

SYNTHESIS OF THE BULGARIAN PROTOZOOLOGICAL INVESTIGATIONS OF SOUTH SHETLAND ISLANDS (THE ANTARCTIC)

VASSIL GOLEMANSKY

*Bulgarian Academy of Sciences, Sofia-1000, 1, Tsar Osvoboditel blvd., Bulgaria;
E-mail: v.golemansky@abv.bg; golemansky@zoology.bas.bg*

Received 7 January 2016 | Accepted 15 February 2016 | Published online 17 February 2016.

Abstract

Within the range of the Bulgarian Antarctic Program (1987-2010) some parasitological investigations on the coccidian parasites of 3 penguin species from South Shetland Islands (Livingston and St George Islands) were conducted, namely: *Pygoscelis antarctica*, *P. papua* and *P. adeliae*. Three coccidian species were found, one of which was described as a new species: *Eimeria pygosceli* Golemansky, 2003. It was isolated from the fecal samples of all 3 penguin species, living at South Shetland Islands. The other not identified coccidian's were of the genera *Eimeria* and *Isospora*. (Table 1). The great number of coccidian's oocysts in some of the examined birds (from 80 – 220 oocysts in one microscopic field) suggest for their pathogenic role on the penguin's population.

As a result of our studies on some soil, moss and freshwater habitats from Livingston Island a total of 48 rhizopods were found. Three of them were naked amoebae of the genera *Hartmanella*, *Thecamoeba* and *Vannella* and the other 45 species were testate amoebae of 17 genera. Data on their occurrence frequency (pF) and dominance frequency (DF) were given (Table 2). The research on the marine interstitial testate amoebae from the supralittoral of the Antarctic region of Chile and Livingston Island shows the presence of 17 species of psammobiotic and psammophilous testate amoebae (Table 3).

Key words: Antarctic, South Shetland Islands, Coccidia, Penguins, *Pygoscelis*, testate amoebae, naked amoebae.

Introduction

Bulgarian National Antarctic Program started at 1987-1988 years with the establishment of an Antarctic Research Station in Livingston Island (South Shetland Islands: 62°27'–62°48'S and 59°61'–61°15'W). Since 1993-1994 the first field scientific expeditions to South Shetland Islands was also realized with the participation of Bulgarian researches from different fields as zoology, parasitology, ecology, medicine, geology, physics etc. The collected biological collections from the region of the South Shetland Islands were transported and investigated in Bulgaria by different biological institutes and laboratories. Greater part of the results of the biological investigations were published in a new established scientific series: BULGARIAN ANTARCTIC RESEARCH. I. LIFE SCIENCES", as well in some other national and international journals.

Our protozoological investigations were directed to the study of two groups of protists – the coccidian parasites (Apicomplexa (=Sporozoa: Eucoccidia) of the penguins as a negative factor on their population and the free living terrestrial and marine naked and testate amoebae (Amoebozoa & Cercozoa). The aim of the proposed article is to summarize the results of our studies so far and to draw some conclusions of biological and ecological interest on the biodiversity of South Shetland Islands.

Intestinal coccidian parasites of the penguins

Introduction. Three species of penguins of the family Pygoscelidae live at Livingston and King George Islands, namely the Gentoo Penguin (*Pygoscelis papua* (Forester, 1781), the Chinstrap penguin (*P. antarctica* (Forester, 1781) and the Adeliae Penguin (*P. adeliae* (Hombron a. Jacquinot, 1841). Among them *P. adeliae* is relatively rare at South Shetland Islands and inhabits the region mainly in December. *P. papua* and *P. antarctica* are widely distributed on the islands and they have nesting colonies there (Del Hoyo et al., 1992).

