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Sibling Species Within *Paramecium jenningsi* Revealed by RAPD

Bogumiła SKOTARCZAK, Ewa PRZYBOŚ[2], Beata WODECKA[1] and Agnieszka MACIEJEWSKA[1]

[1]Department of Genetics, Faculty of Biology, Szczecin University, Szczecin; [2]Institute of Systematics and Evolution of Animals, Polish Academy of Sciences, Kraków, Poland

Summary. The studies with application of the classical genetic method (strain crosses) and a molecular technique (RAPD-PCR fingerprinting, primer Ro 460-04) revealed the presence of separate sibling species within *P. jenningsi*. One of these is confined to six genetically related strains originating from Japan, separated from other group (results of strain crosses) and showing only one characteristic band pattern. The other of these includes strains from India, Saudi Arabia and China (Przyboś *et al*. 2003). We wanted to verify the RAPD markers specific for *P. jenningsi* and to prove the existence of two sibling species within it (revealed by one primer only), by application of others Ro-type primers. The conducted RAPD-PCR fingerpring analysis revealed that the genomes of the studies strains of *P. jenningsi* have polymorphic DNA sequences complementary to the four applied primers. The band patterns can be used as species-specific markers for *P. jenningsi.* DNA amplification by four primers used differentiates *P. jenningsi* into two groups of strains, one of which includes the Indian, Saudi Arabian and Chinese strains, the other the Japanese strains. Our results are congruent with inter-strain crossing experiments in which reproductive isolation was found when continental and Japanese strains were tested. This is further evidence for the existence of two sibling species, whose reproductive isolation is genetically induced.

Key words: *Paramecium jenningsi*, primers, RAPD-PCR analysis, sibling species, species markers.

INTRODUCTION

Paramecium jenningsi Diller *et* Earl,1958 was described from India (Bangalore). Later, several strains of this species were also found in different, mainly tropical regions, i.e. Uganda, Madagascar, Florida (USA), Panama, but also from the Kaliningrad district (Russia), Tajikistan and Japan (Przyboś *et al*. 1999). In 1999 and 2000 new strains were collected from Saudi Arabia, China and again from Japan (Table 1). An expansion of the range of *P. jenningsi* prompted studies on intraspecific differentiation, i.e. ascertainment if strains originating from remote and isolated habitats represent one sibling species or more. The existence of one sibling species (syngen) of *P. jenningsi* was accepted (Sonneborn 1958, 1970; Przyboś 1986) and confirmed by genetic, karyological and cytological studies (Przyboś 1975, 1978, 1980, 1986; Jurand and Przyboś 1984) carried out on strains from India, Uganda and Madagascar. Biochemical investigations of esterases and acid phosphotases by Allen *et al*. (1983) were performed on strains from India, Panama and the USA (Florida).

However, a preliminary analysis of *P. jenningsi* strains from India, Madagascar, Uganda, Florida and one

Address for correspondence: Ewa Przyboś, Institute of Systematics and Evolution of Animals, Polish Academy of Sciences, Sławkowska 17, Kraków 31-016, Poland; Fax: (48) 12 422 42 94; E-mail: przybos@isez.pan.krakow.pl

strain from Japan (Przyboś *et al*. 1999) using the RAPD-PCR method (primer Ro 460-04) revealed the existence of three different DNA genotypes (band patterns) within the species. Three groups of strains were distinguished; one group consisting of strains from India and Madagascar, the second one of strains from Uganda and Florida, and a third from Japan (Hagi).

The differentiation of subsequent strains from Saudi Arabia, China, six strains from Japan and one from India (Bangalore) was investigated (Przyboś *et al*. 2003). These studies were carried out with the classical genetic method (strain crosses) and a molecular technique (RAPD-PCR fingerprinting, primer Ro 460-04). They revealed the presence of separate sibling species within *P. jenningsi*. One of these is confined to six genetically related strains originating from Japan, separated from other groups (results of strain crosses) and showing only one characteristic band pattern. The other sibling species of *P. jenningsi* includes strains from India, Saudi Arabia and China.

