

New Stands of Species of the *Paramecium aurelia* Complex in Africa and Europe

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The relevance of geographical distribution and the roles of dispersal and spatial isolation during the speciation of microorganisms are nowadays of great interest. The *Paramecium aurelia* species complex is a perfect model system to explore these questions given its long history as a study subject and broad distribution. However, the world-wide distribution of the *Paramecium aurelia* complex (Ciliophora, Protista) still needs study, e.g. sampling in the southern hemisphere has been quite limited, while Europe has been investigated for years, with the majority of *aurelia* species isolated from here. Recently, new stands of species of the *P. aurelia* complex were found in southern Europe (Malta, Bulgaria, Cyprus) and in the Czech Republic (*P. primaurelia*, *P. triaurelia*, *P. octaurelia*). In Africa (Republic of South Africa), new stands of *P. primaurelia*, *P. triaurelia*, and *P. octaurelia* were found. Interestingly, the rare species *P. triaurelia*, and *P. octaurelia* were found to co-occur both in South Africa (SA 13) and the Czech Republic (CKV 8). Newly established strains were identified to species by crossing with the test strains (the reference strains for the particular species).

Key words: protists; ciliates; *Paramecium aurelia* species complex; biogeography.

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The relevance of geographical distribution and the roles of dispersal and spatial isolation during the speciation of microorganisms are nowadays of great interest to scientists. It's obvious that the sites in which a microorganism is present reflect the way in which areas were gradually colonized, so intraspecific diversity often has a geographic pattern, and spatial isolation gives rise to new species.

The dispersal of microorganisms is generally not due to active movement, but rather to migration with the help of macro- objects they are bound to, which is especially true for species with resting stages. These mechanisms include water and atmospheric flows, macroorganisms (including man and human activity, e.g. vehicles), dust, flotsam, etc. Theoretically, this allows microorganisms to form invasive populations and occupy virtually all ecologically-fit biotopes across huge territories. This view has been characterized as “everything - everywhere” (BEIJERINCK 1913) and is now embraced – in a milder form – by other scientists who

have documented instances of such distributions (e.g. FENCHEL & FINLAY 2004, 2006). However, others, including FOISSNER and his followers, insist that some level of spatial isolation has played an important role in the speciation of microorganisms (FOISSNER 2006; COTTERILL *et al.* 2013). Studies using biomolecular techniques examine intraspecific diversity and sister-species distributions: in some cases (yeasts) allopatric speciation is well demonstrated (KUEHNE *et al.* 2007). But it is unclear how general this mechanism is in other microorganisms.

The *Paramecium aurelia* species complex is a perfect model system to explore this subject. The species complex includes 15 sibling species that cannot be distinguished by morphological criteria but have strict reproductive isolation (SONNEBORN 1975; AUFDERHEIDE *et al.* 1983). The inter- and intraspecific biodiversity of the *P. aurelia* complex has been intensively investigated, sibling species can be identified by either physiological characteristics – the ability to conjugate – or by using

various well-developed and characterized molecular markers (PRZYBOŚ *et al.* 2012). Comparisons of groups revealed by crosses and molecular markers demonstrate general congruence of these two approaches, nevertheless, there are some discrepancies between conjugation ability and genetic profiles of the strains (CATANIA *et al.* 2009; PRZYBOŚ *et al.* 2012). Analysis of silent-site polymorphism in *Paramecium* revealed a high level of diversity that was interpreted as a consequence of high effective population size (SNOKE *et al.* 2006).

The *P. aurelia* species complex is cosmopolitan and distributed on all continents in many freshwater reservoirs. Correlation of geographic distribution and genetic diversity is still questionable. Molecular comparison of several genes from a widely dispersed collection of *P. aurelia* sibling species did not support the presumption of a huge global *P. aurelia* population, as the same alleles were found in strains originating from different continents (CATANIA *et al.* 2009). It was recently shown that genetic control of mating types in *P. tetraurelia* and *P. septaurelia* is under the control of different genes and has independently evolved on the basis of the *scn*-RNA pathway (SINGH *et al.* 2014). Involvement of the *scn*-RNA pathway in mating control may also influence the species and population genetics.