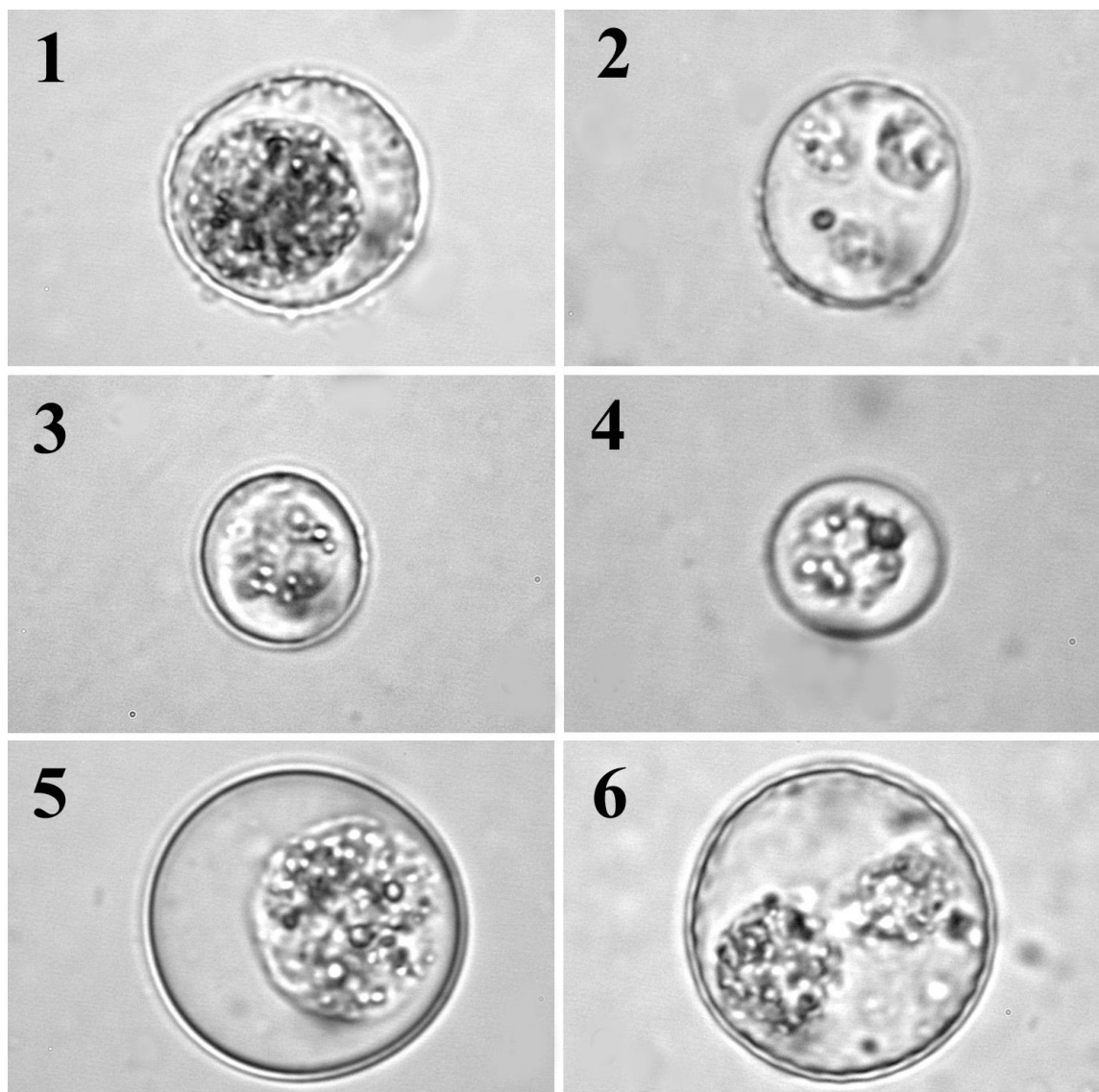
The information on the coccidian parasites and parasitic diseases of the penguins of genus *Pygoscelis*, as well as their pathogenic effect on the penguin populations is scarce and insufficient (Obendorf and McCool, 1980, Clarke and Kerry, 1993, Duignan, 2001, Duszynski and Upton, 2003, Barbosa and Palacios, 2009 etc.). Our studies on the intestinal coccidians of the penguins, living at Livingston and St George Islands started at 1994 year, including all 3 species of genus *Pygoscelis* from this region.

