

Species of the *Paramecium aurelia* complex in Russia, Lower Volga Basin

Ewa Przyboś¹, Maria Rautian² and Alexey Potekhin²

¹ Department of Experimental Zoology, Institute of Systematics and Evolution of Animals, Polish Academy of Sciences, Kraków, Poland

² Laboratory of Protozoan Karyology, Biological Research Institute, St. Petersburg State University, Saint Petersburg, Russia

Summary

The Lower Volga Basin is very rich in species of the *Paramecium aurelia* complex. The presence of the following species has been revealed there: *P. primaurelia*, *P. biaurelia*, *P. triaurelia*, *P. pentaurelia*, *P. sexaurelia*, *P. septaurelia*, and *P. novaurelia*. *P. septaurelia* was recorded in the Volga Basin for the first time in Europe (Przybos et al., 2004); *P. pentaurelia* and *P. sexaurelia* are rare in Europe. In some sampling places several (up to four in one sample) species can occur in one and the same population*. Special attention is devoted to the frequent occurrence of *P. septaurelia* in the region of investigation. The idea of Schewiakoff (1893) about the role of birds' and other animals' migrations in dispersal of ciliates is discussed in the context of the data obtained.

Key words: *Paramecium aurelia* species complex, distribution of species, species competition, species expansion

Introduction

Among 15 species of the *Paramecium aurelia* complex known world-wide (Sonneborn, 1975; Aufderheide et al., 1983), the following have been found in Europe: *P. primaurelia*, *P. biaurelia*, *P. triaurelia*, *P. tetraurelia*, *P. pentaurelia*, *P. sexaurelia*, *P. novaurelia*, and *P. tredecaurelia* (cf. Sonneborn, 1975; Przyboś, 1993; Przyboś and Fokin, 2000). Recently, the presence of *P. dodecaurelia* (Przyboś and Fokin, 2003) and *P. septaurelia* (Przyboś et al., 2004) has also been revealed in Europe. *P. primaurelia*, *P. biaurelia*, and *P. novaurelia* are common in Europe. The occurrence of some species, such as *P.*

triaurelia, *P. tetraurelia*, *P. pentaurelia*, and *P. sexaurelia* seems to be confined to certain environments, and in the case of *P. tredecaurelia*, *P. dodecaurelia*, and *P. septaurelia*, even to habitats (Przyboś, 2005).

In the European part of Russia the following species have been recorded: *P. primaurelia*, *P. biaurelia*, *P. novaurelia* in Moscow, *P. primaurelia* with *P. novaurelia* in St. Petersburg or its vicinity (Komala and Dubis, 1966), and *P. biaurelia* in Stary Peterhof, St. Petersburg environ (Przyboś and Fokin, 1996). *P. triaurelia* has

* Editor's note: Responsibility for the use of the term "population" in this article lies entirely with the authors.

been recorded in the Volga River (Astrakhan Nature Reserve) together with *P. novaurelia* (cf. Kościuszko, 1985), and *P. pentaurelia* in the Belgorod region (Fokin and Ossipov, 1986). Our previous paper (Przyboś et al., 2004) was concerned with the occurrence of species of the *P. aurelia* complex in the Lower Volga Basin, i.e. Astrakhan Nature Reserve and Natural Reserve Complex Volga-Akhtuba flood lands, Volgograd region. The region is very interesting from biological point of view and, as was demonstrated in the previous paper (Przyboś et al., 2004), is also very rich in species of the *P. aurelia* complex. The presence of the following species has been revealed there: *P. primaurelia*, *P. biaurelia*, *P. triaurelia*, *P. pentaurelia*, *P. sexaurelia*, *P. septaurelia*, and *P. novaurelia*. *P. septaurelia* had been known before only from the territory of the USA and was recorded in Europe for the first time (Przyboś et al., 2004); *P. pentaurelia* and *P. sexaurelia* are rare in Europe.

The present paper is a continuation of studies of the *P. aurelia* species complex in the Lower Volga Basin.

Material and Methods

Water samples (15–40 ml each) were collected in typical habitats of *Paramecium* (Table 1). The same day paramecia were isolated from the whole sample volume, and clones were established. Samples (different bottles) often were collected at a very short distance from each other (from bow to stern of a small stationary boat). In these cases paramecia from different samples were considered as the same population. The strain index includes letters (AZ for Astrakhan Nature Reserve or V for Natural Reserve Complex Volga-Akhtuba) and numbers, the first number representing the population and the second one (after the dash), the isolated paramecium cell. E.g., AZ11-13 means strain number 13 collected in Astrakhan Nature Reserve, population 11.

