

Two species of the *Paramecium aurelia* complex (Ciliophora, Protista) from the Black Sea region (Russia) with their RAPD-PCR fingerprints characteristics

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Summary

The presence of *Paramecium tetraurelia* and *Paramecium pentaurelia* of the *Paramecium aurelia* complex was revealed in the Black Sea region, Russia. *P. tetraurelia* was found for the first time on the Russian territory. RAPD-PCR fingerprints were obtained for the newly identified *P. tetraurelia* and *P. pentaurelia* strains and compared with the fingerprints of other strains of these two species. *P. tetraurelia* had high polymorphism of fingerprints of strains of different geographical origin, while *P. pentaurelia* showed 100% similarity of fingerprints of strains within the species.

Key words: Distribution of *Paramecium aurelia* species complex, intra-species polymorphism, RAPD-PCR fingerprinting.

Introduction

Among 15 species of the *Paramecium aurelia* complex known world-wide (Sonneborn, 1975; Aufderheide et al., 1983), the following have been found in Europe: *P. primaurelia*, *P. biaurelia*, *P. triaurelia*, *P. tetraurelia*, *P. pentaurelia*, *P. sexaurelia*, *P. septaurelia*, *P. novaurelia*, *P. dodecaurelia*, and *P. tredecaurelia* (Sonneborn, 1975; Przyboś, 2005); *P. primaurelia*, *P. biaurelia*, and *P. novaurelia* are common there. Some species, such as *P. triaurelia*, *P. tetraurelia*, *P. pentaurelia*, and *P. sexaurelia*, seem to be confined to certain environments, and some, such as *P. tredecaurelia*, *P. dodecaurelia*, and *P. septaurelia*, even to certain habitats (cf. Przyboś, 2005).

In the European part of Russia, *P. primaurelia*, *P. biaurelia* and *P. novaurelia* have been recorded in Moscow, St. Petersburg or in their vicinity (Komala and Dubis, 1966; Przyboś and Fokin, 1996; Przyboś et al., 2006), *P. triaurelia* together with *P. novaurelia* in the Volga River (Astrakhan Nature Reserve) (cf. Kościuszko, 1985), *P. triaurelia* with *P. primaurelia* in Kaliningrad (Przyboś et al., 2006), *P. pentaurelia* in the Belgorod region (Fokin and Ossipov, 1986), and *P. novaurelia* in Vladimir (Przyboś et al., 2006). Our previous papers (Przyboś et al., 2004, 2005c) concerned the occurrence of species of the *P. aurelia* complex in the Lower Volga Basin, including the Caspian coast, which turned out to be very rich in species of the *P. aurelia* complex. The presence of

P. primaurelia, *P. biaurelia*, *P. triaurelia*, *P. pentaurelia*, *P. sexaurelia*, *P. septaurelia*, and *P. novaurelia* was revealed there. *P. septaurelia* was recorded there (Przyboś et al., 2004) for the first time in Europe, having been known before only from the USA, and *P. pentaurelia* and *P. sexaurelia*, species rare in Europe, were found to be frequent there.

In the present paper we report the new findings of species of the *P. aurelia* complex in the Eastern part of the Black Sea coast (Krasnodar region, Russia). RAPD-PCR (randomly amplified polymorphic DNA - polymerase chain reaction) fingerprints were obtained for the *P. tetraurelia* and *P. pentaurelia* strains recorded there and compared with the fingerprints of other strains of these two species.

Material and Methods

The study region of the Black Sea coast is situated at the foot of Caucasus Mountains. The water samples (15-30 ml each) with plankton were collected in two water reservoirs in September 2004; water temperature and ambient temperature were about 24°C. Paramecia were isolated and strains were established. The strains studied and the collection sites where paramecia were found are presented in Table 1.

the method of daily isolation lines). The occurrence of the desired stage of autogamy (individuals at the stage of two macronuclear anlagen) was examined on preparations stained with acetocarmine. Survival of clones in both generations was estimated. According to Chen (1956), the clones could be recognized as surviving after passing 6-7 fissions during 72 hours after separation of partners of conjugation or postautogamous caryonids.

P. multimicronucleatum and *P. caudatum* were identified by analyzing the type and number of their micronuclei (Vivier, 1974) on slides stained with acetocarmine (Sonneborn, 1950).

