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European Journal of Protistology 49 (2013) 298–311

European Journal of PROTISTOLOGY

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# Ontogeny and molecular phylogeny of a new marine ciliate genus, *Heterokeronopsis* g. n. (Protozoa, Ciliophora, Hypotricha), with description of a new species

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Received 7 April 2012; received in revised form 23 August 2012; accepted 30 August 2012
Available online 16 October 2012

## Abstract

The morphology and morphogenesis of a new ciliate, *Heterokeronopsis pulchra* g. n., sp. n., isolated from a mangrove wetland near Shenzhen, southern China, were investigated using live observation and protargol impregnation methods. *Heterokeronopsis* g. n. is characterized by having a bipartite adoral zone, an extremely shortened paroral membrane, frontal cirri arranged in an indistinct bicorona, a midventral complex composed of midventral pairs and midventral row(s), one left and one right marginal row, bipolar dorsal kineties, and buccal cirri; frontoterminal, transverse, and caudal cirri are absent. The single species *H. pulchra* sp. n. has a long, slender body that is brownish in colour, two kinds of cortical granules, one posteriorly located contractile vacuole, one buccal cirrus, usually six frontal cirri, three dorsal kineties, and one midventral row. The main morphogenetic features are: (i) the old oral apparatus is completely replaced by new structures derived from the oral primordium of the proter which originates de novo on the dorsal wall of the buccal cavity, (ii) the posteriormost frontal-midventral-transverse cirral anlage generates a midventral row, (iii) no frontoterminal and transverse cirri are formed, (iv) the anlagen for the marginal rows and the dorsal kineties are formed intrakinetally, and (v) the macronuclear nodules fuse into a mass at the middle stage. Phylogenetic analyses based on SSU rRNA gene sequence data reveal that *Heterokeronopsis pulchra* is a member of the family Pseudokeronopsidae and is most closely related to *Nothoholosticha fasciola*.


***Keywords:*** Ciliophora; *Heterokeronopsis*; Hypotricha; New genus; Ontogeny; Phylogeny

## Introduction

Urostylid ciliates are characterized, inter alia, by a zigzag arrangement of the midventral cirri (Berger 2006). Hitherto, about 160 species have been reported based on morphological information (e.g. Berger 2006; Chen et al. 2010, 2011a,b; Kumar et al. 2010; Li et al. 2011; Liu et al. 2009, 2010; Paiva et al. 2012; Shao et al. 2007, 2008, 2011; Song et al. 2006; Wang et al. 2011; Wilbert and Song 2005; Xu et al. 2011). However, ontogenesis and molecular phylogeny have been studied for only a few species (Berger 2006; Song et al. 2011; Xu et al. 2011; Yi et al. 2009, 2010; Yi and Song 2011). The family Pseudokeronopsidae comprises a relatively small group of urostylid ciliates that are characterized

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http://dx.doi.org/10.1016/j.ejop.2012.08.008

by their slender body, conspicuous pigments, and frontal cirri arranged in conspicuous arcs called the bicorona (Berger 2006). Recent studies, however, have shown that the diversity of cirral patterns in pseudokeronopsids is greater than previously expected (Chen et al. 2011a; Li et al. 2009).

In the spring of 2011, we isolated a novel hypotrich ciliate from a mangrove wetland near Shenzhen, southern China, and successfully maintained it in uniprotistan culture giving the opportunity to study its morphology, morphogenesis, and molecular phylogeny based on its small subunit rRNA (SSU rRNA) gene sequence. These studies indicate that this species represents a new genus in the family Pseudokeronopsidae.

## Material and Methods

### Collection, isolation, and cytological methods

Samples of water with some leaves were collected on 13 April 2011 from a small puddle (water temperature 26 °C, pH 7.3, salinity ca. 19‰) in a mangrove wetland near Shenzhen (22°32′N, 114°01′E), China. Ciliate cells were isolated using a micropipette and maintained for several weeks as uniprotistan cultures in filtered seawater obtained in situ with rice grains added to enrich the bacterial food.

