

Morphology and molecular phylogeny of *Aegyria foissneri* sp. n. and *Lynchella minuta* sp. n. (Ciliophora, Cyrtophoria) from brackish waters of southern China

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Abstract

The morphology, ciliary pattern and small subunit rDNA (SSU rDNA) sequences of two new cyrtophorian ciliates, *Aegyria foissneri* sp. n. and *Lynchella minuta* sp. n., isolated from brackish waters in southern China, were investigated. *Aegyria foissneri* sp. n. is characterized as follows: cell size $85\text{--}170 \times 45\text{--}80 \mu\text{m}$ in vivo; body inverted oval with a protrusion and a dark pigment spot on anterior left part; 42–77 somatic kineties; one preoral and three to six circumoral kineties; five to eight transpodial segments; 31–44 nematodesmal rods; 12–16 contractile vacuoles; and single oval macronucleus. *Lynchella minuta* sp. n. is distinguished from its congeners by having a cell size of $20\text{--}30 \times 15\text{--}20 \mu\text{m}$ in vivo, oval body outline; four preoral and 14 or 15 postoral kineties, three circumoral kineties; ca. 11 nematodesmal rods; one finger-like tentacle on the ventral side; and two diagonally located contractile vacuoles. Molecular phylogenetic analyses support the genus assignment of *Aegyria foissneri* sp. n. and indicate the monophyly of the genus. While *Lynchella minuta* sp. n. clusters with *Coeloperix* species, which indicates that *Lynchella* is non-monophyletic.

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Keywords: *Aegyria*; Cyrtophorians; *Lynchella*; Morphology; SSU rDNA

Introduction

The cyrtophorians are a highly specialized and divergent group of ciliates, which can be found in a wide variety of biotopes, such as soil, freshwater, brackish water and marine habitats (Deroux 1970; Gong and Song 2009; Gong et al.

2008; Kahl 1931; Song and Wilbert 1989). There is still some confusion in terms of species separation and identification. The reasons for this are: (i) some useful living features for species circumscription (e.g., contractile vacuole, pellicular structure) are either insufficiently described or unreported in many early studies; (ii) many species descriptions are based only on living observations, and thus the exact kinety patterns are unavailable; and (iii) intraspecific variations and interspecific similarities have not been considered seriously

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(Song and Wilbert 2002). Therefore, extensive investigations with modern methods are needed.

During the past twenty years more than 40 cyrtophorian ciliates, including new taxa and little-known species, have been described (e.g., Gong and Song 2004; Pan et al. 2012, 2013, 2016; Qu et al. 2015a,b,c; Shao et al. 2008; Song and Packroff 1997; Xu et al. 2016), supporting the hypothesis that the species diversity of this taxon was greatly underestimated. At the same time, phylogenetic relationships within the Ciliophora were also revealed based on small subunit rDNA (SSU rDNA) sequences and other molecular data (e.g., Chen et al. 2016; Gao et al. 2012, 2016; Snoeyenbos-West et al. 2004; Yi et al. 2016; Zhang et al. 2014).

During a survey of ciliates in the brackish waters of southern China, two unusual cyrtophorian species were isolated. Subsequent investigations on the morphology and small subunit rDNA sequence demonstrate that they represent two new species.

Material and Methods

Sample collection, observations and identification

The species were isolated from Guangdong province, southern China (Fig. 1). *Aegyria foissneri* sp. n. was collected from a mangrove wetland in Zhanjiang (21°22'27"N, 110°24'49"E; Fig. 1B) on 5 December 2013 (water temperature ca. 24.8 °C, pH ca. 6.7, and salinity ca. 17.0‰). *Lynchella minuta* sp. n. was isolated from water from a sandy beach of an estuary of the Pearl River in Zhuhai (22°15'48"N, 113°35'00"E; Fig. 1A) on 26 May 2014 (water temperature ca. 29.2 °C, pH ca. 7.9, and salinity ca. 2.5‰).

Sample treatment was in accordance with that described previously (Pan et al. 2015). In short, samples were treated immediately or maintained in the laboratory in Petri dishes at room temperature of 20–25 °C, with rice grains added to the in situ water to enrich bacterial food. Living cells were observed using a light microscope equipped with differential interference contrast (BH-2, Olympus). The protargol preparation method used to reveal the ciliary pattern and nucleus apparatus basically follows the protocol by Foissner (2014). Measurements were made at a magnification of 1000×. Terminology and classification follow Lynn (2008).

DNA extraction and gene sequencing

Several cells of *Aegyria foissneri* sp. n. and *Lynchella* sp. n. were isolated and washed from the non-clonal cultures, and then were used for DNA extraction. The DNeasy Tissue Kit (Qiagen, Hilden, Germany) was used to extract genomic DNA following the user instructions. The SSU rDNA was amplified using Q5® Hot Start High-Fidelity DNA Polymerase (Cat. #M0493L, New England Biolabs Inc., USA)

with universal primers (Medlin et al. 1988). Cloning and sequencing were performed as described by Chen et al. (2016).

Phylogenetic analyses

Other than the SSU rDNA sequences of *Aegyria foissneri* sp. n. (KX364493) and *Lynchella minuta* sp. n. (KX364494), 66 sequences used in this paper were obtained from GenBank database (accession numbers as shown in Fig. 5). Six suctorians were selected as out-group taxa, namely *Acineta compressa* (FJ865205), *Discophrya collini* (L26446), *Ephelota gemmipara* (EU600180), *Heliophrya erhardi* (AY007445), *Prodiscophrya* sp. (AY331802), and *Tokophrya lemnae* (AY331720). Sequences were aligned online by MUSCLE on the European Bioinformatics Institute web server (<http://www.ebi.ac.uk>) with default parameters, resulting in a matrix of 1796 characters. Maximum-likelihood (ML) analyses were performed using RAxML-HPC2 version 8.2.4 (Stamatakis et al. 2008) on the CIPRES Science Gateway (Miller et al. 2010) with the model of GTR + I + G being the optimal choice. Support for the best ML tree was from 1000 bootstrap replicates. A Bayesian inference (BI) analysis was carried out under MrBayes 3.2.6 (Ronquist and Huelsenbeck 2003) on the CIPRES Science Gateway using the GTR + I + G as the model (selected by MrModeltest2.2; Nylander 2004). Markov chain Monte Carlo simulations were run with two sets of four chains for 4,000,000 generations and a sample frequency of 100 generations, with the first 25% discarded as burn-in. The rest of the trees were used to calculate posterior probabilities using a majority rule consensus.

