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Morphological and molecular information of a new species of *Geleia* (Ciliophora, Karyorelictea), with redescriptions of two *Kentrophoros* species from China

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Abstract

The morphology and infraciliature of three karyorelictean ciliates, *Geleia sinica* spec. nov. and two poorly known *Kentrophoros* species, *K. flavus* and *K. gracilis*, isolated from the intertidal zone of a beach at Qingdao, China, were investigated. *Geleia sinica* spec. nov. is distinguished from its congeners by the following combination of characters: body medium-sized and slender-cylindrical; with a conspicuous prebuccal fossa; 28–34 somatic kineties; about 40 short adoral polykineties; intrabuccal kinety composed of 25–34 dikinetids; paroral kineties composed of closely spaced dikinetids. The comparison with similar congeners clearly supports the validity of this new species based on morphological and small subunit (SSU) rRNA gene sequence data. In light of these new data the "well-known" morphotype, *Geleia simplex* (Fauré-Fremiet, 1951), is redefined. Two *Kentrophoros* species are redescribed and improved diagnoses are supplied. *Kentrophoros flavus* Raikov and Kovaleva, 1968 is mainly characterized by having about 33 macronuclei and 12 micronuclei forming a row that extends along the cell meridian, and 12–19 ciliary rows on the right side of the cell. *Kentrophoros gracilis* Raikov, 1963 is characterized by having about 14 macronuclei, 13 micronuclei and 10–13 kineties on the right side of the cell.
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Keywords: Karyorelictea; Marine ciliate; New species; SSU rRNA; Taxonomy

Introduction

The class Karyorelictea Corliss, 1974 is widely believed to represent the nature of the ancestral ciliate lineage (Lynn 2008). Karyorelicteans are characterized by their elongated, vermiform, often flattened body and numerous non-dividing macronuclei that arise from the division of a micronucleus and are often clustered around a micronucleus (Lynn 2008). Karyorelicteans are common in marine interstitial environments (Alekperov et al. 2007; Al-Rasheid 1996, 1997, 1998, 2001; Al-Rasheid and Foissner 1999; Dragesco 1960). Hitherto, 17 genera and about 130 morphospecies of karyorelicteans have been reported (Carey 1992; Foissner 1996, 1997a, 1997b; Foissner and Al-Rasheid 1999a, 1999b; Foissner and Dragesco 1996a, 1996b; Lynn 2008; Mazei et al. 2009). Molecular data for karyorelicteans,

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however, are limited and gene sequences are available for only 26 morphotypes (Andreoli et al. 2009; Gao et al. 2010).

In the present study, one new species of *Geleia* and two poorly known *Kentrophoros* species, isolated from the intertidal zone of a sandy beach at Qingdao, China, are described or redescribed based on their living morphology and infraciliature which, in each case, was revealed for the first time following silver impregnation. The small subunit (SSU) rRNA gene was also sequenced for the new species of *Geleia*. The implications of our findings for the definitions of the order Protoheterotrichida Nouzarède, 1977 and the family Geleiidae Kahl, 1933 are briefly discussed.

Material and Methods

Ciliates were isolated from the intertidal zone of the No. 1 Beach at Qingdao (36°06′N; 120°32′E), China. Sampling was mainly according to Fan et al. (2010). Briefly, a 15 cm-deep hole was dug in the sand into which seawater gradually seeped. The sample comprised a mixture of seawater and sand from the bottom of the hole.

