

Polymorphism within *Paramecium sexaurelia* (Ciliophora, Oligohymenophorea) and Description of a New Stand of the Species in China

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The presence of *Paramecium sexaurelia* from the *Paramecium aurelia* complex was recorded for the first time in China (Beijing). RAPD fingerprints (band patterns) of *P. sexaurelia* strains, the new strain from China and others from Asia, as well as from Europe and Puerto Rico, showed polymorphism within the species as several groups of genotypes characterized by different band patterns.

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

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The *Paramecium aurelia* complex is composed of 15 species (SONNEBORN 1975; AUFDERHEIDE *et al.* 1983). Some species are cosmopolitan, others known from some regions or from single habitats only.

P. sexaurelia is a cosmopolitan species occurring in the tropical zone, extending into the temperate zone; it occurs in the eastern USA as far north as Pennsylvania, in India around Bangalore, in Puerto Rico, and Kenya (SONNEBORN 1975; PRZYBOŚ & FOKIN 2000a). Later it was recorded from Thailand (DAGGETT 1978; PRZYBOŚ & FOKIN 2000b), Japan (PRZYBOŚ & FOKIN 2001) and in China at present. The occurrence of species of the *P. aurelia* complex has not been studied on Chinese territory and this large country is still “*terra incognita*”, apart from this report (no papers were found in the accessible literature). *P. sexaurelia* in Europe (PRZYBOŚ 2005) was found mainly in the southern zone, in the following countries: Spain (PRZYBOŚ 1990), Germany (PRZYBOŚ & FOKIN 1997), Greece (PRZYBOŚ 1996; PRZYBOŚ & LEKKA 2000), Croatia (PRZYBOŚ 2003), and Russia (PRZYBOŚ *et al.* 2004).

Species of the *P. aurelia* complex are characterized by various degrees of inbreeding (SONNEBORN

1957; LANDIS 1986), having an effect on intra-specific differentiation. Intra-specific differentiation within the particular species may be studied via genetic analyses, i.e. classical inter-strain crosses (DIPPELL 1954; KOŚCIUSZKO 1965) in which survival is evaluated as the percentage of surviving inter-strain hybrid clones, or by molecular methods – RAPD, RFLP, ARDRA (STOECK *et al.* 1998, 2000; PRZYBOŚ *et al.* 2005, 2006a, 2007) presenting different band patterns characteristic for the particular genotypes within species.

Genetic studies carried out on *P. sexaurelia* have shown that this species is characterized by extreme inbreeding. A low percentage of surviving clones (40% and 11 %) was observed in F2 of hybrids of the Spanish strains with strain 159 from Puerto Rico (PRZYBOŚ 1990), and in F2 of hybrids (41% and 18.5%) of the Croatian strains with strain 159 (PRZYBOŚ 2003). RAPD-PCR analysis also revealed the existence of different genotypes within *P. sexaurelia* with specific DNA bands which were strictly confined to certain geographical regions (STOECK *et al.* 1998) when only some strains of the species were studied.

The aim of the present paper is the evaluation of intraspecific polymorphism within *P. sexaurelia*, with the inclusion of a new strain from China and also several other strains of the species originating from distant and isolated regions, even different continents.

Material and Methods

Material

Seven strains (designated CB1 to CB7) were collected by M. Rautian in June 2005 in China, in the neighborhood of Beijing, from natural ponds situated at a distance of about 10 to 30 km from each other.

Methods

1. Culturing and identification of paramecia

Paramecia were cultured on a lettuce medium inoculated with *Enterobacter aerogenes* and identified according to the methods of SONNEBORN (1970). Clones mature for conjugation were mated with the reactive mating types of the standard strain (159) of *Paramecium sexaurelia*.

2. Strain crosses

The F1 generation was obtained by conjugation and F2 by autogamy (using the method of daily isolation lines). The occurrence of the desired stage of autogamy (specimens at the stage of two macronuclear anlagen) was examined on preparations stained with aceto-carmin. Survival of clones in

both generations was determined as a percentage. 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. The methods were described in detail in PRZYBOŚ (1975).

