PL-ISSN 0015-5497 (print), ISSN 1734-9168 (online)
Folia biologica (Kraków), vol. **58** (2010), No 1-2

doi:10.3409/fb58_1-2.55-60

# *Paramecium sexaurelia* – Intra-Specific Polymorphism and Relationships with other *Paramecium aurelia* spp., Revealed by Cytochrome *b* Sequence Data

Ewa PRZYBOŚ, Dana BARTH, and Thomas U. BERENDONK

*Accepted September 15, 2009*

PRZYBOŚ E., BARTH D., BERENDONK T. U. 2010. *Paramecium sexaurelia* – intra-specific polymorphism and relationships with other *Paramecium aurelia* spp., revealed by cytochrome *b* sequence data. Folia biol. (Kraków) **58**: 55-60.

Genetic distances among strains of *P. sexaurelia* were compared by sequencing the mitochondrial cytochrome *b* gene. The relationships of *P. sexaurelia* with representative strains of other species of the *P. aurelia* complex and related species, i.e. *P. schewiakoffi*, *P. jenningsi* and *P. multimicronucleatum* were evaluated. All investigated *P. sexaurelia* and *P. dodecaurelia* strains grouped together with high support. This *P. sexaurelia*/*P. dodecaurelia* cluster was furthermore composed of three distinct, strongly supported subgroups. Two of these groups contained both *P. sexaurelia* and *P. dodecaurelia* strains, suggesting that these species are not monophyletic. The third branch was composed of strains from Sevilla, Spain and was deeply separated (12-14 % p-distance) from the other *P. sexaurelia*/*P. dodecaurelia* clades. This illustrates the urgent need for further work and more intense sampling of these "rare" species, in order to understand the status and the relationships of *P. sexaurelia* and *P. dodecaurelia* within the *P. aurelia* species complex. We recommend that general investigations on the speciation process be conducted within and between species of the *P. aurelia* complex due to the high genetic variation combined with observations that for some of the species possible cryptic speciation may have occurred. This is emphasized by data showing that for some species within this complex, species status may be less static and more dynamic than originally thought.

Key words: *Paramecium aurelia* species complex; intra-specific polymorphism, relationships of species, sequencing cytochrome *b*.

*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*
*Dana BARTH, Institute of Biology II, Molecular Evolution and Animals Systematics, University of Leipzig, Talstrasse 33, 04 103 Liepzig, Germany.*
*E-mail: tberendonk@rz.uni-leipzig.de*
*E-mail: dbarth@rz.uni-leipzig.de*
*Thomas U. BERENDONK, Institute of Hydrobiology, Dresden University of Technology, 01069 Dresden, Germany.*

The *Paramecium aurelia* complex composed of 15 sibling species (SONNEBORN 1975; AUFDERHEIDE *et al*. 1983) is a model system for speciation processes (NANNEY *et al*. 1998; COLEMAN 2005; BARTH *et al*. 2008; CATANIA *et al*. 2009). Sibling species of this complex probably originated as a result of an explosion of speciation events which coincided with the most recent whole-genome duplication in paramecia as indicated by phylogenetic analysis (AURY *et al*. 2006), this rapid speciation was confirmed using cytochrome *b* sequences (BARTH *et al*. 2008).

Several sibling species show intra-specific genetic variability. Intra-specific differentiation within some species of the *P. aurelia* complex was studied by classical inter-strain crosses and molecular analyses, first RAPD only (STOECK & SCHMIDT 1998; STOECK *et al*. 1998, 2000), later also by RAPD-PCR fingerprinting, ARDRA riboprints and RFLP-PCR analysis (PRZYBOŚ *et al*. 2007a). For some species diagnostic rDNA and/or mtDNA genes were also sequenced (TARCZ *et al*. 2006; PRZYBOŚ *et al*. 2008a, b; BARTH *et al*. 2008; PRZYBOŚ *et al*. 2009a, b).

RAPD studies showed that all species of the complex possessed characteristic band patterns, and the majority were also polymorphic intra-specifically. A correlation exists between the degree of inbreeding characteristic for each species (SONNEBORN 1957; LANDIS 1986), and the differentiation of DNA "genotypes" revealed by RAPD (STOECK *et al*. 1998, 2000; PRZYBOŚ *et al*. 2007a).

