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Morphological Redescriptions of *Aspidisca magna* Kahl, 1932 and *A. leptaspis* Fresenius, 1865 (Ciliophora, Euplotida), with Notes on Ontogenesis in *A. magna*

Liqiong LI[1,2], Qianqian ZHANG[2], Khaled A. S. AL-RASHEID[3], Choon Bong KWON[1], Mann Kyoon SHIN[1]

[1]Department of Biology, University of Ulsan, Ulsan, Korea; [2] Laboratory of Protozoology, KLM, Ocean University of China, Qingdao, China; [3] Zoology Department, King Saud University, Riyadh, Saudi Arabia

Summary. The largest known *Aspidisca* species, *A. magna* Kahl, 1932, was found from coastal waters near Qingdao (Tsingtao), northern China, and investigated using both the "wet" silver nitrate and protargol staining method. Based on the living observation and impregnated individuals, improved diagnosis and morphometric data are provided. As an additional contribution, the morphogenesis in *Aspidisca magna* during binary fission was revealed and summarized as follows: 1) the parental adoral zone of membranelles and paroral membrane are retained by the proter; 2) the oral primordium of the opisthe develops hypoapokinetally behind the posterior part of the adoral zone of membranelles; 3) five frontoventral-transverse cirral anlagen are formed *de novo*, initially as primary primordia, and develop into 3:3:2:2:1 cirri from left to right, respectively in both dividers; 4) the leftmost frontoventral cirrus is generated from an independently formed cirral anlage in both dividers. In the present work, Chinese and Korean populations of *A. leptaspis* Fresenius, 1865 were investigated respectively to support the importance of living characteristics in identification of *Aspidisca* species. The isolation of *A. magna* and *A. leptaspis* from other congeners are also firmly demonstrated by the SSU rRNA gene sequence alignments.

Key words: *Aspidisca*, Euplotida, morphogenesis, morphology, silver staining.

INTRODUCTION

The well-known genus *Aspidisca* comprises about 60 morphologically similar and confusing species (e.g. Stein 1859; Buddenbrock 1920; Mansfeld 1923; Kahl 1932; Dragesco 1960, 1963; Tuffrau 1964; Borror 1972; Curds 1977; Wu and Curds 1979; Fernandez-Leborans and Castro de Zaldumbide 1987; Berger 2001; Shen *et al.* 2010). Morphological and molecular studies on this group of ciliates with rigid cortex have ignored and/or not recorded the peculiarities *in vivo*, such as dorsal ridges, thorns, and spurs, which are important diagnostic characters. Considering the accurate ciliary pattern, which is indispensable for ciliates identification and circumscription, some of the nominal forms still need sufficient redescription based on both living and silver-stained specimens, as well as the small subunit ribosomal RNA gene sequence homology (Hartwig 1973;

Address for correspondence: Mann Kyoon Shin, University of Ulsan, Ulsan 680-749, Korea; E-mail: mkshin@ulsan.ac.kr

Agamaliev 1974, 1975; Foissner 1982, 1994; Fernandez-Leborans and Castro de Zaldumbide 1987; Song and Wilbert 1997, 2002; Song 2003; Li *et al.* 2008).

Since *Aspidisca magna* was first reported by Kahl (1932), this species has been reported times and again under different names (Delphy 1938, Vacelet 1961). However, except for the silverline system on the ventral side, the infraciliature and division process still remain unknown (Tuffrau 1964, Wu and Curds 1979). As regards *A. leptaspis*, the available morphological and morphogenetic information is comprehensive (Song and Wilbert 1997, Song 2003), however, some misidentifications leading to confusions have been also reported. Therefore, the aims of the present study are to: 1) redescribe *A. magna* based on observations of specimens both *in vivo* and following silver impregnation including drawings, photomicrographs, and morphometric data, 2) provide improved diagnoses and gene sequence comparison for both species, and 3) document in detail the main morphogenetic processes in *A. magna*.

MATERIALS AND METHODS

Chinese populations of *Aspidisca magna* and *A. leptaspis* were collected on September 17 and 28, 2007 respectively from open mussel-farming waters near Qingdao, China with a salinity of 29–31‰, water temperature about 16°C, pH ca. 8.0. Whereas, another two populations of *A. leptaspis* were collected on February 19 and April 18, 2009 from the estuarine littorals of Taehwa River (2–20‰ salinity) in Ulsan, Korea. All of the samples were collected using framed slides which were left immersed at a depth of 1–1.5 m for about 10 days to allow colonization by ciliates to occur. Then slides with original water were maintained in Petri dish at room temperature (ca. 22°C) for raising ciliates. After about one week both forms emerged with other accompanying species. Short-term raw cultures for both forms were established using rice grains to enrich bacteria as food source. (Li *et al*. 2010).

Observations on live specimens were carried out using differential interference microscopy. The protargol staining method of Wilbert (1975) was used to reveal the infraciliature and nuclear apparatus. The Chatton-Lwoff method (Wilbert and Song 2008) was followed for revealing the silverline system of *A. magna.*

Counting and measurements were made at a magnification of 1250 ×. Drawings were made with the help of a camera lucida. To illustrate the changes occurring during morphogenesis, parental cirri are depicted by contour whereas new ones are shaded black. Terminology is mainly according to Borror (1972) and Wu and Curds (1979).

Voucher slides of *A. magna* have been deposited in the Natural History Museum, London, UK, with registration number 2008:5:16:1. Other voucher slides of both species have been deposited in the Laboratory of Protozoology, Ocean University of China and Lab of Biodiversity, Department of Biology, University of Ulsan, Korea.

