Folia biologica (Kraków), vol. **55** (2007), No 3-4

Strains of *Paramecium decaurelia* (Ciliophora, Protozoa) from Russia with Molecular Characteristics of other Known Strains of the Species

Ewa PRZYBOŚ, Magdalena GRECZEK-STACHURA, Alexey POTEKHIN and Maria RAUTIAN

Accepted April 16, 2007

PRZYBOŚ E., GRECZEK-STACHURA M., POTEKHIN A., RAUTIAN M. 2007. Strains of *Paramecium decaurelia* (Ciliophora, Protozoa) from Russia with molecular characteristics of other known strains of the species. Folia biol. (Kraków) **55**: 127-132.

The presence of *Paramecium decaurelia* from the *Paramecium aurelia* species complex was demonstrated in Yaroslavl, Russia, (European part, northwestern Russia) and in the Altai Mts (Asiatic part of Russia, western Siberia). RAPD-PCR fingerprints of the newly identified strains of *P. decaurelia*, rare throughout the world, were compared to those characteristic for the other known strains of the species. *P. decaurelia* strains show some polymorphism within species, strains from Russia have 60% similarity of band patterns, and strains from USA and Japan about 70% similarity of band patterns.

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

Ewa PRZYBOŚ, Department of Experimental Zoology, Institute of Systematics and Evolution of Animals, Polish Academy of Sciences, Sławkowska 17, 31-016 Kraków, Poland.
E-mail: Przybos@isez.pan.krakow.pl
Magdalena GRECZEK-STACHURA, Institute of Biology, Educational Academy, Podbrzezie 3, 31-054 Kraków, Poland.
E-mail: magresta@onet.pl
Alexey POTEKHIN, Maria RAUTIAN, Laboratory of Protozoan Karyology, Department of Invertebrate Zoology, Biological Research Institute, St. Petersburg State University, Oranienbaumskoye shosse 2, 198504 St. Petersburg, Russia.
E-mail: aurelia@fromru.com, mrautian@mail.ru

Among 15 species of the *Paramecium aurelia* complex known world-wide (SONNEBORN 1975; AUFDERHEIDE *et al*. 1983), *P. primaurelia, P. biaurelia, P. tetraurelia,* and *P. sexaurelia* are cosmopolitan, while the occurrence of some other species seems to be limited to certain temperature zones, environments or even special habitats (cf PRZYBOŚ 2005). *P. decaurelia* had been known only from Florida, USA (SONNEBORN 1975), and was later also recorded in Japan (PRZYBOŚ & NAKAOKA 2002; PRZYBOŚ *et al*. 2003a).

In the European part of Russia, *P. primaurelia, P. biaurelia* and *P. novaurelia* have been recorded in Moscow and in St. Petersburg or their vicinity (KOMALA & DUBIS 1966; PRZYBOŚ & FOKIN 1996; PRZYBOŚ *et al*. 2006a), *P. triaurelia,* together with *P. novaurelia* in the Volga River (Astrakhan Nature Reserve) (cf KOŚCIUSZKO 1985), and *P. triaurelia* with *P. primaurelia* in Kaliningrad (PRZYBOŚ *et al*. 2006a), *P. pentaurelia* in the Belgorod region (FOKIN & OSSIPOV 1986), and *P. novaurelia* in Vladimir (PRZYBOŚ *et al*. 2006a). The Lower Volga Basin turned out to be very rich in species of the *P. aurelia* complex, as the presence of *P. primaurelia, P. biaurelia, P. triaurelia*, *P. pentaurelia, P. sexaurelia, P. septaurelia*, and *P. novaurelia* has been revealed there (PRZYBOŚ *et al*. 2004, 2005a). Among these species, *P. septaurelia* was found for the first time in Europe, known before only from the USA, and also *P. pentaurelia* and *P. sexaurelia,* rare species in Europe, were found to be frequent there. *P. tetraurelia* and *P. pentaurelia* were recorded in the Eastern part of the Black Sea coast (PRZYBOŚ *et al*. 2007b).

