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Morphology and morphogenesis of *Apoholosticha sinica* n. g., n. sp. (Ciliophora, Hypotrichia), with consideration of its systematic position among urostylids

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Abstract

This paper investigates the morphology, morphogenesis and SSU rRNA gene-based phylogeny of *Apoholosticha sinica* n. g., n. sp., isolated from mangrove wetland in Shenzhen, southern China. The new genus *Apoholosticha* is characterized by its bipartite adoral zone, clearly differentiated frontal cirri arranged in a bicorona, midventral complex composed of midventral pairs only, one marginal cirral row on each side, presence of frontoterminal and transverse cirri, and the lack of a buccal cirrus and caudal cirri. The type species, *Apoholosticha sinica* n. sp. is diagnosed by the elongated body shape and two kinds of cortical granules. Its main morphogenetic features are similar to that of *Pseudokeronopsis* except for (1) no buccal cirrus is formed and (2) its macronuclear nodules fuse into a single mass during cell division. Phylogenetic analyses for the new taxon indicate that *Apoholosticha* n. g. is most closely related to *Nothoholosticha* and *Heterokeronopsis*, and falls into the family Pseudokeronopsidae within the core Urostylida clade. In addition, a species that had been misidentified in previous literature is here recognized and assigned to the new genus as *Apoholosticha sepetibensis* (Wanick and Silva-Neto, 2004) n. comb. (basionym: *Pseudokeronopsis sepetibensis* Wanick and Silva-Neto, 2004).

Keywords: *Apoholosticha sinica* n. g., n. sp.; Marine ciliates; Morphogenesis; Phylogeny; SSU rDNA; Taxonomy

Introduction

Urostylid ciliates are characterized, inter alia, by a zigzag-arrangement of the ventral cirri differentiated from a longitudinal field of many oblique frontal–ventral–transverse streaks during cell division. They appear to be one of the most diverse groups (Berger 2006). Recently, this group has been revealed to be even more diverse than originally supposed and it is likely that many more species will need to be discovered and analyzed before the group is fully understood (e.g. Chen et al. 2011a,b, 2013; Jiang et al. 2013; Paiva et al. 2012; Pan et al. 2013; Shao et al. 2011; Yi and Song 2011).

Among the urostyloids, the family Pseudokeronopsidae is one of the most diverse groups. Borror and Wicklow (1983)

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established the family and united *Pseudokeronopsis* (including *Uroleptopsis* as a synonym) and *Thigmokeronopsis* within it (Berger 2006). Later, three new genera, *Nothoholosticha*, *Heterokeronopsis* and *Apokeronopsis*, were assigned into this family (Li et al. 2009; Pan et al. 2013; Shao et al. 2007). Recent studies reveal that the family Pseudokeronopsidae is not monophyletic and suggest that some pseudokeronopsids should be transferred to the family Urostylidae (Yi and Song 2011). Chen et al. (2011a), for instance, transferred *Thigmokeronopsis* and *Apokeronopsis* to the family Urostylidae *sensu* Lynn (2008).

In April 2011, a novel urostylid ciliate was isolated from the mangrove wetland in Shenzhen, southern China. The isolate was successfully maintained in culture giving the opportunity to study its morphology, morphogenesis, and molecular phylogeny based on its small subunit ribosomal RNA (SSU rRNA) gene sequence. These studies demonstrated it to be a new genus that should be assigned to the family Pseudokeronopsidae.

Material and Methods

Sampling and cultivation

Samples were collected from the mangrove wetland in Shenzhen (22°31′29″ N, 114°00′55″ E), southern China on April 13, 2011 with the water temperature being 27.8 °C and salinity 5.6‰. Non-clonal cultures were maintained for several weeks at room temperature (20 °C) in Petri dishes containing filtered seawater with squeezed rice grains to enrich the bacterial food (Foissner 2012).

Morphology and morphogenesis

Living cells were isolated and observed using bright field and differential interference contrast microscopy at 100–1000×. Protargol impregnation (Wilbert 1975) was used to reveal the infraciliature and nuclear apparatus. Counts and measurements of impregnated specimens were performed with an ocular micrometer. Drawings were made with the help of a camera lucida (Shao et al. 2013a). To illustrate the changes occurring during the morphogenetic process, parental cirri are depicted with contour lines, whereas new ones are shaded black (Shao et al. 2013b). Terminology is according to Berger (2006).

DNA extraction, PCR amplification, and sequencing

Genomic DNA extraction, PCR amplification, and sequencing of the SSU rRNA gene were performed according to Huang et al. (2012). The SSU rRNA gene was amplified by PCR with primers Euk A and Euk B (Medlin et al. 1988). Cycling parameters were as follows: 5 min initial denaturation (94 °C), followed by 35 cycles of 45 s at 94 °C, 1 min at 56 °C, and 1 min at 72 °C, with a final extension of 5 min at 72 °C. The PCR product was purified and sequenced directly using primers Euk A, Euk B and three internal primers. Sequencing in both directions was carried out on an ABI 3700 sequencer.

Phylogenetic analyses

Preliminary Maximum Likelihood (ML) analyses showed that different selections of representative taxa (e.g. 51, 54 or 65 taxa) and different outgroups generally resulted in trees with similar topologies. We selected a set of 51 SSU rDNA sequences for further phylogenetic analyses using two choreotrichs and two oligotrichs as the outgroup species (for accession numbers, see Fig. 6).

Sequences were aligned with MUSCLE v3.7 with default parameters. The resulting alignment was manually edited using the program Bioedit 7.0 (Hall 1999). Ambiguously aligned regions and gaps were excluded prior to phylogenetic analyses. The program MrModeltest v.2.0 (Nylander 2004) selected the GTR + I (=0.4405) + G (=0.4667) as the best model with Akaike Information Criterion (AIC), which was then used for Bayesian inference (BI) analysis. A Bayesian inference (BI) analysis was performed with MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003), with a run of 1,000,000 generations at a sampling frequency of 100 and a burn-in of 2500 trees (25%). All remaining trees were used to calculate posterior probabilities using a majority rule consensus. Maximum Likelihood (ML) analyses were conducted online on the CIPRES Science Gateway (The CIPRES Portals. URL: http://www.phylo.org/sub_sections/portal) with RaxML-HPC BlackBox (7.2.8) (Stamatakis et al. 2008). Node support came from 100 bootstrap replicates. TREEVIEW v1.6.6 (Page 1996) and MEGA 4.0 (Tamura et al. 2007) were used to visualize tree topologies. The systematic classification mainly follows Lynn (2008) and Chen et al. (2011a).

Results

Order Urostylida Jankowski, 1979

Family Pseudokeronopsidae Borror and Wicklow, 1983

Genus *Apoholosticha* n. g.

Diagnosis. Urostylids having a bipartite adoral zone; clearly differentiated frontal cirri arranged in an indistinct bicorona; midventral complex composed of midventral pairs only; one marginal cirral row on each side of cell; frontoterminal and transverse cirri present; buccal and caudal cirri absent.

Type species. *Apoholosticha sinica* n. sp.

Etymology. Composite of the Greek prefix *apo-* (from, off, away, after, without, separated) and the genus-group name