Protist, Vol. 163, 263–273, March 2012
http://www.elsevier.de/protis
Published online date 27 July 2011

Protist

ORIGINAL PAPER

A Two-locus Molecular Characterization of *Paramecium calkinsi*

Ewa Przyboś[a], Sebastian Tarcz[a,1], Alexey Potekhin[b], Maria Rautian[c], and Małgorzata Prajer[a]

[a]Department of Experimental Zoology, Institute of Systematics and Evolution of Animals, Polish Academy of Sciences, Kraków 31-016, Sławkowska 17, Poland
[b]Department of Microbiology, Faculty of Biology and Soil Science, St. Petersburg State University, Saint Petersburg 199034, Universitetskaya nab. 7/9, Russia
[c]Laboratory of Protozoan Karyology, Biological Institute of St. Petersburg State University, Saint Petersburg 198504, Oranienbaumskoye shosse 2, Russia

Submitted February 18, 2011; Accepted June 25, 2011
Monitoring Editor: C. Graham Clark

Paramecium calkinsi **(Ciliophora, Protozoa) is a euryhaline species which was first identified in freshwater habitats, but subsequently several strains were also collected from brackish water. It is characterized by clockwise spiral swimming movement and the general morphology of the "*bursaria* type." The present paper is the first molecular characterization of *P. calkinsi* strains recently collected in distant regions in Russia using ITS1-5.8S- ITS2-5′LSU rDNA (1100 bp) and *COI* (620 bp) mtDNA sequenced gene fragments. For comparison, our molecular analysis includes *P. bursaria,* exhibiting a similar "*bursaria* morphotype" as well as species representing the "*aurelia* type," i.e., *P. caudatum*, *P. multimicronucleatum*, *P. jenningsi*, and *P. schewiakoffi*, and some species of the *P. aurelia* species complex (*P. primaurelia*, *P. tetraurelia, P. sexaurelia,* and *P. tredecaurelia*). We also use data from GenBank concerning other species in the genus *Paramecium* and *Tetrahymena* (which used as an outgroup). The division of the genus *Paramecium* into four subgenera (proposed by Fokin et al. 2004) is clearly presented by the trees. There is a clear separation between *P. calkinsi* strains collected from different regions (races). Consequently, given the molecular distances between them, it seems that these races may represent different syngens within the species.**

Key words: *COI* mtDNA; intraspecific polymorphism; ITS1-5.8S-ITS2-LSU rDNA; molecular phylogeny; *Paramecium calkinsi*.

Introduction

The genus *Paramecium* comprises 12 relatively widely distributed valid species and 4 endemic African species (Fokin et al. 2004; Wichterman 1986). Variation in SSU rDNA (Strüder-Kypke et al. 2000a, b), *COI* mtDNA (Strüder-Kypke and Lynn 2010), and morphometric and biological data (Fokin 2001) show that species within the genus form a monophyletic cluster divided into four subgenera, which "reflects the phylogeny of the genus and can also be seen in a large set of morpho-biological features of the ciliates" (Fokin et al. 2004). According to Fokin et al. (2004), the first subgenus of the genus *Paramecium*, named *Chloroparamecium* due to its prominent symbiosis with the green alga *Chlorella*, consists only of *P. bursaria.* This species occupies the basal position in the tree presenting all the species of the genus. The second

[1]Corresponding author; fax +48 12 422 42 94
e-mail starcz@isez.pan.krakow.pl (S. Tarcz).

doi:10.1016/j.protis.2011.06.006

subgenus, *Helianter*, includes *P. putrinum* and *P. duboscqui*, both of which branch off in the genus after *P. bursaria*. The third subgenus, *Cypriostomum*, encompasses *P. woodruffi, P. nephridiatum, P. calkinsi*, and *P. polycaryum*. This subgenus is placed in the middle of the genus tree. Finally, the fourth subgenus, named *Paramecium* due to the fact that all of its species have a cigar-shape, contains the following species: the *P. aurelia* species complex, *P. jenningsi, P. schewiakoffi, P. caudatum, P. multimicronucleatum*, and some rare species, i.e., *P. wichtermani*, *P. africanum*, *P. jankowski*, and *P. ugandae*.

