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Species of the *Paramecium aurelia* Complex in Russia (Western Region of European Russia) with Molecular Characteristics of *Paramecium novaurelia*

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The presence of *P. primaurelia*, *P. biaurelia*, *P. triaurelia*, and *P. novaurelia* of the *P. aurelia* complex was revealed in the studied region of Russia. RAPD-PCR fingerprints (band patterns) of newly identified *P. novaurelia* strains from Russia were compared to those characteristic for the other chosen European strains of the species. The strains revealed intraspecific polymorphism as several groups of genotypes confirming the existence of polymorphism within *P. novaurelia*.

Key words: *Paramecium aurelia* species complex, distribution of species, species expansion, RAPD-PCR fingerprinting, intraspecific polymorphism within *P. novaurelia*.

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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* (cf. SONNEBORN 1975; PRZYBOŚ 2005). *P. primaurelia, P. biaurelia*, and *P. novaurelia* are common in Europe. The occurrence of some species, such as *P. triaurelia*, *P. tetraurelia*, *P. pentaurelia*, and P. sexaurelia seems to be limited to certain environments, and in the case of *P. tredecaurelia, P. dodecaurelia*, and *P. septaurelia* even to habitats (cf. PRZYBOŚ 2005).

In the European part of Russia the following species have been recorded: *P. primaurelia, P. biaurelia, P. novaurelia* in Moscow, *P. primaurelia* with *P. novaurelia* in St. Petersburg or its vicinity (KOMALA & DUBIS 1966), and *P. biaurelia* in the vicinity of Stary Peterhof, St. Petersburg (PRZYBOŚ & FOKIN 1996). The presence of *P. triaurelia* has been recorded in the Volga River (Astrakhan Nature Reserve) together with *P. novaurelia* (cf KOŚCIUSZKO 1985), and *P. pentaurelia* in the Belgorod region (FOKIN & OSSSIPOV 1986). Previous papers (PRZYBOŚ *et al*. 2004, 2005) investigated the occurrence of species of the *P. aurelia* complex in the Lower Volga Basin which turned out to be very rich in species of this complex. The presence of *P. primaurelia*, *P. biaurelia*, *P. triaurelia*, *P. pentaurelia, P. sexaurelia, P. septaurelia*, and *P. novaurelia* was revealed there. Among these species, *P. septaurelia* was recorded (PRZYBOŚ *et al.* 2004) for the first time in Europe, known before only from the territory of the USA, and *P. pentaurelia* and *P. sexaurelia* are rare species in Europe. *P. septaurelia* strains originating from the Lower Volga Region showed substantial intraspecific polymorphism being characterized by different band patterns (genotypes) revealed by the applied primer Ro 460-04 (Roth, Karsruhe, Germany). The first genotype appeared in two strains from Astrahan Nature Reserve, the second genotype in the other strain from the same region, and the third genotype in the strain from the Natural Reserve Complex Volga-Ahtuba (PRZYBOŚ & TARCZ 2005).

The present paper presents new localities of species of the *P. aurelia* complex in the Western region of the European part of Russia with molecular (RAPD-PCR fingerprints – randomly amplified polymorphic DNA-polymerase chain reaction) characteristics of *P. novaurelia* strains. Previously, 14 strains of *P. novaurelia* originating from Europe (Spain, Germany, Scotland, Poland, Czech Republic, Ukraine) and one from Turkey were used in the studies (STOECK *et al*. 2000), including a combination of classical inter-and intra-strain crosses by mating reactions and RAPD-PCR fingerprints. The studies revealed four different genotypes within the species which were able to mate showing a high percentage of surviving clones in both generations, F1 (obtained by conjugation) and F2 (obtained by autogamy). The characteristic genotypes seemed to not be totally connected with the geographical origin of the studied strains. It seemed also interesting to study RAPD fingerprints (band patterns) of the newly identified strains of *P. novaurelia* from Russia and to compare them with band patterns of other European strains of this species.

Material and Methods

Material

The studied strains are presented in Tables 1 and 2.