The strains designated AZ were collected in Astrakhan Nature Reserve (Fig. 1) near Damchik (45°83' N/ 47°85' E) and at the Caspian coast (45°7' N/ 47°9' E).

The strains designated V were collected in the Natural Reserve complex Volga-Akhtuba flood lands, Volgograd region (approximately 48.7 N/ 44.7 E; Fig.1).

Paramecia cultivation and identification were performed according to Sonneborn (1970). The paramecia were cultivated on a lettuce medium inoculated with *Enterobacter aerogenes*. The species of the *P. aurelia* complex were identified by mating the strains under investigation with mating types of standard strains of the particular species. The following standard strains were used:

- P. primaurelia*, strain 90,
- P. biaurelia*, strain Rieff, Scotland,
- P. triaurelia*, strain 324,

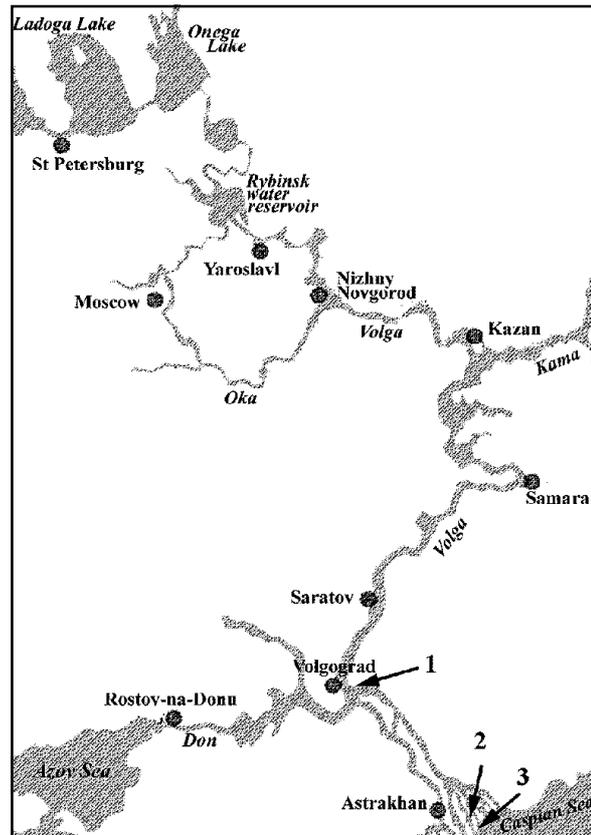


Fig. 1. Map of the Volga Basin. The sites of sampling are marked by arrows. 1 - Natural Reserve Complex Volga-Akhtuba flood lands; 2 - Astrakhan Nature Reserve, Damchik; 3 - Astrakhan Nature Reserve, Caspian coast.

- P. tetraurelia*, strain from Sydney, Australia
- P. pentaurelia*, strain 87,
- P. sexaurelia*, strain 159,
- P. septaurelia*, strain 38,
- P. octaurelia*, strain 138.

Other species of the *Paramecium* genus and other ciliates present in the water samples were also identified (Table 1).

Results and Discussion

The presence of several species of the *P. aurelia* complex was revealed in the regions studied (Table 1).

In the Volgograd region *P. primaurelia*, *P. biaurelia*, *P. triaurelia*, *P. pentaurelia*, *P. septaurelia*, and *P. novaurelia* were recorded. Namely: *P. primaurelia* - strain V7-13; *P. biaurelia* - strain V1-5; *P. triaurelia* - strain V10-12; *P. pentaurelia* - strains V2-1, V2-4, V2-7, V2-10, *P. septaurelia* - strain V5-13, and *P. novaurelia* - strain V9-6.

As a results of studies presented in the previous paper (Przyboś et al., 2004), the following species were

Table 1. Occurrence of species of the *Paramecium aurelia* complex and characteristics of the habitats examined.

