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Taxonomic studies on seven species of *Dysteria* (Ciliophora, Cyrtophoria), including a description of *Dysteria paraprocera* sp. n.

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Abstract

The living morphology and infraciliature of seven *Dysteria* species isolated from the seas around China were investigated by observation of both living cells and specimens after protargol impregnation. *Dysteria paraprocera* sp. n. is characterized as follows: cell size 110–150 × 30–40 μm in vivo; body elongate rectangular and slender; a yellow-brown to dark red coloured pigment spot located at anterior end of body; three right kineties, with rightmost two extending apically to dorsal margin and innermost one starting at level of cytostome; eight or nine short left kineties at equatorial area. *Dysteria nabia*, *D. proraefrons*, *D. brasiliensis*, *D. cristata*, *D. derouxi* and *D. crassipes* basically correspond well with previous studies and therefore only brief descriptions are presented. Discussions of these species are helpful, however, in understanding the circumscription of *Dysteria* morphotypes. After careful comparison, *Dysteria procera* sensu Liu et al. (2008, Acta Hydrobiol. Sin. 32 (suppl.), 84–89 (in Chinese with English abstract)) was verified as a new species, *D. subtropica* sp. n., mainly because the innermost right kinety starts at mid-body. Small-subunit (SSU) rRNA genes were sequenced for four species of *Dysteria*, namely, *D. paraprocera* sp. n., *D. subtropica* sp. n., *D. proraefrons* and *D. nabia*. Sequence comparisons and phylogenetic analyses indicate that these species are well outlined and cluster with their congeners.

Keywords: *Dysteria*; Dysteriidae; Marine ciliates; Morphology; Phylogeny; SSU rRNA gene

Introduction

The cyrtophorids are a group of highly specialized and divergent ciliates with dorsoventrally or laterally flattened bodies (Gao et al. 2012; Kahl 1931). More than 150 cyrtophorid species have been reported to date, with most of these being free-living marine forms (e.g. Foissner et al. 1991; Gong and Song 2009; Park and Min 2014; Song et al. 2009; Zhao et al. 2014). Over the last two decades a taxonomical survey of ciliates in marine biofilm environments in China has reported more than 40 new or poorly-known cyrtophorids, meaning that this group is more species rich than had previously been thought (Chen et al. 2012; Fan et al. 2014; Gong and Song 2006a, b; Gong et al. 2005, 2008; Pan et al. 2012, 2013a,b; Qu et al. 2015).

The species-rich genus *Dysteria*, a group of cyrtophorids with highly bilaterally compressed bodies and cilia restricted

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to a narrow ventral groove between two lateral plates, has been found worldwide in the periphyton or ectocommensally (e.g. Agamaliev 1983; Borror 1972; Carey 1992; Deroux 1976; Dragesco 1966; Jankowski 1967; Lepsi 1927; Tucolesco 1962). Over 30 nominal species have been reported so far, with information regarding infraciliature available for 20 of these (e.g. Chen et al. 2011; Deroux 1965; Dragesco and Dragesco-Kernéis 1986; Fauré-Fremiet 1965; Gong and Song 2003, 2004; Gong et al. 2002, 2003, 2007; Hu and Suzuki 2005; Pan et al. 2011; Park and Min 2014; Petz et al. 1995; Song and Packroff 1997; Song and Wilbert 2002; Wilbert 1971; Wilbert and Song 2005). Previous studies have suggested that the pattern of infraciliature, especially the ventral ciliary structure, is a critical species-specific character and is therefore reliable for species identification (Al-Rasheid 1997; Gong et al. 2003).

In the present work, seven cyrtophorids belonging to the genus *Dysteria* were isolated and identified, including a new species, *Dysteria paraprocera* sp. n. For *Dysteria procera* sensu Liu et al. (2008), the new species *D. subtropica* is established. The small-subunit (SSU) rRNA genes of *Dysteria paraprocera* sp. n., *D. subtropica* sp. n., *D. proraefrons* and *D. nabia* were sequenced and used for phylogenetic analyses.

