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Strains of *Paramecium quadecaurelia* from Namibia, Africa; Genetic and Molecular Studies

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Summary. New strains of *Paramecium quadecaurelia* were found in Namibia, Africa. Previously, this species from the *P. aurelia* complex was known only from Australia, Emily Gap. Namibian strains were identified by mating reaction; their relationship with the Australian strain was studied by classical strain crosses (survival in F1 and F2 generations) and by comparison of cytosol-type *hsp70* gene sequences. Phylogenetic trees of the Namibian and Australian strains of *P. quadecaurelia* and the other species of the *P. aurelia* complex were generated based on the maximum-likelihood method.

Key words: breeding system, genetic relationships, geographical distribution, *hsp70* gene sequences, *Paramecium, Paramecium aurelia* species complex, phylogenetic tree of the *P. aurelia* spp. complex.

INTRODUCTION

Among 15 known species of the *Paramecium aurelia* complex (14th characterized by Sonneborn, 1975 and 15th - *P. sonneborni* by Aufderheide *et al*. 1983), *P. quadecaurelia* was presented as a species, which "has been found only once, at Emily Gap, Australia" (Sonneborn 1975). The mentioned strain, designated 328 is a reference (standard) strain of the species. *P. quadecaurelia* according Sonneborn (1975) "is characterized by its inability to react or conjugate with any other species". Mating type inheritance is by the caryonidal system. The species is distinguished from the other *P. aurelia* spp. complex electrophoretically examined by two cathodal bands and no anodal bands for esterase C (Allen *et al.* 1973). The species belongs to the group of the largest ones in the *P. aurelia* complex: length of the cells reaches 175 µm.

The paper presents new strains of the species, found in Africa in Namibia.

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MATERIALS AND METHODS

Strains. The strains were established from the water sample collected by Dr S. Dobretzov in 2001 in Vindhoek, capital of Namibia, from pond in a park.

The strains were designated AN1-1, AN1-2, AN1-3, and AN1-6. The strain AN1-2 was identified as *Paramecium multimicronucleatum*,

the others as species of the *P. aurelia* complex, by the type and number of micronuclei (Vivier 1974). The preparations were stained by acetocarmine (Sonneborn 1970) and by Giemsa's stain and Feulgen reaction (after fixation and hydrolysis, Przyboś 1978).

Strain cultivation and identification by mating reaction. Cultivation and identification of the established strains of the *Paramecium aurelia* spp. were carried out according to Sonneborn (1950, 1970). The species was identified by mating the investigated strains with the mating types of the standard strains of known species of the *P. aurelia* complex. The following standard strains were used: strain 90 of *P. primaurelia*; the Rieff strain, Scotland, of *P. biaurelia*; strain 324 of *P. triaurelia*; strain 159 of *P. sexaurelia*; strain 325 of *P. septaurelia*; strain 138 of *P. octaurelia*; strain 510 of *P. novaurelia*; strain 223 of *P. decaurelia*; strain 219 of *P. undecaurelia*; strains 321 and 209 of *P. tredecaurelia*, and strain 328 of *P. quadecaurelia*.

The paramecia were cultivated on a lettuce medium inoculated with *Enterobacter aerogenes* (Sonneborn 1970).

Strain crosses. In the intra- and inter-strain crosses, F1 generation was received by conjugation and F2 by autogamy (by the method of daily isolation lines). The occurrence of the desired stage of autogamy (specimens in the stage of two macronuclear anlagen) was examined on preparations stained with acetocarmine. Survival of 100 clones in both generations was estimated after 72 h after separation of partners of conjugation or postautogamous caryonids (cf Chen 1956).

The percentage of surviving hybrid clones in the inter-strain crosses (Namibian strains x Australian strain) was compared. Methods were described in details by Przyboś (1975).