Material and methods. The material (fresh faecal samples) were collected on place every year during the seven years Bulgarian Antarctic campaigns from December to March (1994 – 2010). A total of 360 fecal samples were collected and investigated from the three species: *P. antarctica* – 92 samples, *P. papua* (239) and *P. adeliae* (10). The major part of the samples were collected from penguins populations on Livingston Island (318 samples) and only 42 samples were from St George's population of *P. papua*. The samples were collected from nesting and free birds from the vicinities of the Bulgarian, Spanish and Polish Antarctic stations of both islands. The fresh fecal samples were preserved *in situ* in sterile flacons in a 2.5% solution of Potassium dichromate (K₂Cr₂O₇) and investigated in our Institute's laboratory. Oocyst detection was accomplished by the Fülleborn's method. Positive fecal samples were placed in Petri dishes with moist filter paper at room temperature (22 – 25° C). It is important to note that the sporulation process in European laboratory room conditions started normally, but very few of the oocysts achieved the phase of 2 or 4 sporoblasts. The same problems with the sporulation process of coccidian oocysts from genera *Eimeria* and *Caryospora* from Antarctic birds was observed also by Obendorf and McCool (1980) and Upton (1998, personal communication). Microscopically studies were accomplished at a NU-2 microscope (Zeiss) and the photomicrographs were made with an OLYMPUS-500 digital camera.

Results. In the investigated 3 species of penguins from Livingston and St George islands a total of 3 species of coccidians were established. One of them was identified and described as new taxon: *Eimeria pygosceli* (Golemsky, 2003). Because of the incomplete sporulation of the oocysts and sporocysts the other two species were identified at genus level only, as *Eimeria sp.* and *Isospora sp.* The total prevalence of the coccidian infection was high (35%). For the investigated penguin species the coccidian prevalence vary from 25.8% in *P. papua*, 34.5% in *P. adeliae* and 35.01% in *P. papua*. We suppose the relatively high coccidian prevalence is due to the fact that the studied penguins nest colonially on relatively limited sites where the possibilities for mutual infection were high. The three observed coccidians (*E. pygosceli*, *Eimeria sp.* and *Isospora sp.*) were found in mixed infections in all 3 penguin species. This fact is attributed to the close phylogenetic relationship of the three examined penguin species, as well to the reason that they inhabit the same localities in the islands and have a similar feeding and reproductive biology. Nevertheless that *P. adeliae* occurs in smaller populations on Livingston and St George islands it is in close contact with the dominant *P. antarctica* and *P. papua*, so the exchange of their parasites is well possible. The three coccidian parasites, their hosts and general morphometric characteristics of the oocysts are presented in Table 1. The microphotographs of their unsporulated oocysts are presented in Fig. 1.

Discussion. Generally, the problem of pathogenesis from the coccidian infection on the penguin populations in Antarctic regions is not well studied so far. There are some publications, based on anatomical and pathological investigations too and they prove the pathogenic effect of intestinal and renal coccidian infections of penguins: Obendorf and McCool (1980), Masson et al. (1991), Harrigan (1991), Clarke and Kerry (1993), Duignan (2001), Rose (2005), Barbosa and Palacios (2009) etc. During our investigations we observed in more than 20 specimens of all three penguin species a high number of coccidian oocysts- from 80 to 220 in one microscopical field with a magnification about 150 x. In all cases dominated the oocysts of both eimerian species (*E. pygosceli* and *Eimeria sp.*), while the biggest oocysts of *Isospora sp.* were

relatively rare. We considered this fact as an indirect indicator of the possible pathogenic role of the observed coccidians on the natural populations of the penguins from South Shetlands Islands.



Figures 1-6. Coccidian parasites from the penguins of *Pigoscels* spp. **1-2** – unsporulated oocysts of *Eimeria pygosceli* Golemansky, 2003; **3-4** - unsporulated oocysts of *Eimeria* sp.; **5-6** - unsporulated oocysts of *Isospora* sp.