Results and Discussions

Order Dysteriida Deroux, 1976

Family Hartmannulidae Poche, 1913

Aegyria Claparède and Lachmann, 1859

Aegyriafoissneri sp. n. (Figs 2A–H, 3A–I; Tables 1, 2)

Diagnosis. Cell size 85–170 × 45–80 μm in vivo; body inverted oval with a protrusion and a dark pigment spot on anterior left part; 42–77 somatic kineties; one preoral and three to six circumoral kineties; five to eight transpodial segments; 31–44 nematodesmal rods; 12–16 contractile vacuoles; single oval macronucleus.

Type locality. Water with algae and decayed leaves from a mangrove wetland in Guandu, Zhanjiang (21°22'27"N, 110°24'49"E; Fig. 1B), PR China. Water temperature ca. 24.8 °C, pH ca. 6.7, and salinity ca. 17.0‰.

Dedication. We dedicate this new species to Prof. Wilhelm Foissner, University of Salzburg, Austria, in recognition of his significant contributions to the taxonomy of ciliates.

Type deposition. A permanent slide with the holotype specimen (marked with a black circle, Figs 2C, D, 3D) and two other slides with paratype specimens were deposited

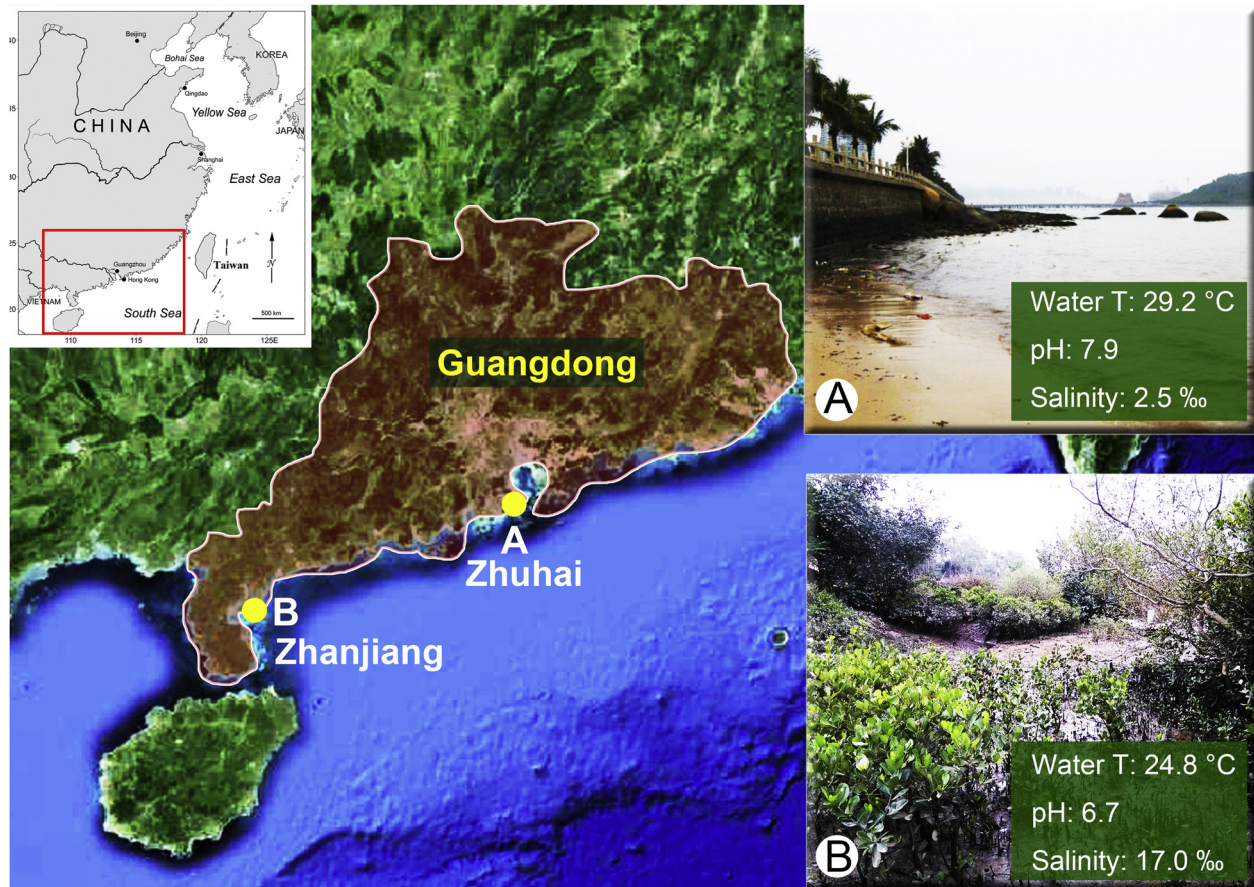


Fig. 1. Sample sites in Guangdong province, southern China. (A) The estuary of the Pearl River, Zhuhai (21°22'27"N, 110°24'49"E; from Qu et al. 2015a). (B) A mangrove wetland in Zhanjiang (22°15'48"N, 113°35'00"E).

in the Laboratory of Protozoology, Ocean University of China, with registration numbers of QZS2013110501-1, QZS2013110501-2, and QZS2013110501-3, respectively.

SSU rDNA sequence. The length (bp), GC content and GenBank accession number of *Aegyria foissneri* sp. n. are 1623, 44.55%, and KX364493.