The aim of the present study was the verification of RAPD markers specific for *P. jenningsi*. We also wanted to prove the existence of two separate species within *P. jenningsi* (revealed by one primer only) by the application of four Ro-type primers. Therefore, the adequacy of the primers in differentiating strains within the species could be assessed.

MATERIALS AND METHODS

Paramecium DNA was isolated from the 9 strains listed in Table 1 (two cultures each) using QIAamp DNA Mini Kit (Qiagen, Germany) as described by Przyboś *et al*. (2003). Random amplified polymorphic DNA-PCR (RAPD-PCR) was performed with primers Ro 460-04 (5' GCAGAGAAGG 3'), Ro 460-06 (5' GTAGCCATGG 3'), Ro 460-07 (5' AACGTACGCG 3') and Ro 460-10 (5' CTAGGTCTGC 3') (Roth, Karsruhe, Germany) by the method described by Stoeck and Schmidt (1998), using Taq polymerase (Fermentas, Lithuania). The products of the PCR reaction were separated by electrophoresis in 1.5% agarose for 3.5 h at 85 V together with a DNA molecular weight marker (SmartLadder, Bioline, Germany), stained with ethidium bromide and visualized in UV light. The images were stored in computer memory using the program Biocapt (Vilbert Lourmat, France).

All RAPD-PCR reactions for each strain and culture were repeated several times for verification. Phylogenetic analysis was performed by comparing the molecular weight of DNA obtained by the RAPD-PCR method using the Bio 1D program (Vilbert Lourmat, France). The similarity index according to Nei and Li (1979) applied in the program was calculated by the equation: a=2nxy/(nx+ny); where: nx and ny describe the numbers of bands appearing in the RAPD patterns of x and y, respectively; while nxy describes the number of DNA-bands with similar molecular weight shared by the two patterns. Phylogenetic trees were constructed on the basis of a similarity matrix with the values from the above equations.

RESULTS

RAPD-PCR fingerprints (band patterns) revealed by primers Ro 460-04, Ro 460-06, Ro 460-07 and Ro 460-10 from nine strains (two cultures each) of *P. jenningsi* are presented in Figs 1-4. Schematic band patterns were constructed on the basis of photographs of gel separation products, characterizing strains from particular geographic localities, and also the applied primers. The approximate molecular weight of RAPD-PCR products reveals 100% concordance in all 9 strains (two cultures per strain).

RAPD-PCR fingerprint analysis for the Ro 460-04 primer (Fig. 1) revealed a pervasive 483 bp band for all strains, and another band (1466 bp) appearing in all samples with the exception of China. A band of 900 bp occurred in all strains except the Arabian and Japanese Okinawa strain. Another band (206 bp) common to all but two Japanese strains (Shinnamyou and Okinawa) was recorded. Specific Japanese markers of *P. jenningsi* with a molecular weight of 1348 and 743 bp were also discovered. Three non-Japanese strains (India, Saudi Arabia and China) shared common bands of 1090, 750, 700 and 645 bp.

Analysis of RAPD-PCR fingerprints for the Ro 460-06 primer did not reveal a common band for the entire species (Fig. 2). All continental strains and two of the Japanese strains (Hagi and Ube) shared a 1272 bp band, as well as a 1156 bp band in all continental strains and two of the Japanese strains (Yamaguchi and Ube). Moreover, four of the continental strains and one Japanese (Okinawa) had specific bands not present in other strains and recognized as markers. The Indian band had a molecular mass of 2253 and 1824 bp, the Saudi Arabian a 2370 and 2000 bp band, the Chinese a 852 and 448 bp band, and 685 and 331 bp band in the Okinawa strain.

Results of RAPD-PCR fingerprints for primer Ro 460-07 (Fig. 3) were almost identical to Ro 460-06 (Fig. 2). A common band for the entire species was not present. Neither the Japanese, nor the continental strains shared bands. Continental strains and some of the Japanese strains shared bands, as in fingerprints for Ro 460-06.

Table 1. *Paramecium jenningsi* strains.