Strain index	Species of the <i>P. aurelia</i> complex	Characteristics of the habitat	Main other ciliates present in the sample		
AZ2-2 AZ2-10 AZ2-14	<i>P. septaurelia</i>	Small permanent pond	<i>P. caudatum</i>		
AZ4-2 AZ4-4	<i>P. septaurelia</i>	Small river duct	<i>P. bursaria</i>		
AZ5-1 AZ5-2 AZ5-3	<i>P. sexaurelia</i> <i>P. septaurelia</i>	River duct	<i>Stentor sp.</i> , <i>Stylonychia sp.</i>		
AZ6-25 AZ6-26	<i>P. pentaurelia</i>	River Bystraya, Lotus field	<i>P. caudatum</i>		
AZ8-11 AZ8-6 AZ8-4 AZ8-21	<i>P. primaurelia</i> <i>P. pentaurelia</i> <i>P. sexaurelia</i>	Mouth of river Bystraya, lotus field	<i>P. bursaria</i>		
AZ8-3 AZ8-8 AZ8-10 AZ8-16	<i>P. septaurelia</i>				
AZ9-2	<i>P. pentaurelia</i>			Coast, rotting grass	<i>P. caudatum</i> , <i>P. bursaria</i>
AZ12-17 AZ12-20 AZ12-22	<i>P. primaurelia</i> <i>P. sexaurelia</i>			Coast, slime	<i>P. bursaria</i>
AZ13-2 AZ13-4 AZ13-6	<i>P. pentaurelia</i>	Coast, bay of a small island, duckweed grass	<i>P. caudatum</i> , <i>P. bursaria</i> , <i>Stylonychia sp.</i> , <i>Tetrahymena sp.</i>		
AZ15-12 AZ15-13 AZ15-17 AZ15-19 AZ15-20 AZ15-22	<i>P. primaurelia</i>	Coast, near an island	<i>P. caudatum</i>		
AZ17-13 AZ17-14	<i>P. pentaurelia</i>	Outfall of a duct, water chestnut and other water plants	<i>P. caudatum</i> , <i>P. bursaria</i>		
AZ19-4	<i>P. septaurelia</i>	Canal Beshenyi	<i>Stentor sp.</i> , <i>Euplotes sp.</i>		
AZ20-6	<i>P. septaurelia</i>	Canal Beshenyi	<i>P. caudatum</i> , <i>P. bursaria</i>		
AZ21-1 AZ21-4	<i>P. septaurelia</i>	Canal Beshenyi, lotus field	<i>P. caudatum</i> , <i>P. bursaria</i> , <i>Stylonychia sp.</i>		
AZ22-1 AZ22-2 AZ22-3	<i>P. septaurelia</i>	Canal Beshenyi, algae	<i>P. caudatum</i>		
AZ24-4	<i>P. septaurelia</i>	Lotus field	<i>Stylonychia sp.</i>		
AZ25-2	<i>P. biaurelia</i>	Backwater, water plants	<i>Stentor sp.</i>		
V1-5	<i>P. biaurelia</i>	Canal Verblud, near the dam	<i>P. caudatum</i> , <i>P. bursaria</i>		
V2-1 V2-4 V2-7 V2-10	<i>P. pentaurelia</i>	Small separated pool	<i>Stylonychia sp.</i> <i>Dileptus sp.</i> , <i>Stentor sp.</i>		
V5-13	<i>P. septaurelia</i>	Small river	<i>P. caudatum</i>		
V7-13	<i>P. primaurelia</i>	Piscicultural pond	<i>P. caudatum</i>		
V9-6	<i>P. novaurelia</i>	Pond	<i>P. caudatum</i> , <i>P. bursaria</i> , <i>Stentor sp.</i> , <i>Didinium sp.</i> , <i>Spirostomum sp.</i>		
V10-12	<i>P. triaurelia</i>	Lake, reeds	<i>P. caudatum</i> , <i>Dileptus sp.</i>		

recorded in the samples mentioned: *P. primaurelia* (strain V7-6) together with *P. triaurelia* (strains V7-8, V7-11) in population V7; *P. biaurelia* (strains V1-3, V1-4); *P. triaurelia* (strains V10-6, V10-7 and V3-1), and *P. novaurelia* (strain V9-1).

In the Astrakhan Nature Reserve the presence of five species of the *P. aurelia* complex, namely *P. primaurelia*,

P. biaurelia, *P. pentaurelia*, *P. sexaurelia*, and *P. septaurelia* was recorded. *P. septaurelia* was recorded for the first time in Europe (Przyboś et al., 2004), it had been known before (Sonneborn, 1975) only from the territory of the USA. *P. pentaurelia* and *P. sexaurelia* are rare in Europe. Only *P. primaurelia* and *P. biaurelia* are common in Europe (Przyboś and Fokin, 2000) and even cosmo-

politan (Sonneborn, 1975). The aquatic environment at the sites explored in the Astrakhan Nature Reserve seems to be eutrophic, which explains the occurrence of different species at a small distance. It is amazing that in some populations (e.g., AZ8) four species occur: *P. primaurelia*, *P. pentaurelia*, *P. sexaurelia*, and *P. septaurelia*. The latter is the most common in the territory studied and is known in Europe from that place only.

