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New Stands of Species of the *Paramecium aurelia* Complex; is the Occurrence of Particular Species Limited by Temperature Barriers?

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The occurrence of ciliates, especially the *Paramecium aurelia* complex, has not yet been studied in many parts of the world, or sampling was done only occasionally. Generally, the southern hemisphere still awaits investigation. In North America only the USA was studied in greater detail; the majority of species of the complex were there recorded. In Asia, more frequent sampling was performed only in Japan and Asiatic Russia. Europe was studied more carefully, however, a different number of habitats was studied in particular zones of Europe, the least in the southern zone. New stands of *P. tetraurelia* , *P. sexaurelia*, *P. octaurelia*, and *P. novaurelia* were revealed as a result of the present investigations carried out in Africa (Mozambique – *P. tetraurelia*, *P. sexaurelia*), Asia (Indonesia – *P. sexaurelia*), borderland of Asia and Europe (Georgia – *P. octaurelia*), and Europe (Macedonia – *P. tetraurelia* and Romania – *P. novaurelia*). Are climatic zones the main factor limiting the occurrence of species of the *P. aurelia* complex? Analysis of data on the distribution of the *P. aurelia* species complex in warm "tropical" zones on different continents may suggest such preferences for some species, including *P. sexaurelia*, *P. octaurelia*, *P. tredecaurelia*, *P. quadecaurelia*. The first two of these species were recorded herein in warm or "tropical" zone.

Key words: Ciliates, *Paramecium aurelia* species complex, biogeography, climatic zones.

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Some protists, and among them ciliates, have a complicated species structure because morphological species are composed of sibling species, genetically isolated from each other (SONNEBORN 1975; NANNEY & MCCOY 1976; PRZYBOŚ 1986; FENCHEL 2005; GENTAKAKI & LYNN 2012; FERNANDES & DA SILVA NETO 2013). Sibling species can be recognized by mating reactions in the case of sexually reproducing species and/ or by molecular investigations or only by the latter in the case of asexual species (SONNEBORN 1937, 1957, 1974, 1975; STRŰDER-KYPKE & LYNN 2010; KHER *et al.* 2011; CARON 2013; STOECK *et al*. 2014). In this respect, one of the best studied ciliate genera is *Paramecium* (BARTH *et al.* 2008), and within it the *Paramecium aurelia* species complex (Protista, Oligohymenophorea), (PRZYBOŚ *et al*. 2007, 2010; CATANIA *et al* 2009; TARCZ *et al*. 2013). The *P. aurelia* complex is composed of 15 species known world-wide (SONNEBORN 1975; AUFDERHEIDE *et al*. 1983), some are cosmopolitan, others were recorded only in a few or single habitats. The occurrence of ciliates, and especially the *Paramecium aurelia* complex, has not yet been studied in many parts of the world, or sampling was done only occasionally (PRZYBOŚ & FOKIN 2000; PRZYBOŚ & SURMACZ 2010; FOKIN 2010/ 2011). Generally, the southern hemisphere is in need of investigation. Central and South America, Australia, and some parts of Asia were studied in a very limited extent, at random. In North America, only the USA was studied in greater detail (SONNEBORN 1975), the majority of species (12 among 15 known species) of the complex were recorded, only *P. novaurelia*, *P. tredecaurelia* and *P. quadecaurelia* were found on other continents. Among species occurring in the USA, the following are known only from the southern states: *P. septaurelia*, *P. octaurelia*, *P. decaurelia*, P. *undecaurelia*, and *P. dodecaurelia* (SONNEBORN 1970). In Asia, more frequent sampling was done only in Japan and Asiatic Russia (PRZYBOŚ *et al*. 2013a). Europe was studied more extensively, the largest dataset on the distribution and frequency of occurrence of species of the *P. aurelia* complex concerns this continent (Przyboś & Surmacz 2010; Przyboś *et al*. 2014a). However, a different number of habitats was studied in particular zones of Europe, the fewest in the southern part of the continent. Under - sampling is the main factor limiting our understanding of the geographical distributions of protists (Foissner 2008). It is therefore important to investigate strains from southern Europe, as well as from parts of Asia and Africa sampled only at random, and where investigations are still very limited in number. This was the aim of the present paper.

Material and Methods

Materials

The studied strains are presented in Table 1.

Methods

Sonneborn's (1950, 1970) methods were used for cultivation, induction of conjugation, autogamy and strain crosses. 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.

Species of the *P. aurelia* complex were identified by mating reaction. 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, Great Britain, *P. biaurelia*;
strain 324, Florida, USA, *P. triaurelia*;
strain Sydney, Australia and strain 51, Indiana, USA, *P. tetraurelia*;
strain 87, Pennsylvania, USA, *P. pentaurelia*;
strain 159,Puerto Rico, *P. sexaurelia*;
strain 138, Florida, USA, *P. octaurelia*;
strain 205, Edinburgh, Scotland, UK, *P. novaurelia*;
strain 223, Florida, USA, *P. decaurelia*;
strain 248, Mississippi, USA, *P. dodecaurelia*.