Cell cultivation in molecular studies. *Paramecium* strains were in cultivated in 1.25% (w/v) lettuce juice in modified Dryl' s solution (Dryl 1959), in which KH_2PO_4 was replaced with $NaH_2PO_4 \cdot 2H_2O$, and inoculated with a nonpathogenic strain of *Klebsiella pneumoniae* at 25°C, as described previously (Fujishima *et al*. 1990).

Strains used in molecular studies: strain HV15-1 of *P. primaurelia*, strain 51 of *P. tetraurelia*, strain 87 of *P. pentaurelia*, strain 91 YB 1-3 of *P. novaurelia*, strain 321 of *P. tredecaurelia*, strain G3 from Japan of *P. caudatum*.

Amplification of the cytosol-type *hsp70* gene and sequencing. Cells of each strain in stationary phase were washed with modified Dryl' s solution. Ten to twenty cells were used as a template after boiling for 10 min at 95°C. The amplification protocol consisted of denaturation at 94°C for 2 min, followed by 30 cycles of denaturation at 94°C for 45 s, annealing at 50°C for 60 s, and extension at 72°C for 60 s, with final extension at 72°C for 5 min. To isolate the cytosol-type *hsp70* gene, we utilized the specific nucleotide primers, 5-GAGGAGAAGATTTCGATAAC-3 (sense, corresponding to the amino acid sequence GEDFDN) and 5-GCTTCATCTGGGTTGATTGA-3 (antisense, corresponding to the amino acid sequence SINPDE), which yielded a PCR product of 416 bp.

PCR products were cloned into the T/A plasmid vector pGEM-T easy (Promega Biotech). Plasmids were purified with Wizard Plus SV Minipreps DNA Purification system (Promega Biotech). Genomic clones were sequenced on both strands with the dideoxy termination methods using a Thermo Sequenase Cycle Sequencing Kit (Amersham Pharmacia Biotech Inc). The resulting nucleotide sequences were analyzed with the GENETYX-SV/RC 9.0 program (Genetyx Corp.).

Phylogenetic analysis. The *hsp70* sequences determined in this study were aligned by the GENETYX-SV/RC 9.0 program. Phylogenetic trees were generated using the nucML of molphy program package version 2.3 based on the maximum-likelihood method (Felsenstein 1981, Adachi *et al*. 1996).

To investigate the relationships within the *P. aurelia* spp. complex and other *Paramecium* species, we used cytosol type *hsp70* sequences of *Paramecium caudatum* (AB048692), *P. primaurelia* (AB100846), *P. tetraurelia* (AB100847), *P. pentaurelia* (AB100848), *P. novaurelia* (AB100851), *P. tredecaurelia* (AB100852) and *P. quadecaurelia* (AB106336).

RESULTS AND DISCUSSION

The strains AN1-1, AN1-3, and AN1-6, three clones in each strain, were identified as *P. quadecaurelia* by mating reaction with the reference specimens of the strain 328 from Australia and by checking vitality of the inter-strain hybrid clones in F1 and F2.

A very strong conjugation (95 - 100% of conjugating pairs) was observed after mixing the complementary mating types of the studied strains and the appropriate standard ones.

Conjugation appeared within the strains from Namibia and within the strain from Australia on 6th and 7th days of culturing clones in 24°C.

Autogamy was observed in the Namibian strains, the Australian strain, and inter-strain hybrids after 15 - 18 fissions (the growth rate of cultures was 3 fissions per day) in the daily isolation lines cultivated at 24°C (5 to 6 days).

A high viability was observed in both generations in inter-strain crosses (Table 1). The results of crosses between strain from Australia and strains from Africa of *Paramecium quadecaurelia* show that in those species exists a type of breeding system, which can be called moderate inbreeding. This means inbreeding with high fertility in crosses of geographically distant populations. There is probability of gene flow among populations, which are not genetically isolated.