Table 1

Occurrence of species of the *P. aurelia* complex in the Western region of European Russia

Collection place	Strain designation	Species of the *P. aurelia* complex
St. Petersburg	TR9	*P. novaurelia*
	RR2-1	*P. biaurelia*
Kaliningrad	KK2-7	*P. primaurelia*
	Ko3-5	*P. triaurelia*
Vladimir	VL4-8	*P. novaurelia*

Table 2

Strains of *Paramecium novaurelia* used in RAPD-PCR fingerprinting

Strain designation	Strain origin
SB	Spain, Bassotes
510	Scotland, Edinburgh
PT	Poland, Tyniec
RV	Russia, Volgograd region
RP	Russia, St. Petersburg
RB	Russia, Vladimir

Methods

1. Culture and identification of paramecia

Culture and identification of paramecia (Table 1) were performed according to SONNEBORN (1970). The paramecia were cultivated on a lettuce medium inoculated with *Enterobacter aerogenes.* The species of the *P. aurelia* complex were identified by mating the investigated strains with mating types of standard strains of the particular species. The following standard strains were used:

P. primaurelia , strain 90,

P. biaurelia , strain Rieff, Scotland,

P. triaurelia, strain 324,

P. novaurelia, strain 510.

2. Molecular methods

RAPD-PCR fingerprint analysis for *Paramecium novaurelia* was generally as in (STOECK & SCHMIDT 1998). Details are described in PRZYBOŚ *et al*. 2003. DNA was isolated from 6 strains of *P. novaurelia* (Table 2) using the QIAamp™ DNA Mini Kit (Qiagen™, Germany). RAPD-PCR was performed with primers: Ro-460 04 (5' –GCAGAGAAGG- 3', Roth, Karsruhe, Germany) and S83 (5' –GAGCCCTCCA- 3' , IBB PAN, Poland[1] using Taq polymerase (Qiagen). The Ro-460-04 primer was selected (STOECK & SCHMIDT 1998) after testing several dozen oligonucleotide primers as the one giving "robust band patterns" in the *P. aurelia* species complex. It was also used in other studies carried out on the *P. aurelia* species complex (STOECK *et al*. 1998, 2000) and on *P. jenningsi* strains (PRZYBOŚ *et al.* 1999, 2003; SKOTARCZAK *et al.* 2004 a, b) and *P. schewiakoffi* (FOKIN *et al*. 2004). The S83 primer was selected from a group of primers which were used by KAI & ZHE-MIN (2003).

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

[1] IBB PAN – Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland.