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. In the intra- and inter-strain crosses, the F1 generation was obtained by conjugation and F2 by autogamy (using the method of daily isolation lines). 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 hybrids (F1 and F2 generations, Table 2) in inter-strain crosses (between the studied strains and standard strain of a particular species) was investigated and 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 post-autogamous caryonids.

Table 1

New stands of species of the *Paramecium aurelia* complex

Strain index	Place of collection	Coordinates of collection place	Kind of water body	Collector's name and date of collection	Species identified
Indo 1.7I	Indonesia, Sulawesi, Lake Kalimpa'a	1°1935S/120°1832E	small lake	S. Krenek, February 2008	*P. sexaurelia*
Indo 11.5I	Indonesia, Sulawesi, Lake Lindu	1°1957S/120°36E	big lake		*P. sexaurelia*
GBK1	Georgia, Batumi reg, Kobuleti	41°38 19N/ 41° 38 14E	stream	A. Kaczanowski, September 2014	*P. octaurelia*
Moz13BIII	Mozambique, Mafambiasse	19°3249S/34°3842E	small pond	S. Krenek, June 2010	*P. sexaurelia*
Moz 13CXI	Mozambique, Mafambiasse	19°3249S/34°3842E			*P. tetraurelia*
MO	Macedonia	41° 00N/ 20° 45 E	Lake Ohrid	E. Przyboś, June 2014	*P. tetraurelia*
RP	Romania, Petresti de Jos, Apuseni Mts, Cluj region	46°.5716N/23°666E	Meander of a river Hădane	T. Postawa, September 2013	*P. novaurelia*

Table 2

Survival of the *Paramecium aurelia* spp. interstrain hybrids

Species	Crossed strains: studied x standard of particular species	Percentage of surviving clones	
		F1	F2
P. tetraurelia	Mozambique (13 CXI) x 51/4 (Indiana, USA)	97	75
	Macedonia (MO) x S/4 (Sydney, Australia)	96	93
P. sexaurelia	Indonesia (Indo 1.71)) x 159/6 (Puerto Rico)	100	70
	Indonesia (Indo 11.5I) x 159/6	96	100
	Mozambique (13BIII) x 159/6	90	63
P. octaurelia	Georgia (GBK1) x 138/ 8 (Florida, USA)	100	90
P. novaurelia	Romania (RP) x 205 /9 (Edinburgh, Scotland, GB)	83	80

Results and Discussion

New stands of *P. tetraurelia, P. sexaurelia*, *P. octaurelia*, and *P. novaurelia* were revealed in in Africa (Mozambique), Asia (Indonesia), borderland of Asia and Europe (Georgia), and Europe (Macedonia and Romania), Table 1. 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. Lower survival (63%) was only observed in the F2 generation of *P. sexaurelia* hybrids, when strains from Mozambique (13BIII) and from Puerto Rico (159) were crossed. This may reflect high genetic divergence among these strains.

P. tetraurelia is a cosmopolitan species of the *P. aurelia* complex occurring in temperate and subtropical climatic zones, recorded in North, Central and South America, Asia, Australia and Europe (SONNEBORN 1975; PRZYBOŚ & SURMACZ 2010). Herein, the species was recorded in Mozambique, it is the first record of this species in Africa, however, investigations concerning the occurrence of species of the *P. aurelia* complex carried in Africa are very rare. This species was also recorded at present in Macedonia in Lake Ohrid. This is a very interesting stand, as the lake preserves a unique ecosystem with many endemic and relict forms. The lake originated from a geotectonic depression during the Pliocene epoch five million years ago, is very deep (maximal depth 288m), oligomictic type similar to alpine lakes but subtropical (STANKOVIC 1960).

P. sexaurela is also considered a cosmopolitan species and can be found in Africa, Asia, Europe and the Americas (SONNEBORN 1975; PRZYBOŚ & SURMACZ 2010). This study revealed its presence in Asia (Indonesia) and in Africa (Mozambique). Both countries were sampled the first time for *P. aurelia* species.

P. octaurelia was recorded at present in Georgia. This species was revealed already in tropical and subtropical Americas, Africa, and Asia (SONNEBORN 1975; PRZYBOŚ & SURMACZ 2010; RAUTIAN *et al.* 2014) and was also recorded in Europe (PRZYBOŚ *et al.* 2009a; PRZYBOŚ *et al.* 2014a; RAUTIAN *et al.* 2014). In Europe, it was found in Kaiserslautern in Germany, (PRZYBOŚ *et al.* 2009a), Vienna in Austria, Cheboksary in Russia (PRZYBOŚ *et al.* 2014a), Karlove Vary in the Czech Republic (RAUTIAN *et al.* 2014), however, it seems rather rare on this continent.

According to SONNEBORN (1975), *P. novaurelia* can only be found in Europe, and is quite frequent there (PRZYBOŚ & SURMACZ 2010), at present it was recorded in Romania in Petresti de Jos, Apuseni Mts , Cluj region. The species was also already recorded in this country near Cluj, Bucharest and Constanta (PRZYBOŚ 1968).