Moderate inbreeders such as *P. pentaurelia* (STOECK *et al*. 2000), *P. decaurelia*, *P. tredecaurelia*, and *P. quadecaurelia* (PRZYBOŚ *et al*. 2007a) showed a high similarity of "genotypes" (band patterns) and extreme inbreeders such as *P. sexaurelia* (STOECK *et al*. 1998) and *P. dodecaurelia* revealed exceptional polymorphism (PRZYBOŚ *et al*. 2007a).

*P. sexaurelia* is recognized as a cosmopolitan species (SONNEBORN 1975; PRZYBOŚ & FOKIN 2000) known from Puerto Rico, Asia, and Europe. However, in Europe its occurrence seems to be limited to warm and temperate climatic zones, and the species was reported from only 10 of 483 investigated suitable habitats (cf. PRZYBOŚ *et al.* 2008c).

The problem of intra-specific differentiation of *P. sexaurelia* was previously studied by classical inter-strain crosses and RAPD-PCR fingerprinting (STOECK *et al*. 1998). The following strains were used: strain 159 from Puerto Rico (at present designated 126), strains from Spain (132 and 133), Greece (129), Germany (131), and another strain from Germany that perished later. As a result, four different types of "genotypes" were obtained, i.e. I – characteristic for strains from Spain, II – for strains from Germany, III – for the strain from Greece, and IV – for the strain from Puerto Rico. The obtained "genotypes" were strictly confined to certain geographical regions. Such great intraspecific differentiation was connected with extreme inbreeding, characteristic for *P. sexaurelia*.

Afterwards a new strain of *P. sexaurelia* from China was obtained, the problem was studied again, and the Chinese strain was compared with the previously known ones (PRZYBOŚ *et al*. 2007b) by RAPD analysis. Strains with the following origins were used: China (designated CB), Thailand (127), Japan (128), Russia (137), Croatia (135), Spain (132, 133) Germany (131), and Puerto Rico (126). Distinct polymorphisms were shown as several groups of "genotypes" (different band patterns). Strains from Spain had similar band patterns, strains from Croatia and Greece grouped together, other strains had very different band patterns (each strain belonged to a different group), the most divergent was the strain from China.

Genetic distances among strains of *P. sexaurelia* were again compared by sequencing the mitochondrial cytochrome *b* gene, and the results are presented in this paper.

## Material and Methods

### Material

The list of strains studied is given in Table 1.

### Methods

#### 1. Identification and cultivation of strains

Paramecia cultivation and identification were performed according to SONNEBORN (1970). The paramecia were cultivated on a lettuce medium inoculated with *Enterobacter aerogenes*.

Identification of species of the *P. aurelia* complex was carried out on the basis of 95-100% conjugation between reactive (mature for conjugation) complementary mating types of the investigated strains with the mating types of the standard strains of the particular species of the *P. aurelia* complex. The survival of the hybrid clones was observed in generations F1 (obtained by conjugation) and F2 (obtained by autogamy, using the method of daily isolation lines).

*P. schewiakoffi*, *P. jenningsi* and *P. multimicronucleatum* strains were used as an outgroup.

#### 2. Molecular methods

For DNA extraction, 10-20 cells (paramecia) were incubated with 10% Chelex for 20 min at 99°C. A fragment of the mitochondrial cytochrome *b* gene was amplified using PCR primers as described in BARTH *et al*. (2008). For the sequence analysis, 618 bp fragments were used. A Neighbor Joining tree was constructed with MEGA 4.0 (TAMURA *et al.* 2007), 2000 bootstrap replicates were performed. GenBank accession numbers are given in Table 1.

## Results and Discussion

Figure 1 presents a phylogenetic tree of the *P. sexaurelia* strains and their relationships with representative strains of other species of the *P. aurelia* complex and related species, i.e. *P. schewiakoffi*, *P. jenningsi* and *P. multimicronucleatum*, which was used as an outgroup.

