ISSN 0015-5497, e-ISSN 1734-9168

Folia Biologica (Kraków), vol. 65 (2017), No 2
https://doi.org/10.3409/fb65_2.127

New Stands of Species of the *Paramecium aurelia* Complex (Ciliophora, Protista) in Europe, Asia, and Oceania (Guam)

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Accepted May 16, 2017 *Published online September 07, 2017* *Published September 20, 2017*

PRZYBOŚ E., RAUTIAN M. 2017. New stands of species of the *Paramecium aurelia* complex (Ciliophora, Protista) in Europe, Asia, and Oceania (Guam). Folia Biologica (Kraków) **65**: 127-131.

New stands of species of the *Paramecium aurelia* complex in Europe (Poland – *P. novaurelia*, Spain – *P. primaurelia*), Asia (Russia, Altay – *P. biaurelia*, *P. novaurelia* and Kamchatka – *P. pentaurelia*, Iran – *P. primaurelia*), and Oceania (Guam island – *P. octaurelia*) are presented in the paper. Especially interesting is a stand on Guam, the largest and southernmost island in the Marianas Archipelago (Australia with Oceania), as well as a stand in Ispahan, Iran. Both regions were sampled for the first time for the presence of the *P. aurelia* spp. Results of the paper are in agreement with the opinion that some species of the *P. aurelia* complex are cosmopolitan (as *P. primaurelia* and *P. biaurelia*), other species such as *P. octaurelia* seem limited to tropical or subtropical regions, and *P. novaurelia* can also be found outside of Europe. Besides the *P. aurelia* spp., other species of *Paramecium* genus were identified in the collected water samples, i.e. *P. caudatum* (Ispahan, Iran and Białowieża, Poland); *P. polycaryum* (Guam), and *P. multimicronucleatum*-like (Guam).

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

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Paramecia belonging to the *Paramecium aurelia* spp. complex are model organisms applied in several studies concerning general biology, among other also evolution, and possibility of adaptation to different environmental conditions and colonization of some fresh water bodies. Knowledge of biogeography seems fundamental for understanding evolution (LOWE 2015). Studies on the occurrence and distribution of particular species of the *P. aurelia* complex have been carried out for many years (SONNEBORN & DIPPELL 1946; BEALE & PREER 2008) and revealed that among 15 known (SONNEBORN 1975; AUFDERHEIDE *et al*. 1983) species of the complex, some are cosmopolitan (as *P. primaurelia*, *P. biaurelia*, *P. tetraurelia*, and *P. sexaurelia*), and others limited to particular regions, environments, or habitats (SONNEBORN 1975). However, only some parts of the world have been sampled intensively, such as Europe (PRZYBOŚ & SURMACZ 2010; PRZYBOŚ *et al*. 2014a; RAUTIAN *et al*. 2014; PRZYBOŚ & PRAJER 2015), and the USA in North America where the majority of species of the *P. aurelia* complex were recorded (SONNEBORN 1975; BEALE & PREER 2008). Other continents were studied only occasionally, including Central and South America, Australia, and Africa (PRZYBOŚ & FOKIN 2000; PRZYBOŚ & SURMACZ 2010; PRZYBOŚ *et al*. 2014b; RAUTIAN *et al*. 2014; PRZYBOŚ & PRAJER 2015). Huge territories of Asia are still unexplored, more intensive sampling was done only in Japan and the Asiatic part of Russia (PRZYBOŚ *et al*. 2013a; RAUTIAN *et al*. 2015). The southern hemisphere, generally, still awaits investigation (FOKIN 2010/2011).

Different models of ciliate distribution have been proposed, including the models of ubiquity (FINLAY & FENCHEL 2004; FENCHEL 2005) and moderate endemicity (FOISSNER 2006; FOISSNER

et al. 2008). Some molecular studies also support the latter model (EPSTEIN & LOPEZ-GARCIA 2008), i.e., the concordance of some protist species distributions with biogeography. The dispersal of paramecia is also a very important aspect concerning their biogeography. Paramecia can only be transferred by animals or human activities with some drops of water, as cysts are unknown (LANDIS 1988; GUTIERREZ *et al*. 1998; BEALE and PREER 2008). BEALE and PREER (2008) presented a hypothesis that *Paramecium* might be an old species which already existed before the separation of continents from Pangea and distributed by movement of the continents. The same argument (split of Pangea) was suggested by FOISSNER (2006) and FOISSNER *et al*. (2008) concerning dispersal of micro-organisms.

The other aspect is the existence of climatic zones limiting the occurrence of some sensitive species, suggested by SONNEBORN (1975), and recently proven (PRZYBOŚ & PRAJER 2015). The authors analyzed new and published data (Table 3 in PRZYBOŚ & PRAJER 2015) and confirmed that some species were recorded only in warm climate zones, including *P. quadecaurelia* (PRZYBOŚ *et al*. 2013b), *P. sonneborni* (PRZYBOŚ *et al*. 2014a; PRZYBOŚ *et al*. 2015), as well as, e.g., *P. sexaurelia*, *P. octaurelia*, and *P. tredecaurelia* (PRZYBOŚ & PRAJER 2015). Knowledge on paramecium species occurrence around the world and filling all existing "white spots" may increase our understanding of the geographical distribution of protists.