Origin and collector's name	References
Saudi Arabia, neighbourhood of Riyadh; K.A.S. AL-Rasheid, July 1999	Fokin *et al.* 2001
India, Bangalore; [1, 2] P.B. Padmavathi, 1955	Diller and Earl 1958, Przyboś 1975, 1978, 1986
China, Shanghai; M. Fujishima, November 1999	Fokin *et al.* 2001
Japan, Honshu Island, Yamaguchi prefecture, Ube City; M. Fujishima, October 2000	Present paper
Japan, Honshu Island, Yamaguchi prefecture, Nagato City; M. Fujishima, September 2000	Present paper
Japan, Honshu Island, Yamaguchi prefecture, Shinnamyou; M. Fujishima, October 2000	Present paper
Japan, Honshu Island, Yamaguchi prefecture, Hagi City; [1]S. Fokin, September 1997	Przyboś *et al*. 1999
Japan, Honshu Island, Yamaguchi prefecture, Yamaguchi City; S. Fokin, November 1999	Fokin *et al*. 2001
Japan, Okinawa Island, Okinawa prefecture, Hujigawa; M. Fujishima 2000	Present paper

[1]Strains studied by RAPD-PCR previously (Przyboś *et al.* 1999); [2]Strain from India was studied genetically and karyologically (Przyboś 1975, 1978, 1986).

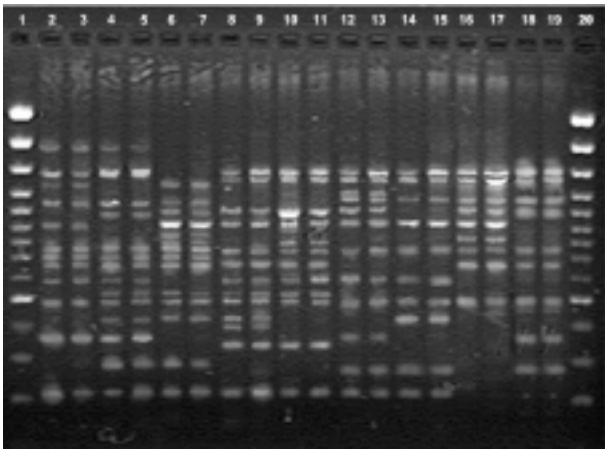

Fig. 1. RAPD reaction products for primer Ro 460-04 separated in an agarose gel. Lanes: 1 and 20 - weight marker; 2, 3 - Indian strain (Bangalore); 4, 5 - Saudi Arabian strain; 6, 7 - Chinese strain (Shanghai); 8, 9 - Japanese strain (Hagi); 10, 11 - Japanese strain (Yamaguchi); 12, 13 - Japanese strain (Ube); 14, 15 - Japanese strain (Nagato); 16, 17 - Japanese strain (Shinnamyou); 18, 19 - Japanese strain (Okinawa, Hujigawa).

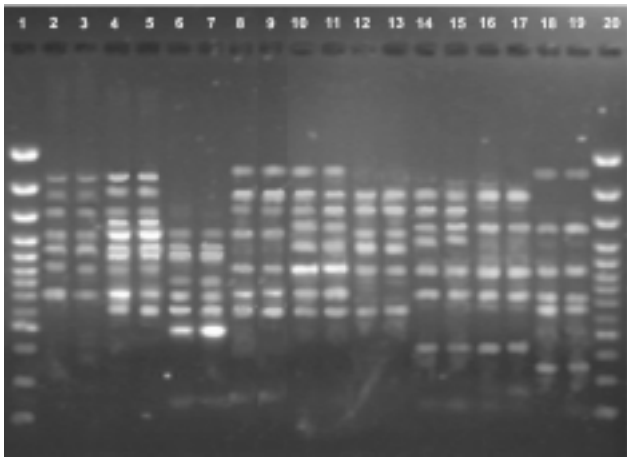

Fig. 2. RAPD reaction products for primer Ro 460-06 separated in an agarose gel. Lanes: 1 and 20 - weight marker; 2, 3 - Indian strain (Bangalore); 4, 5 - Saudi Arabian strain; 6, 7 - Chinese strain (Shanghai); 8, 9 - Japanese strain (Hagi); 10, 11 - Japanese strain (Yamaguchi); 12, 13 - Japanese strain (Ube); 14, 15 - Japanese strain (Nagato); 16, 17 - Japanese strain (Shinnamyou); 18, 19 - Japanese strain (Okinawa, Hujigawa).