All investigated *P. sexaurelia* strains and *P. dodecaurelia* strains (199 and 197) grouped together with high support (Fig. 1). This *P. sexaurelia*/*P. dodecaurelia* cluster was furthermore composed of three distinct, strongly supported subgroups. Two of these groups (A, B) contained both *P. sexaurelia* and *P. dodecaurelia* strains, suggesting that these species are not monophyletic. The third branch (C) was composed of the strains 132 and 133 (both from Sevilla, Spain), and was deeply separated (12-14% p-distance) from the other *P. sexaurelia*/*P. dodecaurelia* clades. The genetic distance between 132/133 and the other *P. sex/dodecaurelia* clades was as high as interspecies distances within the *P. aurelia* species complex. This raises the question of whether the Spanish

Table 1

Origin and accession numbers of the *Paramecium* strains used in this study

| Species | Strain | Origin | Accession Number |
|---|---|---|---|
| *P. sexaurelia* | 126 (159 SONNEBORN) | Puerto Rico | AM949765 |
| | 132 | Spain, Sevilla, America Square | FN293375 |
| | 133 | Spain, Sevilla, Maria Luiza Park | FN293376 |
| | 131 | Germany, Stuttgart | FN293374 |
| | 135 | Croatia, Krka River | FN293372 |
| | 129 | Greece, Ioannina Lake | FN293371 |
| | 137 | Russia, Astrakhan Nature Reserve | FN293373 |
| | 128 | Japan, Yamaguchi | AM949764 |
| | CB | China, Beijing | FN293370 |
| *P. primaurelia* | 1 | USA, Pennsylvania | AM949780 |
| *P. biaurelia* | 34 | Russia, Irkutsk | AM949784 |
| *P. triaurelia* | 78 | USA, Florida | AM949778 |
| *P. tetraurelia* | 92 | Australia, Sydney | AM949771 |
| *P. pentaurelia* | 106 | USA, Pennsylvania | AM949782 |
| *P. septaurelia* | 162 | Russia, Astrakhan Nature Reserve | AM949768 |
| *P. septaurelia* | 144 | USA, Florida | AM949766 |
| *P. octaurelia* | 168 | USA, Florida | AM949767 |
| *P. octaurelia* | 169 | Israel, Ein Affek | AM949772 |
| *P. novaurelia* | 175 | Ukraine, Gorgany Mts | AM949776 |
| *P. decaurelia* | 194 | USA, Florida | AM949769 |
| *P. undecaurelia* | 196 | USA, Texas | AM949783 |
| *P. dodecaurelia* | 197 | USA, Mississippi | AM949763 |
| *P. dodecaurelia* | 199 | USA, Hawaii | AM949762 |
| *P. tredecaurelia* | 205 | Israel, Kiryat Motzkin | AM949761 |
| *P. quadecaurelia* | 207 | Namibia, Windhoek | AM949774 |
| *P. sonneborni* | AU-208 | USA, Texas | AM949786 |
| *P. jenningsi* | S-AU | Saudi Arabia | AM949758 |
| *P. schewiakoffi* | Sh1 | China, Shanghai | AM949759 |
| *P. multimicronucleatum* | GMA | Germany, Martinfeld | AM949757 |
| *P. multimicronucleatum* | ISN-11 | Italy, Naples | AM949756 |

*P. sexaurelia* strains are members of a cryptic species. Interestingly, a similar picture was observed in the study of CATANIA *et al.* (2009) in which one *P. primaurelia* strain branched off separate to all other *P. aurelia* strains.

It seems that one *P. sexaurelia* cluster (A) is mainly composed of strains from Europe (Croatia, Russia, Greece, Germany) plus the *P. dodecaurelia* strain from Hawaii; the second (B) cluster is composed of strains from Asia (Japan, China), and Puerto Rico plus the *P. dodecaurelia* strain from the USA (Mississippi).

Our results are only partly in concordance with the RAPD data of PRZYBOŚ *et al*. (2007b). In this study, only strains from Spain had similar band patterns, whereas strains from Croatia and Greece grouped together. The mating data (survival in F1 and F2 generations of inter-strain hybrids) also do not correspond to the molecular data presented in the above mentioned publication. Gene sequences of the cytosolic type *Hsp70* in *P. sexaurelia* strains from Spain, Greece and Puerto Rico have also been compared (HORI *et al*. 2006). Only slight genetic differences were observed between strains (Fig. 1 in the cited paper).