Identification of new strains of species of the *P. aurelia* complex collected in Europe, Asia, and Oceania (Guam) was the aim of this paper. Especially interesting is the strain isolated from Guam, the largest and southernmost island in the Marianas Archipelago (Australia with Oceania, GŁODEK 1967), as well as the strain from Ispahan, Iran. Both regions were sampled for the first time.

Material and Methods

Material

Paramecia studied in the present paper representing species of the *P. aurelia* complex are listed in Table 1. They were collected in Russia, Altay

Table 1

New stands of species of the *Paramecium aurelia* complex

Species	Clone index	Collection place		Coordinates	Collector, date of collection	Remarks
P. novaurelia	Alt 16-3	Asia	Russia, Altay Mts, small river (ca 1300 a.s.l.)	51°53′N/ 85°52′E	M. RAUTIAN	
P. biaurelia	Alt 28-5		Russia, Altay Mts, ditch near Chuysky tract (ca 1500 a.s.l.)	51°56′N/85°54′E		
P. biaurelia	Alt 28-7					
P. biaurelia	Alt 265-2		Russia, Altay Foreland, river Soloviha	52°03′N/83°35′E		Symbionts in cytoplasm
P. pentaurelia	Kam 87-3		Russia, Kamchatka, Ust' Bolsheretsk, small pond with a lot of green algae	52°49′N/156°18′E	M. RAUTIAN, A. BELIAVSKAIA	
P. novaurelia	Alt 268-10		Russia, Altay Foreland, river Eltsovka	52°15′N/83°31′E	M. RAUTIAN	
P. novaurelia	Alt 279-9		Russia, Altay Foreland, river Charysh	52°65′N/82°13′E		
P. novaurelia	Alt 268-9		Russia, AltayForeland, river Eltsovka	52°15′N/83°31′E		
P. novaurelia	Alt 283-3		Russia, Altay Forelasnd, Lake Bakhmatovskoie	52°42′N/81°56′E		
P. novaurelia	Alt 291-1		Russia, Altay Foreland, lake Krestianskoie	52°47′N/81°81′E		Symbionts in cytoplasm
P. novaurelia	Alt 291-2					
P. primaurelia	IrI		Iran, Ispahan, artificial pond	32°39′N/51°40′E	E. PRZYBOŚ 2015	
P. novaurelia	PB	Europe	Poland, Białowieża, pond	52°42′N/23°52′E	P. WOJTAL 2015	
P. primaurelia	Spa 17-1		Spain, Malaga, Alhambra, fountain	36°72′N/4°41′E	M. RAUTIAN, A. BELIAVSKAIA 2015	Symbionts in cytoplasm
	Spa 21-1		Spain, Malaga, River Guadalmedina	36°71′N/4°43′E	M. RAUTIAN , A. BELIAVSKAIA 2015	
P. octaurelia	G3-7	Australia and Oceania	Guam (unincorporated territory of the USA), Guam, river Asan	13°47′N/144°71′E	M. RAUTIAN 2016	

region and Kamchatka; Iran, Ispahan; Poland, Białowieża; and Spain, Malaga.

Clone cultures from Russia (Altay, Kamchatka), Spain (Malaga) and Guam were deposited in CCCS (Culture Collection of Ciliates and their Symbionts, Collection registered in WFCC, #1024) in St. Petersburg State University.

Methods

SONNEBORN's methods (1950, 1970) of cultivation and identification of strains were used. 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. 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.

The following standard strains were used:

strain 90, Pennsylvania, USA, *P. primaurelia*;

strain Rieff, Scotland, Great Britain, *P. biaurelia*;

strain 51, Indiana, USA, *P. tetraurelia*;

strain 87, Pennsylvania, USA, *P. pentaurelia*;

strain 138, Florida, USA, *P. octaurelia*;

strain 205, Edinburgh, Scotland, UK, *P. novaurelia.*

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

In the inter-strain crosses, the F_1 generation was obtained by conjugation and F_2 by autogamy (using the method of daily isolation lines). Inter-strain crosses were carried out between recently identified strains from Spain, Iran, Russia, Guam and Poland representing particular species of the *P. aurelia* complex and standard strains of the species (Table 2). 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 both generations was estimated as percentages. According to CHEN (1956), clones can be considered as surviving after passing 6-7 fissions during 72 hours after separation of partners of conjugation or postautogamous caryonids.

Other species of *Paramecium* were also identified in the studied samples based on a comparison of cell size and morphology, number and type of micronuclei (VIVIER 1974), and also morphology of contractile vacuoles and number of CV pores (FOKIN 2010/2011).