Analysis of RAPD-PCR fingerprints revealed a 1031 bp band, present in all strains, when the primer Ro 460-10 was applied (Fig. 4) and it can also be used as a molecular marker for *P. jenningsi*. RAPD-PCR profiles in all strains shared a band of 1304 bp, with the exception of those from the Arabian locality. Analogously, all strains except the Japanese Yamaguchi strain had a band of 677 bp. Another band (513 bp) was present in all strains except for the Japanese Hagi and Yamaguchi. All continental strains (India, Saudi Arabia, China) possessed a 1200 bp band, acknowledged as a geographical marker. Additionally, all continental strains and two of the Japanese strains (Ube and Okinawa) shared a 912 bp band. Amplification with primer Ro 460-10 did not produce a band specific only for Japanese strains. A 1975 bp band was revealed in all Japanese strains except for Okinawa. Only the Chinese strain had a specific 1129 bp band, which can be used as a marker for this strain. Two of the continental strains (India and Saudi Arabia) had a conspicuous 443 bp band.

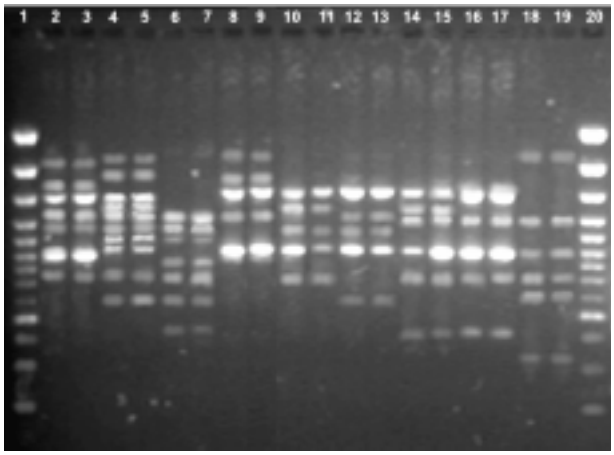

Fig. 3. RAPD reaction products for primer Ro 460-07 separated in an agarose gel. Lanes: 1 and 20 - weight marker; 2, 3 - Indian strain (Bangalore); 4, 5 - Saudi Arabian strain; 6, 7 - Chinese strain (Shanghai); 8, 9 - Japanese strain (Hagi); 10, 11 - Japanese strain (Yamaguchi); 12, 13 - Japanese strain (Ube); 14, 15 - Japanese strain (Nagato); 16, 17 - Japanese strain (Shinnamyou); 18, 19 - Japanese strain (Okinawa, Hujigawa).

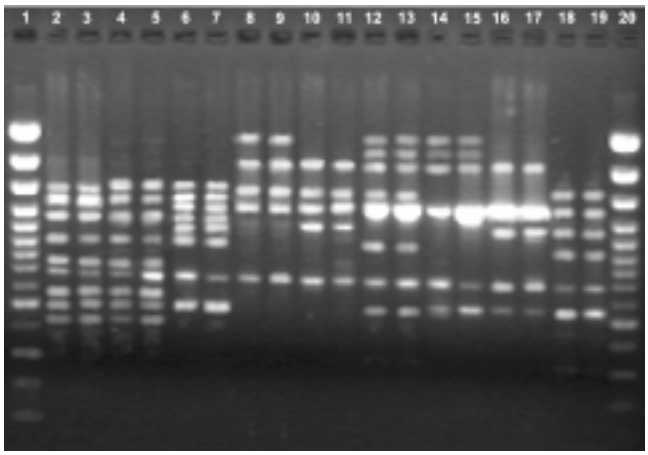

Fig. 4. RAPD reaction products for primer Ro 460-10 separated in an agarose gel. Lanes : 1 and 20 - weight marker; 2, 3 - Indian strain (Bangalore); 4, 5 - Saudi Arabian strain; 6, 7 - Chinese strain (Shanghai), 8, 9 - Japanese strain (Hagi), 10, 11 - Japanese strain (Yamaguchi); 12, 13 - Japanese strain (Ube), 14, 15 - Japanese strain (Nagato), 16, 17 - Japanese strain (Shinnamyou), 18, 19 - Japanese strain (Okinawa, Hujigawa).