Terrestrial testate and naked amoebae of Livingston Island

Material and methods. The object of our study on the terrestrial naked and testate amoebae were a total of 22 samples from soil and rock moss and lichens (9 samples), small glacial lakes (7 samples) and superficial soil samples (6 samples), collected during the Antarctic summer campaigns (1994-1995) from different stations of Livingston Island. A new series of samples (moss samples – 16, soil samples – 13 and glacial lakes – 12), collected during January, 1996 – February, 1997) were also studied. And, the last series of 81 samples (moss communities – 30, soils – 23 and small lakes – 29) were collected and investigated during the Bulgarian Antarctic campaigns carried out in the 1998-2003 period. So, the present overview is based on a total of 144 samples (moss, soil and freshwater), collected during a period of 9 years. The living moss and soil samples were washed with 100 ml distilled water in the Institute laboratory in Sofia and treated by the floatation method of Bonnet and Thomas (1958). Microscopically analysis were made by light microscopes NU-2 and Amplival (Zeiss).

Table 1. Coccidian parasites of *Pygoscelis* spp. from South Shetland Islands (the Antarctic).

Coccidian parasites	Hosts	Shape of oocysts	Size of oocysts (in μm)	Oocyst wall	Micro-pyle	Polar granules	Oocyst residuum	Localities
<i>Eimeria pygosceli</i>	<i>P. antarctica</i>	round, subspherical	21.0 – 32.0	double,	-	1–3	+	Livingston I.
	<i>P. papua</i>			smooth,				King George I.
	<i>P. adeliae</i>			rugged				
<i>Eimeria</i> sp.	<i>P. papua</i>	round,	16.2 – 21.0	double,	-	1-2	+	Livingston I.
	<i>P. adeliae</i>	subspherical		smooth				King George I.
<i>Isospora</i> sp.	<i>P. papua</i>	round,	30.2 – 40.6	double,	-	1-4	+	Livingston I.
	<i>P. antarctica</i>	subspherical		smooth				King George I.

A biocenological analysis was fulfilled by using the indices of occurrence frequency (pF) and dominance frequency (DF) (De Vries, 1937). The frequency of occurrence (pF) of the particular species was calculated by the formula $pF = (m/n) \cdot 100$, where m is the number of the samples in which the species was found and n is the total number of samples. Depending of their pF index, the species were classified in 3 categories as follows: 1 – constant, recorded in more than 50% of samples; 2 – incidental, recorded in 25 – 50% of samples and 3 – accidental, found in less than 25% of the samples.

The dominance frequency (DF) of the particular species was calculated by the formula $DF = (d/n) \cdot 100$, where d is the number of the samples in which the species dominates, and n is the total number of the samples.

Results. As a result of our studies a total of 48 testate and naked amoebae were found at Livingston Island so far (Todorov and Golemansky, 1996, 2004, Golemansky and Todorov, 2004). The complete list of the observed taxa, their frequency of occurrence (pF) and dominance frequency (DF) in the terrestrial and freshwater habitats studied are presented at Table 2.

The species diversity of testate and naked amoebae in moss is twice bigger in the soil and freshwater habitats. A total of 41 taxa (3 naked amoebae and 38 testate amoebae) were observed in the investigated moss samples. The only living naked amoebae found in the humid moss samples were *Hartmannella vermiformis*, *Thecamoeba striata* and *Vanella simplex* with a low frequency of occurrence and dominance frequency, variant from 3.3 – 10.0% (pF) and from 0.0 to 3.3 (DF).

The most common testate amoebae were from 2 genera – *Euglypha* (11 species) and *Centropyxis* (6 species) (Table 2). *Euglypha rotunda*, *Trinema lineare* and *Centropyxis aerophila* were the constant species in moss habitats with highest frequency of occurrence (pH), 77%, 60% and 57% respectively. The cited species have the highest dominance frequency (DF) also: *T. lineare* (20.0%), *E. rotunda* (13.3%) and *C. aerophila* (13.3%). With a high frequency of occurrence (pH), changing between 25% and 50% were the next six species- *Assulina muscorum*, *Euglypha laevis*, *E. compressa* f. *glabra*, *Trinema pulchellum*, *Microchlamys patella* and *Diffflugia lucida*.