Results and Discussion

New stands of *P. primaurelia*, *P. biaurelia*, *P. pentaurelia*, *P. octaurelia* and *P. novaurelia* were recorded in the studied localities (Table 1). Results of pioneer sampling in Iran (Ispahan) and Guam seemed especially intriguing where only single strains of the *P. aurelia* spp. were collected. *P. primaurelia* (Iran) and *P. octaurelia* (Guam), respectively, were recorded there, however, the occurrence of the other species of the *P. aurelia* complex in these regions cannot be excluded. *P. octaurelia* seems rather rare, limited to the tropical and subtropical regions, being found in Panama, Uganda, Israel and Georgia (SONNEBORN 1975; PRZYBOŚ & PRAJER 2015). A new stand of *P. primaurelia* was also recorded in Spain (Malaga), which was not sampled before. Previously, several species of the complex were already found in Spain, such as *P. primaurelia, P. biaurelia, P. triaurelia, P. tetraurelia, P. pentaurelia, P. sexaurelia* , and *P. novaurelia*, as the result of sampling carried out in different regions of the country

Table 2

Percentage of surviving hybrid clones in inter-strain crosses of the studied species of the *Paramecium aurelia* complex

Species	Crossed strains, studied x standard of particular species	Percentage of surviving clones in	
		F1 (obtained by conjugation)	F2 (obtained by autogamy)
P. primaurelia	Spa 17-1 (Spain, Malaga) x 90/1 (USA, Pennsylvania)	100	94
P. primaurelia	IrI (Iran, Ispahan) x 90/1 (USA, Pennsylvania)	97	66
P. biaurelia	Alt 265-2 (Russia, Altay) x Scotland, Rieff	90	72
P. pentaurelia	Kam 87-3 (Russia, Kamchatka) x 87/5 (USA, Pennsylvania)	98	74
P. octaurelia	G 3-7 (Guam) x 138/8 (USA, Florida)	98	82
P. novaurelia	Alt 292-1 (Russia, Altay) x 205/9 (Scotland, Edinburgh)	90	80
P. novaurelia	PB (Poland, Białowieża) x 205/9 (Scotland, Edinburgh)	97	84

and in different years (PRZYBOŚ 2005). *P. primaurelia* was the most frequent species in this country. *P. novaurelia* was recorded in Białowieża, Poland, in a recently collected water sample from a local pond. The same species was recorded many years ago in this locality in the river Narewka (KOMALA *et al*. 1960). In Altay, Russia, the presence of *P. biaurelia* (3 strains), at foothills (dry climate, very cold winter and mild summer) and *P. novaurelia* (7 strains), in foreland (hot summer) was revealed recently. The *P. aurelia* spp. were not found in the upper part of the Altay Mts (about 2000-2500 m. a.s.l.), and *P. caudatum* was very rare as only found in 6 samples among 200 collected. Studies carried out previously in the Altay region revealed *P. biaurelia* and *P. pentaurelia* in the Altay Foreland and in the Altay Mts (POTEKHIN *et al*. 2006). One stand of *P. pentaurelia* was found in Kamchatka, Ust Bolsheretsk, at present. Previously, other species of the *P. aurelia* spp. were recorded on this territory, namely *P. primaurelia*, and *P. dodecaurelia* (PRZYBOŚ *et al*. 2013a).

The viability of the offspring of the hybrid exconjugant clones in the inter-strain crosses was high in F1 and F2 generations (Table 2) in the studied species, a lower (66%) percentage of surviving clones was observed in F2 hybrids of the Iran strain x standard strain of *P. primaurelia* from the USA. This may be caused by high genetic divergence between strains originating from these remote habitats.

Besides the *P. aurelia* spp., other species of *Paramecium* genus were also identified in the collected water samples, i.e. *P. caudatum* (Iran, Ispahan and Poland, Białowieża); *P. polycaryum* (Guam, strains G6-10, G5-1), and *P. multimicronucleatum* -like (Guam, strains G6-4, G6-14, G1-2).

"Only a small percentage of protist diversity can be discovered when single samples are taken from surroundings. While undersampling may be the cause (FOISSNER 2006), our limited understanding is the problem".

Conclusions

The results of the paper are in agreement with the opinion that some species of the *P. aurelia* complex are cosmopolitan (such as *P. primaurelia* and *P. biaurelia*), other species such as *P. octaurelia* seem limited to tropical or subtropical regions, and *P. novaurelia* can also be found outside Europe.

Acknowledgements

The authors are grateful to D. Sc. Piotr WOJTAL from the Institute of Systematics and Evolution of Animals, Polish Academy of Sciences, who collected water samples with *Paramecium aurelia* spp. from Białowieża, Poland, and Aleksandra BELIAVSKAIA from St. Petersburg University, Russia for help in the expedition to Kamchatka.
Research was partly supported by grant RNF 14-24-00155 and RFBR 17-04-02156A.
The authors are grateful to RC „Molecular and Cellular technologies and RC „Cultivation of Microorganisms" of St. Petersburg State University.

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