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Systematic Analyses of the Genus *Architricha* and *Pleurotricha curdsi* (Ciliophora, Oxytrichidae), with Redescriptions of Their Morphology

Yuan XU [1], Lifang LI[2], Xinpeng FAN[3], Hongbo PAN[4], Fukang GU[3], Saleh A. AL-FARRAJ[5]

[1] State Key Laboratory of Estuarine and Coastal Research, East China Normal University, Shanghai 200062, China; [2] Marine College, Shandong University at Weihai, Weihai, 264209, China; [3] School of Life Sciences, East China Normal University, Shanghai 200062, China; [4] College of Fisheries and Life Science, Shanghai Ocean University, Shanghai, 201306, China; [5] Zoology Department, College of Science, King Saud University, Riyadh 11451, Saudi Arabia

Abstract. Two oxytrichids *Architricha indica* Gupta *et al*., 2006 and *Pleurotricha curdsi* (Shi *et al*. 2002) Gupta *et al*., 2003 collected in East China, were studied using live observation and the silver staining method. The description and morphometric characterization of the new populations were supplied. The Shanghai population of *A. indica* differs from the Indian population in the number of cirri in the third right marginal row (average of 16.8 vs. 21.1). The Shanghai population of *P. curdsi* corresponds well with the Indian population, but it differs from the other Chinese population in the number of right marginal rows (two vs. three). The early process of reorganization of *A. indica* was studied, and a difference on the formation of anlage V was found compared to the original report. The small subunit rRNA genes of both species were sequenced for the first time. The phylogenetic analyses based on SSU rRNA gene sequence data revealed that *Architricha* is sister to the assemblage of *Pseudouroleptus caudatus* and two *Strongylidium*, while *P. curdsi* clusters with its congener *P. lanceolata* and is located in Stylonychinae.

Key words: *Architricha indica*, morphology, oxytrichids, *Pleurotricha curdsi*, phylogeny, SSU rRNA gene.

INTRODUCTION

Hypotrichous ciliates display a diverse range of morphology resulting in challenging and unsolved systematic problems. While detailed morphological characteristics and morphogenesis events have improved the situation to a great extent (Foissner *et al*. 2002; Berger 2006; Shao *et al*. 2008a, b), problems still exist regarding population variations and different systematic classification ideas. In recent years, molecular analyses using marker genes have given rise to new evolutionary clues and hypotheses regarding this group, which have gradually proved to have a positive effect on modifying taxonomists' subjective judgments (Foissner and Stoeck 2011, Küppers *et al*. 2011, Chen *et al*. 2013a, Modeo *et al*. 2013, Kumar *et al*. 2014).

The family Oxytrichidae contains nearly 40 genera and over 200 species, usually having 18 fronto-ventral-transverse cirri (Berger 1999, 2011, Lynn 2008, Shao *et al*. 2015). Although knowledge has been accumulated regarding their morphology, ontogenesis and gene sequences, the evolution and classification of this group

Address for correspondence: Xinpeng FAN, School of Life Sciences, East China Normal University, Shanghai 200062, China; E-mail: xpfan@bio.ecnu.edu.cn

remain highly controversial, calling for more integrated data combining molecular information and ontogenesis (Chen *et al*. 2013b, Lv *et al*. 2013, Jung *et al*. 2015).

Architricha and *Pleurotricha*, as well as *Coniculostomum*, *Onychodromopsis*, *Ponturostyla*, *Allotricha*, and *Parurosoma* are oxytrichids that have more than one marginal cirri row on each side. Such oxytrichids were placed in separated families by Berger (1999) and Lynn (2008), but they were assigned to the family Pleurotrichidae by Shi (2000), Song (2001) and Wang *et al*. (2002). However, these assignments were never confirmed by molecular evidence, as the gene sequences of these genera, i.e. *Architricha*, *Coniculostomum*, and *Parurosoma* were missing (Petz and Foissner 1996, Shi *et al*. 2002, Gupta *et al*. 2003, 2006).

Based on populations collected in East China, the present paper provides morphological redescriptions of *Architricha indica* and *Pleurotricha curdsi*, and supplies the phylogenetic position of the genus *Architricha*, based on SSU rRNA gene sequences, for the first time.

MATERIALS AND METHODS

Sampling and morphological methods. *Architricha indica* and *Pleurotricha curdsi* were collected from a small eutrophic pond in Changfeng Park (31°13′N; 121°24′E), Shanghai, China, where the water temperature was 16°C. The upper 2 cm layer of sediment on the edge of the pond, including rotten leaves and mud, was collected together along with freshwater. The targeted cells were then picked out from the field sample using a fine pipette, and transferred to a Petri dish containing boiled water from the sample site in order to set up a uniprotistan culture that was not from a single clone. To enrich bacterial food, some crushed wheat was added to the culture (Chen *et al*. 2013a). Cells isolated from the culture were used for live observation and protargol staining (Wilbert 1975). Drawings of stained specimens were made with the help of a camera lucida. Measurements were made under 100–1250 × magnification. To illustrate changes during the regenerative processes, parental cirri were depicted in contour. Voucher slides of *A. indica* and *P. curdsi* with protargol-impregnated specimens were deposited in the Laboratory of Protozoology, School of Life Sciences, East China Normal University, Shanghai, China with registration numbers fxp20120607-01 and fxp20120321-01 respectively. Terminology and systematics are according to Berger (1999, 2011) and Lynn (2008).

DNA extraction and sequencing. Total genomic DNA was extracted using a DNeasy Tissue kit (Qiagen, Valencia, CA) according to the methods described by Gao *et al*. (2014). TaKaRa ExTaq (TaKaRa, Otsu, Japan) was used to amplify the SSU rRNA gene using the universal oligonucleotide primers (forward 5'-AACCTGGTTGATCCTGCCAGT-3'; reverse 5'-TGATCCTTCTGCAGGTTCACCTAC-3') designed by Elwood *et al*. (1985) and Medlin *et al*. (1988). Cloning and sequencing were performed as reported in Gao *et al*. (2014).