The identification of new stands of *P. aurelia* strains will facilitate the exploration of *P. aurelia* species structure and diversity. In this publication newly established strains from South Africa and southern Europe were identified to species by crossing with the test strains (the reference strains for the particular species). The 15 known species of the *P. aurelia* complex (SONNEBORN 1975; AUFDERHEIDE *et al.* 1983) differ in frequency and occurrence. Some are considered cosmopolitan, such as *P. primaurelia*, *P. biaurelia*, *P. tetraurelia* and *P. sexaurelia* (SONNEBORN 1975; PRZYBOŚ & FOKIN 2000; PRZYBOŚ & SURMACZ 2010), whereas others have been recorded in only a few habitats (e.g. *P. tredecaurelia* and *P. quadecaurelia*; PRZYBOŚ *et al.* 2013a,b). According to FOISSNER (2006) and FOISSNER *et al.* (2008), ciliates including *Paramecium* represent “the moderate endemism model” as concerns their distribution. However, various parts of the world have not been studied to the same degree. The USA (SONNEBORN 1975) has been carefully surveyed, and the majority of species of the complex have been recorded. Similarly, Europe (cf. PRZYBOŚ & SURMACZ 2010) has been investigated for many years, many water bodies sampled, and the majority of species are also recorded there. However, different numbers of habitats were studied across Europe, the most numerous in the central zone, less numerous in the northern and the southern zones. In the

southern zone of Europe only 62 habitats were studied (cf. PRZYBOŚ *et al.* 2008; PRZYBOŚ & RAUTIAN 2012; PRZYBOŚ *et al.* 2012; PRZYBOŚ *et al.* 2013a), including Spain, Italy, Croatia, Greece, Bulgaria, Romania, Cyprus, and the European part of Russia.

Data concerning the occurrence of species of the *P. aurelia* complex in Africa are still extremely rare. *P. primaurelia* and *P. triaurelia* were recorded in Morocco (PRZYBOŚ & RAUTIAN 2012), *P. sexaurelia* was recorded in Kenya, *P. octaurelia* in Uganda (SONNEBORN 1975), *P. tredecaurelia* in Madagascar (RAFALKO & SONNEBORN 1959; PRZYBOŚ *et al.* 2013b), and *P. quadecaurelia* in Namibia (PRZYBOŚ *et al.* 2003).

Our further sampling has recently identified new stands of *P. aurelia* species in southern Europe (Malta, Bulgaria, Cyprus) and in Czech Republic, as well as in the Republic of South Africa. These findings are recorded and presented in this paper.

Material and Methods

Paramecia were collected from water bodies situated in the locations in Table 1. Small samples of water (50 ml) were taken and analyzed by microscopy. If paramecia were found, single cells were isolated by micropipette, washed several times to remove associated protozoans, and transferred to a micro-aquarium with a drop of culture medium. After several (3-5) cell fissions the clone was transferred to a tube and cultured as usual. Clone cultures were deposited in CCCS (Culture Collection of Ciliates and their Symbionts, Collection registered in WFCC, #1024) in St. Petersburg State University. The studied strains are presented in Table 1. All newly identified strains of the *P. aurelia* species complex belong to the Culture Collection of Ciliates and their Symbionts (CCCS) of St. Petersburg State University (http://www.wfcc.info/ccinfo/collection/by_id/1024). At present this collection is deposited in RC “Microbial Collections” of St. Petersburg State University.