Geleia sinica spec. nov. was collected on 29 April 2009, when the water temperature was 11 °C and salinity 32‰. *Kentrophoros flavus* was collected on 23 November 2006, water temperature 15 °C, salinity 26‰. Two populations of *K. gracilis* were collected, one on 26 November 2006 (pop I) the other on 18 September 2009 (pop II); the salinity was about 25‰ on both occasions, water temperature about 15 °C and 20 °C respectively. Cells were isolated using the method described by Fauré-Fremiet (1951), i.e. in order to stabilize the cells, a 12% (w/v) $MgCl_2$ solution was added to the sample to give a final concentration of 2.5% $MgCl_2$. Cells were then picked up with a capillary pipette. Living cells were studied by bright field and differential interference microscopy (100× to 1000× magnifications). The infraciliature was revealed by the protargol impregnation method (Wilbert 1975) using the following fixative: 10 ml saturated, aqueous mercuric chloride and 3 ml Bouin's solution, mixed just before use. Counts and measurements of stained specimens were performed at a magnification of 1250×. Drawings were made with the help of a camera lucida. Terminology is mainly according to Dragesco (1999) and Foissner (1998).

Since some structures found in certain karyorelicteans are not commonly known, the following terms are briefly explained:

Adoral polykineties (Ap). An oral ciliary field located on the left side of the buccal field, consisting of numerous, densely arranged fragment-like rows.

Fossa (="dimple" after Dragesco 1999**)**. A cavity or depression located subapically on the ventral surface, anterior of the buccal field.

Intrabuccal kinety (Ibk). A single-rowed structure, located on the right side of the buccal field.

Paroral kineties (Pk). An oral ciliary field located on the right side of the buccal cavity consisting of numerous rows of di- or polykinetids.

Preoral kinety (Prek). A fragment-like, single-rowed ciliary structure consisting of dikinetids, located on the right side of the fossa and within the preoral suture.

DNA extraction and polymerase chain reaction (PCR) amplification were performed according to Shen et al. (2010). In brief, cells were isolated and repeatedly washed using sterilized seawater. DNA was extracted using an REDExtract-N-Amp Tissue PCR Kit (Sigma, St. Louis, MO, USA) according to the manufacturer's protocol, with the slight modification that only 1/10 of the volume suggested for each reagent solution was used (Gong et al. 2009). DNA samples were stored at −20 °C. Amplification of the SSU rDNA using the universal eukaryotic primers EukA (5′-AACCTGGTTGATCCTGCCAGT-3′) and EukB (5′-TGATCCTTCTGCAGGTTCACCTAC-3′) (Medlin et al. 1988) failed. However, the SSU-ITS2 region was successfully amplified using the primers EukA and Rev3 (5′-GCATAGTTCACCATCTTTCG-3′). Direct sequencing of PCR products was performed on an ABI-PRISM 3730 Automated DNA Sequencer (Applied Biosystems Inc., Foster City, CA), using the original PCR primers and four additional primers (+B: 5′-GGTTAAAAAGCTCGTAGT-3′; +C: 5′-GTATGGTCGCAAGDCTGAAACTTA-3′; RevD: 5′-TCAGGCTCCYTCTCCGGAAY-3′).

Results and Discussion

Genus *Geleia* Foissner, 1998

The genus *Geleia* was first established by Kahl (1933) who failed to designate a type species. Consequently Foissner (1998) declared *Geleia* a nomen nudum and re-established it as a new genus, designating *G. fossata* Kahl, 1933 as the type species. Based on the redescriptions by Dragesco (1999), Dragesco and Dragesco-Kernéis (1986), Nouzarède (1977) and the present work, an improved diagnosis is here supplied.

Improved diagnosis of *Geleia*: Geleiidae with cylindrical body shape; cells completely ciliated with longitudinal rows consisting of dikinetids; buccal field located subapically; preoral kinety present; typical geleiid oral structure with dominant conspicuous adoral polykineties that comprise numerous long rows of kineties.

Remarks: Based on the findings of Nouzarède (1977) and Dragesco (1999), Foissner (1998) described geleiids as being "completely ciliated and with oral monokinetids forming a right and left oral ciliary field". Furthermore he considered these 'paracytostomal' monokinetids to be the most important autapomorphy of the geleiids (Foissner 1998). Lynn (2008) likewise considered the monokinetid nature of the oral ciliature to be the defining character of the order Protoheterotrichida, which was established by Nouzarède (1977) for the family Geleiidae. According to Dragesco

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