3. Molecular analysis – randomly amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR)

Paramecium genomic DNA was isolated (200 µl of cell culture was used for DNA extraction) from vegetative cells at the end of the exponential phase using the Qiamp DNA Kit (Qiagen™, Germany) from the strains of *P. sexaurelia*, the strains are presented in Table 1.

The RAPD – PCR fingerprint method was applied in general as in STOECK and SCHMIDT (1998), details are described in PRZYBOŚ *et al.* (2003). RAPD-PCR was performed with a 10mer random primer Ro-460 04 (Roth, Karlsruhe, Germany), with nucleotide sequence: 5' –GCAGAGAAGG-3', using Taq polymerase (Qiagen). The 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; PRZYBOŚ *et al.* 2005, 2006a, 2007). The RAPD-PCR was done in a Biometra thermocycler, products of PCR reactions were separated by electrophoresis in 1% agarose gels for 1.5 h at 85V together with the molecular weight marker VI™ (Roche™, France), then stained with ethidium bromide and visualized in UV light. The images were stored in computer memory using the Scion Image™ program (Scion Corporation™, USA). Three repetitions of the

Table 1

Paramecium sexaurelia strains

Strain designation	Geographical origin	References
159 (standard of the species)	Puerto Rico	SONNEBORN 1975
SA	Spain, Sevilla, America Square	PRZYBOŚ 1990
SL	Spain, Sevilla, Maria Luiza Park	PRZYBOŚ 1990
GS	Germany, Stuttgart	PRZYBOŚ & FOKIN 1997
CK	Croatia, Krka River	PRZYBOŚ 2003
GJ	Greece, Joannina Lake	PRZYBOŚ 1996
RA	Russia, Astrakhan Nature Reserve	PRZYBOŚ <i>et al.</i> 2004
TP	Thailand, Phuket Island	PRZYBOŚ & FOKIN 2000b
JY	Japan, Yamaguchi	PRZYBOŚ & FOKIN 2001
C B	China, Beijing	present paper

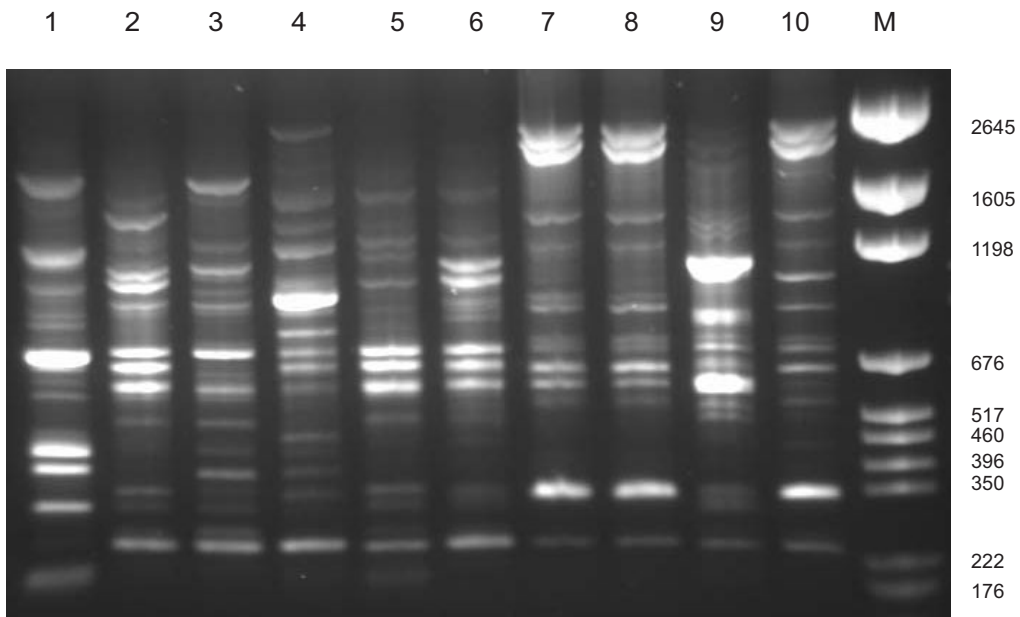


Fig. 1. RAPD fingerprints of *Paramecium sexaurelia* strains originating from: 1 – China, 2 – Thailand, 3 – Japan, 4 – Russia, 5 – Croatia, 6 – Greece, 7 – Spain (SA), 8 – Spain (SL), 9 – Germany, 10 – Puerto Rico. M – molecular pGEM marker, molecular weight of the marker DNA bands are given in bp.