The investigations carried out on *P. triaurelia* and *P. sexaurelia* (Stoeck *et al*. 1998) and later on *P. pentaurelia* and *P. novaurelia* (Stoeck *et al*. 2000) in which combination of classical strain crosses and molecular technique (RAPD-PCR) was used, showed that those species differ in their degree of inbreeding. The species of the *P. aurelia* can be arranged in some order as far as degree of inbreeding is concerned, from

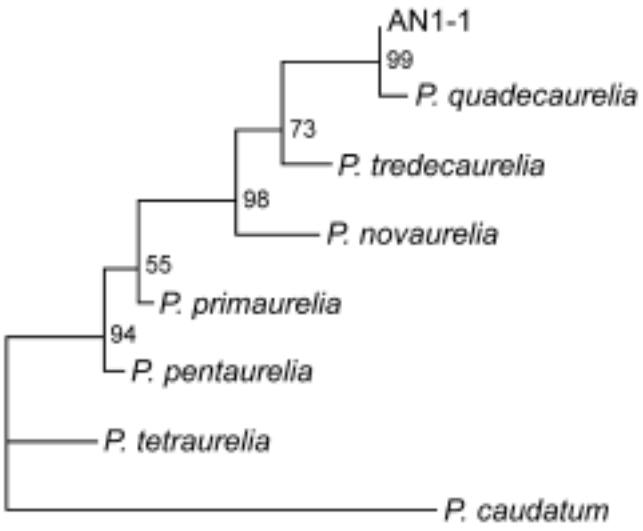

Fig. 1. Phylogeny of the *Paramecium aurelia* spp. complex, based on the comparison of cytosol type hsp70 sequences. Bootstrap probabilities are given as percentages near the individual nodes.

extreme inbreeders (*P. tetraurelia*, *P. sexaurelia* and to some extent *P. primaurelia*), moderate inbreeders (*P. novaurelia*, *P. triaurelia*), and at the other end, weak inbreeders (*P. pentaurelia*). It is based on the mentioned studies (Stoeck *et al*. 1998, 2000).

Inbreeding which is generally characterising for species of the *P. aurelia* complex (Landis 1986) is leading to a molecular differentiation of local populations showed by karyological, antigenic (cf Przyboś 1986) and molecular studies (Stoeck *et al*. 1998, 2000). Strains, however, maintain a similar morphology and possibility to cross and produce lively offspring.

It is interesting to mention that the electrokaryotype of the strain AN1-6 (Potekhin *et al*. 2002), obtained by pulsed-field gel electrophoresis (PFGE) of macronuclear DNA, differed from PFGE profiles characteristic for the previously studied strains of the *P. aurelia* complex (Rautian and Potekhin 2002). The dominant high molecular weight band (2000kb) and specific banding of the molecular spectrum were the special features for the strain AN1-6 (Potekhin *et al*. 2002), identified presently as *P. quadecaurelia*.

The strain AN1-1, AN1-3 and AN1-6 have the same base sequence for the cytosol type *hsp70* gene. The Genbank accession number for An1-1 is AB106337; AN1-3 and An1-6 have not been registered because their sequences are the same. The base sequence of these strains was 99.2% identical to the cytosol type *hsp70* gene of the Australian (328) strain of *P. quadecaurelia* (AB106336). The sequences of the

Table 1. Percentage of surviving clones in *Paramecium quadecaurelia* strain, crosses in F1 and F2 generations

Strain	F1 by conjugation	F2 by autogamy
328 (Australia) x 328	100	96
AN1-1 x AN1-1 (Africa, Namibia)	100	98
AN1-3 x AN1-3 (Africa, Namibia)	100	100
AN1-6 x AN1-6 (Africa, Namibia)	100	100
AN1-1 x 328 (Namibian x Australian)	100	96
AN1-3 x 328 (Namibian x Australian)	98	98
AN1-6 x 328 (Namibian x Australian)	100	100

Namibian and Australian strains of *P. quadecaurelia* were compared with those of *P. primaurelia* (AB100846), *P. tetraurelia* (AB100847), *P. pentaurelia* (AB100848) and *P. tredecaurelia* (AB100852), however, the homology was 93.7, 89.9, 92.9 and 96.0 %, respectively. Furthermore, phylogenetic tree suggests that the strains from Namibia belong to *P. quadecaurelia* (Fig.1).