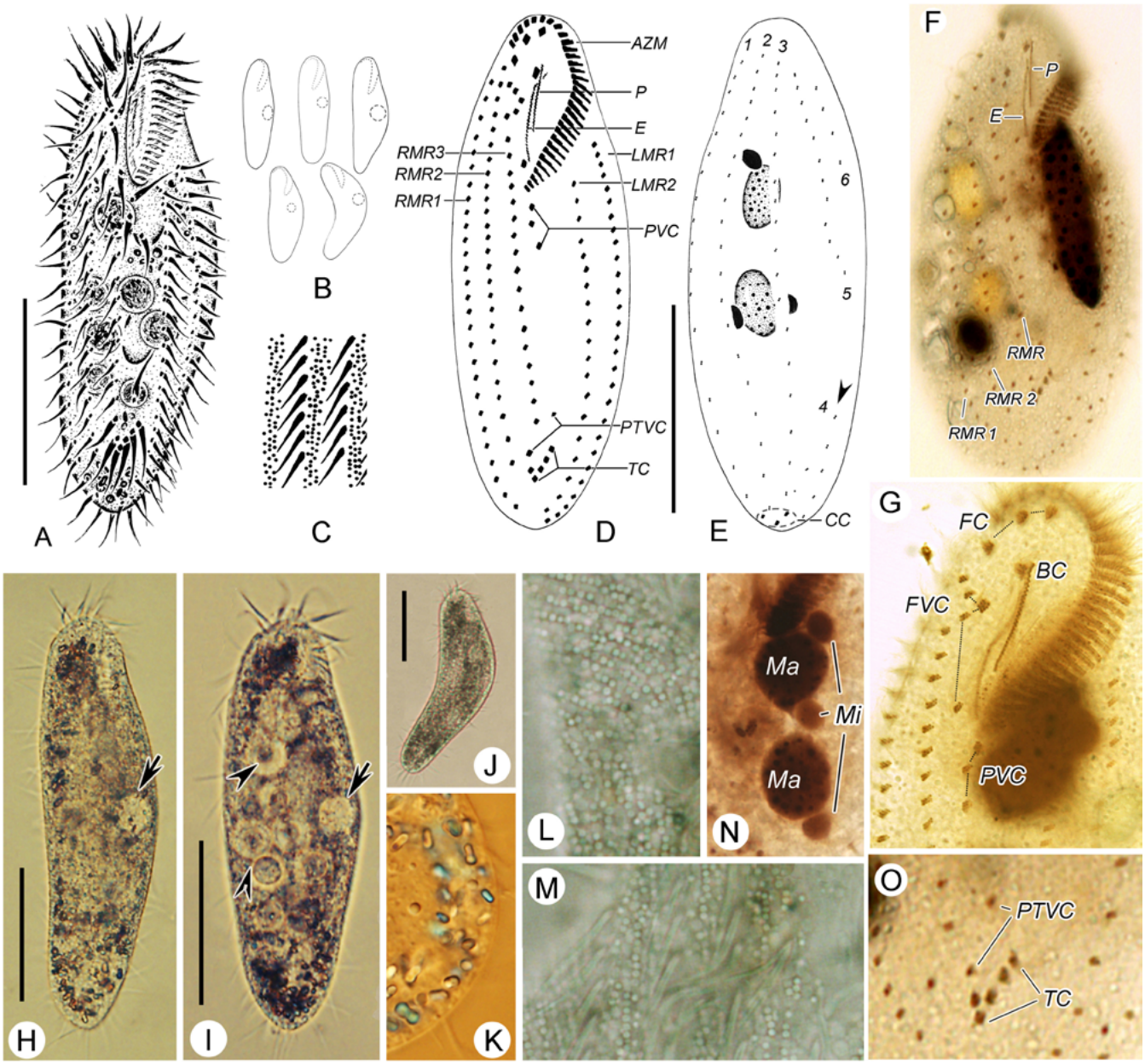
Phylogenetic analyses. The phylogenetic analyses within Oxytrichidae comprised SSU rRNA gene sequences of the two newly sequenced species along with 60 other taxa obtained from the NCBI database (see Fig. 6 for accession numbers). *Parabirojimia similis* and *P. multinucleata* were chosen as the outgroup taxa. Sequences were first aligned using the GUIDANCE algorithm (Penn *et al*. 2010a) with default parameters in the GUIDANCE web server (Penn *et al*. 2010b) and further modified manually using BioEdit 7.0 (Hall 1999). A final alignment of 1,779 characters was used to construct phylogenetic trees using the following methods. Briefly, the maximum likelihood (ML) tree was constructed in PhyML V2.4.4 (Guindon and Gascuel 2003) using a nonparametric bootstrap method with 1,000 replicates, with the best model TrN + I (= 0.5945) + G (= 0.5221), selected by Modeltest v.3.4 (Posada and Crandall 1998). The Bayesian inference (BI) analysis was performed with MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003) using GTR+I+G as the best model selected by MrModeltest v.2.0 (Nylander 2004). Four simultaneous Markov Chain Monte Carlo algorithms were run for 1,000,000 generations, sampling every 100th generation. The first 2,500 trees were discarded as burn-in. The remaining trees were used to calculate the posterior probabilities, applying majority rule consensus.

