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Phylogenetic position of the marine ciliate, *Cardiostomatella vermiforme* (Kahl, 1928) Corliss, 1960 inferred from the complete SSrRNA gene sequence, with establishment of a new order Loxocephalida n. ord. (Ciliophora, Oligohymenophorea)

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Abstract

The small subunit rRNA (SSrRNA) gene was sequenced for *Cardiostomatella vermiforme*, a large marine ciliate the systematic position of which is uncertain but which has been regarded as a scuticociliate for about forty years. The present work indicates that this organism, together with a closely related species, *Dexiotrichides pangi*, always form a separate assemblage as a sister group to the scuticociliates sensu stricto. The fact that the clade comprising *Cardiostomatella* and *Dexiotrichides* branches between the typical scuticociliates and Hymenostomatia, and shares a series of morphological and morphogenetical characters with both, supports the conclusion that it belongs to an intermediate group between the two. We suggest that this group represents a new order, Loxocephalida n. ord. within the subclass Scuticociliatia, which possibly contains all taxa within the families Loxocephalidae and Cinetochilidae and with Loxocephalidae as the type family.

Keywords: SSrRNA; Phylogenetic position; *Cardiostomatella vermiforme*; Loxocephalida n. ord.; Scuticociliates

Introduction

Over the past four decades, numerous studies have been carried out on the taxonomy and cell development of the scuticociliates (Corliss 1979; Lynn 1979; Small 1967; Song 2000; Song and Wilbert 2000). Based mainly on morphological and morphogenetical data, Lynn and Small (1997) divided the scuticociliates into 3 orders: Philasterida, Pleuronematida and Thigmotrichida. Comparatively few studies have been performed on the systematics of this group (Lynn and Small 1997), despite the fact that the morphology and morphogenesis of many species, especially those in the order Philasterida, have been investigated in detail using modern methods (Grolière 1980; Hu et al. 1996; Song 2000; Morade and Small 1994; Song and Wilbert 2000).

Molecular methods, in particular the determination of small subunit rRNA (SSrRNA) sequences, have been commonly used to re-evaluate the phylogenetic relationships of many ciliate groups in recent years (Chen and Song 2001; Elwood et al. 1985; Ragan et al. 1996; Shang

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et al. 2002, 2003; Stoeck et al. 1998). Several of these studies provided different conclusions from those based on morphological and/or ontogenetic characters (Chen and Song 2002; Ragan et al. 1996; Strüder-Kypke et al. 2000). Nevertheless, sequence data for poorly known groups such as the *Cardiostomatella–Dexiotrichides*-complex remain comparatively rare and incomplete.

As a contribution to the analysis of ciliate phylogeny we have sequenced the SSrRNA gene from the marine ciliate, *Cardiostomatella vermiforme*, in order to ascertain its systematic position with molecular biological methods, particularly as the placement of the *Cardiostomatella–Dexiotrichides* complex within the true scuticociliates is now thought to be highly questionable (Song et al. 2005).

Material and methods

Ciliate collection and identification

Cardiostomatella vermiforme (Kahl 1928) Corliss 1960 was collected from the coast of Qingdao, China in November 2004. Clonal cultures were established and maintained at room temperature in autoclaved marine water with the appropriate prey. Observations on living cells were carried out using differential interference microscopy. Protargol (Wilbert 1975) and pyridinated silver carbonate (Fernández-Galiano 1976) impregnation techniques were applied to reveal the infraciliature.

Extraction of genomic DNA and PCR amplification

Ciliates were starved overnight, rinsed with sterile artificial sea water and then sedimented by centrifugation. 50 μl lysis buffer (Shang et al. 2003) was added to 20 μl of the concentrated cells and the mixture incubated at 56 °C for 1–2 h to extract DNA, and then at 94 °C for 15 min to denature protein. The PCR reaction steps were performed according to Medlin et al. (1988) and with the primers used by Chen and Song (2002).

Cloning and sequencing of SSU rRNA gene

The PCR products were extracted with UNIQ-5 DNA Cleaning Kit (Sangon Bio. Co., Canada) and cloned into a pUCm-T vector according to the manufacturer's protocol. The plasmid DNA was extracted using the mini-prep spin column kit (Sangon Bio. Co., Canada), according to the manufacturer's protocol. The SSUrDNA gene for *Cardiostomatella vermiforme* was double strand-sequenced with three forward and three reverse modified 18S sequencing primers (Elwood et al. 1985; Medlin et al. 1988) and the vector based primers, RV-M and M13-20 primers, using the ABI Prism 377 Automated DNA Sequencer (Applied Biosystems Inc.).

Sequence availability

The SSrRNA gene sequences of 33 other taxa were obtained from the GenBank/EMBL databases under the following accession numbers: *Cyclidium glaucoma* Z22879, *Cyclidium plouneouri* U27816, *Entodiscus borealis* AY541687, *Entorhipidium pilatum* AY541689, *Entorhipidium tenue* AY541688, *Miamiensis avidus* AY550080, *Philasterides dicentrarchi* AY642280, *Plagiopyliella pacifica* AY541685, *Pleuronema coronatum* AY103188, *Schizocaryum dogieli* AF527756, *Thyrophylax vorax* AY541686, *Frontonia vernalis* U97110, *Lembadion bullinum* AF255358, *Epicarchesium abrae* DQ190462, *Zoothamnopsis sinica* DQ190469, *Zoothamnium arbuscula* AF401523, *Pseudovorticella punctata* DQ190466, *Vorticella fusca* DQ190468, *Anophryoides haemophila* U51554, *Cohnilembus verminus* Z22878, *Parauronema longum* AY212807, *Dexiotrichides pangi* AY212805, *Paranophrys magna* AY103191, *Metanophrys similis* AY314803, *Mesanophrys carcini* AY103189; *Uronema marinum* Z22881, *Pseudocohnilembus hargisi* AY212806, *Glaucoma chattoni* X56533, *Tetrahymena asiatica* X56167, *Tetrahymena hyperangularis* X56173, *Tetrahymena vorax* AF364038, *Lambornella* sp. AF364043, *Glaucoma scintillans* AJ511861, *Glaucomides bromelicola* AJ810077, *Bromeliophrya brasiliensis* AJ810075, *Colpidium campylum* X56532, *Ophryoglena catenula* U17355, *Ichthyophthirius multifiliis* U17354, *Pseudomicrothorax dubius* X65151, *Obertrumia georgiana* X65149, *Furgasonia blochmanni* X65150, *Colpoda inflata* M97908, *Sorogena stoianovitchae* AF300285, *Euplotes woodruffi* AF492707, *Paramecium bursaria* AF100314, *Paramecium tetraurelia* X03772, *Paramecium nephridiatum* AF100317, *Dysteria derouxi* AY378112, *Tokophrya quadripartita* AY102174, *Tetrahymena corlissi* U17356, and a karyorelictid ciliate, *Loxodes striatus* L24248 was selected as the outgroup species.

Data analysis

The SSrRNA gene sequences of the various taxa were aligned using Clustal W, V.1.80 (Thompson et al. 1994), and then refined through consideration of the conserved primary structures. The computer program, MrBayes v3.0b4 (Huelsenbeck and Ronquist 2001) was used for the Markov chain Monte Carlo (MCMC) algorithm to construct a Bayesian tree. The chain length for our analysis was 10,000,000 generations with trees sampled every 50 generations. PHYLIP V.3.57c (Felsenstein 1995) was used to calculate the sequence similarity and evolutionary distances between pairs of nucleotide