RAPD-PCR fingerprint analysis of 13 *P. aurelia* spp. strains (Table 2) was performed as described earlier (Przyboś et al., 2003), according to modified protocol proposed by Stoeck and Schmidt (1998). DNA was isolated from strains of each species, *P. tetraurelia* and *P. pentaurelia* (Table 2), using a QIAamp™ DNA Mini Kit (Qiagen™, Germany). RAPD-PCR was performed with primer Ro 460-04 (5'-GCAGAGAAGG-3', Roth, Karlsruhe, Germany) using Taq polymerase (Qiagen). Stoeck and Schmidt (1998) selected the Ro 460-04 primer after testing several dozen oligonucleotide primers as the one giving "robust band patterns" in the *P. aurelia* species complex. Later this primer was used in other studies

Table 1. Occurrence of the *Paramecium aurelia* complex species in two places of the Black Sea region (Russia)

Sampling place	Species of <i>Paramecium aurelia</i> complex	Strain index	Habitat	Additional <i>Paramecium</i> spp.
Novorossiysk (sea level)	<i>P. pentaurelia</i>	Nr1-9	Sewage pool, near municipal beach	<i>P. caudatum</i>
		Nr1-10		
	<i>P. tetraurelia</i>	Nr7-1	Eutrophic pond overgrown with aquatic plants, in the town	Not present
		Nr7-5		
Verkhnebakanskiy, Krasnodar region (500 m a.s.l.)	Not present		Small duck pond with aquatic plants	<i>P. multimicronucleatum</i>

Culturing and identification of paramecia were performed according to Sonneborn (1970). Paramecia were cultivated on a lettuce medium inoculated with *Enterobacter aerogenes*. Species of the *P. aurelia* complex were identified by mating the strains under investigation with mating types of standard strains. The following standard strains were used:

P. tetraurelia, strain from Sydney, Australia

P. pentaurelia, strain 87 from Pennsylvania, USA.

In the inter-strain crosses, F1 generation was obtained by conjugation and F2 by autogamy (using

carried out on the *P. aurelia* species complex (Stoeck et al., 1998; Stoeck et al., 2000; Przyboś et al., 2005a, b; Przyboś et al., 2006, 2007), on *P. jenningsi* strains (Przyboś et al., 1999; Przyboś et al., 2003; Skotarczak et al., 2004 a, b), and on *P. schewiakoffi* (Fokin et al., 2004).

The RAPD-PCR was done in Biometra thermocycler using the PCR conditions as described by Stoeck and Schmidt (1998). The products of the PCR reactions were separated by electrophoresis on 1.8% agarose gels for 2.5 h at 85V together with molecular weight marker XIV™ (Roche™, France), stained with

Table 2. List of strains and species of the *Paramecium aurelia* complex used in genetic studies

Species	Strain designation	Geographical origin	References
<i>P. tetraurelia</i>	S (standard of the species)	Australia, Sydney	Sonneborn 1974
	J	Japan, Honshu Island	Przyboś and Fokin 2001
	PK	Poland, Kraków	Komala and Przyboś 2000
	SM	Spain, Madrid	Przyboś 1980
	ST	Slovakia, Carpathians, Tatras, Strbske Lake	Dubis and Komala 1963
	IT	Israel, Tabga	Przyboś 1995
	Nr7-1	Black Sea region, Novorossiysk	Present paper
<i>P. pentaurelia</i>	87 (standard of the species)	USA, Pennsylvania	Sonneborn 1974
	Nr1-9	Russia, Black Sea region, Novorossiysk	Present paper
	Nr1-10	Russia, Black Sea region, Novorossiysk	Present paper
	ISN	Italy, Sicily, Giardini Naxos	Przyboś et al. 2005a
	ALT	Russia, Altai, plains	Przyboś et al. 2005a
	HB	Hungary, Balatonfüzfo	Kościuszko 1964

ethidium bromide, and visualized in UV light; the program Scionimage™ (Scion Corporation™, USA) was used to store images obtained in computer memory. Three repetitions of the PCR reaction were performed in order to assess the reproducibility of the data.

Analysis of phylogenetic similarity was carried out by comparing the band patterns (i.e., molecular mass of PCR products obtained by the RAPD method) using the Bio1D++™ program, Vilbert Lourmat, France, according to the Nei and Li (1979) similarity coefficient. Dendrograms were produced using the UPGMA (unweighted pair group match average) algorithm.