It is interesting that *P. dodecaurelia* strains cluster with *P. sexaurelia* strains (BARTH *et al.* 2008, Fig. 1), since mating between *P. sexaurelia* and *P. dodecaurelia* strains was never observed by us or SONNEBORN (1975). This indicates that the mating reaction and genetic profile are in conflict in some *P. aurelia* species. For the other species within the aforementioned cluster, *P. dodecaurelia*, considerable intraspecific genetic variation

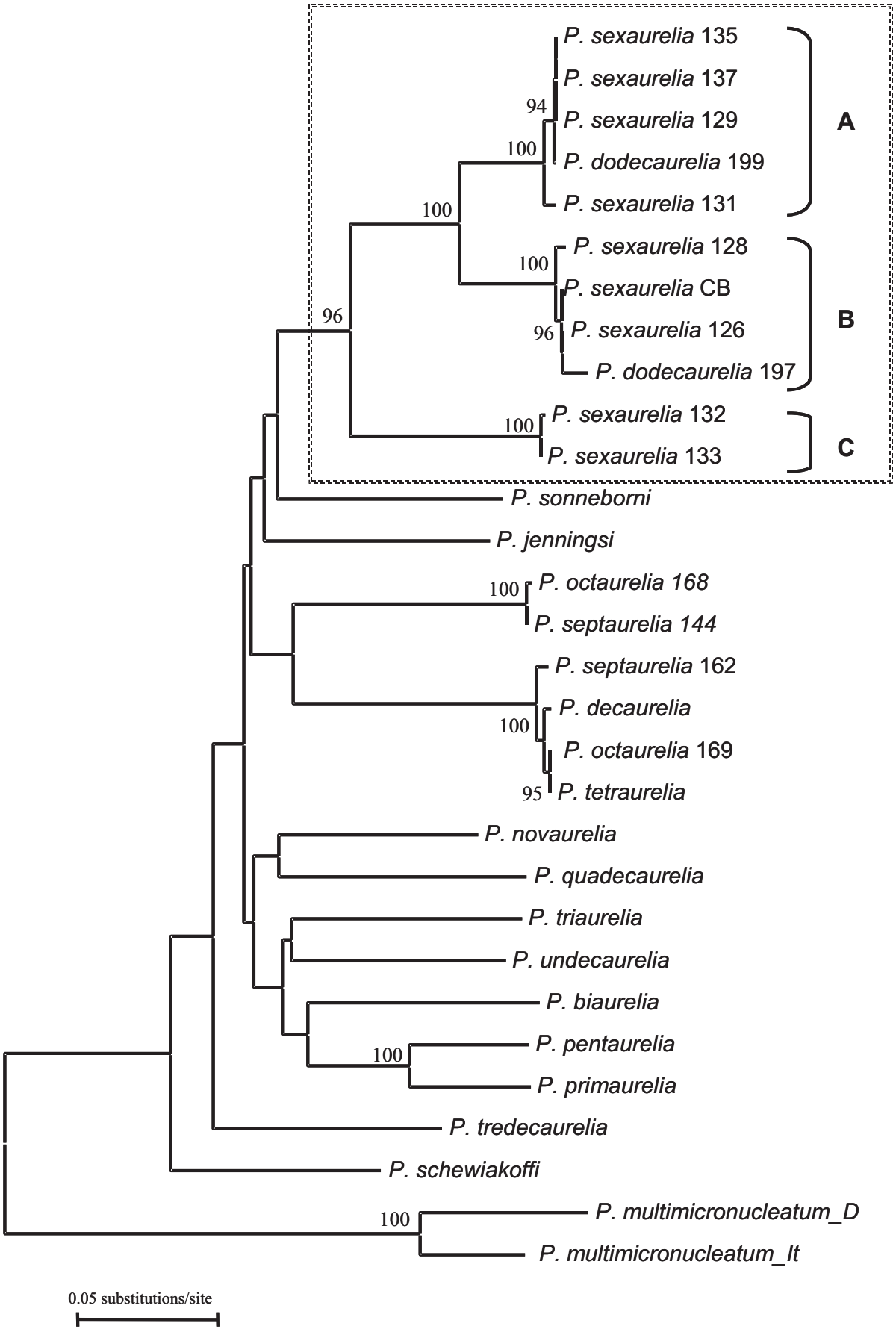


Fig. 1. Neighbor Joining tree of the *Paramecium aurelia* species complex. Only bootstrap support values above 75 are shown.