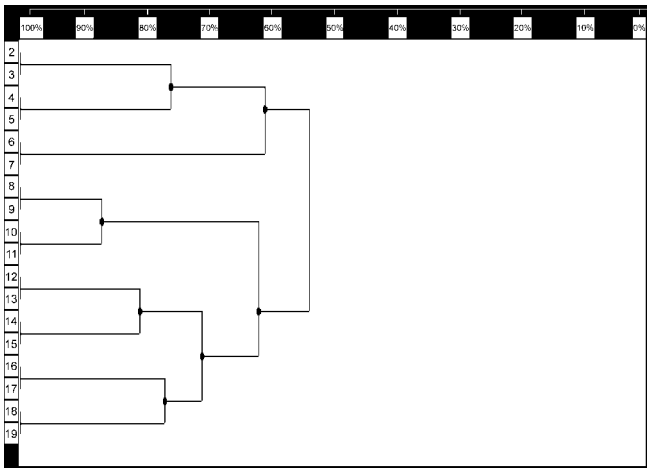

Fig. 5. Dendrogram representing phylogenetic relationships among strains of *P. jenningsi* with RAPD fingerprint data from the Ro 460-04, Ro 460-06, Ro 460-07, Ro 460-10 primers (2, 3 - Indian strain; 4, 5 - Saudi Arabian strain; 6, 7 - Chinese strain; 8, 9 - Japanese strain from Hagi; 10, 11 - Japanese strain from Yamaguchi; 12, 13 - Japanese strain from Ube; 14, 15 - Japanese strain from Nagato; 16, 17 - Japanese strain from Shinnamyou; 18, 19 - Japanese strain from Okinawa).

Results of genetic kinship analysis. On the basis of band patterns obtained from RAPD-PCR fingerprinting with primers Ro 460-04, Ro 460-06, Ro 460-07 and Ro 460-10, using the program Bio 1D (Vilber Lourmat), dendrograms were constructed depicting the genetic relatedness of the different geographical strains of *P. jenningsi* following Nei and Li's (1979) similarity coefficients.The confidence limit of the performed analyses was 1%.

The Fig. 5 depicts a dendrogram obtained from the results of RAPD fingerprinting with the primers Ro (460-04, 460-06, 460-07, 460-10). The 9 strains of *P. jenningsi* are divided into 2 main groups: the first large group includes all continental strains; the second encompasses the Japanese strains (similarity value of 54%). The first group contains an Indian and Arabian subgroup and another with only the Chinese strain (similarity of 62%). The Japanese strains are additionally divided into two subgroups (similarity of 63%): the first includes the Hagi and Yamaguchi strains, while the second involves two lines composed of Ube / Nagato and Shinnamyou / Okinawa strains, respectively (similarity of 73%).

DISCUSSION

Species from the *Paramecium aurelia* group, which includes *P. jenningsi*, show morphological similarity

making them difficult to identify. *Paramecium* is acknowledged as a model organism in many fields of experimental biology, and also considered an indicator in ecological studies, i.e. in littoral zone dynamics of aquatic ecosystems. Therefore, a precise and quick method is needed to identify the species of *Paramecium*. The primers applied in this study can be considered suitable for the identification of *P. jenningsi* and also can be used to analyze genetic relatedness and phylogenetic relationships amongst strains originating from remote and isolated geographic localities. The conducted RAPD-PCR fingerprint analysis revealed that the genomes of most of the 9 studied strains of *P. jenningsi* have polymorphic DNA sequences complementary to the 4 applied primers. The observed genetic variability did not occur among individuals belonging to the same strain, which was repeatedly confirmed by studies involving many isolates descending from cultures of different strains of *P. jenningsi*. The band patterns obtained from the RAPD-PCR analysis can be used as species-specific markers for the separation of *P. jenningsi* from other species in the genus and also as strain-specific markers determining the geographic origins of strains in a sample.