The shallow soils under tufts of the grass *Deschampsia antarctica* were inhabited by 21 species of testate amoebae with 2 dominant species: *Trinema lineare* (pH = 60.6 %; population density (DF = 17.3) and *Centropyxis aerophila* (pH = 56.6 %); population density (DF = 8.6).

In the aquatic habitats a total of 22 species from 10 genera were identified. The major part of the observed taxa – 15 species were from 3 genera: *Centropyxis* (6), *Diffflugia* (5) and *Euglypha* (4). The other 7 genera were represented by one species only. The most frequent testate amoebae were *Trinema lineare* (pF = 27.5%) and *Diffflugia lucida* (pF = 17.2%). Their population density (DF) was 6.9 and 3.4 respectively.

Discussion. The fauna of naked and testate amoebae from terrestrial and rocky moss, soil and some freshwater habitats of Livingston Island is comparatively poor and composed mainly by cosmopolitan and ubiquitous species. The most common are *Trinema lineare*, *Centropyxis aerophila*, *Euglypha rotunda*, *E. laevis*, *Assulina muscorum*, *Corythion dubium*, *Diffflugia lucida*, *Microchlamys patella*, *Trachelocorythion pulchellum*. Our results confirmed the hypothesis of Smith (1982, 1986, 1987) and Smith and Wilkinson

Table 2. Check-list of the rhizopods, their frequency of occurrence (pF) and dominance frequency (DF) in moss, terrestrial and freshwater habitats on Livingston Island. ¹

Taxa	Habitats		
	Moss (30 samples)	Soil (23 samples)	Aquatic (29 samples)
Naked amoebae			
* <i>Hartmanella vermiformes</i> Page	3.3/0.0	-	-
* <i>Thecamoeba striata</i> (Penard)	6.6/3.3	-	-
* <i>Vannella simplex</i> (Wohlfarth-Bottermann)	10.0/0.0	-	-
Testate amoebae			
<i>Arcella arenaria</i> Greeff	13.3/0.0	-	-
* <i>A. arenaria</i> var. <i>sphagnicola</i> Deflandre	3.3/0.0	-	-
<i>Assulina muscorum</i> Greeff	37.0/3.3	21.6/0.0	6.9/0.0
<i>Centropyxis aculeata</i> (Ehrenberg) Stein	-	-	6.9/0.0
<i>C. aerophila</i> Deflandre	57.0/13.3	56.6/8.6	6.9/0.0
<i>C. aerophila</i> var. <i>sphagnicola</i> Deflandre	6.6/0.0	-	3.4/0.0
<i>C. cassis</i> Deflandre	3.3/0.0	8.6/0.0	6.9/0.0