In the Asiatic part of Russia in Western Siberia, recently, the presence of *P. primaurelia* was recorded in Omsk, *P. biaurelia* in Krasnoyarsk and in the Altai Mountains, *P. triaurelia* in Krasnoyarsk, and *P. pentaurelia* in Novosibirsk, Altai Foreland, and Altai Mountains (POTEKHIN *et al*. 2006). Some sampling was also carried out earlier in several sites in eastern Siberia and demonstrated the presence of *P. primaurelia* in the Kamchatka

peninsula (DAGGETT 1978), *P. biaurelia* on Sakhalin Island (PREER *et al*. 1974), in Nahodka (KOŚCIUSZKO 1985), and in Irkutsk (PRZYBOŚ & FOKIN 1996).

This paper presents new stands of *P. decaurelia*, globally a very rare species, and recorded for the first time in Russia. One of these stands is situated in the European part of Russia in Yaroslavl in the Volga River in northwestern Russia, and the second in the Asiatic part of Russia, western Siberia in Altai Mts. The molecular (RAPD-PCR fingerprints – random amplified polymorphic DNA – polymerase chain reaction) characteristics of known *P. decaurelia* strains is also presented.

Material and Methods

Material

The water samples (15-30 ml each) with plankton were collected in several sites in the Yaroslavl district (European part of Russia) and in a small lake in the Altai Mountains (Asiatic part of Russia), these regions are separated by more than 3000 km.

In the Yaroslavl district, one sample was collected near Uglich, another three in different sites in the vicinity of Borok (100-120 km apart) from small rivers and a pond, one of the rivers was polluted by a milk farm discharging a high amount of organic substances to the water.The collecting sites are presented in Table 1.

Water temperature as well as ambient temperature were about 20°C.

Methods

1. Culture, identification, and strain crosses. Culture and identification of paramecia (Table 1) were performed according to SONNEBORN (1950, 1970). Paramecia were cultivated on a lettuce medium inoculated with *Enterobacter aerogenes*. *P. decaurelia* was identified by mating the investigated strains with the mating types of standard strain (223) of the species. The following species of the *P. aurelia* complex were used during testing:

P. primaurelia, strain 90 from Pennsylvania, USA

P. biaurelia, strain Rieff from Scotland

P. triaurelia, strain 324 from Florida, USA

P. tetraurelia, strain from Sydney, Australia

P. pentaurelia, strain 87 from Pennsylvania, USA

P. sexaurelia, strain 159 from Puerto Rico

P. septaurelia, strain 38 from Florida, USA

P. novaurelia, strain 510 from Scotland

P. decaurelia, strain 223 from Florida, USA

P. dodecaurelia, strain 246 from southern state, USA.

In the intra and inter-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-carmine. Survival of clones in

Table 1

Occurrence of *Paramecium decaurelia* in Russia

Geographical region	Sampling site	Index of sample	Number of isolated strain	Other *Paramecium* species	Type of habitat
north-western Russia, Yaroslavl district (European part)	Uglich	YE65	YE65-8*, YE65-9,YE65-13		small river
	Borok 1	YM93	YM93-21		small river
	Borok 2	YD127	YD127-3, YD127-4		pond
	Borok 3	YB150	YB150-1	*P. bursaria*	small river
		YB151	YB151-1		pond polluted by milk farm
Altai Mts South part of western Siberia (Asiatic part)	Lake at the foot of Babyrgon mountain	GA1	GA1-12, GA1-15*	none	small mountain lake, 2000 m a.s.l.

* strains analyzed by RAPD-PCR method.

Table 2

List of strains of *Paramecium decaurelia* used in genetic studies

Strain designation	Geographical origin	References
223 (standard of the species)	Florida, USA	SONNEBORN 1974
JN	Nara, Japan	PRZYBOŚ *et al*. 2003a
RA (strain GA1-2)	Russia, Altay Mts	present paper
RY (strain YE65-8)	Russia, Yaroslavl	present paper