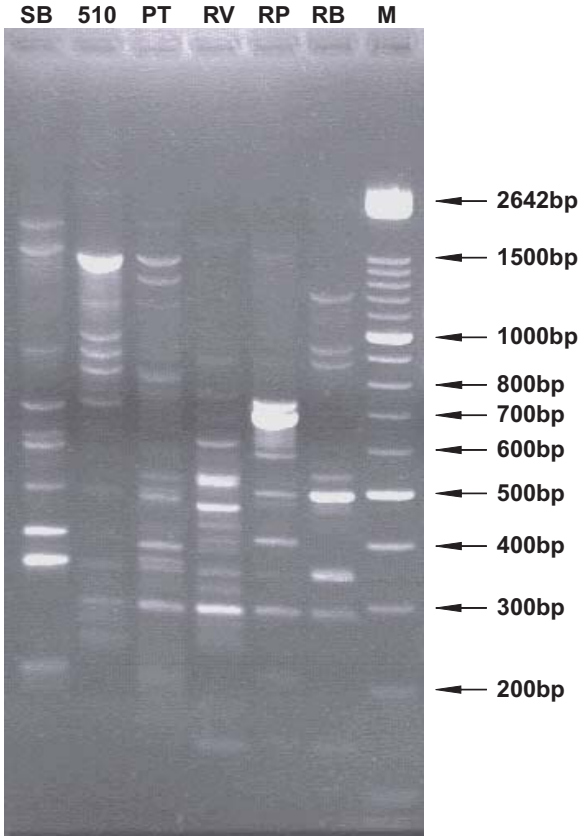

Fig. 1. RAPD fingerprints (revealed by primer Ro 460-04) of the studied strains belonging to *Paramecium novaurelia*: SB – strain from Spain, Bassotes; 510 – strain from Scotland, Edinburgh; PT – strain from Poland, Tyniec; RV – strain from Russia, Volgograd region; RP – strain from Russia, St. Petersburg; RB – strain from Russia, Vladimir. M – molecular marker, molecular weight of the marker DNA bands in bp. Gel.

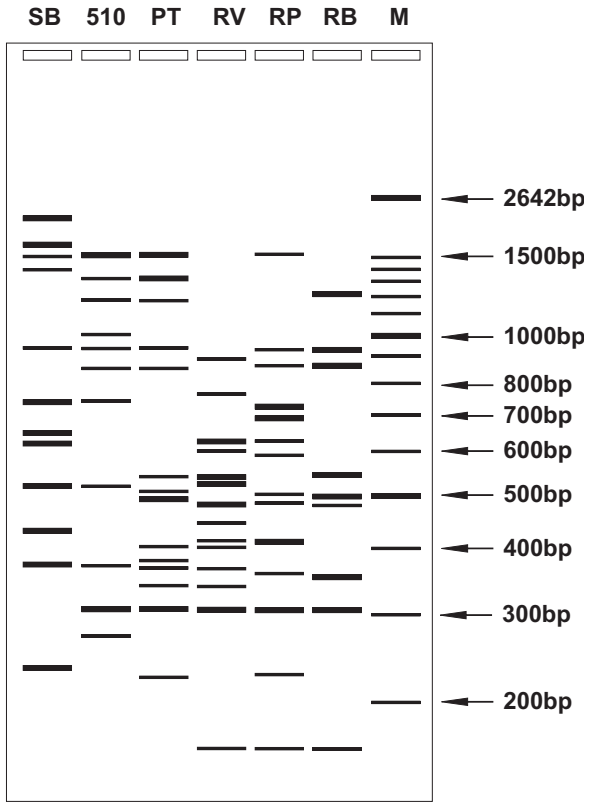

Fig. 2. Schematic representation of Fig.1 showing specific band patterns representing different genotypes as revealed by RAPD-fingerprints.

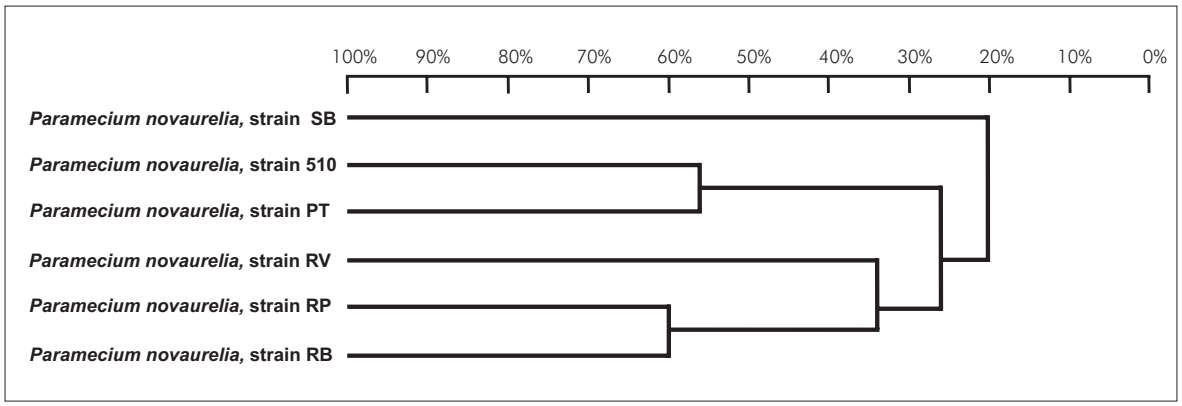

Fig. 3. Intraspecific dendrograms of *P. novaurelia* strains based on RAPD fingerprinting.

stained with ethidium bromide and visualized under UV light. The images were stored in computer memory using the program Scionimage™ (Scion Corporation™, USA). 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 molecular mass of DNA band patterns obtained by the RAPD method (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

The presence of *P. primaurelia, P. biaurelia, P. triaurelia, and P. novaurelia of the P. aurelia* complex was revealed in the studied region (Table 1) on the basis of strong conjugation between the studied and the standard strains of the particular species.