RESULTS

Family Oxytrichidae

Architricha indica Gupta *et al*., 2006 (Figs 1–4 and Table 1)

Morphological description of Shanghai population. Cells *in vivo* measuring 100–140 µm × 30–40 µm. Body flexible, usually elongated ellipsoid in outline (Fig. 1A, B, H–J). Adoral zone of membranelles (AZM) not prominent, occupying 1/4–1/3 body length, with widest part of membranelles about 15 µm (Fig. 1A, B). Thin Pellicle, with colorless, round cortical granules, 0.5 µm in diameter, on both ventral and dorsal sides: on ventral side, arranged in lines alongside the marginal cirral rows; and on dorsal side, spread evenly in rows (Fig. 1C, L, M). Several food vacuoles about 10 µm across often present (Fig. 1A, I). Single contractile vacuole located in equatorial region near left body margin, approximately 12 µm across, contracting at intervals of about 20 s; collecting canals undetectable (Fig. 1H, I). Colorless cytoplasm, usually with many lipid droplets and dumbbell-like crystals, up to 8 µm long (Fig. 1K). Two oval macronucleus nodules, each nodule about 10 × 6 µm in size, located at mid-body; usually two to six micronuclei, seven or eight in a few individuals (Fig. 1E, N). Infraciliature as shown in Fig. 1D–G, O. AZM with 28 membranelles on average. Straight and short Paroral membrane, longer endoral membrane

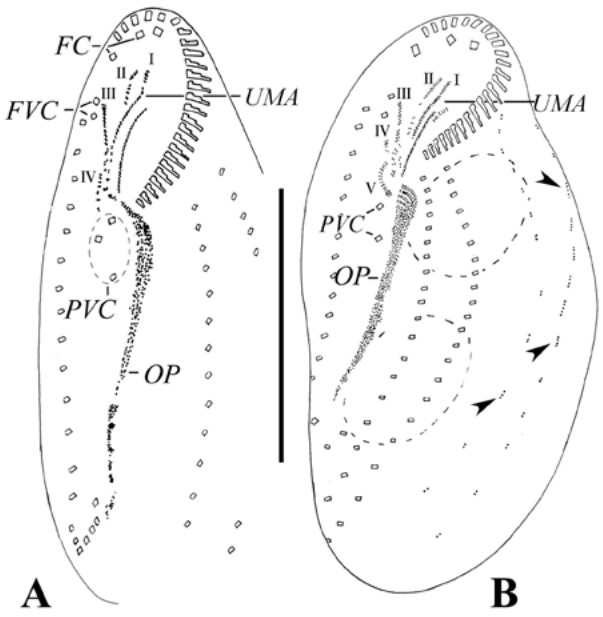


Figs 1A–O. Line drawings and photomicrographs of *Architricha indica* during trophophase from life (A–C, H–M) and after protargol impregnation (D–G, N, O). **A, H–J** – ventral views of typical individuals, arrows in (H, I) indicate the contractile vacuole and arrowheads in (I) refer to the food vacuoles; **B –** different body shapes; **C –** distribution of cortical granules between right marginal rows; **D, F** – ventral view of infraciliature; **E** – arrangement of dorsal ciliature, caudal cirri and nuclei, arrowhead indicates the anteriorly shortened dorsal kinety 4; **G, O** – details of infraciliature, showing frontal cirri, buccal cirrus, frontal-ventral cirri, and postoral ventral cirri in (G), and showing pretransverse ventral cirri and transverse cirri in (O); **K** – detail of crystals in the cytoplasm; **L, M** – cortical granules on dorsal (L) and ventral (M) side; **N** – macronuclei and micronuclei. 1–6 – dorsal kineties 1–6, AZM – adoral zone of membranelles, BC – buccal cirrus, CC – caudal cirri, E – endoral membrane, FC – frontal cirri, FVC – frontal ventral cirri, LMR1, 2 – left marginal row 1 and 2, P – paroral membrane, PVC – postoral ventral cirri, PTVC – pretransverse ventral cirri, RMR1, 2, 3 – right marginal row 1, 2 and 3, TC – transverse cirri. Scale bars: 50 µm.

Table 1. Morphometric characterization of *Architricha indica* (upper line) and *Pleurotricha curdsi* (lower line). All data are based on protargol-impregnated specimens. Measurements in µm. AZM – adoral zone of membranelles, CV – coefficient of variation in %, FVT – frontoventral-transverse, LMR 1, 2 – left marginal rows 1 and 2, Max – maximum, Mean – arithmetic mean, Min – minimum, n – number of individuals examined, n – number of specimen examined, no. – number, RMR 1, 2, 3 – right marginal rows 1, 2 and 3, SD – standard deviation.

Character	Min	Max	Mean	SD	CV	n
Body, length	74	200	153.0	29.4	19.2	18
	132	220	169.6	20.7	12.2	21
Body, width	28	77	59.7	11.8	19.8	18
	63	100	81.3	11.8	14.5	21
AZM, length	43	67	52.4	7.7	14.7	18
	58	103	81.8	10.8	13.2	21
Frontal cirri[a], no.	8	8	8.0	0	0	18
	9	9	9.0	0	0	19
Ventral cirri, no.	5	5	5.0	0	0	18
	6	8	6.2	0.5	8.6	19
Transverse cirri, no.	5	5	5.0	0	0	18
	5	6	5.1	0.3	6.2	19
FVT cirri, no.	18	18	18.0	0	0	18
	20	22	20.2	0.6	2.8	19
Membranelles, no.	23	33	28.3	2.5	8.9	16
	44	63	51.7	5.7	11.0	24
Cirri in LMR 1, no.	22	29	25.8	2.1	8.1	16
	15	26	22.2	2.6	11.9	23
Cirri in LMR 2, no.	17	23	19.3	1.9	9.8	16
	–	–	–	–	–	–
Cirri in RMR 1, no.	22	29	25.6	2.3	8.9	16
	21	36	27.7	3.5	12.5	23
Cirri in RMR 2, no.	21	29	24.3	2.4	10.0	16
	5	22	14.8	4.4	29.8	22
Cirri in RMR 3, no.	14	21	16.8	2.0	11.6	16
	–	–	–	–	–	–