<i>C. constricta</i> (Ehrenberg) Deflandre	3.3/0.0	-	3.4/0.0
<i>C. elongata</i> (Penard) Thomas	3.3/0.0	-	3.4/0.0
* <i>C. minuta</i> Deflandre	-	4.3/0.0	-
<i>C. sylvatica</i> (Deflandre) Bonnet & Thomas	10.0/0.0	-	-
<i>Corythion aerophila</i> Decloitre	20.0/3.3	8.6/0.0	-
<i>C. dubium</i> Taranek	23.3/6.6	20.0/3.3	3.4/0.0
<i>Cryptodiffugia compressa</i> Penard	10.0/0.0	-	-
* <i>Cyclopyxis eurytoma</i> Deflandre	10.0/0.0	-	-
<i>Diffugia ampullula</i> Playfair	3.3/0.0	-	3.4/0.0
<i>D. lacustris</i> (Penard) Ogden	-	-	3.4/0.0
<i>D. lucida</i> Penard	30.0/3.3	4.3/0.0	17.2/3.4
<i>D. penardi</i> Hopkinson	-	-	3.4/0.0
<i>D. pristis</i> Penard	3.3/0.0	-	3.4/0.0
<i>Diffugiella oviformis</i> (Penard) Bonnet & Thomas	13.3/3.3	4.3/0.0	3.4/0.0
<i>Euglypha bryophila</i> Brown	3.3/0.0	-	-
<i>E. ciliata</i> (Ehrenberg) Leidy	10.0/0.0	8.6/0.0	-
<i>E. ciliata</i> f. <i>glabra</i> Wailes	6.6/0.0	8.6/0.0	-
<i>E. compressa</i> Carter	6.6/0.0	-	3.4/0.0
<i>E. compressa</i> f. <i>glabra</i> Wailes	30.0/0.0	13.0/0.0	-
<i>E. cristata</i> Leidy	6.6/0.0	-	-
<i>E. denticulata</i> Brown	13.3/0.0	4.3/0.0	-
<i>E. laevis</i> Perty	47.0/6.6	13.0/0.0	6.9/0.0
<i>E. rotunda</i> Wailes & Penard	60.0/13.3	16.6/0.0	6.9/0.0
<i>E. strigosa</i> (Ehrenberg) Leidy	-	-	3.4/0.0
* <i>E. strigosa</i> f. <i>glabra</i> Wailes	13.3/0.0	-	-
<i>E. tuberculata</i> Dujardin	3.3/0.0	-	-
<i>Heleopera sylvatica</i> Penard	6.6/0.0	8.6/0.0	-
<i>Microchlamys patella</i> (Clap. & Lachm.) Cockerell	30.0/6.6	4.3/0.0	3.4/0.0
<i>Microcorycia flava</i> Greeff	6.6/0.0	-	-
<i>Padaungiella lageniformes</i> (Penard)	6.6/0.0	-	3.4/0.0
* <i>Plagiopyxis callida</i> var. <i>grandis</i> Thomas	3.3/0.0	-	-
* <i>P. declivis</i> Thomas	-	4.3/0.0	-
<i>Phryganella acropodia</i> (Hertw. & Less.) Hopkinson	10.0/3.3	21.6/0.0	-
<i>Trachelocorythion pulchellum</i> (Penard) Bonnet	33.3/3.3	4.3/0.0	3.4/0.0
* <i>Trinema complanatum</i> Penard	3.3/0.0	-	-
<i>T. enchelys</i> (Ehrenberg) Leidy	13.3/0.0	-	-
<i>T. lineare</i> Penard	77.0/20.0	60.6/17.3	27.5/6.9
<i>T. lineare</i> var. <i>truncatum</i> Chardez	-	10.0/0.0	-
Total taxa: 48	41	21	22