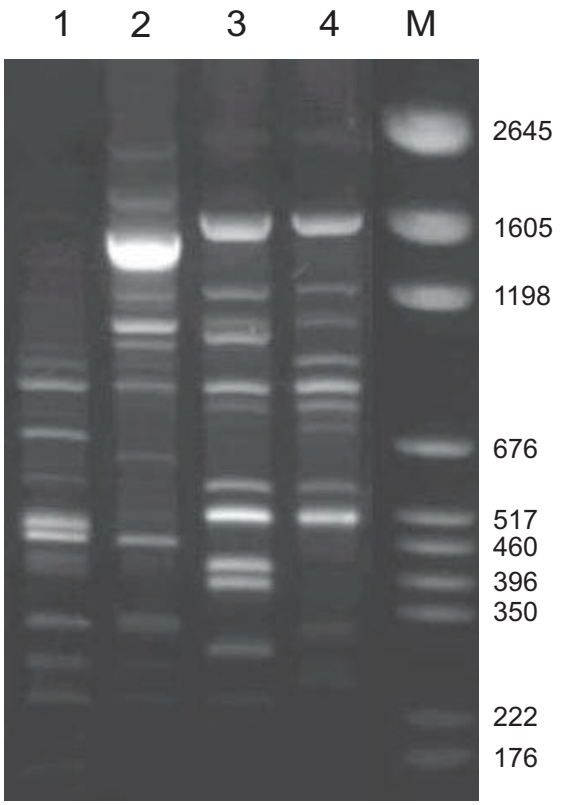

Fig. 1. RAPD fingerprints of *Paramecium decaurelia* strains: 1 – Russia, Yaroslavl; 2 – Russia, Altay Mts; 3 – USA, Florida (strain 223); 4 – Japan, Nara. M – pGEM narker. Molecular weight of the marker DNA bands is given in bp.

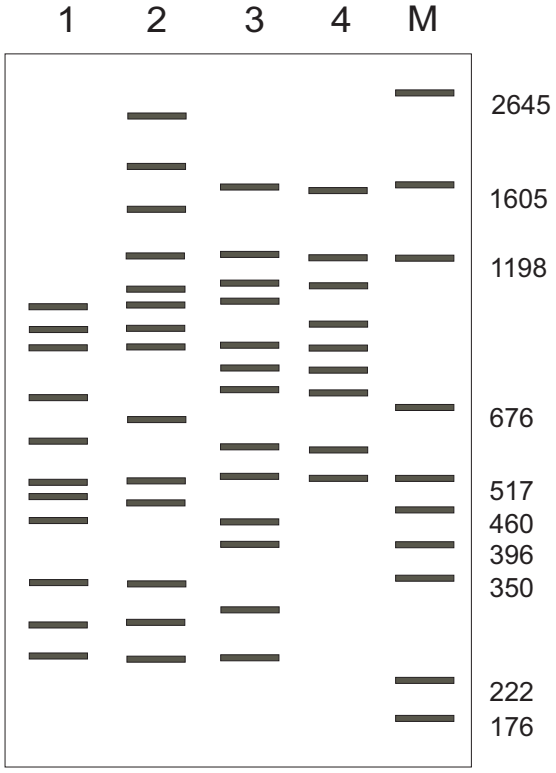

Fig. 2. Diagram presenting band patterns of the particular strains (explanation as in Fig. 1.).

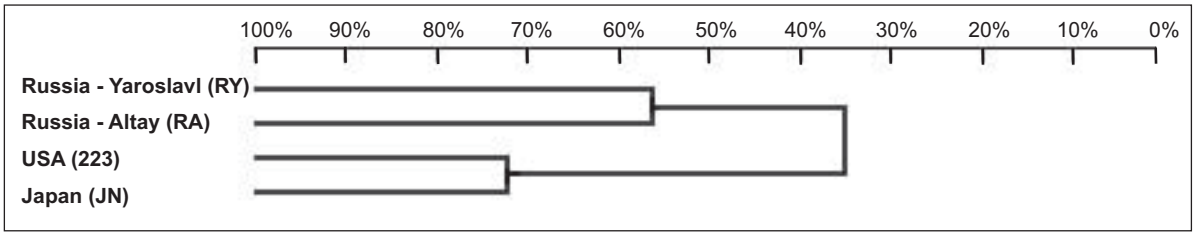

Fig. 3. Dendrogram presenting similarity of band patterns of the studied strains.

both generations was estimated. According to CHEN (1956), the clones were regarded as surviving after passing 6-7 fissions during 72 hours after separation of partners of conjugation or postautogamous caryonids.