The alignment of SSU rRNA gene sequences for the *Aspidisca* spp. was performed using Clustal W implemented in BioEdit Ver. 7.00 (Hall 2004). The nucleotide sequences of *A. magna* and *A. leptaspis* which were recently reported by Yi *et al.* (2009) were obtained from the GenBank/EMBL databases with the accession numbers EU880598 and EU880597. Sequences of other *Aspidisca* spp. were available in GenBank/EMBL databases: *A. aculeata* (EF123704), *A. orthopogon* (EU430745), *A. steini* (AF305625). Sequence identities were calculated using BioEdit Ver. 7.00 (Hall 2004).

RESULTS

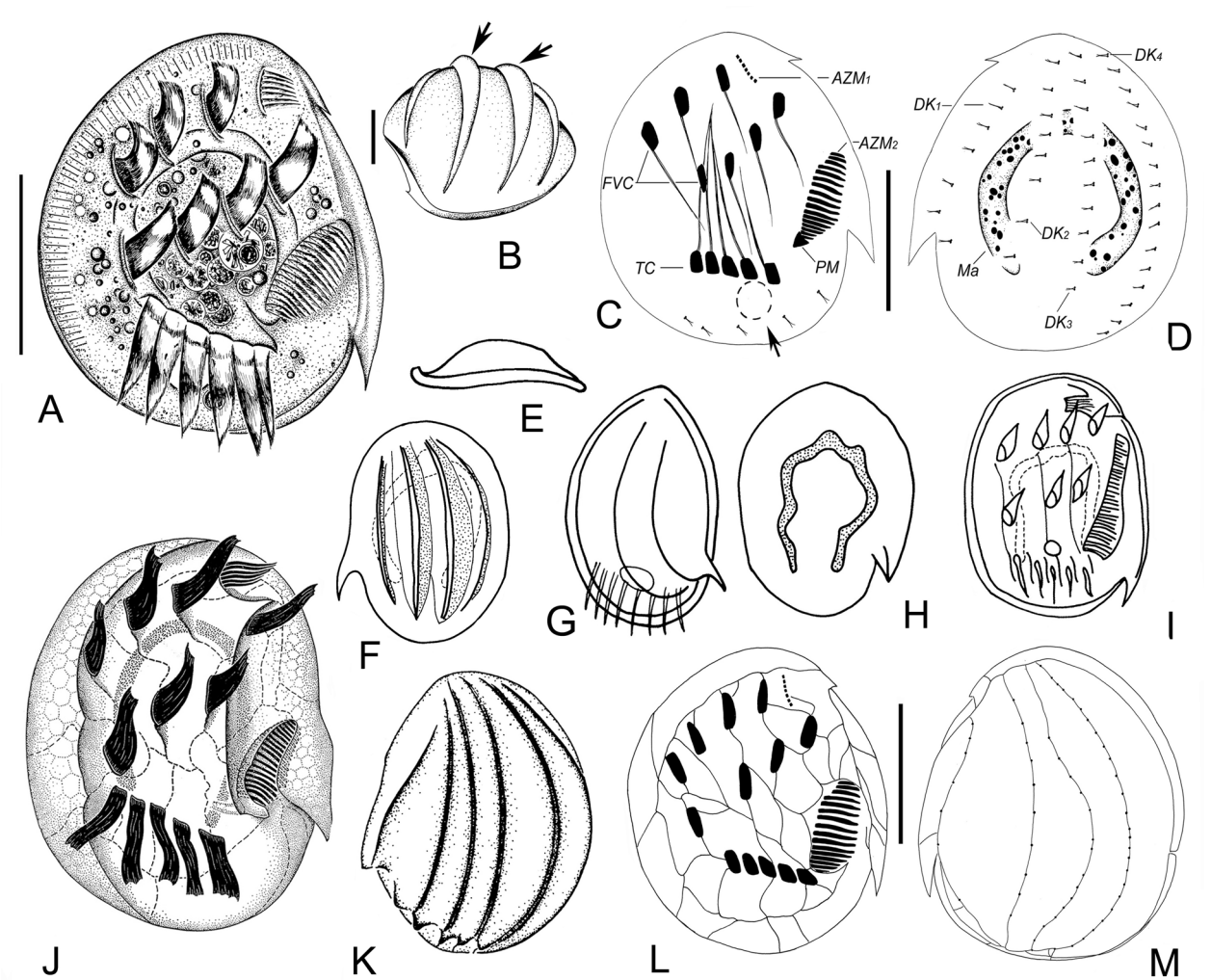
Aspidisca magna Kahl, 1932

Syn. *A. pelvis* Delphy, 1938
A. maxima Vacelet, 1961

Remarks: As reported firstly by Kahl (1932), *A. magna* can be distinctly identified by its large size (135 μm), two medial wing-like and two low lateral dorsal ribs, one peristomial spur. Our organism corresponds well with the original and later descriptions in respect of the general ciliary pattern, body outline, and dorsal ribs, thus can be confirmed conspecific (Vacelet 1961, Tuffrau 1964, Curds and Wu 1979). The smaller body size of our isolate (length 50–100 μm vs. 135–157 μm), could be ascribed to intraspecific variation between populations or the nutritional deficiency. The synonyms suggested by Curds and Wu (1979) under the names *A. pelvis* and *A. maxima* respectively are basically agreed with. Since only vague descriptions were given till now, here we present an improved diagnosis and a redescription based on previous and current investigations.

Improved diagnosis: Marine *Aspidisca*, body 50–160 × 40–115 μm *in vivo*; body broadly oval with one beak-like peristomial spur posteriorly located on the left border; four dorsal ridges, the middle ones slightly more prominent than the others; seven equally strong frontoventral cirri; five transverse cirri, four dorsal kineties, with on average 8, 10, 11, 19 dikinetids; 7–8 membranelles in AZM_1, about 16 membranelles in AZM_2.