Are climatic zones the main factor limiting the occurrence of species of the *P. aurelia* complex? Recent records of new stands of *P. quadecaurelia* (PRZYBOŚ *et al.* 2013b) and *P. sonneborni* (PRZYBOŚ *et al.* 2014b) in warm climate zones confirms that the occurrence of the *P. aurelia* complex may be restricted by temperature barriers. Analysis of data collected in Table 3, concerning the distribution of the *P. aurelia* species complex in warm "tropical" zones on different continents, may suggest such preferences for some species such as *P. sexaurelia*, *P. octaurelia*, *P. tredecaurelia*, *P. quadecaurelia*. The first two mentioned species were recorded at present in warm or "tropical" zones (Table 1).

The characteristics of species should contain morphological, genetic, ecological and molecular features, as was postulated by SCHLEGEL & MEISTERFELD (2003), WEISSE (2008), CARON (2013), DUNTHORN *et al.* (2014) and others. Geographical distribution, ecological and temperature preferences may also be important. Large physiological and ecological diversity may be hidden at the morphological level.

Table 3

Species of the *Paramecium aurelia* complex in the warm regions of Central and Southern America, Africa, Australia, Asia

Continent/ Country	Species of the *P. aurelia* complex designated by numbers															References
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	P.s.	
Central America																
No details of occurrence	+			+		+										Sonneborn 1975
Panama								+								Sonneborn 1975
Mexico, (Cuernavaca Valley)													+			Rafalko & Sonneborn 1959
Southern America																
No details on occurrence	+	+		+												Sonneborn 1975
Brazil				+												Przyboś & Surmacz 2010
Ecuador	+						+							+		Przyboś *et al.* 2013b; Przyboś *et al.* 2014a
Africa																
Tenerife												+				Przyboś *et al.* 2008
Uganda								+								Sonneborn 1975
Kenya						+										Sonneborn 1975
Morocco	+		+													Przyboś & Rautian 2012
Namibia														+		Przyboś *et al.* 2003
Madagascar													+			Rafalko & Sonneborn 1959; Przyboś *et al.* 2013c
Mozambique				+		+										Present paper
Australia																
Australia		+		+	+									+		Sonneborn 1974, 1975; Przyboś 2008; Przyboś & Surmacz 2010
Asia																
Israel	+	+		+				+					+			Przyboś 1995; Przyboś *et al.* 2002
Lebanon		+														Sonneborn 1975
Georgia								+								Present paper
India	+			+	+											Sonneborn 1975; Przyboś *et al.* 2009b
Thailand						+							+	+		Przyboś *et al.* 2013 b,c
Indonesia						+										Present paper
Europe																
Macedonia				+												Present paper
Romania									+							Present paper
Cyprus															+	Przyboś *et al.* 2014 b

The occurrence and distribution of protists, and among them ciliates, is still a debated problem. There are two opinions: the model of ubiquity (Finlay & Fenchel 2004; Fenchel 2005), and the model of moderate endemicity (Foissner 2006; Foissner *et al.* 2008). According to Foissner (2008), one third of free-living protist species, among about 300 000 extant species, show a restricted distribution. Epstein and Lopez-Garcia (2008) also supposed that molecular, recent data also support the idea …"on protists as a world of diverse species with biogeographies".

Protist biogeography and diversity is similar to one characteristic for macroorganisms (Bass *et al.* 2007; Weisse 2008). According to Foissner *et al.* (2008) the distribution pattern of microorganisms is "similar to those known from higher plants and animals, with increased proportion of cosmopolites and these pattern reflect … split of Pangea and ecological conditions, … as Gondwanan and Laurasian ciliate communities". The supercontinent Pangea existed 250-160 million years ago from the end of the Paleozoic to the middle of the Mesozoic Era (WILEY & LIEBERMAN 2011). Some protists have a long evolutionary history (FOISSNER 2008). For example, the speciation of the *P. aurelia* complex took place about 320 million years ago (MCGRATH *et al.* 2014) at the recent whole-genome duplication. Species of the complex (as was supposed by AURY *et al.* 2006 based on *P. tetraurelia* studies) passed through successive three whole-genome duplications, followed by reciprocal silencing of duplicated genes in different populations. Later, isolation of populations and explosive radiation took place.

A very important aspect is the dispersal of species of the *P. aurelia* complex. SMITH *et al.* (2014) supposed that dispersal events are "… the primary initiators of geographical isolation and speciation", this statement concerns birds in the Neotropical landscape but could be true for other organisms. *Paramecia*, however, can be transferred always with some drops of water, as cysts are unknown (LANDIS 1988; GUTIERREZ *et al.* 1998; BEALE & PREER 2008). Human activities also played a very important role in the dispersal of microorganisms (FOISSNER 2008).

Further more extensive sampling especially in the southern hemisphere may put forth new data on the occurrence of species of the *P. aurelia* complex.

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