Description. Cell size 85–170 × 45–80 μm in vivo. Body shape inverted oval, ratio of body length to width ca. 2:1; both right and left margin convex and a distinct protrusion at anterior left portion, with dark spot containing numerous tiny pigments (Figs 2A, E, 3A–C). Cytostome ca. 30 μm wide, supported by 31–44 nematodesmal rods (Figs 2G, 3I). Dorsoventral thickness 35–45 μm; ventral side flat, while dorsal side strongly vaulted (Figs 2B, 3E). A longitudinal groove on dorsal side (Figs 2E, 3C). Macronucleus ellipsoidal and juxtaposed heteromorous, ca. 30 × 20 μm in vivo, centrally located (Figs 2A, D, E, 3G). Micronucleus not detected. 12–16 contractile vacuoles (n=3), up to 4 μm across and with a contraction interval of 20–30 s (Fig. 2F). Contractile vacuole pores difficult to detect after protargol preparation. Podite ca. 8–10 μm long in vivo, located at about 83% of body length (Figs 2A, B, 3B). Cilia ca. 8 μm in vivo. Cytoplasm colorless or grayish and containing many 1–5 μm-sized lipid droplets. Numerous bar-shaped bacteria (1–2 μm long) dis-

tributed along somatic kineties on cell surface, especially on unciliated areas (Fig. 3F). Usually slowly crawling on substrate, sometimes rotating around body axis in water; thigmotactic.

About 42–77 somatic kineties; right-most 12–27 kineties extending onto dorsal surface; all kineties on right side, except for the right-most one, extending to posterior end of cell (Fig. 2C, arrow). Posterior end of kineties left side gradually shortened from right to left; ca. five to eight kineties at posterior end “interrupted” by podite and hence forming a cilia-barren area, leaving five to eight transpodial segments (Figs 2C, 3D). Terminal fragment composed of ca. 35 basal bodies, positioned in left portion of frontal end of cell on dorsal side (Fig. 2D).

Oral ciliature consisting of three to six circumoral kineties and one preoral kinety (Figs 2H, 3D, I). Circumoral kineties almost in parallel with cytostome and positioned right ahead of cytostome; preoral kinety positioned left ahead of cytostome.

Comparison. *Aegyria* was established by Claparède and Lachmann (1859) but no type was specified. For a long time *Aegyria* was thought to be a synonym of *Dysteria* Huxley, 1857 (Deroux 1974; Kahl 1931). Deroux (1974) established *Aegyriana* and assigned *Aegyriana oliva* (basonym *Aegyria*