2. Molecular methods

RAPD-PCR fingerprint analysis of *Paramecium decaurelia* strains (Table 2), carried out in general as in STOECK and SCHMIDT (1998), is described in PRZYBOŚ *et al*. 2003a. DNA was isolated from strains using a QIAamp™ DNA Mini Kit (Qiagen™, Germany). RAPD-PCR was performed with primer Ro 460 04 (5-'GCAGAGAAGG- 3', Roth, Karsruhe, Germany) 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; PRZYBOŚ *et al*. 2005b,c, 2006a, 2007), on *P. jenningsi* strains (PRZYBOŚ *et al*. 1999, 2003a; SKOTARCZAK *et al*. 2004 a,b), and on *P. schewiakoffi* (FOKIN *et al*. 2004).

The RAPD-PCR was done in a Biometra thermocycler using the PCR conditions as described in 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 a molecular weight marker XIV™ (Roche™, France), stained with ethidium bromide, and visualized in UV light, 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 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. decaurelia* of the *P. aurelia* complex was demonstrated at different sites in the Yaroslavl district and also in the Altai Mts (Table 1) on the basis of strong conjugation between the studied and the standard strains of the species. A high percentage of surviving hybrid clones was observed in both generations of inter-strain crosses from the Yaroslavl region (100% in F1 and 94% in F2) and strains from Altai (100% in F1 and 98% in F2) with the standard strain (223) of *P. decaurelia*. *P. decaurelia* was found for the first time on the territory of Russia, it had been known before only from the USA (SONNEBORN 1975) and later was also recorded in Japan (PRZYBOŚ & NAKAOKA 2002; PRZYBOŚ *et al*. 2003a). It was shown that the species has a wider distribution than previously thought and that strains originating from remote regions are genetically differentiated.

In the same collecting site (lake at the foot of Babyrgon mountain) in the Altai Mts in which *P. decaurelia* (sample GA1, strains GA1-12, GA1-15) was recorded at present, *P. pentaurelia* (sample GA1) was identified previously (POTEKHIN *et al*. 2006), showing that these two species can exist in the same population. In the Altai Mts *P. biaurelia* (POTEKHIN *et al*. 2006) was also recorded, but in two different habitats, i.e. a stream in a farmyard and a pond near the Katun river.

Fingerprints (band patterns) of *P. decaurelia* strains from Russia from the Altai and Yaroslavl regions as well as the other known strains, i.e. from Japan and the USA are presented in Figs 1-3. *P. decaurelia* strains show some polymorphism within species, strains from Russia have 60% similarity of band patterns, and strains from USA and Japan about 70% similarity of band patterns. The correlation between the degree of polymorphism revealed by RAPD analysis and degree of inbreeding characteristic for the species, proposed by STOECK *et al*. (1998) when *P. triaurelia* and *P. sexaurelia* were studied, later found (STOECK *et al*. 2000) when *P. pentaurelia* and *P. novaurelia* were investigated, and also when the remaining species of the *P. aurelia* complex (PRZYBOŚ *et al*. 2007) were analysed, was also confirmed at present. *P. decaurelia* seems to be a moderate inbreeder.

According to CORLISS (2002) "our far from complete knowledge of protistan taxonomic and phylogenetic interrelationships, as well as of their ecology, physiology, biochemistry, and molecular and evolutionary biology, hinders rapid progress in better understanding of their multiple roles in sustaining today's biosphere". This also pertains to the problem of the occurrence and distribution of ciliates.

The possibility of the existence of geographic zones with temperature barriers limiting the occurrence of protists has been discussed in the scientific literature, some scientists support, others contradict it (cf. SONNEBORN 1975; FINLAY & FENCHEL 1999; FOISSNER 1999; FINLAY 2004; FOISSNER 2006). FOISSNER (2006) writes, "I suggest historic events (split of Pangaea etc.), limited cyst viability and, especially, time as major factors for dispersal and provinciality of micro-organisms" and "local distribution patterns must exist". For the *Paramecium aurelia* species, much is known

of the well sampled regions (Europe, Northern America) but knowledge is limited for Central Asia, Southern America, and Africa (PRZYBOŚ & FOKIN 2000; PRZYBOŚ 2005). However, we suggest that species show a different frequency of occurrence in different regions (zones) and they may also require different micro-niches. It is also worth noting that cysts are unknown in *Paramecium* (LANDIS 1988; FOISSNER 2006), so their dispersal may be connected with aquatic insects, waterfowl, migratory mammals, or human activities.

Recently, molecular methods have been used in studies concerning ciliate biogeography, i.e. PRZYBOŚ *et al*. (2003b) and DOHERTY *et al*. (2006), they show a very complex picture of haplotype distribution and gene flow.