Description of Qingdao population (Figs 1, 2; Table 1): Size extremely variable: *in vivo* about 50–100 × 40–80 μm, in extreme forms, as reported, might be 160 μm in length (the maximum of body length was 157 μm; in Tuffrau 1964). Body broadly oval with one beak-like peristomial spur posteriorly located on the left border, and an indistinct projection at the level of AZM_1, which could be easily ignored (Figs 1A, 2A, arrow). Well-fed individuals slightly broaden in the

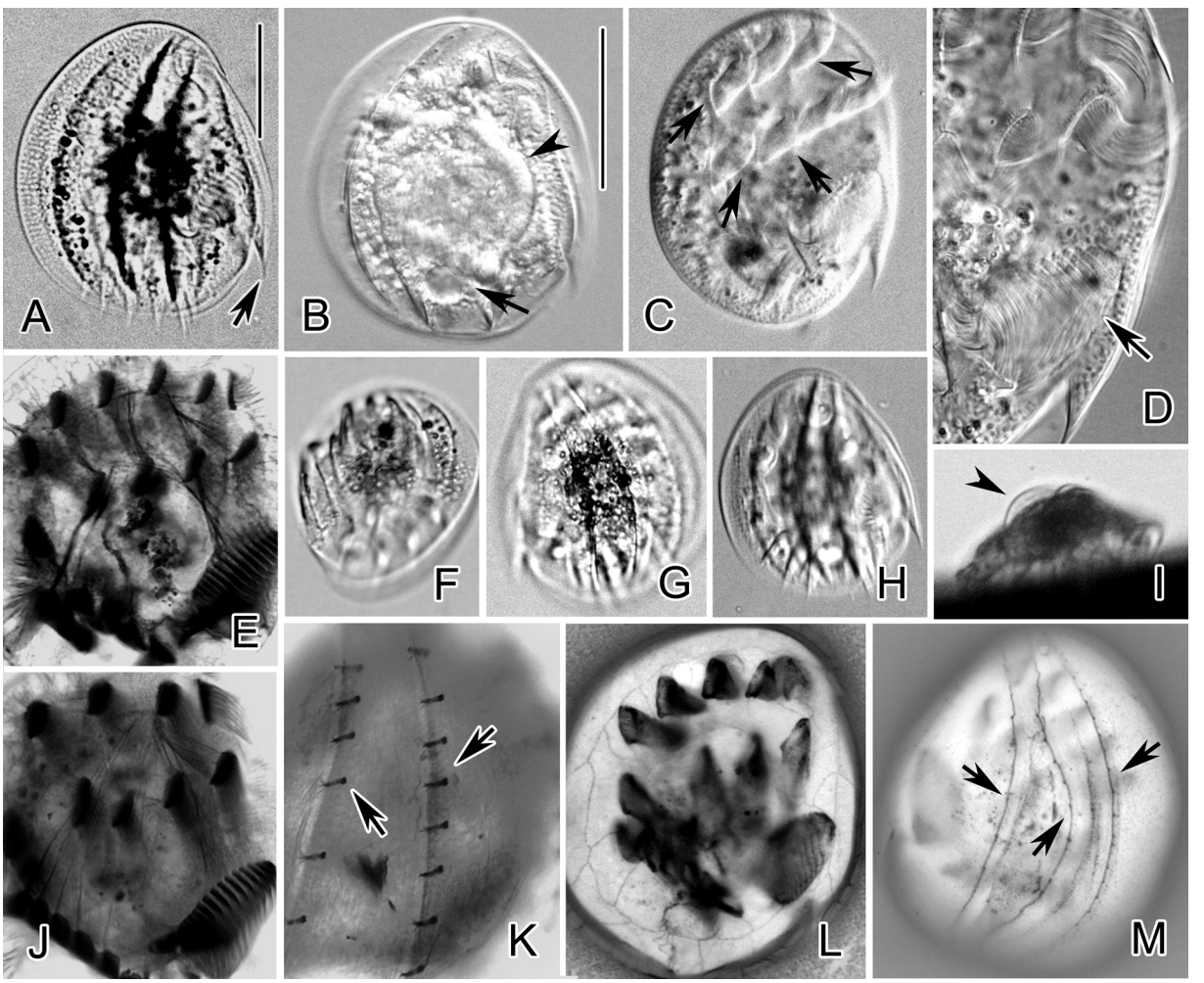


Figs 1A–**M.** Morphology of *Aspidisca magna* from life (A, B, E–I, K), after protargol staining (C, D), and silver nitrate impregnation (J, L, M). **A –** ventral view of a typical individual; **B –** apical view, arrows indicate the two prominent dorsal ridges; **C**, **D –** infraciliature of ventral and dorsal side, arrowhead indicates the contractile vacuole; **E –** lateral view (from Kahl 1932); **F –** dorsal view, note the conspicuous peristomial spur and dorsal ridges (from Kahl 1932); **G**, **H –** ventral views to show the shape, spur, position of contractile vacuole and macronuclear nodule of the synonym *A. pelvis* (from Delphy 1938); **I –** ventral view, showing the general cirral pattern and (from Vacelet 1961); **J –** ventral view showing cirral pattern and silverline system (from Tuffrau 1964); **K –** dorsal view; **L**, **M** – silverline system on ventral and dorsal side. AZM_1 – anterior portion of adoral zone of membranelles, AZM_2 – posterior portion of adoral zone of membranelles, DK – dorsal kineties, FVC – frontoventral cirri, Ma – macronucleus, PM – paroral membrane, TC – transverse cirri. Scale bars: 40 µm.

mid-position to posterior end (Fig. 2B). Dorsoventrally flattened about 3:1 (Fig. 2I). Body inflexible and non-contractile. Under lower magnification, margin of cell transparent and central portion opaque due to hyaline cytoplasm including large food vacuoles and other granules (Figs 2A, G, H). Four conspicuous, longitudinal ridges on dorsal side, the central two of them apparently higher then the marginal ones and wing-like (Figs 2G, I). Macronucleus slim and horseshoe-shaped with conspicuous nucleoli (Fig. 1D). Micronuclei not observed. Contractile vacuole 12 µm across, subcaudally positioned near dorsal side (Figs 1C, arrow; 2B, arrow).

