

New Stands of Species of the *Paramecium aurelia* Complex (Ciliophora, Protozoa) in Europe and South America (Ecuador)

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The occurrence of species of the *P. aurelia* complex has been studied at a large scale in Europe and the majority of known species of the complex have been found there. However, a different number of habitats were studied in particular zones of Europe, the greatest number in the central zone. Herein new stands of several species of the *Paramecium aurelia* complex are presented from Europe including *P. primaurelia*, *P. biaurelia*, *P. triaurelia*, *P. octaurelia*, *P. novaurelia*, and *P. dodecaurelia*. In South America, studies concerning the distribution of the *P. aurelia* species complex were carried out only occasionally and the presence of some cosmopolitan species of the complex has been recorded, i.e. *P. primaurelia*, *P. biaurelia*, and *P. tetraurelia*. Recently, new stands of *P. primaurelia* and *P. septaurelia* were found in Ecuador. Ciliate biogeography and distribution is also discussed.

Key words: Protists, ciliates, *Paramecium aurelia* species complex, biogeography.

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Among the 15 known species of the *Paramecium aurelia* complex (14 characterized by SONNEBORN 1975 and *P. sonneborni* by AUFDERHEIDE *et al.* in 1983), some are cosmopolitan e.g., *P. primaurelia*, *P. biaurelia*, *P. tetraurelia*, and *P. sexaurelia*, while others are limited to certain regions, environments, or even habitats (cf. SONNEBORN 1975). However, only some parts of the world have been sampled intensively, e.g. 536 habitats were studied in Europe (PRZYBOŚ *et al.* 2010; PRZYBOŚ & RAUTIAN 2012; PRZYBOŚ *et al.* 2013a). North America (USA) was also studied in detail (SONNEBORN 1975) where the majority of species of the *P. aurelia* complex were recorded. Other continents were studied only occasionally, including Central and South America, Australia, and Africa (PRZYBOŚ & FOKIN 2000; PRZYBOŚ & SURMACZ 2010).

Several strains of *P. aurelia* spp. collected recently in different parts of Europe and also from South America were studied herein.

Material and Methods

Material

Paramecia were collected from natural pools in several different locations in order to study the biodiversity of the *Paramecium aurelia* species complex. A small sample of water (50 ml) was taken and analyzed with a microscope. If paramecia were found, each single cell was 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, clones of the cells were established, 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.

Table 1

New stands of the *Paramecium aurelia* species complex

Clone index	Collection site	Species	Collector's name and Collection
TG 1-2	Russia, Middle Volga region, Tolyatti region	<i>P. dodecaurelia</i>	M. RAUTIAN 2011, strains deposited in CCCS (Culture Collection of Ciliates and their Symbionts)
PRK 1-4	Russia, Pskov region	<i>P. biaurelia</i>	
PRK 3-2			
ChM3-13	Russia, Cheboksary region	<i>P. octaurelia</i>	K. CUSHKO 2012, strains deposited in CCCS
ChM 3-3			
ChM 3-8			
CyL 7-1	Cyprus, Larnaka, Solten Lake	<i>P. primaurelia</i>	N. LEBEDEVA 2012, strains deposited in CCCS
Sp 7-1	Spain, Barcelona, artificial pond, industrial park	<i>P. primaurelia</i>	N. LEBEDEVA 2012, strains deposited in CCCS
Sp 7-12			
AW 3-2	Austria, Vienna	<i>P. octaurelia</i>	M. RAUTIAN & P. CHETVERIKOV 2012, strains deposited in CCCS
AW 3-33			
AW 3-26			
GB 25-5	Great Britain, London, pond in Hyde Park	<i>P. triaurelia</i>	M. RAUTIAN 2012, strains deposited in CCCS
GB 25-8			
GB 25-9			
GB 4-4			
GB 4-27	Great Britain, Scotland, Blaire Castle "Hercules Gardens"	<i>P. novaurelia</i>	
GB 4-3			
GB 1-5	Great Britain, Scotland, Edinburgh, fountain, sampling point I	<i>P. biaurelia</i>	M. RAUTIAN 2012, strains deposited in CCCS
GB 2-1	Great Britain, Scotland, Edinburgh, fountain, sampling point II	<i>P. biaurelia</i>	
GB 2-2			
GB 2-11			
Abh 4-4	Abkhasia, Ritsa Lake	<i>P. biaurelia</i>	T. LEVINA 2012, strains deposited in CCCS

Methods

Paramecia were cultured at 27°C in a medium made of dried lettuce in distilled water, inoculated with *Enterobacter aerogenes* and supplemented with 0.8 mg/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 Rieff, Scotland, GB, *P. biaurelia*; strain 324, Florida, USA, *P. triaurelia*; strain Sydney, Australia, *P. tetraurelia*; strain 87, Pennsylvania, USA, *P. pentauurelia*; strain 159, Puerto Rico, *P. sexau-*

relia; strain 38, Florida, USA, *P. septaurelia*; strain 138, Florida, USA, *P. octaurelia*; strain Hessen/Rhön, Germany, *P. novaurelia*; strain 246, Mississippi, USA, *P. dodecaurelia*.