Acknowledgements

The expedition to Western Siberia was supported by the Russian Foundation for Basic Research, grant: 02-04-63-105, research grants: 07-04-01755, 06-04-49504, and research grant of Ministry of Science and Education RNP-148.

References

AUFDERHEIDE K. J., DAGGETT P.-M., NERAD T. A. 1983. *Paramecium sonneborni* n.sp., a new member of the *Paramecium aurelia* species-complex. J. Protozool. **30**: 128-131.

CHEN T. T. 1956. Varieties and mating types in *Paramecium bursaria*. II. Variety and mating types found in China. J. exp. Zool. **132**: 255-268.

CORLISS J. O. 2002. Biodiversity and biocomplexity of the Protists and an overview of their significant roles in maintenance of our biosphere. Acta Protozool. **41**: 199-219.

DAGGETT P.-M. Ed. 1978. Protozoa and Algae. (In: Catalogue of Strains. I. Thirteenth edn., the Americam Type Culture Collection, Rockville, Maryland): 1-18.

DOHERTY M., TERRY F. E., COSTAS B. A., MCMANUS G. B., KATZ L. A. 2006. Disentangling the complexities of costal ciliate biogeography. 16th Meeting of the Internat. Soc. for Evolutionary Protistology, 1-5 August 2006, Wrocław, Poland. Abstract Book: 44.

FINLAY B. J. 2004. Protist taxonomy: an ecological perspective. Phil. Trans. R. Soc. London B **359**: 599-610.

FINLAY B. J., FENCHEL T. 1999. Divergent perspectives on protist species richness. Protist **150**: 229-233.

FOISSNER W. 1999. Protist diversity: estimates of the near-impoderable. Protist **150**: 363-368.

FOISSNER W. 2006. Biogeography and dispersal of micro-organisms: a review emphasizing Protists. Acta Protozool. **45**: 11-136.

FOKIN S. I., OSSIPOV D. V. 1986. *Pseudocaedibacter glomeratus* sp.n. – the symbiont of the cytoplasm of *Paramecium pentaurelia.* Cytologia. **28**: 1000-1004. (In Russian).

FOKIN S. I., PRZYBOŚ E., CHIVILEV S. M., BEIER C. L., HORN M., SKOTARCZAK B., WODECKA B., FUJISHIMA M. 2004. Morphological and molecular investigations of *Paramecium schewiakoffi* sp. nov. (Ciliophora, Oligohymenophorea) and current status of distribution and taxonomy of *Paramecium* spp. Europ. J. Protistol. **40**: 225-243.

KOMALA Z., DUBIS K. 1966. Syngens of *Paramecium aurelia* in some regions of Moscow and Leningrad. Folia biol. (Kraków) **14**: 227-228.

KOŚCIUSZKO H. 1985. Species of the *Paramecium aurelia* complex in some regions of the USSR. Folia biol. (Kraków) **33**: 117-122.

LANDIS W. G. 1988. Ecology. (In: *Paramecium.* H.-D. Görz ed., Springer-Verlag, Berlin): 419-436.

NEI L., LI W.-H. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci. **76**: 5260-5273.

POTEKHIN A., RAUTIAN M., NEKRASOVA I., PRZYBOŚ E. 2006. Occurrence of *Paramecium* species in Western Siberia, Russia. Folia biol. (Kraków) **54**: 127-131.

PREER J. R. Jr., PREER L. B., JURAND A. 1974. Kappa and other endosymbionts in *Paramecium aurelia*. Bact. Rev. **38**: 113-163.

PRZYBOŚ E. 2005. Recent data on the occurrence of species of the *Paramecium aurelia* in Europe. Folia biol. (Kraków). **53**: 61-63.

PRZYBOŚ E., FOKIN S. I. 1996. New habitats of species of the *Paramecium aurelia* comlex in Russia and Vietnam. Folia biol. (Kraków) **44**: 105-106.

PRZYBOŚ E., FOKIN S. I. 2000. Data on the occurrence of species of the *Paramecium aurelia* complex world-wide. Protistology **1**: 179-184.

PRZYBOŚ E., FOKIN S. I., STOECK T., SCHMIDT H. J. 1999. Occurrence and ecology of *Paramecium jenningsi* strains. Folia biol. (Kraków) **47**: 53-59.