Locomotion characterized by crawling in circle on substrate, quite slow. When disturbed, always attached to substrate firmly for quite a while.

Frontoventral cirri strong and stiff, seemed to vary in number, mostly 7 (trophic cell at interphase), but sometimes up to 10 observed (newly divided forms



Figs 2A–M. Photomicrographs of *Aspidisca magna* from life (A–D, F–I), after protargol staining (E, J, K), and silver nitrate impregnation (L, M). **A –** ventral view of a typical individual, arrow marks the peristomial spur; **B –** ventral view, arrow indicates the contractile vacuole and arrowhead marks the horseshoe-shaped macronuclear nodule; **C –** ventral view, arrows indicate some frontoventral cirri; **D –** ventral view, arrow indicates AZM_2; **E –** ventral view of a individual with "extra" frontoventral cirri; **F –** apical-dorsal view, showing the dorsal ridges; **G –** dorsal view; **H –** ventral view; **I –** apical view, arrowhead indicates one of the two prominent dorsal ridges; **J –** the general ciliature on ventral side; **K –** dorsal view, showing the dorsal kineties (arrows); **L**, **M –** infraciliature and silverline system (arrows) on dorsal and ventral side. Scale bars: 40 μm.

with late absorption of the parental cirri, or extra cirri derived from the sixth frontoventral-transverse cirral anlage), cilia of which are about 16 μm long (Figs 1C, L, 2C, E). Usually 5 moderately strong transverse cirri, about 20 μm long, tightly arranged in an oblique row, the leftmost one of which often split in living specimens, which appears like two cirri (Fig. 1A). Anterior portion of adoral zone (AZM_1) containing 7–8 membranelles located in deep depression of cell surface; posterior part (AZM_2) with about 16 ones (Fig. 1C). Paroral membrane small, laid adjacent to posterior end of AZM_2. Constantly four dorsal kineties carrying loosely spaced basal body pairs, from left to right 8, 10, 11, 19 in number, respectively, all of which are ciliated (Figs 1D, 2K).

Silverline system on ventral side follows the typical genus pattern (Figs 1L, 2L). Dorsal argyrome is simple, which consists of 4 dorsal kineties with single-cross links connected with a circle along the right margin of cell (Figs 1M, 2M, arrows).

Observations on morphogenesis: Five binary dividers observed at early to late stages prompted us to

Table 1. Morphometric data for *Aspidisca magna* (Chinese population, 1st line) and *A. leptaspis* (Korean population, 2nd line). All data are based on protargol-impregnated specimens. Measurements in µm. Abbreviations: $AZM_{1,2}$ – adoral membranelles in part 1, 2, BB – basal body pairs, CV – coefficient of variation in %, DK – dorsal kinety, FVC – frontoventral cirri, Max – maximum, Min – minimum, n – number of cells measured, SD – standard deviation, TC – transverse cirri.