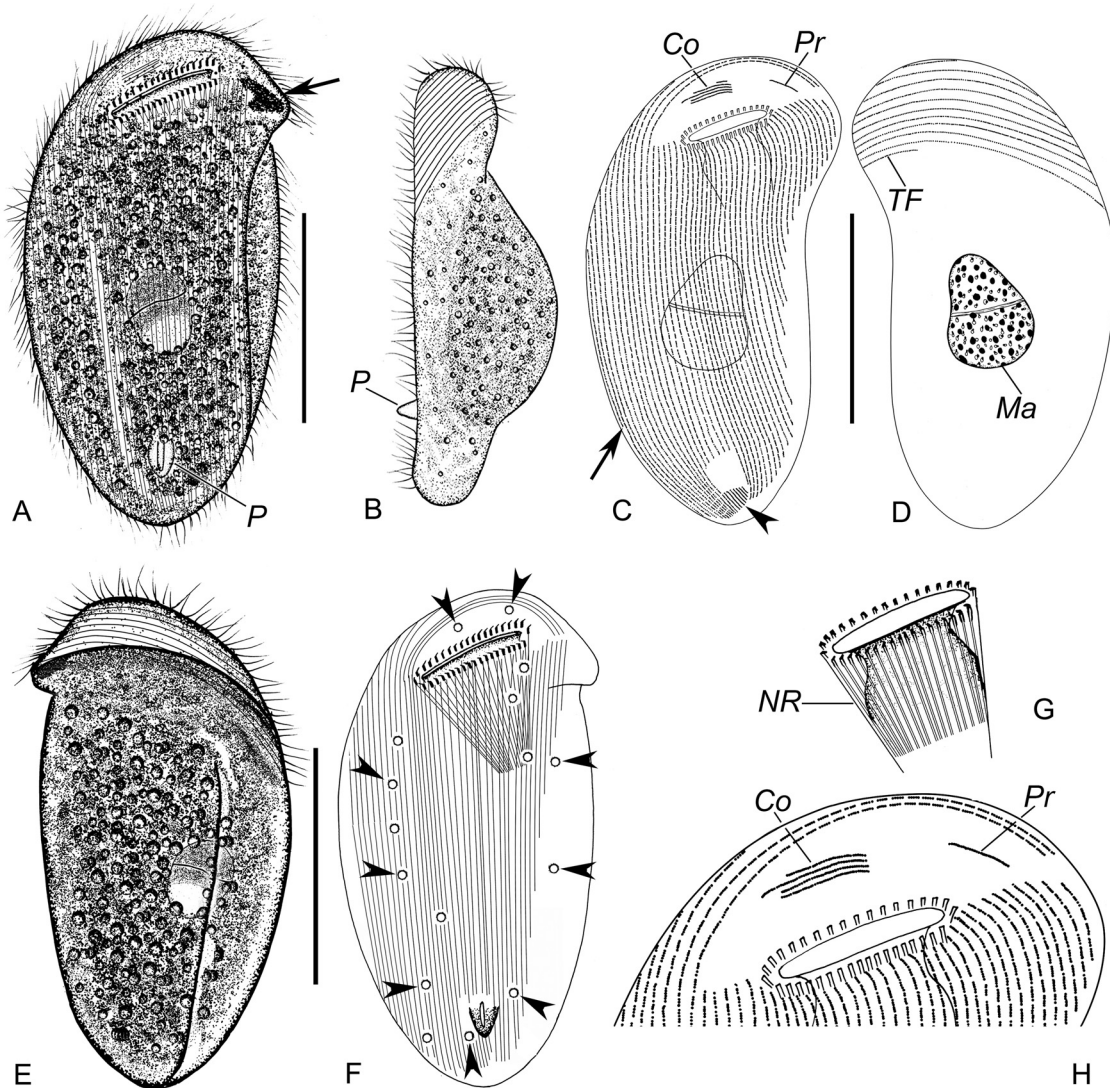


Fig. 2. *Aegyria foissneri* sp. n. from life (A, B, E, F) and after protargol preparation (C, D, G, H). (A) Ventral view, arrow shows the pigment spot in the protrusion. (B) Left lateral view, showing the vaulted dorsal side. (C, D) Ventral and dorsal view of the holotype, arrow marks posterior end of the right-most kinety, and arrowhead points to the transpodial segments. (E) Dorsal view. (F) Ventral view, showing, inter alia, the distribution pattern of contractile vacuoles (arrowheads). (G) Detail of cytostome. (H) Detail of anterior part of the cell, showing oral kineties. Co, circumoral kineties; Ma, macronucleus; NR, nematodesmal rods; Pr, preoral kineties; TF, terminal fragment. Scale bars, 50 μ m.

Table 1. Morphometric data of *Aegyria foissneri* sp. n. based on protargol-impregnated specimens.

Character	Min	Max	Mean	Median	SD	CV	n
Body length (μ m)	90	170	124.4	120.0	18.05	14.5	25
Body width (μ m)	50	80	64.9	65.0	9.23	14.2	25
Somatic kineties, number	42	77	55.6	51.0	10.02	18.0	25
Circumoral kineties, number	3	6	4.4	4.0	0.65	14.7	25
Transpodial segments, number	5	8	6.7	7.0	0.93	14.0	23
Nematodesmal rods, number	31	44	36.4	36.0	4.01	11.0	23
Length of nematodesmal rods (μ m)	30	50	40.0	40.0	5.37	13.4	24
Length of opening of the cyrtos (μ m)	20	40	29.4	30.0	4.44	15.1	25
Macronucleus length (μ m)	30	50	39.2	40.0	5.72	14.6	25
Macronucleus width (μ m)	10	40	30.1	30.0	6.14	20.4	25

CV, coefficient of variation in %; Max, maximum; Mean, arithmetic mean; Min, minimum; n, number of individuals examined; SD, standard deviation.

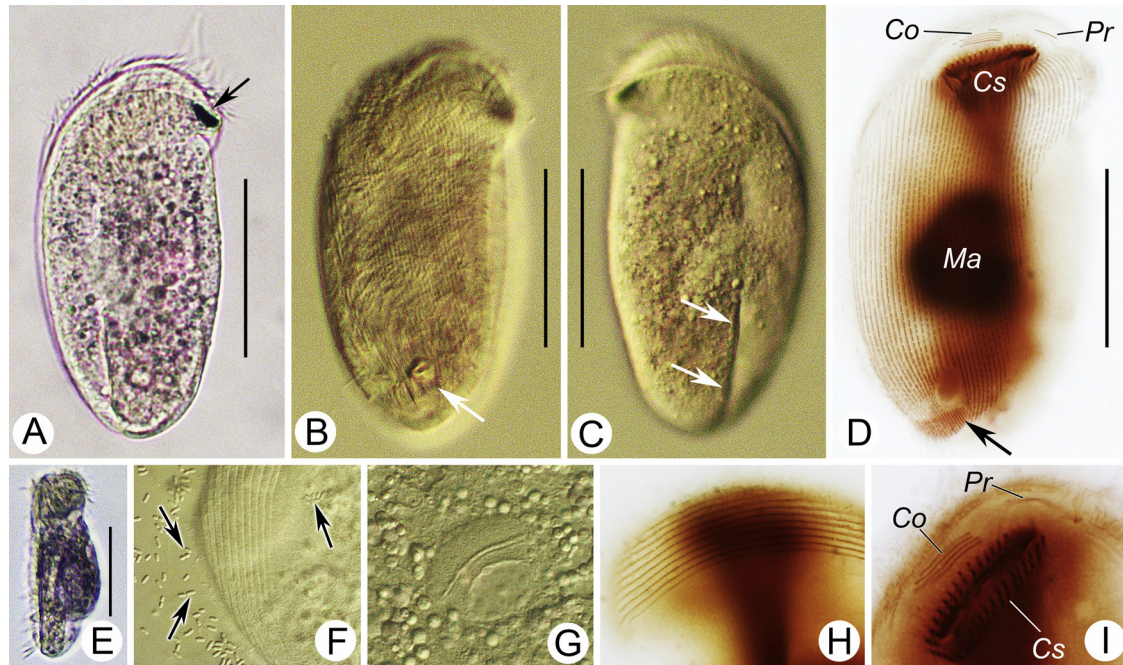


Fig. 3. *Aegyria foissneri* sp. n. in vivo (A–C, E–G) and after protargol preparation (D, H, I). (A, B) Ventral views, arrow in (A) shows the pigment spot, and arrow in (B) marks the podite. (C) Dorsal view, arrows show the gap on dorsal side. (D) Ventral view of the type, arrow illustrates the transpodial segments. (E) Lateral view, showing the vaulted dorsal side. (F, H) Dorsal view of anterior part of the cell, arrows in (F) point to bar-shaped bacteria. (G) Heteromerous macronucleus. (I) Detail of oral kineties. Co, circumoral kineties; Cs, cytostome; Ma, macronucleus; P, podite; Pr, preoral kineties. Scale bars, 50 μ m.

Table 2. Comparison of morphometric data of *Aegyria foissneri* sp. n. and its congeners.