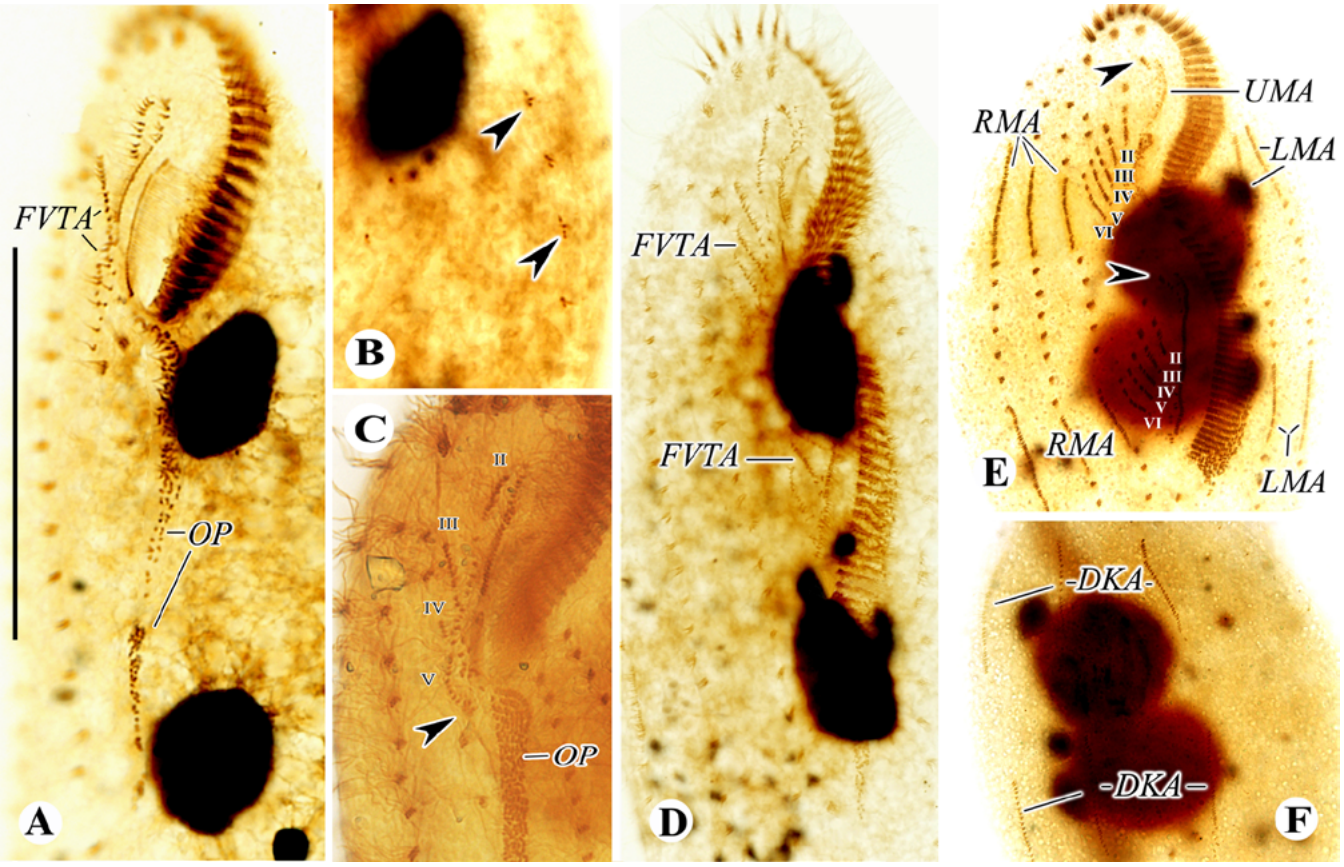
[a] Data include buccal cirrus and frontoventral cirri.



Figs 2A, B. Line drawings of protagol stained cells of *Architricha indica* during early stages of reorganization. **A, B** – ventral views, showing the formation of the oral primordium, I–V anlagen for the frontal-ventral-transverse cirri, and appearance of undulating membranes anlage, arrowheads in (B) indicate anlagen of dorsal kineties. FC – frontal cirri, FVC – frontal ventral cirri, I–V – frontoventral-transverse cirral anlagen I–V, OP – oral primordium, PVC – postoral ventral cirri, UMA – undulating membranes anlage. Scale bar: 50 µm.

gently curved underneath. Three frontal cirri and four frontal ventral cirri. Buccal cirrus located closely to the anterior end of paroral membrane. Ventral cirri in two groups: three postoral ventral cirri arranged in line just beneath the level of posterior end of AZM and two pretransverse ventral cirri located dispersedly near the end of right marginal row 1 and before the transverse cirri. Three dorsal kineties (first to third) extending over entire length of body. Fourth dorsal kinety very short and located near posterior end of body, comprising only several dikinetids. Fifth and sixth dorsal kinety occupying anterior half of body. Three caudal cirri located near posterior end of first, second, and fourth dorsal kineties.