The well-defined species were classified into two groups according the shape of the body, the "aurelia" group and the "bursaria" group (Wenrich 1928). The "aurelia" group includes species characterized by a cigar-shaped body, i.e., the *P. aurelia* species complex, *P. jenningsi, P. caudatum, P. multimicronucleatum*, and *P. schewiakoffi*. The "bursaria" group includes *P. bursaria, P. calkinsi, P. woodruffi, P. nephridiatum, P. polycaryum, P. duboscqui,* and *P. putrinum.* The species are characterized by a shorter and wider body, truncated in its anterior part (Vivier 1974).

Paramecium calkinsi Woodruff, 1921 (Wenrich 1928) is characterized by clockwise spiral swimming movement, which is a unique feature in the genus *Paramecium*. Its general morphology is of the "*bursaria* type," with the anterior part being the broadest (Vivier 1974). Some morphological features of the species were studied by Fokin and Chivilev (1999), who also performed morphometric analysis. According to them, the average size of *P. calkinsi* cells (fixed and stained by Chatton and Lwoff) in four investigated stocks was 115.0 x 38.0 μm, however, the length of living specimens may be greater, reaching 110–140 μm (Vivier 1974). The species shows a variable number of micronuclei (1–5), but usually there are 2 per cell, with a size of 1.7–3.4 μm, generally located close to the macronucleus. The micronuclei are of the "endosomal type"—they have a thin, light periphery, a large chromatin body in the centre, and are spheroid in shape (Fokin 1997).

The main stages of nuclear reorganization in conjugation, the mating types, and the occurrence of selfing were described by Nakata (1958) and also by Fokin et al. (2001). Two syngens with a binary mating system were found in this species (Wichterman 1953, quoted in Wichterman 1986), which is characterized by inbreeding (Fokin et al. 2001).

P. calkinsi (Woodruff 1921) is recognized as a euryhaline species, but was first described as a freshwater one. Subsequently, several strains were collected from brackish water. The species was discovered in North America (original description by Woodruff 1921), Europe and Asia (according to Fokin et al. 2001, 2004). According to literature data (Fokin and Chivilev 1999; Fokin 1997), the European and Asian strains of *P. calkinsi* were established from natural populations collected in Russia (from the White Sea, the Karelian District, the Kandalaksha District, the Barents Sea, the Murmansk District, and the Vladivostok District), in Armenia (from the Sevan lake district), and in Japan (from the coast of the Inland Sea of Japan). However, these strains are no longer in existence.

Molecular studies (PCR-based fingerprint methods and sequenced gene fragments) of the morphological species of the genus *Paramecium* have been conducted mainly for the *P. aurelia* spp. complex using different markers: *cytB* mtDNA (Barth et al. 2008); 10 nuclear and 5 mitochondrial markers (Catania et al. 2009); the ITS1-5.8S-ITS2 region (Coleman 2005); DNA fragments analyzed by PFGE (Nekrasova et al. 2010); RAPD, ARDRA, *hsp70*, and *COI* mtDNA (Przyboś et al. 2007, 2008); RAPD (Stoeck et al. 1998); and the ITS1-5.8S-ITS2-5′LSU rDNA region (Tarcz et al. 2006).

Apart from its application in studies on the *P. aurelia* species complex, RAPD fingerprints have proved useful in identifying the following species: *P. nephridiatum*, *P. dubosqui*, *P. woodruffi*, and *P. calkinsi* (Fokin et al. 1999a, b); *P. jenningsi* (Przyboś et al. 2003); *P. bursaria* (Greczek-Stachura et al. 2010); and *P. caudatum* (Stoeck et al. 2000). Papers concerning molecular analysis of DNA fragments obtained from other *Paramecium* species are rather sparse, and are focused on the rDNA region for *P. bursaria* (Greczek-Stachura et al. 2010; Hoshina et al. 2006); *P. caudatum* and *P. multimicronucleatum* (Barth et al. 2006); and *P. schewiakoffi* (Fokin et al. 2004) and on part of the mitochondrial genome (*COI* gene) for *P. caudatum* and *P. multimicronucleatum* (Barth et al. 2006).

The present paper is the first molecular characterization of *P. calkinsi* strains that were recently collected (2004–2010) in very distant regions in Russia (Fig. 1). The aim of this study is to assess the genetic divergence between *P. calkinsi* and other *Paramecium* species and polymorphism within *P. calkinsi*. The molecular characterization of the strains was conducted with the application of two loci (ITS1-5.8S-ITS2-5′LSUrDNA and *COI* mtDNA), as at least a two-locus approach should be used in molecular phylogenetics (Dunthorn et al. 2011).