Live observations were carried out using bright field and differential interference contrast microscopy. The protargol staining method was used to reveal the infraciliature (Wilbert 1975). Drawings were made with the help of a camera lucida at a magnification of 1250×. To illustrate the changes during morphogenesis, parental cirri are depicted by contour whereas new ones are shaded black. Terminology and systematics basically follow Berger (2006) and Lynn (2008) respectively.

### DNA extraction, PCR amplification, and sequencing

Extraction of genomic DNA was carried out according to the methods described by Jiang et al. (2010). PCR amplifications were performed using a TaKaRa ExTaq DNA Polymerase Kit (TaKaRa Biomedicals, Japan) with two eukaryotic universal SSU rRNA primers, EukA (5′-AACCTGGTTGATCCTGCCAGT-3′) and EukB (5′-TGATCCTTCTGCAGGTTCACCTAC-3′) (Medlin et al. 1988). Cloning and sequencing were performed as reported by Chen et al. (2011b).

### Phylogenetic analyses

The SSU rRNA gene sequence analyses used 60 spirotrichs obtained from the NCBI database (see Fig. 8 for accession numbers) in addition to the new species. Two euplotids, *Certesia quadrinucleata* and *Euplotes charon*, were used as the outgroup taxa. The method used for phylogenetic analyses followed Zhang et al. (2010) with the final alignment of 1602 characters. Briefly, 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 the program MrModeltest v.2.0 (Nylander 2004). Four simultaneous Markov chain Monte Carlo algorithms (MCMC) were run for 1,000,000 generations, sampling every 100th generation. The first 2500 trees were discarded as burn-in. The remaining trees were used to calculate the posterior probabilities, applying the majority rule consensus. A maximum likelihood (ML) tree was constructed with the PhyML V2.4.4 program (Guindon and Gascuel 2003) using a non-parametric bootstrap method for 1000 replicates with the best model GTR + I (=0.5455) + G (=0.5697) selected by the program Modeltest v.3.4 (Yi et al. 2010).

## Results

### Morphology

Order Urostylida Jankowski, 1979
Family Pseudokeronopsidae Borror and Wicklow, 1983
*Heterokeronopsis* g. n.

**Diagnosis**. Pseudokeronopsidae with bipartite adoral zone of membranelles; paroral membrane conspicuously shortened; frontal cirri arranged in an indistinct bicorona; buccal cirrus present; midventral complex composed of midventral pairs and midventral row(s); one marginal row on each side of cell; frontoterminal, transverse, and caudal cirri absent.

**Type species**. *Heterokeronopsis pulchra* sp. n.

**Etymology**. Composite of the Greek adjective *hetero* (different, away from) and the well-known genus group name *Keronopsis*. This indicates that *Heterokeronopsis* has a similar frontal ciliature to *Keronopsis* but lacks midventral cirri. Feminine gender.

**Species assignable**. *Heterokeronopsis pulchra* sp. n.

*Heterokeronopsis pulchra* sp. n.

**Diagnosis**. *Heterokeronopsis* with elongate elliptical cell shape, about 150–250 × 30–45 μm in vivo; two kinds of cortical granules, one colourless, the other light brownish; ca. 50 macronuclear nodules; 31–46 adoral membranelles; usually six frontal cirri; midventral complex comprising 12–21 midventral pairs and one midventral row with on average seven cirri; ca. 40 left and 45 right marginal cirri; three bipolar dorsal kineties; contractile vacuole located slightly below mid-body region.

**Type locality**. A puddle in a mangrove wetland near Shenzhen (22°32′N, 114°01′E), Guangdong Province, China; water temperature 26 °C, pH 7.3, salinity 19.1‰.

**Etymology**. The Latin adjective *pulchra* (beautiful, excellent) refers to the beautiful colour of the pigments on the cell surface.

**Deposition of type specimens**. One protargol-impregnated slide with the holotype specimen