10–11*	13–15*	13–17	14	11
Marginal cirri	Densely ranged, above the TC level	Widely separated	Separated, below the TC level	Separated, below the TC level	Widely separated	Separated, below the TC level	Widely separated	Widely separated, below the TC level
Ma shape	Inverted C-shaped	C-shaped	Curved bar-shaped	C-shaped	C-shaped	Inverted C-shaped	Inverted C-shaped	Curved bar-shaped
Reference	Song and Wilbert (2002)	Present work	Jiang (2010a)	Borror (1968)	Borror (1968)	Carter (1972)	Valbonesi <i>et al.</i> (1997)	Tuffrau (1960)

* Counted from published illustrations.

** Probably from impregnated specimens.

1932, *E. magnicirratus* Kahl, 1932, *E. quinquecarinatus* Gelei, 1950, *E. plicatum* Valbonesi, 1997, *E. trisulcatus* Kahl, 1932 and *E. wilberti* nov. spec. Table 2 summarizes the main characters that define these species. From these data it can be seen that *E. wilberti* nov. spec. is clearly distinguished from its most similar congeners thus justifying its validity as a separate species.

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