Results and Discussion

Though water samples were taken in two localities, the presence of species of *P. aurelia* complex was revealed only in Novorossiysk (Table 1): *P. tetraurelia* and *P. pentaurelia* were identified from two populations on the basis of strong conjugation between the studied strains and the standard ones. A high percentage of surviving hybrid clones was observed in F1 and F2 generations of inter-strain crosses (Table 3) of strains designated Nr7-1 and Nr7-5 with the standard strain (S) of *P. tetraurelia* and strains designated Nr1-9 and Nr1-10 with the standard strain (87) of *P. pentaurelia*. *P. tetraurelia* is a cosmopolitan species (Sonneborn, 1975) known from America, Australia, Asia, and Europe; however, it was found for the first time in Russia. *P. pentaurelia*, a species rare in Europe, was recorded mainly in the southern zone

(Hungary, Romania, Spain, Italy – cf. Przyboś, 2005; Belgorod region, Russia (Fokin and Ossipov, 1986) and the Lower Volga Basin, Russia (Przyboś et al., 2004; 2005c). New finding of this species in the Black Sea region is consistent with its suggested southern distribution.

Table 3. Mean percentage of surviving hybrid clones in crosses of the in crosses of species of the *Paramecium aurelia* complex

Species	Strain	F1 (by conjugation)	F2 (by autogamy)
<i>P. tetraurelia</i>	Nr7-1 x S	100	100
	Nr7-5 x S	100	98
<i>P. pentaurelia</i>	Nr1-9 x 87	100	100
	Nr1-10 x 87	98	96

Fingerprints (RAPD-PCR band patterns) of *P. tetraurelia* and *P. pentaurelia* strains are presented in Fig. 1 and 2, respectively. Strains of *P. tetraurelia* had high polymorphism of band patterns (Fig. 1, A, B), while strains of *P. pentaurelia* (Fig. 2) had no polymorphism within species.

The correlation between the degree of species polymorphism revealed by RAPD analysis and the degree of inbreeding characteristic for the species was suggested by Stoeck et al. (1998) when *P. triaurelia* and *P. sexaurelia* were studied. Later it was confirmed on *P. pentaurelia* and *P. novaurelia* (Stoeck et al., 2000), and also on the remaining species of the