The new strains were identified as particular species of the *P. aurelia* complex on the basis of conjugation between the studied strain and the reference strain of the species.

Results and Discussion

We recorded new stands of several species, i.e. *P. primaurelia*, *P. biaurelia*, *P. triaurelia*, *P. octaurelia*, *P. novaurelia* and *P. dodecaurelia* in Europe, and *P. primaurelia* and *P. septaurelia* in South America (Ecuador), (Table 1).

The occurrence of the *P. aurelia* complex in Europe has been studied at a large scale and the majority of known species of the complex have been found there. However, different numbers of habitats were studied in particular zones of Europe, the greatest number in the central zone (cf PRZYBOŚ *et al.* 2008).

New stands of *P. primaurelia* were recorded in Cyprus (Larnaka) and in Spain (Barcelona). *P. biaurelia* was found in several stands, i.e. in Russia (Pskov), Scotland (Edinburgh), Abkhazia. Both species were recognized as cosmopolitan species. *P. triaurelia* was recorded for the first time in Great Britain (London, England) and also a new stand of *P. novaurelia* was recorded in Blaire Castle, Scotland. This species was described first from Edinburgh, Scotland (BEALE & SCHNELLER 1954) and later was recognized as common in Europe. *P. dodecaurelia* was originally known only from the USA (SONNEBORN 1975), but later was also found in several other places in the world (PRZYBOŚ *et al.* 2012), however, it cannot be recognized as a cosmopolitan species. At present, the species was recorded in Russia (Middle Volga region). The most interesting are the new stands of *P. octaurelia*, a rare species of the complex, in Russia (Cheboksary region) and in Austria. *P. octaurelia* previously was recorded in Europe only in Germany (PRZYBOŚ *et al.* 2009).

In South America, studies on the distribution of the *P. aurelia* species complex were carried out only occasionally, the presence of only some cosmopolitan species of the complex was recorded, i.e. *P. primaurelia*, *P. biaurelia*, and *P. tetraurelia* (SONNEBORN 1975). The last species was also found in Brazil (PRZYBOŚ & SURMACZ 2010). In this study, strains originating from Ecuador were identified as *P. primaurelia* and *P. septaurelia*. This is the first record of the latter species in South America. It is worth mentioning that *P. primaurelia* was found in the same habitat in which *P. quadecaurelia*, a rare species of the complex, was identified (PRZYBOŚ *et al.* 2013b).

Some species of the *P. aurelia* complex may occur in the same habitat at the same time as noted by previous studies (PRZYBOŚ 1993) and also recently (*P. biaurelia* with *P. triaurelia*, and *P. tetraurelia* with *P. dodecaurelia* appeared in one pond during particular seasons) (PRZYBOŚ *et al.* 2011). The species may occupy different niches characterized by various biological and chemical features.

Ciliate biogeography lies between microorganism and plant and animal biogeography. The *P. aurelia* species complex became a model for investigation of complex problems associated with ciliate biodiversity, biogeography and species structure. As comparatively small unicellular organisms, they may be transferred by water flow,

wind, birds or animals, and also by human activities. This is especially important for those species which form cysts. However, species belonging to the genus *Paramecium* do not form cysts (BEALE & PREER 2008), nevertheless they can survive in wet skin or fur of animals, be transferred by water flow, ships and vehicles and thus be subject to long distance dispersal, which is incomparable with their own mobility. This is why they are characterized by an increased proportion of cosmopolitan species. On the other hand, ciliates are eukaryotes with sexual process restricted by species identity, mating types, physiological conditions, age, etc. This complex background favors “the moderate endemicity model” proposed by FOISSNER (2006) and FOISSNER *et al.* (2008). Studies of ZUFALL *et al.* (2012) supported this model, showing that the distribution of *Tetrahymena thermophila* is restricted to the eastern United States of America.

Further sampling, especially in the southern hemisphere, may put forth new data on the occurrence of species of the *P. aurelia* complex. Recently new stands of the rare species of the complex were recorded there, i.e. *P. quadecaurelia* (PRZYBOŚ *et al.* 2013b) in Ecuador and Thailand, and *P. tredecaurelia* in Madagascar and Thailand (PRZYBOŚ *et al.* 2013c).

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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/col_by_country/r/1107). At present this collection is deposited in RC “Microbial Collections” of St. Petersburg State University.

Other strains representing particular species of the *P. aurelia* complex belong to the strain collection of the Institute of Systematics and Evolution of Animals, Polish Academy of Sciences, Kraków, Poland.

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