P. primaurelia (strain AZ8-11), together with *P. pentaurelia* (strain AZ8-6), *P. sexaurelia* (strains AZ8-4, AZ8-21), and *P. septaurelia* (strains AZ8-3, AZ8-8, AZ8-10, AZ8-16) were recorded in the same population AZ8. Previously (Przyboś et al., 2004) *P. septaurelia* (strain AZ8-20) was also recorded in that population. *P. primaurelia* was also recorded in population AZ12 (strains AZ12-17, AZ12-20), as in the previous paper (strain AZ12-19), and in population AZ15 (strains AZ15-12, AZ15-13, AZ15-17, AZ15-19, AZ15-20, AZ15-22), as previously (strains AZ15-3, AZ15-8).

P. biaurelia (strain AZ25-2) was recorded in only one sample. It is interesting that this species common in Europe and cosmopolitan was so rare in the region studied.

P. pentaurelia (strains AZ6-25, AZ6-26) was recorded in population AZ6 (previously - i.e. in Przyboś et al., 2004 - strain AZ6-24), in population AZ9 (strain AZ9-2, and, previously, - strain AZ9-4). Strains AZ13-2, AZ13-4, AZ13-6 were identified as *P. pentaurelia* in population AZ13 (previously - the strain AZ13-3), and strains AZ17-13, AZ17-14 - in population AZ17 (previously - the strains AZ17-19, AZ17-38).

P. sexaurelia (strain AZ5-1) was identified in the same population together with *P. septaurelia* (strains AZ5-2, AZ5-3), in population AZ12 (strain AZ12-22) as well as *P. primaurelia*, and, as mentioned above, in population AZ8 (the strains AZ8-4, AZ8-21). In the previous study (Przyboś et al., 2004), *P. sexaurelia* was found in two populations (strains AZ9-6, AZ11-28, AZ11-14, AZ11-25, AZ11-26).

P. septaurelia, new to Europe, was identified in several samples representing nine natural populations: strains AZ2-2, AZ2-10, AZ2-14, AZ4-2, AZ4-4, AZ5-2, AZ5-3, AZ8-3, AZ8-8, AZ8-10, AZ8-16, AZ19-4, AZ20-6, AZ21-1, AZ21-4, AZ22-1, AZ22-2, AZ22-3, AZ24-4; in the previous studies it was recorded also in populations AZ3 and AZ6 (Przyboś et al., 2004).

The regions studied are very rich in species of the *P. aurelia* complex and worthy of future investigation. It is important to note that in total we have taken 191 water sample, and in 57 of them different *Paramecium* sp. (*P. aurelia* complex, *P. bursaria*, *P. caudatum*) were recorded; representatives of *P. aurelia* complex were found in 31 samples. In the present and previous (Przyboś et al., 2004) studies *P. primaurelia* was found in five AZ populations, and one V population; *P. biaurelia*

appeared in one AZ population and one V population; *P. triaurelia* was found in three V population; *P. pentaurelia* was recorded in five AZ populations, and in one V population; *P. sexaurelia* was identified in five AZ populations, and *P. septaurelia* was the most common species on the territory studied occurring in eleven AZ populations and in one V population.

It is also of interest to analyze the distribution of *P. aurelia* species in different biotops in the Astrakhan Nature Reserve (Table 2). We took samples in three different kinds of environment: small separate ponds; rivers and streams with more or less intensively flowing water; Caspian coast, very eutrophic and warm, without strong water circulation. Though there are not enough data to perform statistical analysis, it seems that *P. septaurelia* prefers habitats with water circulation (streams with more or less rapid current, lotus fields), and is almost not recorded at the coast. *P. primaurelia* was recorded only in the warm non-circulating coastal waters colonized by water plants and algae, and *P. pentaurelia* and *P. sexaurelia* were also preferentially found at the Caspian coast. It is worth to note that the only *P. septaurelia* found in Volgograd region was also collected from the small river with a rather strong water flow.