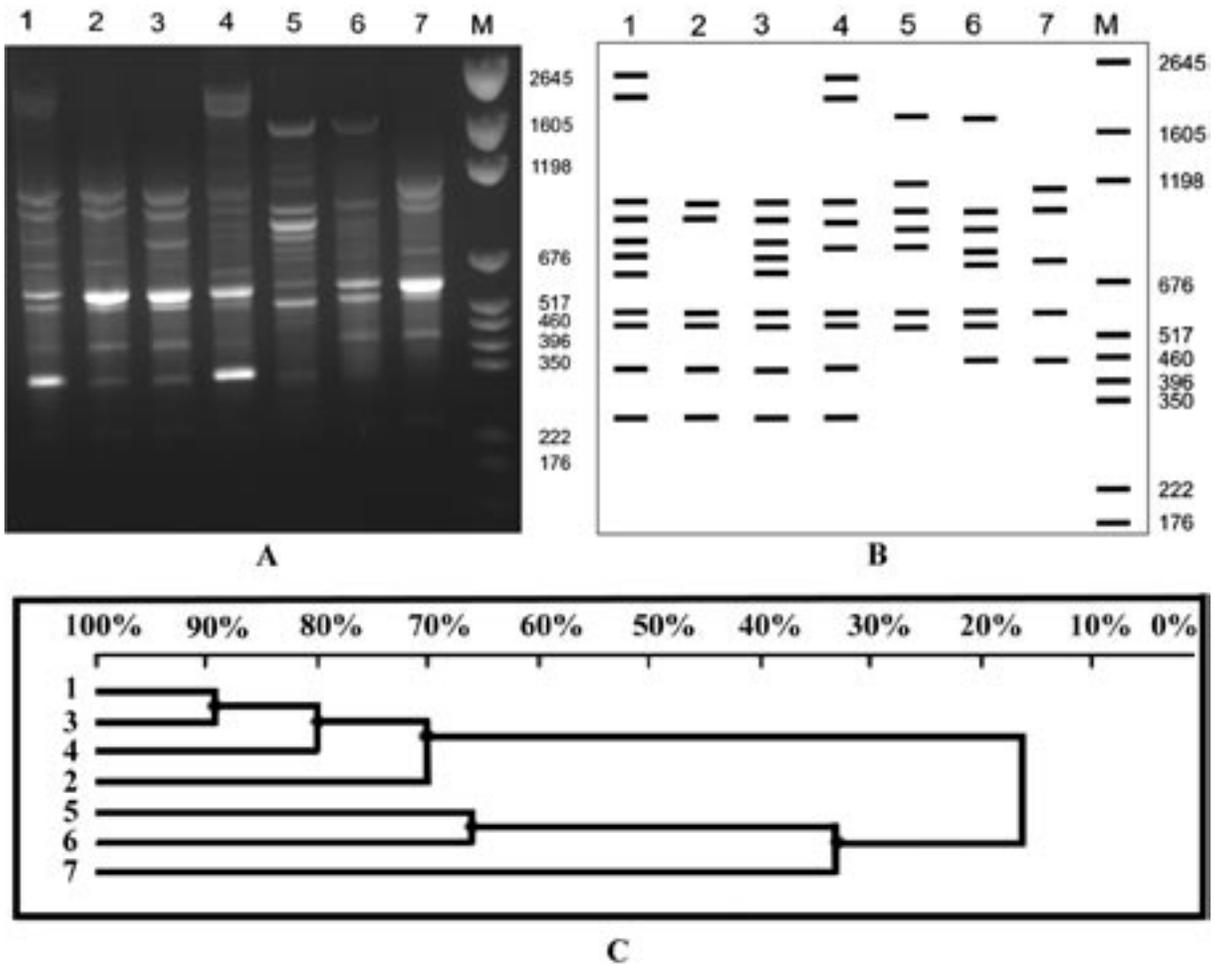


Fig. 1. RAPD fingerprints of *Paramecium tetraurelia* strains. 1 - Sydney, Australia; 2 - Japan; 3 - Poland; 4 - Spain; 5 - Slovakia; 6 - Israel; 7 - Russia, Black Sea region (strain Nr7-1). M – pGEM marker. Molecular weight of the marker DNA bands is given in bp. A – results of electrophoretic separation of RAPD-PCR products, B – a diagram, corresponding to the gel, presenting band patterns of the particular strains, C – a dendrogram presenting similarity of band patterns of the strains studied.

P. aurelia complex (Przyboś et al., 2007). Thus, according to the present results, *P. tetraurelia* should be included into the group of extreme inbreeders, since several groups of genotypes with different band patterns could be seen within the species (Fig. 1, A). The band pattern of new strains from the Black Sea region (only Nr7-1 pattern is presented on gel and diagram (Fig. 1, A, B, lane 7), since the strains Nr7-1 and Nr7-5 are characterized by identical band patterns) is different from patterns of the other European strains from Poland (PK), Slovakia (ST), Spain (SM), as well as from patterns of strains from Israel (IT), Japan (J), and Australia (S). The dendrogram (with homology coefficient 1%, UPGMA) confirms the existence of two groups of strains within *P. tetraurelia* (Fig. 1, C). One group consists of strains from Australia, Poland, Spain, Japan, and another group of strains from

Slovakia, Israel, and the Black Sea region. However, the strain from the Black Sea region (number 7) shows only 33% similarity of band pattern even to strains from Israel and Slovakia, and a low percentage of similarity to the other strains.

On the other hand, *P. pentataurelia* strains revealed 100% similarity of band patterns (Fig. 2) of the studied strains originating from distant places (Russia, Black Sea region – strains Nr1-9, Nr1-10; Russia, Altai - ALT; Italy, Sicily - ISN; Hungary, Balatonfüzfo - HB; USA, Pennsylvania - 87). This species should be included into the group of weak inbreeders. It is very interesting that this species shows no polymorphism (intra-species differentiation): only a single genotype was found in all the strains (Stoeck et al., 2000; Przyboś et al., 2005 a, b; Przyboś et al., 2007) originating from USA and different places in Europe.

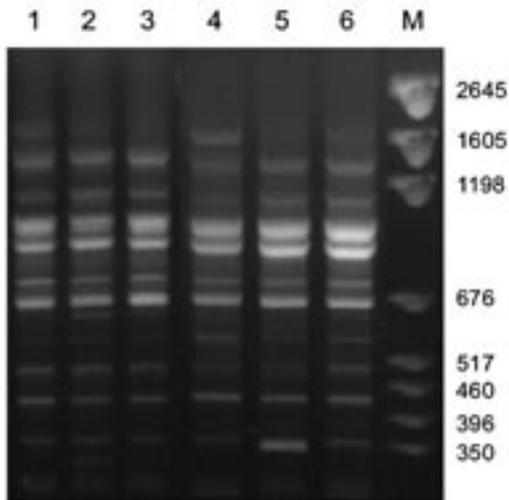


Fig. 2. RAPD fingerprints of *Paramecium pentaurelia* strains: 1 - 87, USA; 2 - Nr1-9, Russia, Black Sea region; 3 - Nr1-10, Russia, Black Sea region; 4 - Italy; 5 - Russia, Altai; 6 - Hungary. M - pGEM marker. Molecular weight of the marker DNA bands is given in bp.

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Editorial responsibility: Sergei Fokin

Added in Proof: One more *P. aurelia* strain 04 T-3 collected in pond in Tonnel'naya close to Novorossiysk was identified later as *P. primaurelia*.