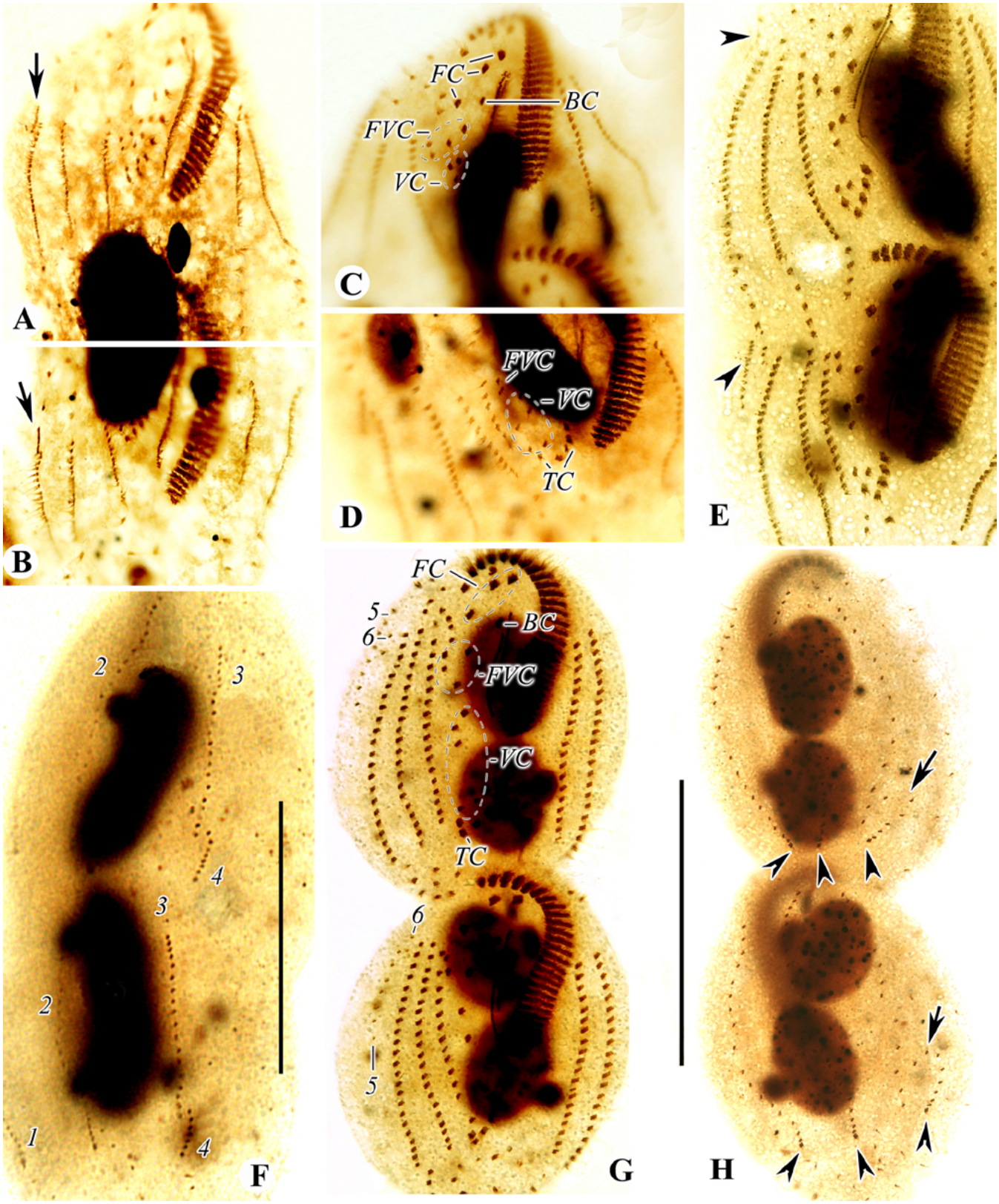
Reorganization. Reorganization commences with the closely arranged basal bodies to form the oral primordium (OP), which occurs *de novo* comprising two narrow basal body groups; at the same stage, cirrus II/2 (buccal cirrus), cirrus III/2, and cirrus IV/3 disintegrate and join in the construction of frontoventral-transverse cirral anlagen II, III, and IV. Dorsal kineties begin to duplicate around the parental basal bodies (Figs 2A, 3A, B). Then, cirrus IV/2 disintegrates and elongates anteriorly to form anlage V, while OP begins to differentiate into membranelles when its anterior part touches the posterior end of parental adoral membranelles; paroral and endoral membranes reorganize at original locations, forming new structures (Figs 2B, 3C).



Figs 3A–F. Photomicrographs of protagol stained cells of *Architricha indica* during regenerative (A–C) and morphogenetic stages (D–F). **A, B** – ventral (A) and detailed dorsal (B) view of cell during early stage of reorganization, showing the formation of oral primordium (OP), the frontoventral-transverse cirral (FVT) anlagen and dorsal kineties anlagen (arrowheads); **C** – anterior part of ventral side of another regenerating cell, showing the anlage V arose from the cirrus IV/2 (arrowhead); **D** – ventral view, showing the six streaks of FVT anlagen; **E, F** – ventral (E) and dorsal (F) detailed view, showing the FVT anlagen dividing into fragments, the dedifferentiation of parental undulating membranes and the origin of multiple marginal rows in (E) and showing the anlagen for dorsal kineties in (F), arrowheads showing the new frontal cirri that came from the undulating membranes anlage. DKA – dorsal kinety anlagen, FVTA – frontoventral-transverse cirral anlagen, I–V – FVT I to V, LMA – left marginal row anlagen, OP – oral primordium, RMA – right marginal anlagen, UMA – undulating membranes anlage. Scale bars: 50 µm.

Morphogenesis. A detailed description of the morphogenesis was reported in the original report (Gupta *et al*. 2006), thus only a brief description based on the Shanghai population is documented here.

The earliest stage observed was the presence of six frontal-mid-ventral-transverse cirral (FVT) anlagen in both opisthe and proter (Fig. 3D). As division progresses, six FVT anlagen keep continue developing, each dividing into fragments (Fig. 3E). In the following stages, the fragments migrate into relative positions and form new frontal cirri, ventral, and transverse cirri, following the typical *Oxytricha* pattern (Fig. 4C–E, G). Undulating membranes of the proter derives from parental structure, and from the OP in opisthe (Fig. 3E). AZM of the opisthe develops anew from the OP and the proter inherits the parental one (Figs 3D, 4E, G). In both dividers, five anlagen arise independently within five parental marginal rows and finally give birth to five new marginal rows (Figs 3E, 4A, B, E). Dorsal kineties (DK) develop with the *Oxytricha* pattern. DK1–3 arise from anlagen and develop separately within parental DK1–3 (Fig. 4F, H). The anlage of DK 3 generates an additional fragment at the posterior part, which generate DK 4 (Fig. 4F, H). Three caudal cirri are generated


A
B
C
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FVC
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at the posterior end of the new DK1, 2, and 4 (Fig. 4H). The anlagen for dorsal kineties 5 and 6 (=dorsomarginal kineties) develop *de novo* near the rightmost marginal row anlage (Fig. 4A, B, E, G).

***Pleurotricha curdsi* (Shi *et al*. 2002) Gupta *et al*., 2003 (Fig. 5 and Table 1)**

This species was originally described by Shi *et al*. (2002) as a member of *Allotricha*. Gupta *et al*. (2003) reinvestigated it and transferred it to the genus *Pleurotricha*. As both of the previous reports documented the morphology and morphogenesis, only a brief description based on the new population is supplied here.