The Lower Volga Basin may be characterized by particular ecological features making possible the occurrence of species rare or unknown (*P. septaurelia*) elsewhere in Europe. The delta of the Volga River is characterized by very continental climate, which means long and hot summer, and rather cold winter. Temperature seems to be one of the most important factors limiting the distribution of species of the *P. aurelia* complex. Sonneborn (1957) associated the occurrence of particular species with the climatic zones which can form the temperature barriers. The range of some species in Europe confirms this conclusion (Przyboś, 2005). For instance, *P. pentaurelia* has been recorded in the warm zone (Hungary, Romania, and Spain), similarly *P. sexaurelia* (Croatia, Greece, Spain, Southern Germany) and in the Lower Volga Basin, presently. Warm climate stimulates the richness of aquatic flora and fauna; the aquatic environment in the Volga delta is especially eutrophic due to the great amount of waterfowl migrating, moulting and nesting there (Isakov and Krivonosov, 1969).

In Europe (Przyboś, 2005), *P. novaurelia* dominates over other species, followed by *P. biaurelia* and *P. primaurelia*. Several species (*P. tetraurelia* and *P. triaurelia*) are rather rare or very rare (*P. pentaurelia* and *P. sexaurelia*). Other species are known from single localities: *P. dodecaurelia* has been recorded in Italy, Elba Island and Germany, Munster, only; *P. septaurelia* in the Lower Volga basin, and *P. tredecaurelia* in France, Paris. It is interesting that *P. tetraurelia* was not recorded

Table 2. Distribution of *Paramecium aurelia* species in different biotops of the Astrakhan Nature Reserve (data from this paper and Przyboś et al., 2004).

Paramecium species	Number of populations in which certain species was recorded			
	Separate pools	Canals and streams	Caspian sea coast	In total
<i>P. primaurelia</i>	-	-	5	5
<i>P. biaurelia</i>	-	1	-	1
<i>P. pentaurelia</i>	-	1	4	5
<i>P. sexaurelia</i>	-	1	4	5
<i>P. septaurelia</i>	2	9	1	12

in the Lower Volga Basin at all, and neither *P. novaurelia*, nor *P. triaurelia* were recorded in the Astrakhan Nature Reserve but only in several populations in Volga-Akhtuba flood lands. Instead of these species, rather common in Europe, other species were dominant in the region studied: *P. septaurelia*, *P. pentaurelia*, and *P. sexaurelia*; only *P. primaurelia* confirmed the status of Europe-universal species. It is unclear how North America-limited species (*P. septaurelia*) could appear in the Lower Volga Basin. Delta of the Volga River is situated on the border between Europe and Asia. In Asia *P. septaurelia* is unknown as well, but, on the other hand, Asia (at least its western and central parts) is almost uninvestigated by ciliatologists, so it could be that European *P. septaurelia* comes from Asia.

The spreading of paramecia is still an unsolved problem. According to the literature, cysts are unknown in *Paramecium* (Landis, 1988; Gutierrez et al., 1998). One possibility of paramecium dispersal could be transfer by insects - for example, water beetles from the family Dytiscidae are characterized by natatory legs with hairs (Razowski, 1996), which could serve as a carrier. The animals may be transferred over long distances with some drops of water by birds. It is well known that birds can serve as transcontinental carriers of viruses, bacteria and some parasitic protozoa (L'vov and Ilyichev, 1979). In fact, the idea that ciliates may be transferred by different migrating animals was first suggested by the Russian protozoologist W.T. Schewiakoff (1893).

It is possible that *P. septaurelia* was transferred to the Volga delta by birds as the Lower Volga Basin is on the way of waterfowl mass migration. There are no direct bird migration routes from North America to Europe, but the complex routes with "transfer points" can exist. One of the possible complex routes connecting Europe and North America could pass via Western and Northern Africa. Some birds (arctic tern) migrate from America to Africa, and there is a mighty bird migration way across Africa to Eurasia (Northern Europe, Central Russia, Siberia), which passes via Northern Caspian coast and Lower Volga basin. Several dozen of waterfowl species (mainly from the orders Anseriformes (goose and ducks), Pelecaniformes (pelicans), and Ciconiiformes (herons) migrate by this route or, at least, parts of it (Mikheev,

1981). It would be of great interest to check this "bird migration - ciliate spreading" hypothesis by investigating the distribution of species of *P. aurelia* complex along this route - in Western and Northern (Nile flood lands) Africa, Arabia and Western Siberia.

