PL-ISSN 0015-5497 (print), ISSN 1734-9168 (online)
Folia Biologica (Kraków), vol. 63 (2015), No 4
doi:10.3409/fb63_4.289

New Stands of Species of the *Paramecium aurelia* Complex in Yakutia, Russia*

Maria RAUTIAN, Ewa PRZYBOŚ and Alexandra BELIAVSKAIA

Accepted September 10, 2015

RAUTIAN M., PRZYBOŚ E., BELIAVSKAIA A. 2015. New stands of species of the *Paramecium aurelia* complex in Yakutia, Russia. Folia Biologica (Kraków) **63**: 289-293.

Paramecium is one of the most studied genera among ciliates. In particular, it is a model organism for investigation of the sibling species problem (also known as the cryptic species problem), spatial distribution, and its role in speciation. The global distribution of *Paramecium* species and of sibling species belonging to the *P. aurelia* species complex (Ciliophora, Protista) still need study, e.g. sampling in some territories has been quite limited, while Europe has been investigated for years with the majority of the *P. aurelia* species isolated from here. The large territory of Yakutia (republic Sakha in the Russian Federation), known for its climate extremes and continuous permafrost that extended over several glacial and interglacial cycles of the Pleistocene, has not been studied before. In the present study we collected paramecia in the central part of Yakutia. Newly established strains were identified to species according to morphology and, in case of the *P. aurelia* complex, by crossing with the test strains (the reference strains for the particular species). New stands of *P. primaurelia*, *P. biaurelia* and *P. novaurelia* were described from the territory of Yakutia.

Key words: Protists; ciliates; *Paramecium aurelia* species complex; biogeography; extreme biological conditions.

Maria RAUTIAN, Alexandra BELIAVSKAIA, Faculty of Biology, St. Petersburg State University, Oranienbaumskoye shosse 2, 198504 St. Petersburg, Russia.
E-mail: mrautian@mail.ru
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

The 15 known species of the *P. aurelia* complex (SONNEBORN 1975; AUFDERHEIDE *et al*. 1983) differ in geographical distribution, frequency, and their character of 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 endemicity model" as concerns their distribution. However, various parts of the world have not been studied to the same degree. The USA (SONNEBORN 1975) in North America has been carefully surveyed, and the majority of species in the complex were recorded. Similarly, Europe (cf PRZYBOŚ & SURMACZ 2010) has been investigated for many years, with many water bodies sampled, and the majority of species have also been recorded there. Some territories are still awaiting exploration, including huge territories in Asia. In this publication the presence of different *Paramecium* species in the central part of Yakutia, Russia, was investigated, and newly established strains of the *P. aurelia* species complex were identified to species by crossing with the test strains (the reference strains for the particular species).

Yakutia, or the Sakha Republic in the Russian Federation, is characterized by a sharply continental climate. It is known for its climate extremes, with the Verkhoyansk Range being the coldest area in the northern hemisphere, with some of the lowest natural temperatures ever recorded. The Northern Hemisphere's Pole of Cold is at Verkhoyansk, where the temperatures reached as low as -67.8°C in 1892, and at Oymyakon, where the temperatures reached as low as -71.2°C in 1926. Even in central parts the winter is long (during 7 months

*The work of M. RAUTIAN, and A. BELIAVSKAIA was partly supported by RFBR grant 13-04-01714.

average temperature is below 0°C) and rather cold; average January temperature: -28°C. Summer is short and comparatively hot, average July temperature in the central part is +19°C. However, it known to be very hot during the day; in Yakutsk in July a temperature of +38.4°C was recorded. Yakutia is a dry region with average annual precipitation of 200 mm in central parts.

Most of Yakutia is covered by permafrost. Calculations indicate that the time required to form the deep permafrost underlying Yakutia was over a half-million years. This extended over several glacial and interglacial cycles of the Pleistocene . The depth of the permafrost can be as much as 1 493 m in the northern Lena and Yana River basins. In central part it is about 300-500 m. Overlying permafrost is a thin active layer that seasonally thaws during the summer. Protozoan life is supported only within the active layer since growth can occur only in fully thawed water for some parts of the year. The thickness of the active layer varies by year and location, but is typically 0.6-3 m. In Yakutia there are vast numbers of lakes and rivers of which the Lena river is the biggest (4310 km), and has hundreds of tributaries.

Fig. 1. Territory of Yakutia marked in red within Russia.

Fig. 2. Sampling points situated along the Lena River in Yakutia, Russia.