Investigations concerning occurrence of species of the *P. aurelia* complex carried out in Africa are very rare. From that continent only *P. sexaurelia* from Kenya and *P. octaurelia* from Uganda (Sonneborn 1975) were previously known. Sonneborn (1975), however, supposed "Further sampling elsewhere may not only yield new species but modify presently known ranges of the 14 species. Present knowledge of geographical distribution is thus of limited value". It was written many years ago, since that time several studies were carried out in Europe and Asia (cf Przyboś and Fokin 2000) but investigations in Africa still are very limited.

It is also very interesting that both localities in which *P. quadecaurelia* was recorded, the one in Australia (Emily Gap situated 15 km east from Alice Springs) and the other one in Africa (Namibia, Vindhoek), are situated in the neighbourhood of the tropic of Capricorn, in the similar geographical latitude. Again Sonneborn's (1957) opinion turned out to be true that climatic zones are the main factor limiting appearance of species of the *P. aurelia* complex.

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REFERENCES

Adachi J., Hasegawa M. (1996) MOLPHY version 2.3: Programs for molecular phylogenetics, based on maximum likelihood. *Computer Sciences Monographs* **28:** 5-150

Allen S. L., Farrow S. W., Golembiewski P .A. (1973) Esterases variations between 14 syngens of *Paramecium aurelia* under axenic growth. *Genetics* **18:** 518-525

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

Dryl S. (1959) Antigenic transformation in *Paramecium aurelia* after homologous antiserum treatment during autogamy and conjugation. *J. Protozool*. **6:** 25

Felsenstein J. (1981) Evolutionary trees from DNA sequences: a maximum likelihood approach. *J. Mol. Evol.* **17:** 368-376

Fujishima M., Sawabe H., Iwatsuki K. (1990) Scanning electron microscopic observation of differentiation from the reproductive short form to the infectious long form of *Holospora obtusa*. *J. Protozool*. **37:** 123-128

Landis W.G. (1986) The interplay among ecology, breeding systems, and genetics in the *Paramecium aurelia* and *Paramecium bursaria* complexes. *Progress in Protist* **1:** 287-307

Potekhin A., Przyboś E., Rautian M. (2002) Identification of the ciliates of genus *Paramecium* by electrokaryotyping. XIV All Russian Symposium "Cell Nucleus Structure and Functions", July 15-17, 2002, Saint-Petersburg, Russia. *Cytology* (*Russian*) **44:** 901 (in Russian)

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. (1986) Species structure in ciliates. *Folia Biol.* (*Kraków*) **34:** 103-132

Przyboś E., Fokin S. (2000) Data on the occurrence of species of the *Paramecium aurelia* complex world-wide. *Protistology* **1:** 179-184

Rautian M. S., Potekhin A.(2002) Electrokaryotypes of macronuclei of several *Paramecium* species. *J. Eukaryot. Microbiol*. **49:** 296-304

Sonneborn T.M. (1950) Methods in the general biology and genetics of *Paramecium aurelia*. *J. Exp. Zool.* **13:** 87-148

Sonneborn T. M. (1957) Breeding systems, reproductive methods, and species problems in Protozoa. In: The Species Problem, (Ed. E. Mayr). AAAS, Washington, 155-324

Sonneborn T. M. (1970) Methods in *Paramecium* research. In: Methods in Cell Biology, (Ed. D. M. Prescott), Academic Press, New York, **4:** 241-339

Sonneborn T. M. (1975) The *Paramecium aurelia* complex of fourteen sibling species. *Trans. Am. Microsc. 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 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

Vivier E. (1974) Morphology, taxonomy and general biology of the genus *Paramecium* In: *Paramecium*, A Current Survey, (Ed. W. I. van Wagtendonk). Elsevier, Amsterdam, 1-89

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