ACKNOWLEDGEMENTS

The authors are greatly indebted to Alexander K. Gorbunov, vice-director of the Astrakhan Nature Reserve, for making collection of material possible and for facilitating work in the Astrakhan Nature Reserve. The work was supported by RFBR (01-04-49386, 02-04-63105, 04-04-48954 to M.R.), and by St. Petersburg grant for postdocs PD04-1.4-254 to A.P.

References

Aufderheide K.J., Daggett P.-M. and Nerad T.A. 1983. *Paramecium sonneborni* n. sp., a new member of the *Paramecium aurelia* species complex. J. Protozool. 30, 128-131.

Fokin S.I. and Ossipov D.V. 1986. *Pseudocaedibacter glomeratus* sp. n. - the symbiont of the cytoplasm of *Paramecium pentaurelia*. Tsitologiya. 28, 1000-1004 (in Russian with English summary).

Gutierrez J.C., Martin-Gonzales A. and Callejas S. 1988. Nuclear changes, macronuclear chromatin reorganization and DNA modifications during ciliate encystment. Europ. J. Protistol. 34, 97-103.

Isakov Yu.A. and Krivonosov G.A. 1969. Waterfowl migration and moult in the Volga delta. Proc. of Astrakhan State Nature Reserve. 12, 1-187 (in Russian).

Komala Z. and Dubis K. 1966. Syngens of *Paramecium aurelia* in some regions of Moscow and Leningrad. Folia biol. (Krakow). 14, 227-228.

Kosciuszko H. 1985. Species of the *Paramecium aurelia* complex in some regions of the USSR. Folia biol. (Krakow). 33, 117-122.

Landis W.G. 1988. Ecology. In: *Paramecium* (Ed. H.-D. Görtz). Springer-Verlag, Berlin. pp. 419-436.

L'vov D.K. and Ilyichev V.D. 1979. Birds migration and transfer of infectious organisms. Nauka, Moscow (in Russian).

- Mikheev A.V. 1981. Migration of birds. Lesnaya promyshlennost', Moscow (in Russian).
- Przyboś E. 1993. Species of the *Paramecium aurelia* complex in Spain. *Microbiologia SEM*. 9, 113-117.
- Przyboś E. 2005. Recent data on the occurrence of species of the *Paramecium aurelia* in Europe. *Folia biol.* (Krakow). (in press).
- Przyboś E. and Fokin S.I. 1996. New habitats of species of the *Paramecium aurelia* complex in Russia and Vietnam. *Folia biol.* (Krakow). 44, 105-106.
- Przyboś E. and Fokin S.I. 2000. Data on the occurrence of species of the *Paramecium aurelia* complex world-wide. *Protistology*. 1, 179-184.
- Przyboś E. and Fokin S.I. 2003. Habitats of *Paramecium dodecaurelia* in Europe. *Protistology*. 3, 136-137.
- Przyboś E., Rautian M. and Potekhin A. 2004. First European record of *Paramecium septaurelia* and the discovery of new European habitats of *P. pentaurelia* and *P. sexaurelia* in Russia (Astrakhan and Volgograd Region). *Folia biol.* (Krakow). 52, 1-4.
- Razowski J. 1996. Dictionary of Insect Morphology. PWN, Warszawa, Krakow. (In Polish).
- Schewiakoff W.T. 1893. Über die Geographische Verbreitung der Süßwasser-Protozoen. *Mem. Acad. Imper. Sci. de St. Petersburg*, VII ser., 41, 1-201.
- Sonneborn T.M. 1957. Breeding systems, reproductive methods, and species problem in Protozoa. In: *Species Problem* (Ed. E. Mayr). AAAS, Washington D.C. pp. 155-324.
- Sonneborn T.M. 1970. Methods in *Paramecium* research. In: *Methods in Cell Physiology*, vol. 4 (Ed. D.M. Prescott). Academic Press, New York, London. pp. 241-339.
- Sonneborn T.M. 1975. The *Paramecium aurelia* complex of fourteen sibling species. *Trans. Amer. Micros. Soc.* 94, 155-178.

Address for correspondence: Maria Rautian. Laboratory of Protozoan Karyology, Biological Research Institute, St. Petersburg State University, Oranienbaumskoye sh. 2, 198504 Saint Petersburg, Russia. E-mail: mrautian@mail.ru

Editorial responsibility: Sergei Fokin