Paramecia were cultured at 27°C in a medium of dried lettuce in distilled water, inoculated with *Enterobacter aerogenes* and supplemented with 0.8 mμ/ml β-sitosterol. Species of the *P. aurelia* complex were identified according to SONNEBORN's methods (1970), by mating reaction. The unidentified strains, mature for conjugation, were mated with reactive-complementary mating types of the reference strains of several species of the *P. aurelia* complex. The following standard strains were used: strain 90, Pennsylvania, USA, *P. primaurelia*; strain 324, Florida, USA, *P. triaurelia*; strain Sydney, Australia, *P. tetraurelia*; strain 87, Pennsylvania, USA, *P. pentaurelia*; strain 138, Florida,

Table 1

New stands of species of the *Paramecium aurelia* complex

Strain designation	Species	Collection place		Coordinates	Collector and date of collection
SA 3-4	<i>P. primaurelia</i>	Africa	Rep. South Africa, Kwazulu Natal, Olivershoek Pass (500m asl)	28.57S/29.05E	M. RAUTIAN, P. CHETVERIKOV 2013
SA13-2	<i>P. triaurelia</i>		Rep. South Africa, Kwazulu Natal, Lemombo Mts, Mkhuze Reserve. Small (3x4m) eutrophic pond	27.36S/30.13E	
SA13-5	<i>P. primaurelia</i>				
SA13-9	<i>P. octaurelia</i>		Africa, Rep. South Africa, close to the Indian ocean coastline, puddle near Mtentu River (27m asl)	31.01S/30.04E	
SA8-4	<i>P. primaurelia</i>				
SA8-8	<i>P. octaurelia</i>				
MAL1-1	<i>P. primaurelia</i>	Europe	Malta, small creek up on hill, near the sea shore	35.95N/14.43E	M. RAUTIAN 2013
MAL1-4	<i>P. primaurelia</i>		Bulgaria, Balchik	43.41N/28.16E	M. RAUTIAN, P. CHETVERIKOV, 2013
BF1-12	<i>P. primaurelia</i>				
BF1-13	<i>P. primaurelia</i>		Cyprus, Larnaca, creek runing to Oraclini lake salty (7-8 pm)	34.96N/33.66E	N. LEBEDEVA 2013
CYL 7-13	<i>P. octaurelia</i>				
CYL7-18a	<i>P. primaurelia</i>		Czech Republic, Karlove Vary, Tepla river	50.24N/12.87E	N. LEBEDEVA 2011, 2012
CKV 8-77	<i>P. octaurelia</i>				
CKV 8-52	<i>P. octaurelia</i>				
CKV 8-22	<i>P. triaurelia</i>				
CKV 8-28					

Table 2

Survival of hybrids in the inter-strain crosses

Species	Strain crossed	F1	F2
<i>Paramecium primaurelia</i>	SA3-4 x 90/1	86	44
<i>Paramecium primaurelia</i>	SA8-4 x 90/1	100	54
<i>Paramecium octaurelia</i>	SA8-8 x 138/8	95	90
<i>Paramecium triaurelia</i>	SA 13-2 x 324/3	96	78
<i>Paramecium primaurelia</i>	SA13-5 x 90/1	95	90
<i>Paramecium octaurelia</i>	SA13-9 x 138/8	90	83
<i>Paramecium primaurelia</i>	MAL1-1 x 90/1	100	74
<i>Paramecium primaurelia</i>	MAL 1-4 x 90/1	100	56
<i>Paramecium primaurelia</i>	BF1-12 x 90/1	92	90
<i>Paramecium primaurelia</i>	BF 1-13 x 90/1	100	90
<i>Paraecium octaurelia</i>	CYL 7-13 x 138/8	100	63
<i>Paramecium primaurelia</i>	CYL 7-18a x 90/1	92	88
<i>Paramecium octaurelia</i>	CKV 8-52 x 138/8	93	60
<i>Paramecium octaurelia</i>	CKV 8-77 x 138/8	93	74

USA, *P. octaurelia*. The standard strains belong to the collection of strains of the Institute of Systematics and Evolution of Animals, Polish Academy of Sciences, Kraków, Poland.