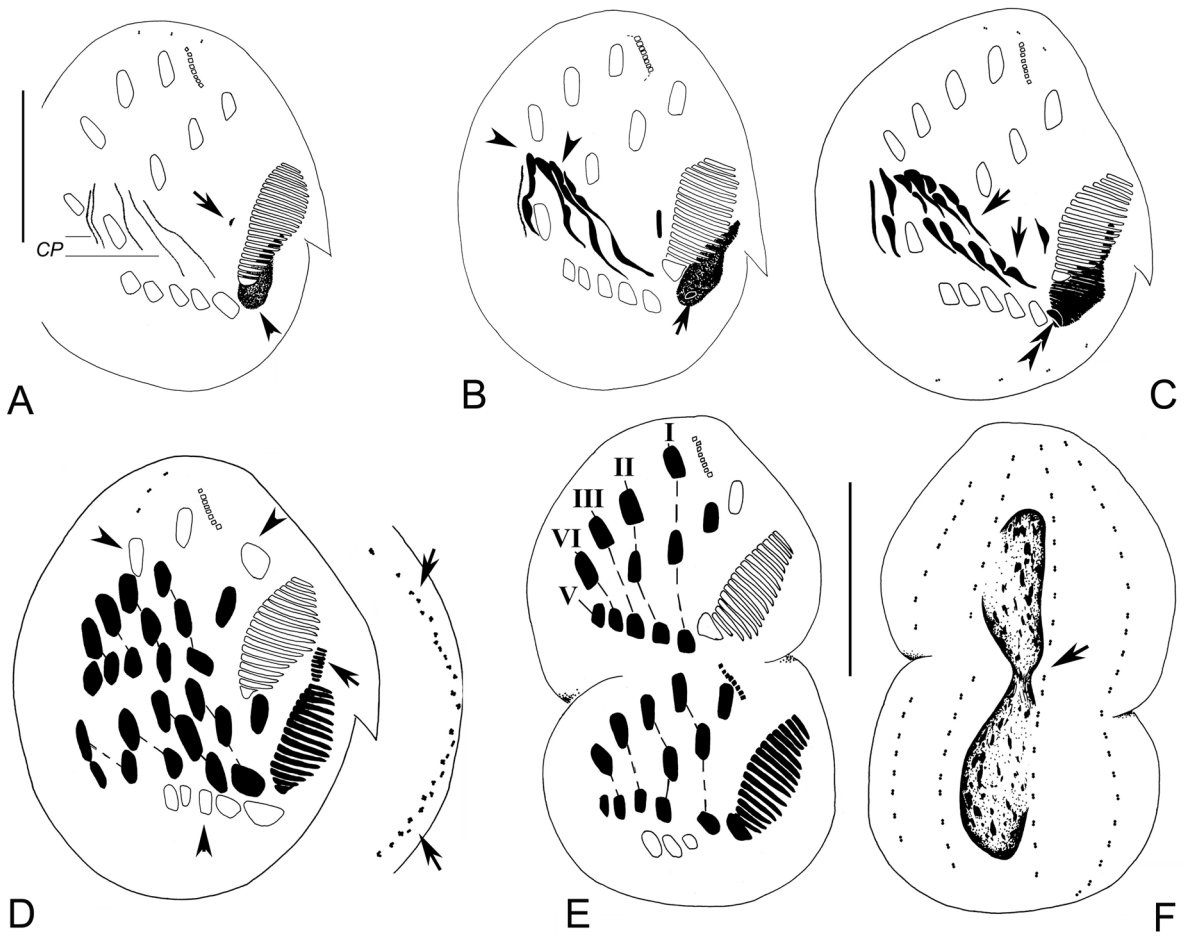
Character	Min	Max	Mean	SD	CV	n
Length of body	68	94	80.9	6.1	7.5	25
	50	80	64.0	7.8	12.2	30
Width of body	52	84	67.7	7.3	10.8	25
	40	55	48.3	3.3	6.8	30
Number of AZM_1	8	8	8.0	0.0	0.0	25
	6	8	7.1	0.5	7.0	30
Number of AZM_2	15	18	16.3	0.7	4.3	25
	14	21	19.1	2.2	11.5	30
Number of FVC	7	9	7.2	0.5	6.9	25
	7	7	7.0	0.0	0.0	30
Number of TC	5	7	5.2	0.6	11.5	25
	5	5	5.0	0.0	0.0	30
Number of DK	4	4	4.0	0.0	0.0	25
	4	4	4.0	0.0	0.0	30
No., BB-pairs in DK_1	6	11	7.9	1.4	17.7	25
	6	10	7.7	1.2	15.6	20
No., BB-pairs in DK_2	8	14	9.5	1.6	16.8	25
	9	11	9.9	0.6	6.1	20
No., BB-pairs in DK_3	8	16	11.1	1.7	15.3	25
	9	14	12.2	1.4	11.5	20
No., BB-pairs in DK_4	16	27	19.2	2.7	14.1	25
	20	26	22.3	1.6	7.2	20

obtain the main morphogenetic process. The earliest cortical stomatogenetic event is the emergence of the oral primordium, which forms *de novo* as a patch of anarchic basal bodies beneath the cortex of the ventral surface, posterior to the AZM_2 and to the left of the transverse cirri (Fig. 3A, arrowhead). Simultaneously, five fine streaks of basal bodies, namely the primary cirral anlagen, appear anterior to the transverse cirri without disorganization of parental structures (Fig. 3A). Additionally, as the new anlage of the leftmost frontoventral cirrus for the proter, a small spot-like cirral anlage also occurs at the outer of cortex, right to the parental AZM_2 (Fig. 3A, arrow). In the next stage, along with the proliferation of basal bodies, the cirral streaks subsequently broaden and aggregate (Fig. 3B, arrowheads) while concurrently, a new independent anlage for the leftmost frontoventral cirrus of the opisthe is formed independently at the surface of cell above the oral primordium (Fig. 3B, arrow). Subsequently, the primary cirral anlagen split longitudinally for both dividers, while the membranelles for the opisthe start to develop posteriad (Fig. 3C). Conspicuously, there is an extra cirral anlage formed in this specimen, which is supposed to give rise to more frontoventral cirri and cause the inconstant number of frontoventral cirri (Fig. 3C, arrows). After that, these two groups of cirral anlagen enlarge and begin to break apart to develop into distinct cirri, as well as the leftmost frontoventral cirrus formed in each divider (Fig. 3D), the new membranelles for the AZM_1 of opisthe migrate onto the cell surface (Fig. 3D, arrow), and the proliferation of new basal bodies occurs within each parental dorsal kinety (Fig. 3D, insert, arrows). At last, five frontoventral-transverse cirral anlagen develop into 3:3:2:2:1 mature frontoventral and transverse cirri from left to right in both proter and opisthe, respectively (Fig. 3E). Likewise, both of the new leftmost frontoventral cirri achieve the final size (Fig. 3E). In the opisthe, the new paroral membrane develops as the formation of the adoral zone of membranelles is completed (Fig. 3E). Eventually, except for the parental AZM_1 and AZM_2 retain in the proter, all the parental cirri are gradually resorbed, while the new oral apparatus and all cirri migrate to their final positions. On the dorsal side of both daughter cells, the new kineties extend to both ends (Fig. 3F). Macronucleus develops in the usual way (Fig. 3F), thus details are not mentioned.