Material and Methods

Sample collection, observation and identification

All samples in the present paper were collected from coastal areas of China. Details of locations, dates, habitats, temperature, pH and salinity are shown in Table 1 and Fig. 1.

Isolated specimens and in situ seawater were maintained in a laboratory for about one week as raw cultures in Petri dishes at about 20 °C, with rice grains added to enrich bacterial food. Living organisms were observed under a light microscope equipped with differential interference contrast. The protargol impregnation method according to Wilbert (1975) was used to reveal the infraciliature and nuclear apparatus. Measurements and counts were made at a magnification of 1000×. Drawings of live cells were based on photomicrographs, and those of silver-impregnated specimens were made with the aid of a camera lucida. Terminology and systematics follow Gong et al. (2007) and Lynn (2008), respectively.

DNA extraction and gene sequencing

Several cells of *Dysteria paraprocera* sp. n., *D. subtropica* sp. n., *D. proraefrons* and *D. nabia* were selected from the non-clonal cultures that were used for morphological studies, respectively, washed three to five times using the filtered and autoclaved marine water and then used for DNA extraction. Genomic DNA was extracted using a DNeasy Tissue kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. TaKaRa ExTaq polymerase (TaKaRa Biomedicals, Japan) was used to amplify the SSU rRNA gene by universal primers (Medlin et al. 1988) with PCR conditions following Gao et al. (2014). Cloning and sequencing were performed as described by Gao et al. (2014).

Phylogenetic analyses

Other than the SSU rRNA gene sequences of *Dysteria paraprocera* sp. n., *D. subtropica* sp. n. *D. proraefrons* and *D. nabia*, 47 representative sequences were obtained from NCBI GenBank database for use in the present analyses (accession numbers as shown in Fig. 8). Six suctorians, namely, *Ephelota gemmipara* (EU600180), *Acineta compressa* (FJ865205), *Prodiscophrya* sp. (AY331802), *Discophrya collini* (L26446), *Tokophrya lemnarum* (AY331720) and *Heliophrya erhardi* (AY007445) were selected as the outgroup species. Sequences were aligned using MUSCLE 3.7 (Penn et al. 2010) with default parameters and manually edited using the program BioEdit 7.0.5.2 (Hall 1999). Ambiguously aligned regions and gaps were excluded prior to phylogenetic analyses, resulting in a matrix of 1726 characters. Maximum-likelihood (ML) analyses were carried out on CIPRES Science Gateway using RAxML-HPC2 version 7.2.8 (Stamatakis et al. 2008) with the model of GTR + G as the optimal choice. Support for the best ML tree came from a majority rule consensus tree of 1000 bootstrap replicates. A Bayesian inference (BI) analysis was performed using MrBayes 3.2.2 (Ronquist and Huelsenbeck 2003) using the GTR + I + G as the best model (selected by MrModeltest v.2.0; Nylander 2004) on CIPRES Science Gateway. Markov chain Monte Carlo simulations were run with two sets of four chains for 1000,000 generations with a sample frequency of 100 generations, and with the first 25% being discarded as burn-in. The remaining trees were used to calculate posterior probabilities using a majority rule consensus.

Results and Discussion

Order Dysteriida Deroux, 1976
Family Dysteriidae Claparède and Lachmann, 1858
Genus *Dysteria* Huxley, 1857

Dysteria paraprocera sp. n. (Table 2; Figs 2A–C, F and 3)

Diagnosis: Large marine *Dysteria*, cell size 110–150 × 30–40 μm in vivo; body elongate rectangular and slender; a yellow-brown to dark red coloured pigment spot located at anterior end of body; three right kineties, with rightmost two extending apically to dorsal margin and innermost one starting at level of cytostome; eight or nine short left kineties in equatorial area; two ventrally located contractile vacuoles.