Morphological description of Shanghai population. Cells *in vivo* about 120–230 µm × 50–110 µm, broadly ellipsoid with rigid pellicle; left side straight, right side convex; posterior end tapering to right (Fig. 5A–C, F). AZM conspicuous, occupying nearly half of body length; distance between anterior body end and distal end of AZM about 25–32 µm (Fig. 5D, E). Cytoplasm containing great number of oil granules, ranging 5–12 µm across, and some large food granules, over 20 µm in length (Fig. 5A–C). No cortical granules. Contractile vacuole about 20 µm in diameter when fully extended (Fig. 5G). Two macronuclei, two or three (rarely four) micronuclei (Fig. 5N). Three frontal cirri, five frontoventral cirri and single buccal cirrus (Fig. 5H, I). Six to eight ventral cirri and five (rarely six) transverse cirri, arranged in two groups of three and two cirri (rarely four and two) (Fig. 5H–K). Frontal cirri about 17–20 µm long, transverse cirri around 22 µm long, ventral cirri about 14–16 µm long, left and right marginal cirri about 13–14 µm long. Cilia of dorsal kineties about 2.5 µm (Fig. 5G). Single left marginal row and two right marginal rows (Fig. 5H). Six dorsal kineties and three caudal cirri (Fig. 5L, M).

Phylogenetic analyses (Figs 6)

The SSU rRNA gene sequences of *Architricha indica* (1,771 nucleotides, 45.06% GC content) and *Pleurotricha curdsi* (1,768 nucleotides, 44.91% GC content) are available under the GenBank accession number KJ000536 and KJ000535. The GC contents are also in the same range as other ciliates.

The phylogenetic analyses based on the SSU rRNA gene with ML and BI showed a basically congruent topology and, thus, they were combined into a single tree using the ML topology for clarity of presentation. As shown in Fig. 7, the family Oxytrichidae was polyphyletic containing several separate clades. *Architricha indica* clustered first, with the clade comprising members of Kahliellidae and Spirofilidae (ML/BI, 79/1.00), and then formed a sister branch to *Oxytricha elegans* and *Hemiurosoma terricola* with relatively low support (ML/BI, 41/0.91). *Pleurotricha curdsi* grouped with two strains of *P. lanceolata* with moderately high support (ML/BI, 82/1.00).

DISCUSSION

Morphological and morphogenetic remarks on *Architricha indica* and *Pleurotricha curdsi*

According to Gupta *et al*. (2006) *Architricha* is monotypic. Compared to the original report, the Shanghai population shows correspondent morphological characters, except for that it has fewer cirri in third right marginal row (average of 16.8 vs. 21.1). Only the middle and late stages of morphogenesis were found in the new population, displaying a consistent ontogenesis pattern, especially the formation of marginal rows, with that of the population reported by Gupta *et al*. (2006). Two specimens during reorganization newly found revealed that the cirri II/2, III/2, and IV/3 give rise to anlagen II, III, and IV, which corresponds well with the formation of the same anlagen in proter during morphogenesis. However, the new finding that cirrus IV/2 gives rise to anlage V in regenerative cells differs from

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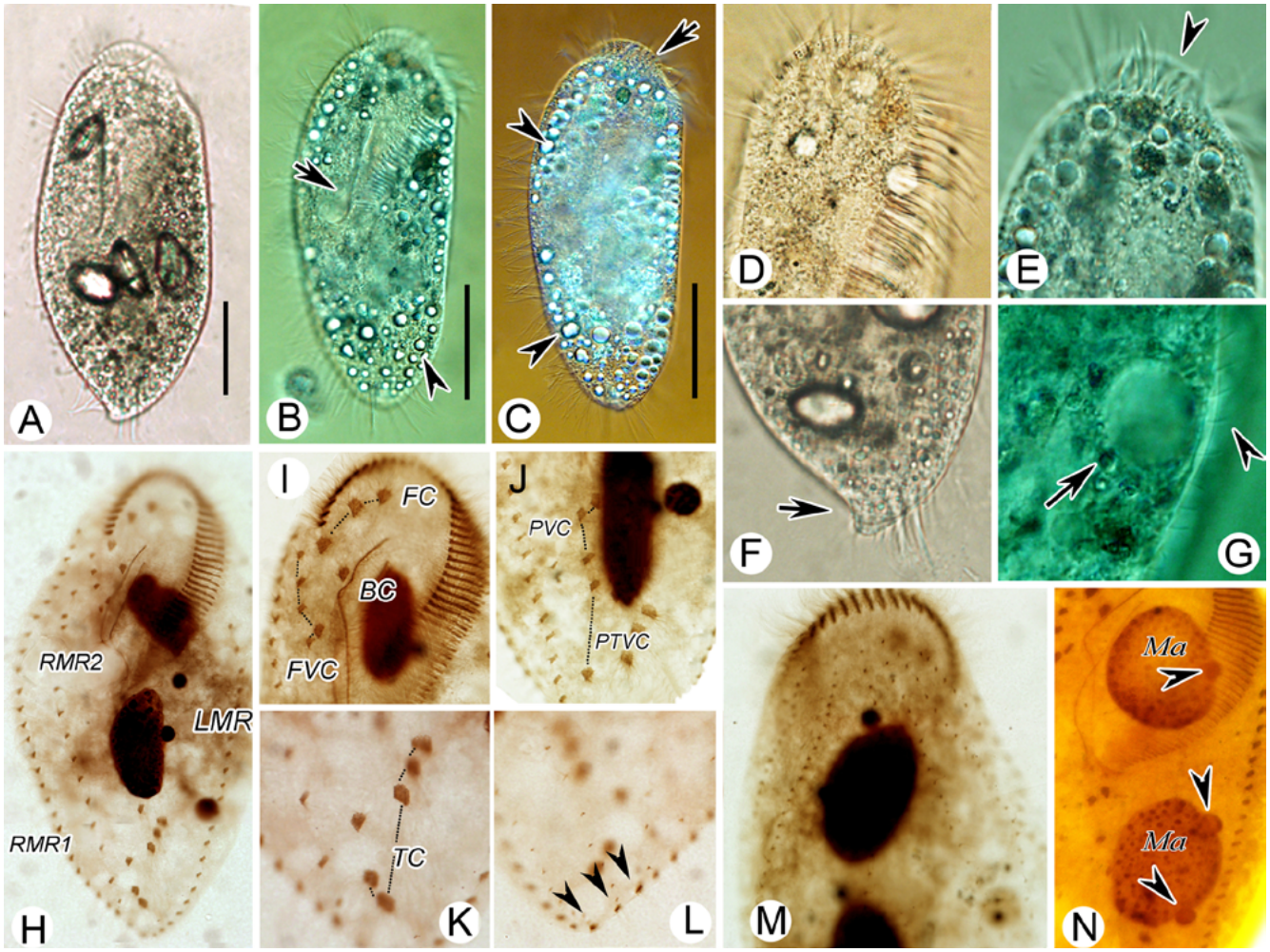
Figs 4A–H. Photomicrographs of protagol stained cells of *Architricha indica* during morphogenesis. **A, B** – ventral view, showing the anlagen of dorsmarginal kineties in both proter and opisthe (arrows); **C, D** – ventral view, showing the migration of new frontal cirri, ventral cirri and transvese cirri; **E, F** – ventral (E) and dorsal (F) view, showing the proliferation of dorsmarginal kinety anlagan (arrowheads) in (E) and showing the development of dorsal kinety anlagan and the formation of dorsal kinety 4 in (F); **G, H** – ventral (G) and dorsal (H) view, showing the development of dorsal kinety anlagen 5 and 6 (=dorsomarginal kineties) and the location of new frontal cirri, ventral cirri, and transverse cirri in (G), showing dorsal kinety 4 separating from dorsal kinety 3 (arrows) and the formation of caudal cirri at the ends of new dorsal kineties 1, 2 and 4 (arrowheads). 1–6 – dorsal kineties 1–6, BC – buccal cirri, FC – frontal cirri, FVC – frontal ventral cirri, TC – transverse cirri, VC – ventral cirri. Scale bars: 50 µm.