Character	<i>A. foissneri</i>	<i>A. oliva</i>	<i>A. rostellum</i>	<i>A. paraoliva</i>	<i>A. minuta</i>
Body length in vivo (μ m)	85–170	40–120	90–150	60–80	40–55
Body shape	Oval	Oval	Triangular	D-shaped	Elliptical
Anterior-left protrusion	Present, obvious	Absent	Present, obvious	Present, inconspicuous	Absent
Somatic kineties, number	41–77	35–44	56–63	33–40	ca. 18
Transpodial segments, number	5–8	6–8	4–5	ca. 8	ca. 7
Nematodesmal rods, number	31–44	20–30	32–42	26–30	–
Contractile vacuoles, number	ca. 12	4–6	Not observed	Not observed	–
Data source	Original	Song et al. (2009)	Chen et al. (2012)	Song and Wilbert (2002)	Deroux (1974)

oliva Claparède and Lachmann, 1859) as the type. Chen et al. (2012) re-activated the genus *Aegyria* and fixed *Aegyria oliva* Claparède and Lachmann, 1859 as the type based on the fact that *Aegyria* was actually not a synonym of *Dysteria*. There are currently four valid species assigned to *Aegyria* (Table 2): *A. oliva* Claparède and Lachmann, 1859 (type species); *A. minuta* (Deroux 1974) Chen et al., 2012; *A. paraoliva* (Song and Wilbert 2002) Chen et al., 2012; and *A. rostellum* Chen et al., 2012.

Aegyria foissneri sp. n. resembles *A. rostellum* in body size (85–170 μ m vs. 90–150 μ m in length), number of somatic kineties (42–77 vs. 56–63), and number of nematodesmal rods (31–44 vs. 32–42). However, the former can be clearly separated from the latter by the following features: (i) body shape (inverted oval vs. triangular or ear-shaped, with obviously narrowed posterior end); and (ii) feature of right

kineties (only the right-most one shortened posteriorly vs. right kineties gradually shortened from left to right) (Chen et al. 2012).

Aegyria oliva is similar to *A. foissneri* sp. n. in terms of cell size in vivo (40–120 μ m vs. 85–170 μ m in length), but differs from the latter in body shape (oval vs. inverted oval), absence of the left-anterior protrusion (vs. presence), and the number of contractile vacuoles (4–6 vs. 12–16) (Song et al. 2009).

Aegyria paraoliva can be clearly separated from the new species by body shape (elliptical or D-shaped vs. inverted oval), body size (60–80 μ m vs. 85–170 μ m in length), and less kineties (33–40 vs. 42–77) (Song and Wilbert 2002).

Aegyria minuta is smaller (ca. 55 μ m long) and has much less somatic kineties (ca. 18 vs. 42–77 in *A. foissneri* sp. n.),

and thus cannot be confused with the new species (Deroux 1974).

Order Chlamyodontida Deroux, 1976

Family Lynchellidae Jankowski, 1968

***Lynchella* Jankowski, 1968**

***Lynchella minuta* sp. n. (Fig. 4A–K; Tables 3, 4)**

Diagnosis. Cell size 20–30 × 15–20 µm in vivo; body oval and consistently with four preoral and 14 or 15 postoral kineties, three circumoral kineties; ca. 11 nematodesmal rods; one finger-like tentacle on the ventral side; macronucleus ovoid; two contractile vacuoles diagonally located.

Type locality. Collected from water of a sandy beach in estuary of the Pearl River, Zhuhai (22°15'48"N, 113°35'00"E; Fig. 1A), PR China. Water temperature ca. 29.2 °C, pH ca. 7.9, and salinity ca. 2.5‰.

Etymology. The species-group name *minut-us*, *-a*, *-um* (Latin adjective; very small, tiny) refers to the fact that the present species is the smallest one of the genus.

Type deposition. A permanent slide with the holotype specimen (marked with a black circle, Fig. 4B, C, H, I) and two other slides with paratype specimens were deposited in the Laboratory of Protozoology, Ocean University of China, with registration numbers of QZS2014052603-1, QZS2014052603-2, and QZS2014052603-3, respectively.

SSU rDNA sequence. The length (bp), GC content and GenBank accession number of *Lynchella minuta* sp. n. are 1678, 44.40%, and KX364494.

Description. Size 20–30 × 15–20 µm in vivo. Body shape oval; both ends broadly rounded and posterior end slightly wider than anterior one; left margin a little concave, right margin slightly convex (Fig. 4A, D–F). Distinctly dorsoventrally flattened. Ventral side flat with inconspicuous depression, dorsal side vaulted. A C-shaped perimeter groove between ventral and dorsal surfaces (ca. 3–4 µm wide) at anterior half of cell (Fig. 4A, G). Two short longitudinal grooves located right and left behind cytostome respectively (Fig. 4A, D, E). Cytostome conspicuous in vivo, positioned at 17% of body length. Extrusomes not observed. One finger-like tentacle, ca. 3 µm long, at 75% of body length (Fig. 4A, F). Cytoplasm colorless and hyaline with several 2–3 µm-sized granules. Two contractile vacuoles (3–4 µm across) diagonally positioned (Fig. 4A, D–F). Two contractile vacuole pores usually obvious after protargol impregnation (Fig. 4B). Macronucleus ellipsoidal and juxtaposed heteromorous, ca. 16 × 10 µm in vivo, at mid-body (Fig. 4A, C, F, G). Micronucleus undetectable. Most cilia ca. 7 µm long, while those at posterior end of cell ca. 10–12 µm long. Usually slowly crawling on substrate, sometimes rotating around body axis; thigmotactic. Feeds mainly on bacteria.

Somatic kineties separated into preoral and postoral portions by cytostome. Consistently four preoral kineties, arched along anterior margin of cell; 14 or 15 postoral kineties, starting behind cytostome level. Left and right kineties forming an inconspicuous suture at posterior part (Fig. 4B, I). A pair of basal bodies located between preoral and postoral on the right (Fig. 4B, I). Kinetosomes in posterior portion of left-most

right kineties densely arranged (Fig. 4B, I). Two terminal fragments: one positioned anteriorly on dorsal side, slightly right of midline, consisting of about five kinetosomes (anterior terminal fragment; Fig. 4C, H, K); the other on anterior left margin of cell, with about four kinetosomes (left terminal fragment; Fig. 4B, K). Equatorial fragment composed of 2–14 kinetosomes (Fig. 4B, I).