Material and Methods

Material

Paramecia were collected in 2013 by M. Rautian and A. Beliavskaia from several water pools in four regions belonging to the Lena basin: Oleokminsk (60°22`46``N/120°24`55``E), which is in the taiga zone, has discontinuous permafrost, Yakutsk (61°40`42``N/129°21`27``E), Sangar (63°55`24``N/127°28`18``E) and Zhigansk (66°7`N/123°3`E; see maps – Figs 1, 2) are in the area of continuous permafrost. Zhigansk is situated beyond the North Polar Circle. In each region 30-60 water samples (50 ml) were taken from different ponds, creeks and river backwaters. The distance between sampling points varied from dozens of meters up to dozens of kilometers. Samples were analyzed for *Paramecium* species presence.

Water samples were analyzed by light microscope. 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.

Paramecia were analyzed on slides stained using aceto-carmine (SONNEBORN 1970) and by DIC microscopy; identification to the particular species of *Paramecium* genus was made according to cell size and morphology, the type and number of micronuclei, morphology of contractile vacuoles and number of CV pores (VIVIER 1974; FOKIN 2010/2011). *Paramecium* species identified in the samples are in Table 1. All paramecia from the *P. aurelia* complex have a nuclear apparatus with two vesicular

Table 1

Paramecium species detected in collected samples

Paramecium species	Collection region	Number of samples with particular species
P. caudatum	Olekminsk	3
	Yakutsk	11
	Sangar	3
P. aurelia complex	Olekminsk	1
	Yakutsk	10
	Sangar	2
P. multimicronucleatum	Yakutsk	1
P. nephridiatum	Olekminsk	1
	Yakutsk	1
P. putrinum	Yakutsk	6
P. bursaria	Olekminsk	3
	Yakutsk	6
	Sangar	1

Table 2a

New stands of species of the *Paramecium aurelia* complex in Yakutia, Russia

Strain designation	Species	Collection place	Coordinates
Ya131-14	*P. primaurelia*	Sangar settlement on the Lena River, small pond	63°55`24``N/ 127°28`18``E
Ya131-13	*P. primaurelia*		
Ya131-16	*P. primaurelia*		
Ya88-7	*P. primaurelia*	Yakutsk city, bayou	62°00`81``N/ 129°43`42``E
111-1	*P. biaurelia*	Yakutsk vicinity, pond	62°00`24``N/ 129°39`28``E
13-2	*P. primaurelia*	Yakutsk city, Pond "Warm"	62°01`N 129°43`E
11-5	*P. novaurelia*		
89-1	*P. novaurelia*	Yakutsk city, ditch	62°00N` 129°42`74``E
91-2	*P. primaurelia*	Yakutsk city, Ditch, dry grass	62°00`69``N 129°42`74``E
189-5	*P. primaurelia*	Yakutsk city, Pond "Warm "	62°01`22``N 129°43`13``E
179-6	*P. primaurelia*	Yakutsk ZOO Pond with water birds	61°40`72``N 129°21272E
100-1	*P. biaurelia*	Way to Pokroskoye, Lena River Bay	61°54`17``N 129°34`22``E
108-20	*P. biaurelia*	Sangar settlement on the Lena River, small pond	61°51`46``N 129°33`25``E
58-21	*P. primaurelia*	Olekminsk town, ditch	60°22`46``N 120°24`55``E
53-20	*P. primaurelia*	Olekminsk town, ditch	60°22`18``N 120°26`21``E
58- 3	*P. primaurelia*	Olekminsk town, pool	60°22`46``N 120°24`55``E

Table 2b

Paramecium aurelia stands in the Far East and China

Strain designation	Species	Collection place	Coordinates
192-2	*P. tetraurelia*	Far East, Lake Levadiyskoye	42°86`13``N 132°66`43``E
CHH7-7	*P. primaurelia*	China, Zhejiang, Hangzhou, West Lake	30°25`63``N 120°15`82``E

micronuclei. The studied strains of the *P. aurelia* complex are presented in Table 2a,b.

Clone cultures 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 mμ/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 Sydney, Australia , *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 strains of the Institute of Systematics and Evolution of Animals, Polish Academy of Sciences, Kraków, Poland.

Table 3a

Survival of hybrids in the inter-strain crosses, strains from Yakutia x standard strains of *P. aurelia* spp.

Species	Strain designation	F1 (obtained by conjugation)	F2 (obtained by autogamy)
Paramecium primaurelia	131-16 (Sangar, Yakutia) x 90/ (Pennsylvania, USA)	95	98
Paramecium biaurelia	111-1 (Yakutsk, Yakutia) x Rieff Scotland, GB/2	94	93
Paramecium novaurelia	11-5 (Yakutsk, Yakutia) x 205/9 (Edinburgh, Scotland, GB)	56	52

Table 3b

Survival of hybrids in inter-strain crosses , strains from Far East and China

Species	Strain designation	F1	F2
Paramecium tetraurelia	192-2 (Far East, Russia) x Sydney/4	88	80
Paramecium primaurelia	CHH7-7 (China,Hangzhou) x 90/1 (Pennsylvania, USA)	98	96

In the intra and inter-strain crosses, the F_1 generation was obtained by conjugation and F_2 by autogamy (using the method of daily isolation lines) (Table 3a, b). 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.