Figs 5A–N. Photomicrographs of *Pleurotricha curdsi* from life (A–G) and after protargol staining (H–N). **A** – a typical individual showing body shape and color; **B, C** – ventral (B) and dorsal (C) view, showing the food granules (arrowheads), arrow in (B) indicates the cytostome, arrow in (C) marks the collar part of adoral zone of membranelles (AZM) on the dorsal side; **D** – anterior part, to show the AZM; **E** – anterior part of dorsal side, to show the AZM in the back collar (arrowhead); **F** – posterior portion, to show the tapered posterior end (arrow); **G** – showing the contractile vacuole (arrow) and cilia of dorsal kinety (arrowhead); **H** – ventral view to show the single left marginal and two right marginal cirri rows; **I** – to show the cirri in frontal area, noting this specimen owning five frontal ventral cirri and three frontal cirri; **J** – postoral ventral cirri and pretransverse and cirri; **K** – transverse cirri; **L** – caudal cirri (arrowheads); **M** – anterior part of dorsal kineties; **N** – macronuclei and micronuclei (arrowheads). BC – buccal cirrus, FC – frontal cirri, FVC – frontal ventral cirri, LMR – left marginal row, Ma – macronucleus, PTVC – pretransverse ventral cirri, PVC – postoral ventral cirri, RMR 1, 2 – right marginal row 1 and 2, TC – transverse cirri. Scale bars: 50 µm.

the finding that during division, anlage V arises from the cirrus V/4 (Gupta *et al*. 2006). It is accepted that reorganization and ontogenesis share the same origin pattern in each anlage (Berger 1999, Hu and Song 2001). Thus, the difference mentioned above needs to be verified with a complete collection of regenerative stages.

Pleurotricha curdsi was originally assigned to the genus *Allotricha* by Shi *et al*. (2002) mainly because it was mis-observed as having a soft body, and at that time, *Pleurotricha* was not clearly diagnosed based on the presence of caudal cirri. Later, Gupta *et al*. (2003) supplied a redescriptions of this species based on an Indian population and transferred it to the genus *Pleurotricha*. Most of our morphometric data overlap with the data reported by Shi *et al*. (2002) and Gupta *et al*. (2003) (Table 2). The only minor difference is the num-