Aspidisca leptaspis Fresenius, 1865

Syn. *A. sedigita* Quennerstedt, 1867; *A. crenata* Fabre-Domergue, 1885; *A. sedigita* sensu Dragesco, 1960; *A. lyncaster* sensu Tuffrau, 1964; *A. baltica* sensu Borror, 1968; *A. psammobiotica* Burkovsky, 1970; *A. lyncaster* sensu Fleury *et al*., 1968

Morphological observation on our populations (Fig. 4): Since, Song and Wilbert (1997) have provided a very comprehensive redescription and updated the diagnosis of this species, we are not repeating most of the characteristics (infraciliature, locomotion), which corresponds well with their population. Here the main diagnostic features, which should be emphasized again or the variation between populations according to our observations are present as follows: size of our populations *in vivo* are a little smaller, about 45–70 × 35–50 µm; specimens from fresh sample or a week old culture always have two small dentations at the anterior left

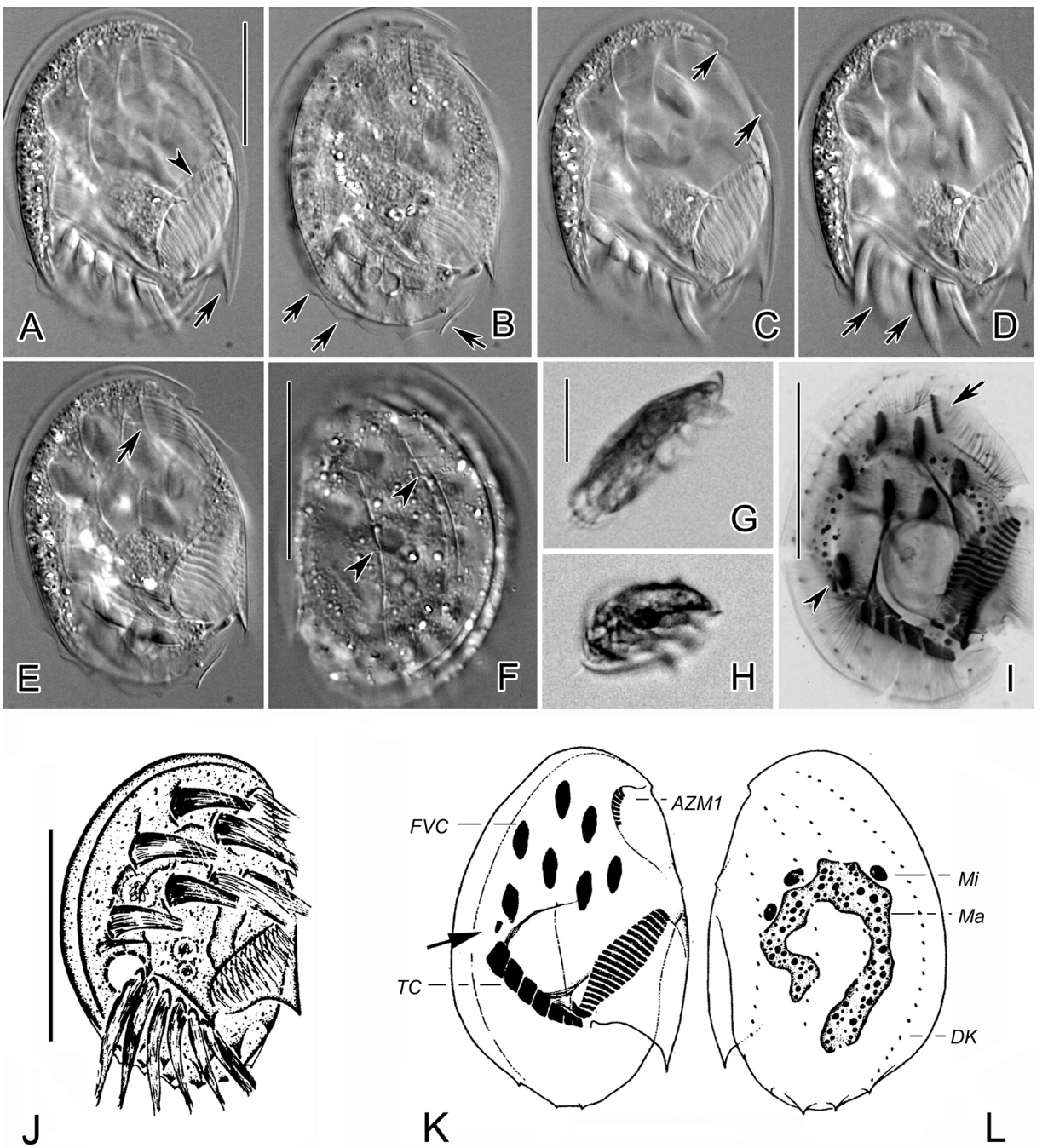


Figs 3A–F. Morphogenesis in *Aspidisca magna* from protargol-impregnated specimens. In (D, E), cirri derived from same anlage are connected by broken lines. **A –** ventral view, to show the *de novo* formed anlage for the leftmost frontoventral cirrus (arrow) and the oral primordium in deep pouch (arrowhead); **B –** ventral side showing the oral primordium (arrow) and five frontoventral-transverse cirral anlagen; **C –** ventral view, arrows show the extra frontoventral-transverse cirral anlage in both dividers, and double-arrowheads mark the independently formed anlage for the leftmost frontoventral cirrus in the opisthe; **D –** ventral side, arrowheads indicate the parental frontoventral and transverse cirri, while arrow marks the anterior portion of adoral zone membranelles (AZM_1) splitting from the newly formed AZM in the opisthe; insert, arrows indicate the dorsal kineties anlagen; **E**, **F –** ventral and dorsal views of the same specimen just before division. Arrow in (F) indicates the separating macronucleus. CP – ciliary primordium, I–V – frontoventral-transverse cirral anlagen. Scale bars: 40 μm.

(Fig. 4C, arrows) and several (4–6) serrations on the posterior border (Fig. 4B, arrows), as well as one thorn-like peristomial spur associated with AZM_2 on left subcaudally (Fig. 4A, arrow). On dorsal side always four inconspicuous to dominant dorsal ridges, which may extend caudally with the notched cell end (Fig. 4H). It is noticeable that for the squeezed specimens, instead of the dorsal ridges, four shallow and longitudinal furrows would appear making a fake character (Fig. 4F, arrowheads).

