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# 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 85–170 × 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; and single oval macronucleus. *Lynchella minuta* sp. n. is distinguished from its congeners by having a cell size of 20–30 × 15–20 μm 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.



***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 lemnarum* (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