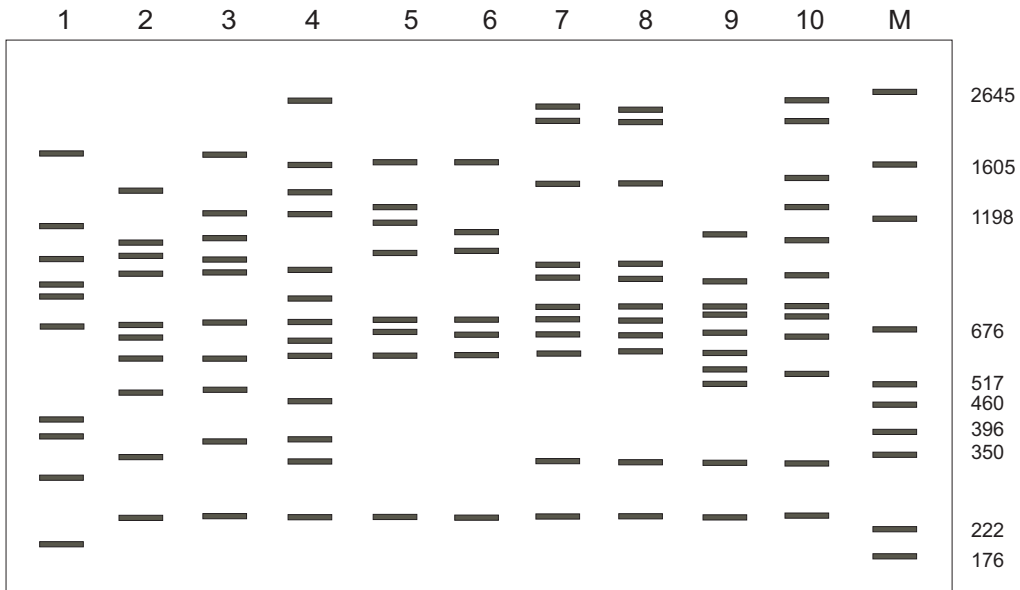


Fig. 2. Schematic representation of Fig. 1 showing specific band patterns representing different genotypes as revealed by RAPD-fingerprints with primer Ro 460-04.

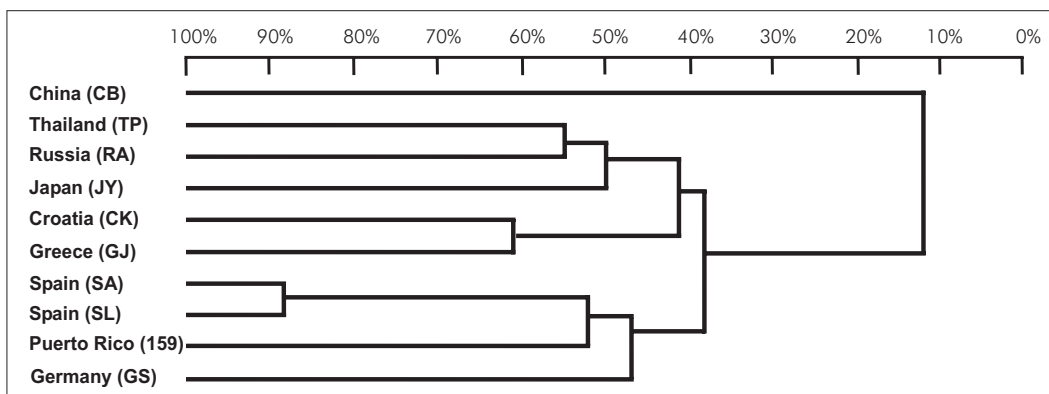


Fig. 3. Tree diagram of the cluster analysis of RAPD fingerprint pattern similarity matrix of the studied *P. sexaurelia* strains. UPGMA method.