Circumoral kineties including one anterior and two posterior rows (Fig. 4B, I); anterior one relatively long and curved, with kinetosomes sparsely arranged, while kinetosomes in posterior two relatively densely arranged. Cytos long, extending to rear body end, composed of ca. 11 nematodesmal rods each ca. 12 µm long (Fig. 4B, C, H).

Comparison. *Lynchella* was firstly established by Kahl (1933) without type. Then Jankowski (1968) made a new diagnosis and fixed *Lynchella gradata* as the type. The genus is distinguished by (i) having perimeter groove but no cross-striated band; (ii) somatic kineties divided into pre- and postoral portions; (iii) three circumoral kineties; and (iv) two terminal fragments (anterior and left terminal fragment) (Deroux 1970; Jankowski 1968). There are currently four valid species within the genus *Lynchella* (Table 4), namely *L. gradata* (Kahl 1933) Jankowski, 1968 (type species); *L. cypris* Jankowski, 1968; *L. fencheli* Jankowski, 1968; and *L. nordica* Jankowski, 1968.

Lynchella gradata, of which the ciliary pattern is unknown yet, can be easily distinguished from the new species by its pointed tail (vs. posterior end broadly rounded) and relatively larger size (50–60 µm vs. 20–30 µm in length) (Kahl 1933).

The new species resembles *Lynchella nordica* in body shape and overall pattern of somatic and oral kineties. However, the latter is larger (40–62 µm long) and has more postoral kineties (23–30 vs. 14 or 15) and finger-like tentacles (3 vs. 1) (Chen et al. 2011; Deroux 1970; Jankowski 1968). Song and Wilbert (2000) suggested a new combination, *Chlamydonella nordica* (Jankowski 1968), formerly *Lynchella nordica*. The authors thought the Y-shaped circumoral kinety in *Chlamydonella* could be fragmented, so the oral structure is not a good criterion for the differentiation of *Lynchella* and *Chlamydonella*. However, the authors ignored the fact that *Lynchella* has two terminal fragments (one anterior and one left vs. one in *Chlamydonella*) apart from their difference in circumoral kineties (one anterior and two posterior rows vs. Y-shaped, fragmented or not in *Chlamydonella*). We thus think their combination is not valid.

Our new species is similar to *Lynchella cypris* in general body shape, pattern of somatic kineties and number of tentacles. However, these two species can be clearly separated by body length (20–30 µm vs. 50 µm) and the number of postoral kineties (14 or 15 vs. 19–22) (Jankowski 1968).

Lynchella fencheli is much larger (ca. 70 µm long) and comprises more kineties (38 or 39), thus cannot be confused with the new species (Jankowski 1968).

Jankowski (2007) established a new genus, *Paralynchella*, and fixed *Lynchella tentaculata* Deroux, 1970 as the type. According to the definition, *Paralynchella* differs from