SSU rRNA gene sequence alignment (Fig. 5)

The dissimilarity of the five *Aspidisca* species is firmly supported by the SSU rRNA gene sequences. *Aspidisca magna* and *A. leptaspis* differ in 131 nucleotides and have sequence identity of 92.1%. Sequence identities between *A. magna* and the other three *Aspidisca* spp. are only 84.1% (*A. aculeata*), 85.5% (*A. steini*), and 80.1% (*A. orthopogon*). While those between *A. leptaspis* and other three *Aspidisca* spp. are 84.5% (*A. aculeata*), 85.3% (*A. steini*) and 80.0% (*A. orthopogon*).



Figs 4A–L. Morphology of *Aspidisca leptaspis* (Chinese population) from life (A–H, J) and after protargol impregnation (I, K, L). **A** – ventral view of a typical individual, arrow indicates the peristomal spur and arrowhead marks the posterior adoral zone of membranelles (AZM_2); **B** – ventral view, to show the spurs on the posterior cell margin (arrows); **C** – ventral view, arrows indicate the spurs on anterior-left cell margin; **D** – ventral view, to show the transverse cirri (arrows); **E** – ventral view, arrow indicates the anterior adoral zone of membranelles (AZM_1); **F** – dorsal view, arrowheads indicate the shallow furrows; **G**, **H** – lateral and apical view; **I** – infraciliature on ventral side, arrow indicates AZM_1 and arrowhead marks the satellite-like cirrus; **J** – ventral view of a typical individual; **K**, **L** – infraciliature on ventral and dorsal side. Scale bars: 20 μm (A, G), 30 μm (F, I, J).

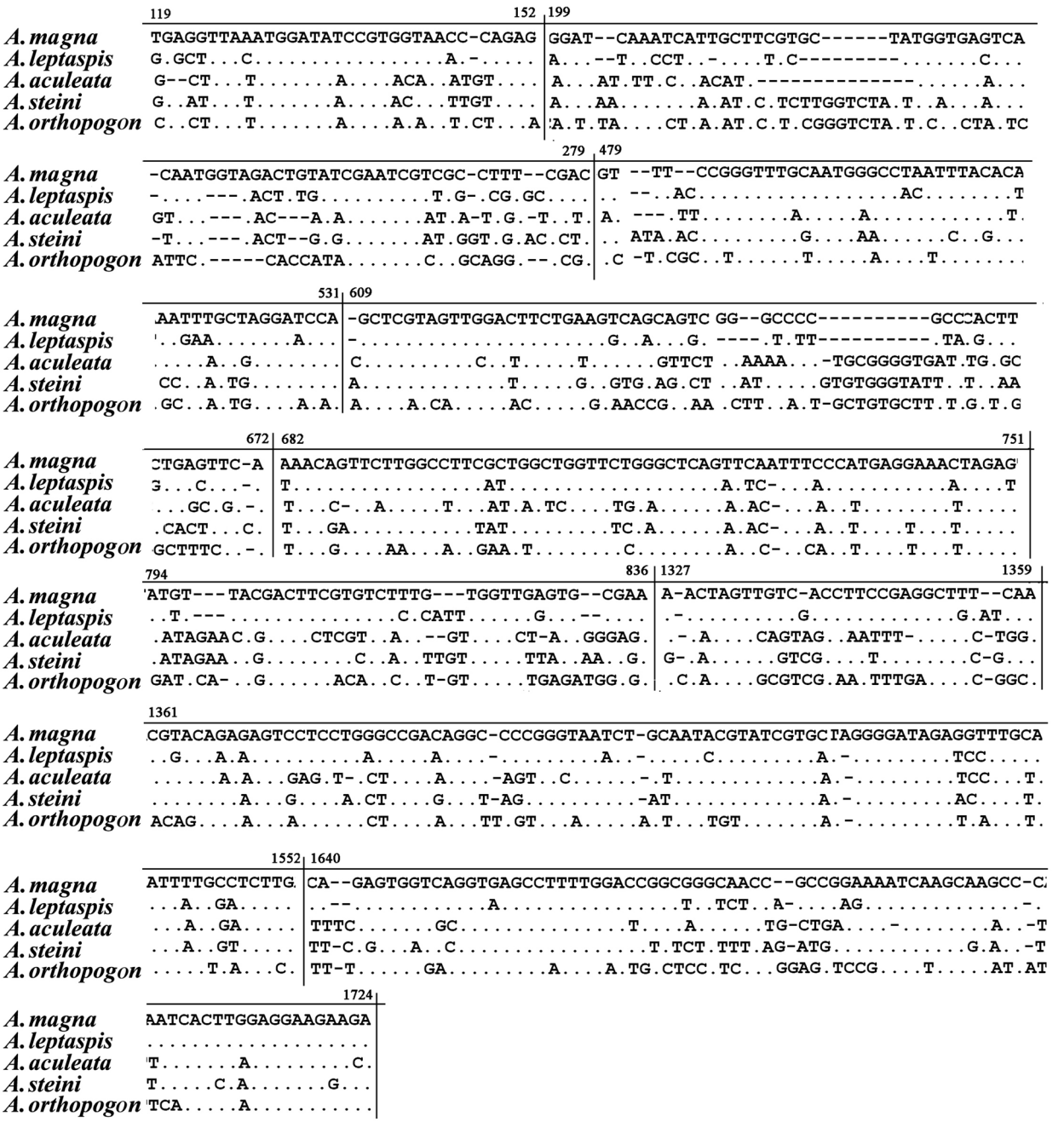


Fig. 5. Small subunit rRNA gene sequence of five *Aspidisca* species. Numbers at the end of lines indicate the number of nucleotides. The differences in sequence length were compensated for by introducing alignment gaps (–) in the sequences. Matched sites are marked with dots.