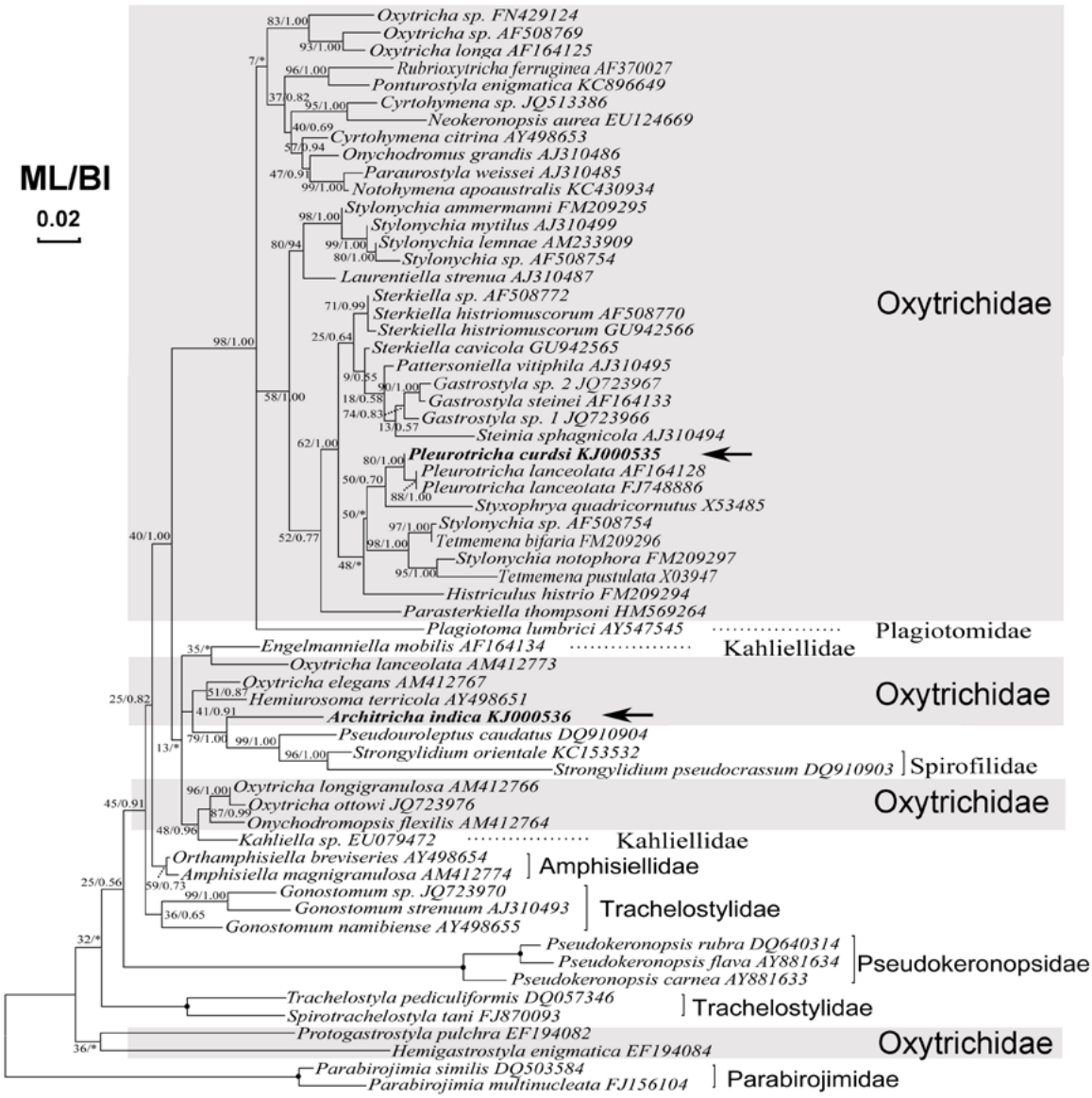


Fig. 6. Maximum likelihood tree inferred from SSU rRNA gene sequences, showing the position of *Architricha indica* and *Pleurotricha curdsi* (bold). Newly sequenced species are marked by arrows. Nodal support for branches in the ML and BI trees are marked in order. "*" indicates bootstrap value disagreement between the ML tree and the reference BI tree at a given node. Black circles indicate full support in all analyses. Bar, 2 substitutions per 100 nucleotide positions. *Parabirojimia similis* and *Parabirojimia multinucleata* are the out group taxa.

ber of right marginal rows (two in the current study vs. three reported by Shi *et al*. 2002). However, according to Gupta *et al*. (2003), this difference is due to the development of right marginal anlage. Therefore, these populations are conspecific.

According to Gupta *et al*. (2003), the type species of the genus, *P. lanceolata*, also has three caudal cirri and it resembles *P. curdsi* in general appearance. Based on our findings and two other populations of *P. curdsi* (Gupta *et al*. 2003, Shi *et al.* 2002), the difference between *P. curdsi* and *P. lanceolata* is mainly the number of FVT cirri (20–22 vs. 17 or 18).

Phylogeny of *Pleurotricha curdsi* and *Architricha*

Previous studies (e.g., Schmidt *et al*. 2007, Chen *et al*. 2013a, b, Lv *et al*. 2013, Kumar *et al*. 2014) have

Table 2. Morphometric comparison among populations of *Pleurotricha curdsi.* All data are based on protargol-impregnated specimens. Measurements in µm. AZM – adoral zone of membranelles, CC – caudal cirri, DK – dorsal kineties, FVT – frontoventral-transverse, LMR – left marginal row, no – number, RMR – right marginal rows.

Characteristics	Present work	Gupta *et al*. 2003	Shi *et al*. 2002
AZM, no.	44–63	46–53	53–61
Frontal cirri[a], no.	9	9	9 or 10
Ventral cirri, no.	6–8	6–7	6–7
Transverse cirri, no.	5 or 6	5 or 6	5
FVT cirri, no.	20–22	20	–
LMR, no.	1	1	1
RMR, no.	2	2	3
DK, no.	6	6	6
CC, no	3	3	3

[a] Data include buccal cirrus and frontoventral cirri.

found that the family Oxytrichidae is not monophyletic, which is supported by our studies. The new population of *Pleurotricha curdsi* clusters well with its congener *P. lanceolata*, the type species of *Pleurotricha*, which validates the accuracy of the transfer based on morphological evidence (Gupta *et al*. 2003), and reveals that the genus *Pleurotricha* is monophyletic. The genus *Architricha* is represented by the sole species *A. indica*, which has a flexible body and typical *Oxytricha* FVT and dorsal ciliature patterns. Shi (2000) and Song (2001) suggested assigning the oxytrichids with multiple marginal rows (MMR) to the family Pleurotrichidae. However, this idea is not supported by our phylogenetic trees, as the genera *Architricha, Pleurotricha*, *Ponturostyla*, and *Onychodromopsis* which possess MMR distribute in different clades of Oxytrichidae. Gupta *et al*. (2006) pointed out the ontogenesis of MMR in oxytrichids includes four modes: *Architricha* mode, *Pleurotricha* mode, *Coniculostomum* mode and *Ponturostyla* mode. But, unfortunately, the topology of the phylogenetic trees does not reflect the relationship of these modes either, For example, *Pleurotricha* and *Onychodromopsis* share the *Pleurotricha* mode, but they were quite far apart in the tree. Gupta *et al*. (2006) also indicated that *Architricha* mode, i.e. five marginal rows generating from five independent primordia, is plesiomorphy of these four modes. However, this opinion is not supported by our phylogenetic trees, as the clade including *Architricha* forms a sister branch to the clade including *Onychodromopsis*. The reason might be that the SSU rRNA gene cannot reflect the evolutionary history of the marginal rows. Considering that recent studies have gradually demonstrated that even very reliable features might evolve convergently, in order to fully understand the systematic position of the members in Oxytrichidae and their closely related taxa, a large sampling range based on multiple gene analyses, along with exploration of morphogenesis patterns, are greatly needed in further studies (Gupta *et al*. 2006,Schmidt *et al*. 2007, Foissner and Stoeck 2008, Jung *et al*. 2014).

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