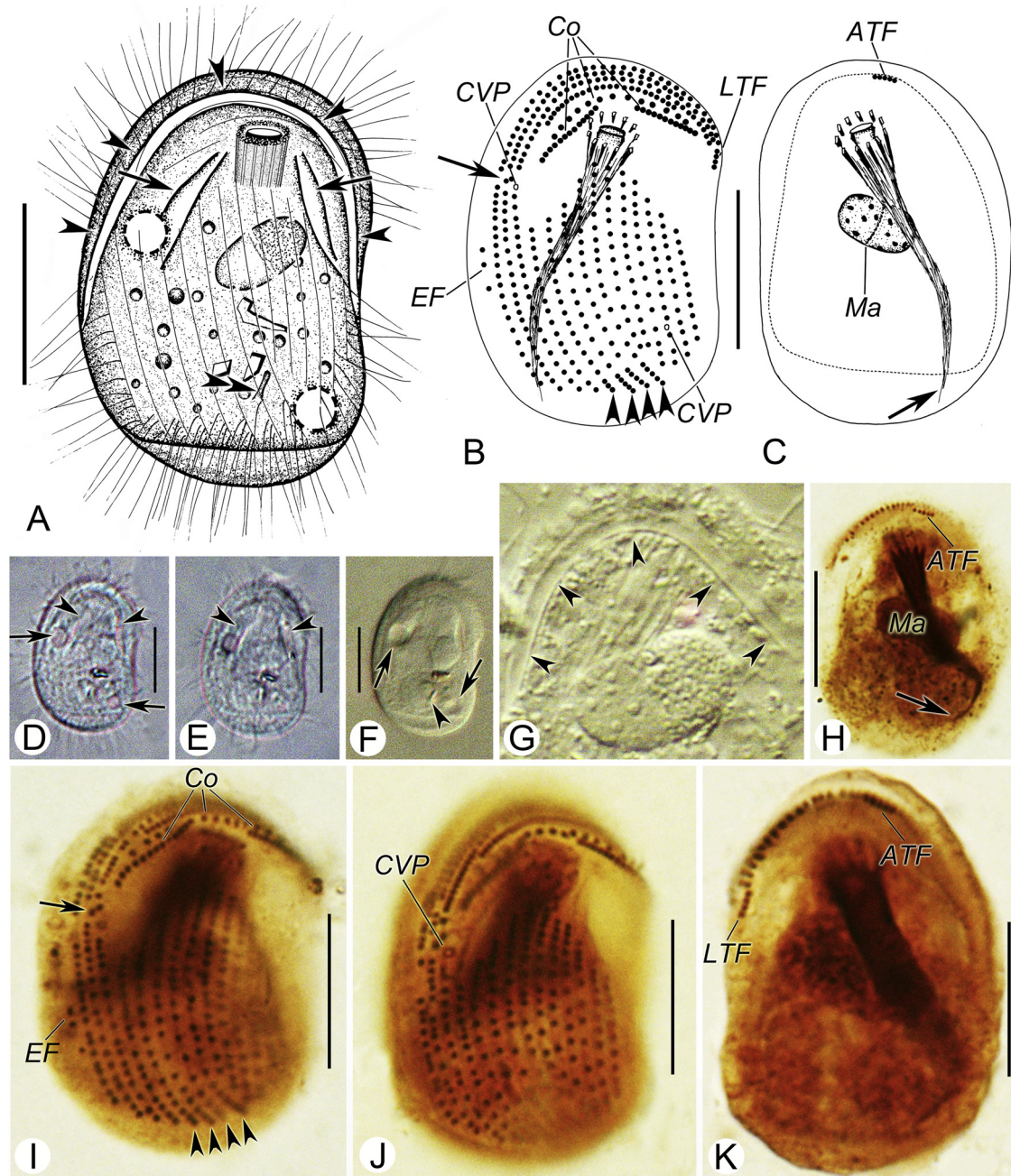


Fig. 4. *Lynchella minuta* sp. n. in vivo (A, D–G) and after protargol preparation (B, C, H–K). (A) Ventral view of a representative specimen, arrows show the longitudinal grooves, arrowheads mark the perimeter groove between ventral and dorsal surface, and double-arrowhead indicates the finger-like tentacle. (B, I, J) Ventral views of the holotype, arrow marks the pair of kinetosomes between preoral and postoral kineties, and arrowheads point to the densely arranged right kineties. (C, H, K) Dorsal views of the holotype, arrow shows the end of the cyrtos. (D–F) Ventral views of representative individuals, arrows show the contractile vacuoles, arrowheads in D, E mark the longitudinal grooves, and arrowhead in F points to the finger-like tentacle. (G) Anterior end of cell, arrowheads show the perimeter groove. ATF, anterior terminal fragment; Co, circumoral kineties; CVP, contractile vacuole pores; EF, equatorial fragment; LTF, left terminal fragment; Ma, macronucleus. Scale bars, 10 μ m.

Lynchella only by the oral ciliary pattern. *Lynchella* has one anterior and two posterior circumoral kineties; the anterior one is long and parallel to the posterior two; while the posterior two are on the same level. *Paralynchella* also has three circumoral kineties, but they are parallel to each other on

different levels. Besides the different oral ciliary pattern, *Lynchella minuta* sp. n. can be separated from *Paralynchella tentaculata* by its smaller body length in vivo (20–30 μ m vs. ca. 75 μ m) and less kineties (14 or 15 vs. 45–50) (Deroux 1970).