The Ro 460-04 primer produced band patterns for each of the 9 different strains and can prove useful as a strain-specific marker for geographic differentiation. Additionally, one band was shared by all strains (483 bp); therefore it is considered a marker for the entire species. Two bands were obtained for Japanese strains (1348 bp and 743 bp) and four bands for continental strains (1090 bp, 750 bp, 700 bp, 645 bp). All of these traits deem the primer Ro 460-04 useful in RAPD analysis for species and strain identification in *P. jenningsi*.

The band pattern acquired in this study as well as in a previous one (Przyboś *et al*. 2003) allowed for strain-specific identification, disclosed a band shared by all strains, and a few bands differentiating continental and Japanese strains. RAPD-PCR fingerprinting with the primer Ro-460-04 in *P. jenningsi* was first carried out in 1999 (Przyboś *et al*. 2003). The study incorporated two Indian strains, the Japanese Hagi strain, Madagascan, Ugandan and Floridian strains. Band patterns were identical for all strains examined and were clearly different from patterns obtained from other *Paramecium* species (*P. aurelia* complex, *P. nephridiatum*, *P. dubosqui*, *P. calkinsi*, *P. woodruffi*, *P. multimicronucleatum*, *P. caudatum*). The diversity in band pattern between the different strains of *P. jenningsi* was considered as genotypic variation in one species. Additionally, this analysis confirmed the results of morphometric and cytological studies allowing the inclusion of the Japanese strain into *P. jenningsi* (Przyboś *et al.* 1999). The current investigation yielded band patterns similar to the ones of Przyboś *et al*. (1999), who applied the 460-04 primer for the Indian and Japanese Hagi strains, used in both studies. However, a difference in RAPD product molecular mass of several tens of base pairs in magnitude was noticed, probably on account of the proximate character of these values estimated by their position relative to the position of the mass marker. Many factors contribute to electrophoretic mobility and the observed differences in mass and band pattern are considered negligible.

The DNA polymorphism revealed by the RAPD technique (Przyboś *et al.* 2003) inclined us to undertake successive studies on *P. jenningsi* using the same strains from India, Saudi Arabia, China and Japan (6 different strains). In the above-cited paper (Przyboś *et al*. 2003), the primer Ro 460-04 disclosed the presence of three types of band patterns (Indian and Arabian strains; Chinese strain; Japanese strains). These band patterns were differentiated by the presence/absence of specific bands. All patterns contained more bands than revealed by the current study. However, the position of the key bands, intensively visible in UV light, was the same in both investigations. The molecular mass of the products was similar and exhibited minimal deviation.

It seems especially meaningful to compare band patterns of *P. jenningsi* with analogous studies carried out on other species of the *P. aurelia* complex (Stoeck and Schmidt 1998, Stoeck *et al*. 1998, 2000). This comparison is further advocated by the close relationship of the two species as revealed by similar reorganization processes in the nucleus (Fokin *et al*. 2001) and sequencing studies incorporating the SSrRNA gene in the subclass *Peniculia* (Strüder-Kypke *et al*. 2000). Differences between *P. jenningsi* and the *P. aurelia* species complex in band pattern expression indicates that the pattern observed for primer Ro 460-04 is specific for *P. jenningsi*. RAPD-PCR fingerprinting with this primer is an excellent tool for fast and accurate identification of this species, i.e. in environmental samples.

Analysis of RAPD-PCR fingerprints for the Ro 460-06 primer did not reveal a common band for the entire species. However RAPD-PCR profiles for the Ro 460-06 primer distinguish between two groups of strains: one containing continental and part of the Japanese strains, the other the remaining Japanese strains. This primer cannot be used to distinguish the species, however, the

profiles show a large degree of variation and are good strain markers, important for confirmation purposes.

RAPD-PCR fingerprints for primer Ro 460-07 were almost identical to Ro 460-06 (Fig. 2). A common band for the entire species was not present. Neither the Japanese, nor the continental strains shared bands. Continental strains and some of the Japanese strains shared bands, as in fingerprints for Ro 460-06. Interestingly, the molecular mass of products from primers Ro 460-06 and 460-07 is very similar, resulting in almost identical band profiles, differing only in the position of one band in the Arabian strain and by the presence of a few additional bands in the Hagi, Yamaguchi and Ube profiles for Ro 460-06.