was also revealed by RAPD, RFLP, ARDRA methods (PRZYBOŚ *et al*. 2007a) as well as by sequencing the 3' end of SSU rRNA-ITS1 and 5' of LSU rRNA and COI mtDNA (TARCZ *et al*. 2006; PRZYBOŚ *et al*. 2008a,b). However, in contrast to the present study sequencing of the histone H4 gene revealed an isolated position of this species within the phylogenetic tree constructed for the species complex of *P. aurelia* (PRZYBOŚ *et al*. 2006). This illustrates an urgent need for further work and more intense sampling of these "rare" species, in order to understand the status and the relationships of *P. sexaurelia* and *P. dodecaurelia* within the *P. aurelia* species complex.

Furthermore, intra-specific differentiation of other *P. aurelia* spp., i.e. *P. novaurelia* (PRZYBOŚ *et al*. 2007c), *P. tetraurelia* (PRZYBOŚ *et al*. 2009a), and *P. octaurelia* (PRZYBOŚ *et al*. 2009b) was detected in sequences of gene fragments of LSU rDNA and COI mtDNA in strains from different continents. Polymorphism of macronuclear electrophoretic karyotypes in the *P. aurelia* species complex was also observed by NEKRASOVA *et al*. (2008).

The high genetic variation within and between species of the *P. aurelia* complex, combined with the observations that for some of the species possible cryptic speciation may have occurred, prompts for a recommendation of general investigations to be undertaken on speciation processes in these species. This is emphasized by data showing that for some species of this complex, species status may be less static and more dynamic than originally thought.

## 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.

AURY J.- M., JAILLON O., DURET L., NOEL B., JUBIN C., PORCEL B. M., SEGUERENS B., DAUBIN V., ANTHOUARD V., AIACH N., ARNAIZ O., BILLAUT A., BEISSON J., BLANC I., BOUHOUCHE K., CAMARA F., DUHARCOURT S., GUIGO R., GOGENDEAU D., KATINKA M., KELLER A.-M., KISSMEHL R., KLOTZ C., KOLL F., Le MOUEL A., LEPERE G., MALINSKY S., NOWACKI M., NOWAK J. K., PLATTNER H., POULAIN J., RUIZ F., SERRANO V., ZAGULSKI M., DESSEN P., BETERMIER M., WEISSENBACH J., SCRAPELLI C., SCHACHTER V., SPERLING L., MEYER E., COHEN J., WINCKER P. 2006. Global trends of whole-genome duplications revealed by the ciliate *Paramecium tetraurelia*. Nature **444** (7116): 171-178.

BARTH D., PRZYBOŚ E., FOKIN S. I., SCHLEGEL M., BERENDONK T. U. 2008. Cytochrome *b* sequence data suggest rapid speciation within the *Paramecium aurelia* species complex. Mol. Phylogenet. Evol. **49**: 669-673.

CATANIA F., WURMSER F., POTEKHIN A. A., PRZYBOŚ E., LYNCH M. 2009. Genetic diversity in the *Paramecium aurelia* complex. Mol. Biol. Evol. **26**: 421-431.

COLEMAN A. W. 2005. *Paramecium aurelia* revisited. J. Eukaryot. Microbiol. **52**: 68-77.

HORI M., TOMIKAWA I., PRZYBOŚ E., FUJISHIMA M. 2006. Comparison of the evolutionary distances among syngens and sibling species of *Paramecium*. Mol. Phylogenet. Evol. **38**: 697-704.

LANDIS W. G. 1986. The interplay among ecology, breeding systems, and genetics in the *Paramecium aurelia* and *Paramecium bursaria* complexes. (In: Progress in Protistology, J. O. Corliss, D. Patterson. eds. Biopress, Bristol): 287-307.