DISCUSSION

Comparison of *Aspidisca magna* with related congeners

Morphologically, *A. herbicola* Kahl, 1932, *A. dentate* Kahl, 1928 and *A. fusca* Kahl, 1928 are the most related congeners with *A. magna* in having the similar body outline (one conspicuous peristomial spur), and seven strong frontoventral cirri. The four species can be easily distinguished by the following differences: 1) body length (50–160 μm in *A. magna* vs. less than 60 μm in the latter three forms); 2) macronuclei (one, horseshoe shaped in *A. magna* vs. two, oval shaped in *A. fusca*); 3)

arrangement of frontoventral-transverse cirri (polystylapattern in *A. magna* vs. lynceus-pattern in *A. herbicola*); 4) habitat (marine in *A. magna* vs. fresh water in *A. herbicola*); 5) number of membranelles in AZM_2 (15–18 in *A. magna* vs. ca. 10 in *A. herbicola*, 6–10 in *A. dentate*); 6) dorsal thorn (absent *A. magna* vs. present in *A. herbicola* and *A. dentate*) (Kahl 1928, 1932; Dragesco 1965; Agamaliev 1967; Wu and Curds 1979).

The comparison of the nucleotide sequences of the SSU rRNA regions (Fig. 5), also supports the morphological conclusion that *A. magna* and *A. leptaspis* are clearly outlined and different forms in *Aspidisca.* Though, the morphological differences among these five *Aspidisca* congeners are distinct, the revealed discrepancies based on SSU rRNA gene sequences are quite larger than expected, which indicates that the species and genetic diversity of *Aspidisca* may be beyond our understanding. Nevertheless, further studies using silver impregnation and molecular techniques are inevitable for testing the doubts on the validities of the nominal morphospecies in *Aspidisca*.

About synonyms of *Aspidisca leptaspis*

As for *A. leptaspis*, Song and Wilbert (1997) provided a very detailed redescription with accurate illustrations, as well as a list of synonyms considering the variation of characteristics, like body outline, split of the left-most transverse cirrus *in vivo*, habitat, the overlooking of the finest frontoventral cirrus and morphometric data. Based on the reports of different populations, the most particular feature of *A. leptaspis*, inter alia, the serrated body outline, no matter less or very conspicuous which is easily observed both on living and fixed specimens is considered stable and diagnostic in the present work (Agamaliev 1974, Song and Wilbert 1997, Song 2003, Jiang *et al.* 2008). Accordingly, *A. caspica* Agamaliev, 1967 and *A. hexeris* Quennerstedt, 1869, which have much less rugged body outlines and no satellite-cirrus should be separated from *A. leptaspis* (Agamaliev 1967, Wu and Curds 1979). *Aspidisca pulcherrima* Kahl, 1932 has almost the same ciliary pattern with *A. leptaspis*, whereas the highly developed spurs, ridges, and the serrated right border at the posterior half portion demonstrate the two forms are not conspecific. Similarly, *A. pulcherrima* var *baltica* Kahl, 1932 and *A. pulcherrima* sensu Tuffrau, 1964 are not regarded as synonyms (Kahl 1932, Tuffrau 1964). For another uncertain synonym *A. tridentata* Dragesco, 1963, it is supposedly impossible that the satellite-like cirrus was ignored by Dragesco (1963), as the original illustration is very precise, therefore, the synonym of this form is excluded temporarily. *A. orthopogon,* which was considered as synonym with doubt has been confirmed as an isolated species recently (Li *et al*. 2008). In conclusion, the left seven forms should remain in the list of synonyms.

Morphogenesis

The investigations on the morphogenetic processes of *A. orthopogon* and *A. leptaspis* are the most detailed and reliable, so far, among the other congeners within this genus (Tuffrau 1964, Song 2003). As Song (2003) discussed, the misinterpretation of morphogenesis on *A. costata* about the 3:3:2:2:2 pattern and the origin of AZM_1 fairly exists (Diller 1975). Combined with the present and previous data, *Aspidisca* spp. share most of divisional features, which can be summarized as follows: 1) the oral primordium of the opisthe develops in a sub-surface pouch and will give rise to both portions of membranelles for the opisthe; 2) both parts of parental adoral zone of membranelles are inherited completely by the proter; 3) five frontoventral-transverse cirral anlagen originate from primary primordia; 4) leftmost frontoventral cirrus (namely buccal cirrus in some literatures) in each divider derived from the isolated anlage; 5) dorsal kineties anlagen formed intrakinetally. The variation consists only in the formation mode of frontoventral-transverse cirri (3:3:2:2:2 in *A. leptaspis* and *A. pulcherrima*; 3:3:2:2:1 in *A. costata*, *A. cicada*, *A. lyncaster*, *A. lynceus*, *A. aculeata* and *A. magna*; 3:3:2:3:1 in *A. orthopogon*), which directly lead to the differences in the number and arrangement of frontoventral cirri, namely, seven equally strong frontoventral cirri, or plus one satellite-like cirrus (totally 8), as regard the latter circumstance, the position of which varies between *A. orthopogon* and *A. leptaspis* + *A. pulcherrima* (Summers 1935; Hamm 1964; Tuffrau 1964; Deroux and Tuffrau 1965; Brown 1966; Diller 1975; Dini and Bacchi 1976; Hill 1979; Wang *et al.* 1992, 1997; Song 2003; Li *et al.* 2008). Recently, the phylogenetic positions of *A. magna* and *A. leptaspis* have been studied in the reconsideration of SSU rRNA gene data of the order Euplotida (Yi *et al.* 2009). They were suggested to form a distinct clade, locating within the genus *Aspidisca* and clustering together with *A. orthopogon* as a sister branch. Therefore, the comparatively conservative ontogenetic pattern also supports the genus *Aspidisca* representing a monophyletic lineage clearly separated from other euplotids.

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