New strains were identified as particular species of the *P. aurelia* complex on the basis of strong conjugation between the studied strain and the reference strain of the species. Survival of hybrids (F1 and F2 generations, Table 2) in inter-strain crosses (between the studied strains and standard strain of a particular species) was investigated.

Results and Discussion

In this study we present new stands of *P. primaurelia*, *P. triaurelia* and *P. octaurelia* in Africa; *P. primaurelia* and *P. octaurelia* in southern Europe; and *P. triaurelia* and *P. octaurelia* in the Czech Republic (Table 1). While Europe has been studied at a large scale and the majority of known species of the complex have been found, the coverage of Europe has not been even, with much of the attention focused on central Europe (cf. PRZYBOŚ *et al.* 2008).

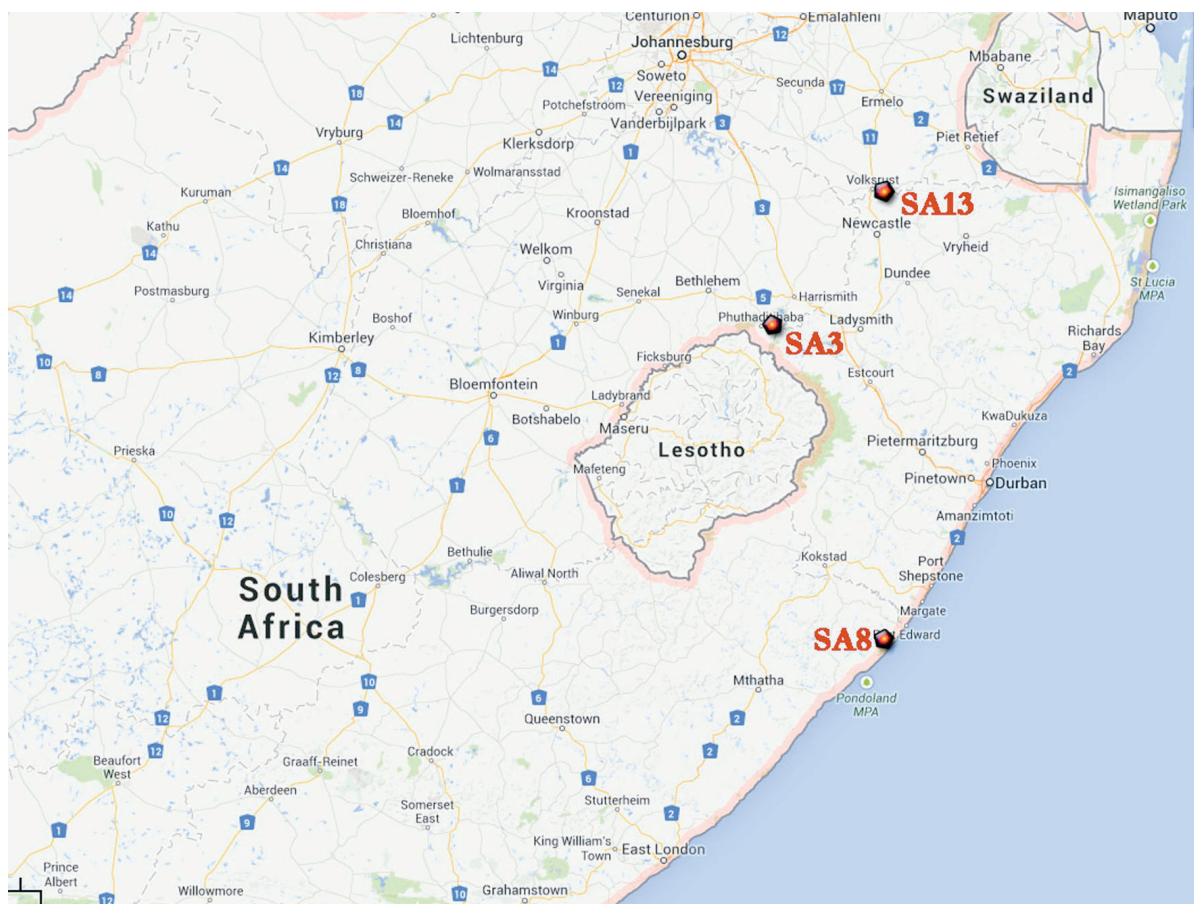


Fig. 1. The map of collecting sites of water samples with plankton in Republic of South Africa.



Fig. 2. Sampling sites (SA 3, SA 8, SA 13) in the Republic of South Africa.

New stands of *P. primaurelia* were recorded in southern countries of Cyprus, Malta, and Bulgaria. The most interesting observation includes the new stands of *P. octaurelia*, considered to be a rare species of the complex, in the Czech Republic (CKV 8, together with *P. triaurelia*) and also in Cyprus (CYL 7, together with *P. primaurelia*). In Europe, *P. octaurelia* was previously recorded only from Germany (PRZYBOŚ *et al.* 2009), Russia (Chebokarsky region) and Austria (PRZYBOŚ *et al.* 2014).

Studies of the distribution the *P. aurelia* species complex in Africa are rare, with the presence of some species of the complex recorded, as mentioned in the introduction. But the majority of the continent remains entirely unexplored. For the first time the Republic of South Africa was studied, with sampling from 3 widely separated sites (see Fig. 1). *P. primaurelia* was recorded in all three habitats (Fig. 2): in one of them alone (Olive-shoek Pass, SA3), in the second (puddle in Mtenu River basin, SA8) with *P. octaurelia*, and another