Analysis of RAPD-PCR fingerprints with the primer Ro 460-10 revealed a common band (1031 bp) present in all strains and it can also be used as a molecular marker for *P. jenningsi*. All continental strains (India, Saudi Arabia, China) possessed a 1200 bp band, acknowledged as a geographical marker. Amplification with primer Ro 460-10 did not produce a band specific only for Japanese strains. However the band pattern acquired in this study allowed for species-specific identification, disclosed a band shared by all strains, so it can be consider as a good general species marker. A single marker obtained for continental strains of *P. jenningsi* and absence of specific one for the Japanese strains does not render it helpful in differentiating between continental and Japanese strains.

This paper is the first to apply the primers Ro 460-06, 460-07 and 460-10 in *P. jenningsi* and consequently, because of the lack of published research concerning their use, a comparative analysis of results is impossible.

Many investigators point out the problems associated with repeatability of RAPD-PCR fingerprinting (Skroch and Nienhuis 1995, Woodburn *et al*. 1995; after Foissner *et al*. 2001). Because of the variation in intensity of bands in parallel samples, interpretation of results is problematic and demands extensive experience, being prone to subjective judgment (Foissner *et al*. 2001). Stoeck and Schmidt (1998) proved that the intensity of bands could change in subsequent experiments in relation to template DNA. Relatively weak bands may not show up at all in successive runs. This is why reliable results required the analysis of several isolates of each strain of *P. jenningsi* in multiple replicates.

Phylogenetic analysis of the 9 strains of *P. jenningsi* based on RAPD-PCR fingerprints permit genetic similarity assessment and the construction of dendrograms reflecting phylogenetic relationships in this species. DNA amplification by four used primers used in RAPD-PCR differentiates *P. jenningsi* into two groups of strains, one of which includes the Indian, Saudi Arabian and Chinese strains, the other the Japanese strains.

An evaluation of the applicability of the primers for identification of *P. jenningsi* demonstrated the usefulness of Ro 460-06, Ro 460-07, Ro 460-10 and the previously tested Ro 460-04. Similarity coefficients (Nei and Li 1979) between particular strains were calculated on the basis of the results of RAPD-PCR fingerprinting with 4 primers. These values were computer analysed and used in dendrogram construction in order to elucidate population structure, the evolutionary history and relationships in *P. jenningsi*. Phylogenetic analysis employing the RAPD data from primer Ro 460-04, Ro 460-06, Ro 460-07 and Ro 460-10 separates the species into two groups: an Indian, Chinese and Saudi Arabian assemblage and a Japanese group. These results agree with an earlier phylogenetic analysis (Przyboś *et al*. 2003), which revealed the presence of two sibling species incorporating the continental and Japanese groups, respectively. In the first group, Indian and Arabian strains had similar band patterns, while the Chinese was slightly different (Przyboś *et al*. 2003).

Our results are congruent with inter-strain crossing experiments in which reproductive isolation was found when continental and Japanese strains were tested (Przyboś *et al*. 2003). This is further evidence for the existence of two sibling species, whose reproductive isolation is genetically induced. Ro primers can be expected to verify these results.

Taking into account the fact that the values of the similarity indexes are not absolute but rather relative - they change depending on the applied primer (Chapco *et al*. 1992, Przyboś *et al*. 1999) - they cannot accurately describe the phylogenetic structure of this species. Landry and Lapoite (1996) concluded that at least 12 primers were needed for appropriate phylogenetic reconstruction. Still, the arrangement of groups and subgroups established on the basis of the similarity matrices is relatively constant and probably reflects actual phylogenetic relationships in *P. jenningsi*.

The genetic structure revealed by RAPD-PCR fingerprinting is closely linked to the life strategy of the species and its evolutionary history. Genetic variation between continental and Japanese strains of *P. jenningsi*,

reflected by the presence of two sibling species, is therefore a result of the geographic dispersal of this species.