Results and Discussion

In three regions, namely, Olekminsk, Yakutsk and Sangar different *Paramecium* species were detected. In some samples there were many *Paramecium* cells, often different species in the same sample (Table 1).

In the Zhigansk region 36 water samples were collected, but none of them contained paramecia. The reason for this large difference between this region and others is unclear. It was the most northern region of the expedition, but other peculiarities (microelements, etc.) of the region cannot be rejected.

As a result of identification of sibling species belonging to the *P.aurelia* species complex, new stands of *P. primaurelia*, *P. biaurelia*, and *P. novaurelia were* recorded in Yakutia, Russia (Table 2a). All strains from Yakutia identified as particular species (*P. primaurelia*, *P. biaurelia*, and *P. novaurelia*) of the *P. aurelia* complex reacted (conjugated) with each other (within particular species).

P. primaurelia was also recorded in China, and *P. tetraurelia* in a stand in the Far East, Lake Levadiyskoye (Table 2b). The samples of water with plankton were collected by M. Rautian in 2013.

The species *P. primaurelia, P. biaurelia, and P. tetraurelia* are cosmopolitan species (SONNEBORN 1975).

Survival of inter-strain hybrids of strains from Yakutia (the studied strain crossed with the standard of the particular species) is presented in Table 3a and hybrids of strains from China and the Russian Far East in Table 3b. In *P. primaurelia*, *P. biaurelia* and *P. tetraurelia* the survival of strain crosses was high. A lower degree of F2 viability was observed in *P. novaurelia* hybrids in crosses of strains Ya11-5 (Yakutia, Russia) x 205 (Scotland, GB) originating from remote habitats. This may reflect the high genetic divergence between the studied strain (from Yakutia) and standard crossing partner. *P. novaurelia* is common in Europe but was never found in Asia. Low survival of progeny was previously established in the case of strong geographic isolation, i.e. for rare species, *P. tredecaurelia* and *P. sonneborni* (PRZYBOŚ *et al.* 2013, 2014). It is also important that *P. novaurelia* was considered as a species limited in occurrence to Europe (SONNEBORN 1975), at present it was found in stands remote from Europe. This species may have been transported by humans, as it was recorded in the town of Yakutsk, which is a commercial centre.

Acknowledgements

All newly identified strains of the *P. aurelia* species complex belong to the Culture Collection of Ciliates and their Symbionts (CCCS) of St. Pe-

tersburg 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.

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.

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.

FOISSNER W. 2006. Biogeography and dispersal of microorganisms: a review emphasizing Protists. Acta Protozool. **45**: 11-36.

FOISSNER W., CHAO A., KATZ L.A. 2008. Diversity and geographic distribution of ciliates (Protista: Ciliophora). Biodivers. Conserv. **17**: 345-363.

FOKIN S.I. 2010/2011. *Paramecium* genus: biodiversity, some morphological features and the key to the main morphospecies discrimination. Protistology **6**: 227-235.

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

PRZYBOŚ E., SURMACZ M. 2010. New, world-wide data on the distribution of species of the *Paramecium aurelia* complex (Ciliophora, Protozoa). Folia Biol. (Kraków) **58**: 185-188.

PRZYBOŚ E., TARCZ S., DUSI E. 2013a. New *Paramecium quadecaurelia* strains (*P. aurelia* spp. complex, Ciliophora) identified by molecular markers (rDNA and mtDNA). Eur. J. Protistol. **49**: 477-486.

PRZYBOŚ E., TARCZ S., RAUTIAN M., LEBEDEVA N. 2014. The first European stand of *Paramecium sonneborni* (*P. aurelia* complex), a species known only from North America (Texas, USA). Europ. J. Protistology **50**: 236-247.

PRZYBOŚ E., TARCZ S., SURMACZ M., SAWKA N., FOKIN S.I. 2013b. *Paramecium tredecaurelia*, unique non-polymorphic species among the *P. aurelia* spp. complex (Oligohymenophorea, Ciliophora). Acta Protozool. **52**: 257-266.

SONNEBORN T.M. 1950. Methods In the general biology and genetics of *Paramecium*. J. Exp. Zool. **113**: 87-148.

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.

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