PCR reaction were performed in order to assess the reproducibility of the data. Analysis of similarity was carried out by comparing the molecular mass of DNA patterns obtained by the RAPD method (the Bio1D++ TM 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

Seven strains from China, Beijing were identified as *P. sexaurelia* on the basis of strong conjugation with the standard strain of the species. A high percentage (96%) of surviving hybrid clones was observed in the F1 generation of inter-strain crosses of the Chinese strains with the standard strain (159) from Puerto Rico, but a low percentage (32%) of hybrid clones was observed in the F2 generation. This is the first stand of the species in China, considered a cosmopolitan species and recorded in other countries in Asia (Japan, Thailand, India).

Fingerprints (band patterns) of the studied *P. sexaurelia* strains, the new strain from China (CB1) and others from Asia, Europe and Puerto Rico (Table 1) revealed by DNA amplification with primer Ro 460 04 are presented in Figs 1-3. Distinct polymorphism within *P. sexaurelia* was shown as several groups of genotypes (different band patterns). Only strains from Spain (both from Seville) have similar band patterns. Similarly, the strains from Croatia and Greece can be grouped together on the basis of similarity of band patterns (60 % similarity). Other strains have very different band patterns, so each strain belongs to a different group. The most divergent is strain from China.

At present, the existence of large intra-specific differentiation within *P. sexaurelia* was confirmed when strains from different continents were compared. Intra-specific differentiation was shown by classical strain crosses as well as by RAPD fingerprinting, which revealed several genotypes (band patterns). As the degree of species-specific polymorphism seems to be connected with the degree of inbreeding characteristic for the species, *P. sexaurelia* should be included into the group of extreme inbreeders. It is worth mentioning the previous studies applying RAPD analysis carried out only on some strains of the species (STOECK *et al.* 1998), i.e. a strain from Puerto Rico, two strains from Spain (Seville, two stands), a strain from Greece, and two strains from Germany (Stuttgart, two stands). They revealed intra-specific polymorphism of *P. sexaurelia* and different genotypes

were strictly confined to certain geographical regions: Spain, Germany, Greece, and Puerto Rico. This is consistent with the point of view presented by SCHLEGEL and MEISTERFELD (2003) concerning the species concept that, "although many free-living protists may be globally distributed, geographical patterns and local distribution also occur". Also, when gene sequences of cytosol-type *hsp70* from *P. sexaurelia* strains from Spain, Greece and Puerto Rico were compared, a slight differences between strains was apparent (Fig. 1 in HORI *et al.* 2006). When strains from different continents, i.e. from Europe (originating from Russia, Croatia, Germany, Spain, and Greece), Asia (from Thailand, Japan, China), and Puerto Rico were taken for RAPD analysis, their band patterns revealed large divergence within species and a "geographical pattern".

Various levels of intraspecific polymorphism were revealed in other species of the *P. aurelia* complex using RAPD, RFLP and ARDRA analyses (PRZYBOS *et al.* 2007), e.g. it was high in *P. dodecaurelia*, moderate in *P. primaurelia*, and low in *P. tredecaurelia*. Very high intraspecific differentiation of *P. dodecaurelia* strains was also confirmed by sequencing fragments of the 3' end of SSU rRNA – ITS1 and the 5' end of LSU rRNA (TARCZ *et al.* 2006). The studies in which H4 histone gene was sequenced (PRZYBOS *et al.* 2006b) also showed a distant position of the species within the phylogenetic tree constructed for the species of the *P. aurelia* complex. No strong correlation was observed between the geographical origin and molecular differentiation of *P. dodecaurelia* strains, however, some kind of correlation exists as a strain from Hawaii was very distant from the other strains of the species, and the European strains were in one cluster (5' LSU rRNA fragment), (TARCZ *et al.* 2006).

Future studies of *P. sexaurelia* strains originating from different continents based on sequencing of fragments of rRNA or mtDNA genes may bring more information concerning their relationships.

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