NANNEY D. L., PARK C., PREPARATA R., SIMON E. M. 1998. Comparison of sequence differences in a variable 23S rRNA domain among sets of cryptic species of ciliated protozoa. J. Eukaryot. Microbiol. **45**: 91-100.

NEKRASOVA I., POTEKHIN A., PRZYBOŚ E., RAUTIAN M. 2008. Polymorphism of macronuclear electrophoretic karyotypes in *Paramecium aurelia* species complex. GDRE *Paramecium* Genomics, 4th Intern. Meeting, 5-8 October 2008, Potzberg, Germany, p. 25.

PRZYBOŚ E., BARTH D., BERENDONK T. U. 2008c. The *Paramecium aurelia* species complex, frequency and co-occurrence across Europe. Folia biol. (Kraków) **56**: 77-81.

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

PRZYBOŚ E., MACIEJEWSKA A., SKOTARCZAK B. 2006. Relationships of species of the *Paramecium aurelia* complex (Protozoa, Ph. Ciliophora, Cl. Oligohymenophorea) based on sequences of the histone H4 gene fragment. Folia biol. (Kraków) **54**: 37-42.

PRZYBOŚ E., PRAJER M., GRECZEK-STACHURA M., SKOTARCZAK B., MACIEJEWSKA A., TARCZ S. 2007a. Genetic analysis of the *Paramecium aurelia* complex by classical and molecular methods. Systematics and Biodiversity **5**: 417-434.

PRZYBOŚ E., RAUTIAN M., GRECZEK-STACHURA M., POTEKHIN S. A. 2007b. Polymorphism within *Paramecium sexaurelia* (Ciliophora, Oligohymenophorea) and description of a new stand of the species in China. Folia biol. (Kraków) **55**: 121-125.

PRZYBOŚ E., TARCZ S., FOKIN S. 2009a. Molecular polymorphism of *Paramecium tetraurelia* (Ciliophora, Protozoa) strains originating from different continents. Folia biol. (Kraków) **57**: 57-63.

PRZYBOŚ E., TARCZ S., GRECZEK-STACHURA M., SURMACZ M., POTEKHIN A., RAUTIAN M. 2008a. Molecular studies on intraspecific differentiation of *Paramecium dodecaurelia*, with description of new stands of the species (Protozoa, Ciliophora). Folia biol. (Kraków) **56**: 249-262.

PRZYBOŚ E., TARCZ S., SCHMIDT H., CHUBATINSKI L. 2009b. First stand of *Paramecium octaurelia* in Europe and molecular characteristics of other known strains of this species. Folia biol. (Kraków) **57**: 65-70.

PRZYBOŚ E., TARCZ S., SKOBLO I. 2007c. First American stand of *Paramecium novaurelia* and intra-specific differentiation of the species. Folia biol. (Kraków) **55**: 53-63.

PRZYBOŚ E., TARCZ S., SURMACZ M. 2008b. A molecular survey of *Paramecium dodecaurelia* (Ciliophora, Protozoa) strains from a single pond. Acta Protozool. **47**: 161-171.

SONNEBORN T. M. 1957. Breeding systems, reproductive methods and species problem in Protozoa. (In: The Species Problem, E. Mayr. ed. AAAS, Washington D.C.): 155-324.

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

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

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., PRZYBOŚ E., SCHMIDT H. J. 1998. A comparison of genetics with inter-strain crosses and RAPD-fingerprints reveals different population structures within the *Paramecium aurelia* complex. Europ. J. Protistol. **43**: 348-355.

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

TAMURA K., DUDLEY J., NEI M., KUMAR S. 2007. MEGA4: Molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol. Biol. Evol. **24**: 1596-1599.

TARCZ S., PRZYBOŚ E., PRAJER M., GRECZEK-STACHURA M. 2006. Intraspecific variation of diagnostic rDNA genes in *Paramecium dodecaurelia, P. tredecaurelia* and *P. quadecaurelia* (Ciliophora, Oligohymenophorea). Acta Protozool. **45**:255-263.