PRZYBOŚ E., FUJISHIMA M., NAKAOKA Y. 2003a. *Paramecium decaurelia* and *Paramecium dodecaurelia* from the *P. aurelia* spp. complex in Japan. Folia biol. (Kraków) **51**: 223-224.

PRZYBOŚ E., HORI M., FOKIN S. I. 2003b. Strains of *Paramecium quadecaurelia* from Namibia, Africa; Genetic and molecular studies. Acta Protozool. **42**: 357-360.

PRZYBOŚ E., NAKAOKA Y. 2002. Thermal tolerance of *Paramecium decaurelia* collected from hot spring in Japan. VIth Asian Conference on Ciliate Biology, July 15-18, 2003, Tsubuka University, Japan. Program and Abstracts: 82.

PRZYBOŚ E., PRAJER M., GRECZEK-STACHURA M., FOKIN S. I., RAUTIAN M., POTEKHIN A. 2005c. New European stands of *Paramecium pentaurelia, Paramecium septaurelia*, and *Paramecium dodecaurelia*, genetic and molecular studies. Folia biol. (Kraków) **53**: 123-128.

PRZYBOŚ E., PRAJER M., GRECZEK-STACHURA M. 2005c. Molecular analysis (RAPD-PCR) of inter-strain hybrids of the *Paramecium aurelia* species complex (Ciliophora, Protozoa). Folia biol. (Kraków) **53**: 115-122.

PRZYBOŚ E., PRAJER M., GRECZEK-STACHURA M., SKOTARCZAK B., MACIEJEWSKA A., TARCZ S. 2007. Genetic analysis of the *Paramecium aurelia* species complex (Ciliophora, Protozoa) by classical and molecular studies. Systematics and Biodiversity.

PRZYBOŚ E., RAUTIAN M., POTEKHIN A. 2004. First European record of *Paramecium septaurelia* and the discovery of new European habitats of *P. pentaurelia* and *P. sexaurelia* in Russia (Astrakhan and Volgograd Region). Folia biol. (Kraków) **52**: 87-90.

PRZYBOŚ E., RAUTIAN M., POTEKHIN A. 2005a. Species of the *Paramecium aurelia* complex in Russia, Lower Volga Basin. Protistology **4**: 129-134.

PRZYBOŚ E., SKOTARCZAK B., WODECKA B. 2003a. Phylogenetic relationships of *Paramecium jenningsi* strains (classical analysis and RAPD studies). Folia biol. (Kraków) **51**: 85-95.

PRZYBOŚ E., TARCZ S., RAUTIAN M., POTEKHIN A. 2006a. Species of the *Paramecium aurelia* complex in Russia (Western region of the European part) with molecular characteristics of *P. novaurelia.* Folia biol. (Kraków) **54**: 43-47.

SKOTARCZAK B., PRZYBOŚ E., WODECKA B., MACIEJEWSKA A. 2004a. Sibling species within *Paramecium jenningsi* revealed by RAPD. Acta Protozool. **43**: 29-35.

SKOTARCZAK B., PRZYBOŚ E., WODECKA B., MACIEJEWSKA A. 2004b. Random amplified polymorphic DNA fingerprinting as a marker for *Paramecium jenningsi* strains. Folia biol. (Kraków) **52**: 117-124.

SONNEBORN T. M. 1950. Methods in the general biology and genetics of *Paramecium aurelia.* J. exp. Zool. **113**: 87-143.

SONNEBORN T. M. 1970. Methods in *Paramecium* research. (In: Methods in Cell Physiology, vol. 4, D. M. Prescott ed., Academic Press, New York, London): 241-339.

SONNEBORN T. M. 1974. *Paramecium aurelia"*.(In: Handbook of Genetics, vol. 2, R. C. King ed., Plenum Press, New York): 469-594.

SONNEBORN T. M. 1975. The *Paramecium aurelia* complex of fourteen sibling species. Trans. Amer. Micros. Soc. **94**: 155-178.

STOECK T., PRZYBOŚ E., SCHMIDT H. J. 1998. A combination of genetics with inter-and intra-strain crosses and RAPD-fingerprints reveal 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.

STOECK T., SCHMIDT H. J. 1998. Fast and accurate identification of European species of the *Paramecium aurelia* complex by RAPD-fingerprints. Microbiol. Ecology **35**: 311-317.