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REFERENCES

Allen S. L., Rushford D. L., Nerad T. A., Lau E. T. (1983) Intraspecies variability in the esterases and acid phosphatases of *Paramecium jenningsi* and *Paramecium multimicronucleatum*: assignment of unidentified paramecia, comparison with the *Paramecium aurelia* complex. *J. Protozool*. **30:** 155-163

Chapco W., Ashton N. W., Martel R. K. B., Antonishyn N. (1992) A feasibility study of random amplified polymorphic DNA in the population genetics and systematics of grasshoppers. *Genome* **35:** 569-574

Diller W. F., Earl P. R. (1958) *Paramecium jenningsi*, n. sp. *J. Protozool*. **5:** 155-158

Foissner W., Stoeck T., Schmidt H., Berger H. (2001) Biogeographical differences in a common soil ciliate, *Gonostomum affine* (Stein), as revealed by morphological and RAPD-fingerprint analysis. *Acta Protozool.* **40:** 83-97

Fokin S. I., Przyboś E., Chivilev S. M. (2001) Nuclear reorganization variety in *Paramecium* (Ciliophora: Peniculida) and its possible evolution. *Acta Protozool.* **40:** 249-261

Jurand A., Przyboś E. (1984) A new method of study of chromosome numbers in *Paramecium*. *Folia Biol. (Kraków)* **32:** 295-300

Landry P. A., Lapointe F. J. (1996) RAPD problems in phylogenetics. *Zool. Scr.* **25:** 283-290

Nei M., Li W.- H. (1979) Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proc. Natl. Acad. Sci. U. S. A.* **76:** 5269-5273

Przyboś E. (1975) Genetic studies of *Paramecium jenningsi* strains (Diller & Earl, 1958). *Folia Biol. (Kraków)* **23:** 425-471

Przyboś E. (1978) Cytological and karyological studies of *Paramecium jenningsi*. *Folia Biol.* (*Kraków*) **26:** 25-29

Przyboś E. (1980) The African strain of *Paramecium jenningsi*. Cytological and karyological investigations. *Folia biol.* (*Kraków*) **28:** 391-397

Przyboś E. (1986) Chromosomes in *Paramecium jenningsi* (Diller & Earl, 1958): A serial section study. *Folia Biol.* (*Kraków*) **34:** 133-160

Przyboś E., Fokin S., Stoeck T., Schmidt H. J. (1999) Occurrence and ecology of *Paramecium jenningsi* strains. *Folia Biol.* (*Kraków*) **47:** 53-59

Przyboś E., Skotarczak B., Wodecka B. (2003) Phylogenetic relationships of *Paramecium jenningsi* strains (classical analysis and RAPD studies). *Folia biol.* (*Kraków*) **51:** 85-95

Skroch P., Nienhuis J. (1995) Impact of scoring error and reproductibility of RAPD data on RABD based estimates of genetic distance. *Theor. Appl. Genet.* **91:** 1068-1091

Sonneborn T. M. (1958) Classification of sibling species of the *Paramecium aurelia- multimicronucleatum* complex. *J. Protozool.* **4(Suppl.):** 17-18

Sonneborn T. M. (1970). Methods in *Paramecium* reserach. In: Methods in Cell Physiology, (Ed. D. M. Prescott). Academic Press, New York, **4:** 242-339

Stoeck T., Schmidt H. J. (1998) Fast and accurate identification of European species of the *Paramecium aurelia* complex by RAPD-fingerprints. *Microb. Ecol*. **35:** 311-317

Stoeck T., Przyboś E., Schmidt H. J. (1998) A combination of genetics with inter- and intra-strain crosses and RAPD-fingerprints reveals different population structures within the *Paramecium aurelia* species complex. *Europ. J. Protistol.* **34:** 348-355

Stoeck T., Przyboś E., Kusch J., Schmidt H. J. (2000) Intra-species differentiation and level of inbreeding of different sibling species of the *Paramecium aurelia* complex. *Acta Protozool*. **39:** 15-22

Strüder-Kypke M. C., Wright A.-D. G., Fokin S. I., Lynn D. H. (2000) Phylogenetic relationships of the subclass Peniculia (Oligohymenophorea, Ciliophora) inferred from small subunit rRNA gene sequences. *J. Eukaryot. Microbiol.* **47:**419-429

Woodburn M. A., Youston A. A., Hilu K. H. (1995) Random amplified polymorphic DNA fingerprinting of mosquito-pathogenic and nonpathogenic strains of *Bacillus sphaericus*. *Int. J. Bact.* **45:** 212-217

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