Paramecium species – *P. polyaryum*; and in the third with *P. triaurelia* and *P. octaurelia* (small pond in Kwazulu Natal, Lemombo Mts, SA 13). The water in the last two habitats was eutrophic due to excrement of antelope or cows. Nevertheless, the finding of three species of the complex in a sample of less than 50 ml is significant. Previously, we have found few samples with this level of species richness (Astrakhan region, PRZYBOŚ *et al.* 2004), and also now in sample CKV 8 from the Czech Republic in which *P. octaurelia* was found together with *P. triaurelia* and other *Paramecium* species, namely *P. multimicronucleatum*, *P. caudatum*.

Another point of interest is the co-occurrence of different species of the complex. *P. primaurelia* was found together with *P. octaurelia* in two samples from RSA, SA8 and SA13 (here also with *P. triaurelia*). This co-occurrence was not surprising because *P. primaurelia* is a ubiquitous species, frequent in Europe, and maybe in Africa also, it was found in all three samples from RSA and in one out of two samples collected from Morocco (PRZYBOŚ & RAUTIAN 2012). Even more interesting is the co-occurrence of two rare species, *P. octaurelia* and *P. triaurelia*, which were found together in South Africa (SA 13), and in the Czech Republic, Karlove Vary, sample CKV 8.

Newly established strains (Table 1) were crossed with the test strains (standard of the particular species) and viability of the progeny was determined (Table 2). The lowest degree of F2 viability was observed in *P. primaurelia* hybrids in crosses of strains from RSA (SA8- 4 and SA3-4) as well as from Malta (MAL1-4) with the reference strain (90) originating from the USA, Pennsylvania. This may reflect the high genetic divergence between these strains and standard crossing partner. This may be expected for strains separated by thousands of kilometers, ocean and the Sahara (in the case of RSA strains) or for island populations (Malta). Interestingly, the strain from the third location in RSA (SA13-5) demonstrates one of the highest degrees of viability. This may be a result of more recent independent invasion of this strain, corresponding with a scenario proposed by CATANIA and coauthors (CATANIA *et al.* 2009). It would be interesting to study these strains using molecular markers.

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