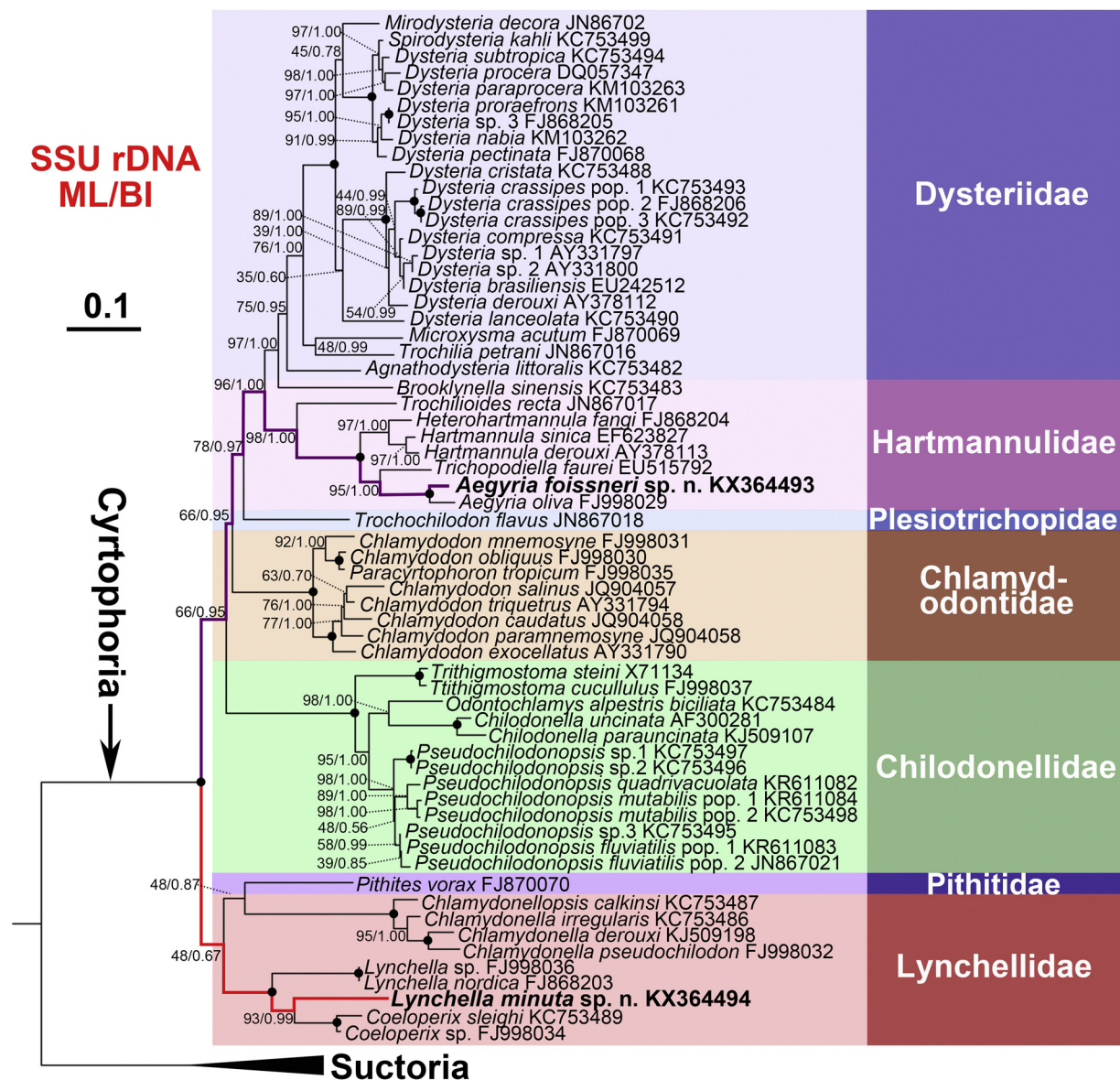


Fig. 5. Maximum-likelihood (ML) and Bayesian inference (BI) analyses based on small subunit ribosomal RNA (SSU rDNA) gene sequences. The new sequences of *Aegyria foissneri* sp. n. and *Lynchella minuta* sp. n. are indicated in bold. Numbers at nodes indicate ML bootstrap values and the BI posterior probability (ML/BI). Nodes that were fully supported (100% ML, 1.00 BI) are represented by solid circles. Bar, 1 substitutions per 10 nucleotide positions.

Coeloperix sleighi Gong and Song, 2004 is similar to *Lynchella minuta* sp. n. in body shape (oval), cell size in vivo (40–50 μ m vs. 30–40 μ m in length), number of somatic kineties (15 or 16 vs. 14 or 15) and nematodesmal rods (12–16 vs. ca. 12). However, the former comprises cross-striated band around the cell periphery (vs. absent), which is the key feature to separate *Coeloperix* from *Lynchella* (Gong and Song 2004).

SSU rDNA trees (Fig. 5)

Aegyria foissneri sp. n. (KX364493) differs from *A. oliva* (FJ998029) by 58 bp, demonstrating the distinctness of *A.*

foissneri as a new species. Surprisingly, the SSU rDNA sequence of *Lynchella minuta* sp. n. (KX364494) differs from those of *L. nordica* (FJ868203) and *L. sp.* (FJ998036) by 192 bp and 191 bp, respectively; while the difference between *L. nordica* and *L. sp.* is 2 bp, which seems to suggest that *L. sp.* is a population of *L. nordica*. *Lynchella minuta* sp. n. (KX364494) differs from *Coeloperix sleighi* (KC753489) and *C. sp.* (FJ998034) by 178 bp and 153 bp, respectively.

The ML and BI trees have similar topologies and thus only the ML tree is presented here and support values of the two trees are mapped onto it. *Aegyria foissneri* sp. n. clusters with *A. oliva* with full support, forming a clade to *Trichopodiella faurei* within the family Hartmannulidae Poche,

Table 3. Morphometric data of *Lynchella minuta* sp. n. based on protargol-impregnated specimens.

Character	Min	Max	Mean	Median	SD	CV	n
Body length (μm)	20	28	24.0	24.0	2.45	10.2	11
Body width (μm)	15	20	17.2	17.0	1.89	11.0	11
Postoral kineties, number	14	15	14.1	14.0	0.30	2.1	11
Preoral kineties, number	4	4	4.0	4.0	0.00	0.0	11
Right kineties, number	8	8	8.0	8.0	0.00	0.0	11
Left kineties, number	6	7	6.1	6.0	0.30	5.0	11
Kinetosomes in LTF, number	2	4	3.6	4.0	0.81	22.2	11
Kinetosomes in ATF, number	3	5	4.4	4.5	0.70	15.9	10
Kinetosomes in EF, number	2	14	7.0	6.5	3.42	48.9	8
Nematodesmal rods, number	10	12	11.2	11.0	0.79	7.0	10
Macronucleus length (μm)	6	10	8.0	8.0	1.33	16.7	10
Macronucleus width (μm)	6	7	6.1	6.0	0.32	5.2	10

ATF, anterior terminal fragment; CV, coefficient of variation in %; EF, equatorial fragment; LTF, left terminal fragment; Max, maximum; Mean, arithmetic mean; Min, minimum; n, number of individuals examined; SD, standard deviation.

Table 4. Comparison of morphometric data of *Lynchella minuta* sp. n. and its congeners.

Character	<i>L. minuta</i>	<i>L. gradata</i>	<i>L. nordica</i>	<i>L. fencheli</i>	<i>L. cypris</i>	<i>Paralynchella tentaculata</i>
Body size in vivo (μm)	20–30	ca. 50	40–62	ca. 70	ca. 50	ca. 75
Body shape	Oval	Oval with pointed tail	Oval	Oval	Oval	Oval
Postoral kineties, number	14–15	–	23–31	38–39	19–22	45–50
Preoral kineties, number	4	–	4	–	–	4
Right kineties, number	8	–	17–22	–	–	ca. 40
Left kineties, number	6	–	6–7	–	–	7
Nematodesmal rods, number	ca. 12	–	12–16	19–20	14–16	20–24
Ventral tentacles, number	1	1	ca. 3	–	–	5–7
Data source	Original	Kahl (1933)	Chen et al. (2011), Deroux (1970) and Jankowski (1968)	Jankowski (1968)	Jankowski (1968)	Deroux (1970)

1913. This is consistent with their morphological features that both the two genera have long and narrow cytostome and interrupted kineties at posterior body. Two main differences are: (i) *Aegyria* has a podite, while *Trichopodiella* lacks a podite but have glandule instead; (ii) *Aegyria* has one preoral kinety and two or more circumoral kineties, while *Trichopodiella* has only one oral kinety (Gong et al. 2008).

Lynchella minuta sp. n. is sister to the subclade of *Coeloperix* sp. and *C. sleighi* with high support (ML/BI, 93%/0.99); then they group with the clade of *L. nordica* and *L. sp.* with full support, which indicates that *Lynchella* is non-monophyletic. This is consistent with the morphological observation because the only difference between *Lynchella* and *Coeloperix* is the cross-striated band, which is lacking in the former. The result also indicates that the cross-striated band may not be a genus-level character that distinguishes *Coeloperix* and *Lynchella* as two genera.

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