The strains from St. Petersburg were identified as *P. biaurelia* (strain RR2-1) and *P. novaurelia* (strain TR9). Both species were recorded earlier in St. Petersburg or its vicinity (KOMALA & DUBIS 1966; PRZYBOŚ & FOKIN 1996) and are also common in Europe, as *P. novaurelia* was recorded in 184 and *P. biaurelia* in 124 among 474 habitats studied in Europe (PRZYBOŚ 2005).

The strain from Vladimir (VL4-8) was identified as *P. novaurelia.*

Two strains from Kaliningrad were identified as *P. primaurelia* (strain KK2-7) and *P. triaurelia* (strain Ko3-5). *P. primaurelia* was recorded earlier in Moscow, St. Petersburg or its vicinity (KOMALA & DUBIS 1966) and *P. triaurelia* is known in the European part of Russia from the Volga River (Astrakhan Nature Reserve) (KOŚCIUSZKO1985) and from the entire Lower Volga Basin (PRZYBOŚ *et al*. 2004, 2005). *P. primaurelia* is a cosmopolitan species (SONNEBORN 1975) common also in Europe, being recorded from 112 habitats and *P. triaurelia* seems to be limited to certain environments, recorded in Europe from 21 habitats among 474 studied (PRZYBOŚ 2005).

P. novaurelia is the most frequent species in Europe and was supposed to be restricted to this continent only (SONNEBORN 1975), however, one habitat of the species was found in Turkey (Asiatic part, Anatolia) by PRZYBOŚ (1998). In Europe, the species was recorded in 184 habitats among 474 studied, in 23% of studied habitats of northern Europe (14 among 60), in 44% of central Europe (161 among 362), and in 17% of southern Europe (9 among 52), (PRZYBOŚ 2005). However, different numbers of habitats were studied in different zones.

RAPD-PCR fingerprints (band patterns revealed by the primer Ro 460-04) of newly identified strains of *P. novaurelia* from Russia, i.e. strains from Petersburg (RP) and Vladimir (RB), were compared with those characteristic for other European strains of this species (Table 2), i.e. the strains designated RV – Russia, Volgograd region (Natural Reserve Complex Volga-Ahtuba), PT – Poland, Tyniec, 510 – Scotland, Edinburgh, SB – Spain, Bassotes (Figs 1 and 2). All studied *P. novaurelia* strains show bands characteristic for the species at 360bp, 500bp, and 950bp. The compared strains revealed, however, intraspecific polymorphism as several groups of genotypes (Fig. 3). The new strains from Russia RP (Petersburg) and PB (Vladimir) may be included in one group as they show 61% similarity of band patterns. The other strain from Russia RV (Volgograd region) shows only 35% similarity of band pattern to the strains from Petersburg and from Vladimir. Comparison of band patterns of RB and RP strains to patterns of the other European strains revealed a low percentage of similarity (Fig. 3). Among the other strains, only the strains from Scotland (510) and Poland (PT) showed 56% similarity of band pattern. The present study confirms the existence of intraspecific polymorphism within *P. novaurelia* revealed earlier (STOECK *et al*. 2000), as well as within the other species of the *P. aurelia* complex (STOECK et al. 1998; PRZYBOŚ & TARCZ 2005; PRZYBOŚ *et al*. 2006). The degree of species polymorphism seems to be connected with the degree of inbreeding characteristic for the species. *P. novaurelia* should be included into the group of moderate inbreeders.

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