¹ Abbreviations - frequency of occurrence (pF); denominator - dominance frequency (DF).

* New rhizopods to the protozoan fauna of the Livingston Island.

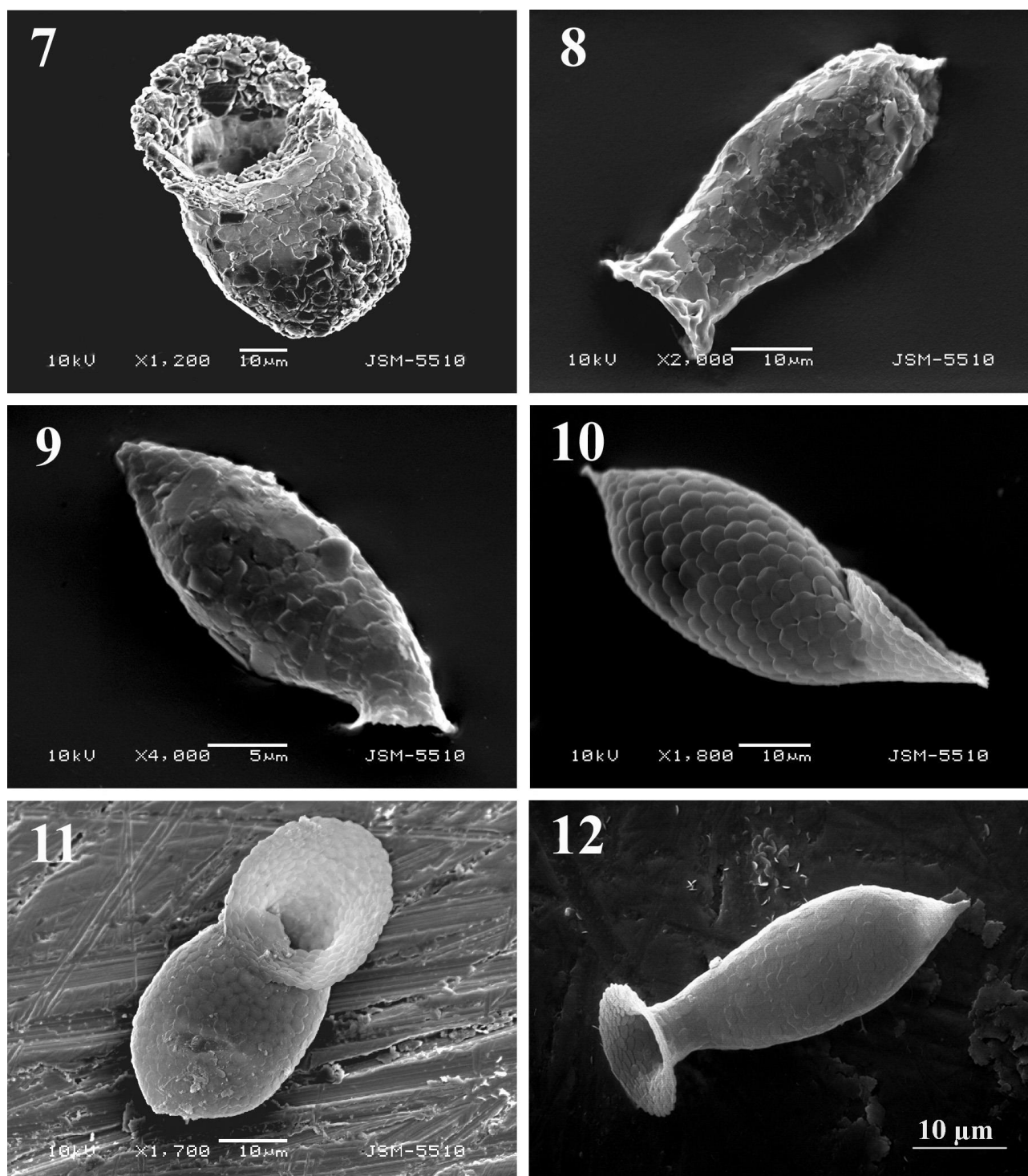
(1986) that there is a trend of decreasing species richness in Antarctic testate rhizopods communities with increasing latitude. The fact is due to the absence of habitats with angiosperm vegetation, long time covered by glass habitats, unfavorable climatic conditions in general. A comparison of testate amoebae fauna from Livingston Island with those of some other sub-Antarctic and Antarctic localities shows the same tendencies: poor taxonomical richness, and dominance of cosmopolitan and ubiquitous species from limited number of genera as *Centropyxis*, *Euglypha*, *Trinema*, *Diffugia*, *Corythion* etc. (Heal, 1965, Grospietsch, 1971, Smith, 1972, 1973, 1974, 1978, 1985, 1992, Bonnet, 1981, Beyens et al., 1995, etc.).

Antarctic marine interstitial testate amoebae

The marine interstitial testate amoebae form a specific ecological association, inhabiting the marine sand and gravel supralittoral over the world. Their presence, species composition and cosmopolitan geographical repartition in the temperate seas and oceans were proved the last 45 years and to now about hundred genera and species were described as new taxa from this habitat (Golemansky, 1969, 1970a, 1970b, 1970c, 1971, 1976, 1980, Chardez, 1972, Chardez et Thomas, 1980, Sudzuki, 1979 etc). During our Antarctic research we studied also the interstitial testate amoebae, inhabiting the underground water of some small sand beaches around the BAS and from the subantarctic of Chile in order to prove their geographical distribution in the cold regions of the world ocean too.

Table 3. Check-list of interstitial testate amoebae found on Livingston Island and Antarctic region of Chile in the Pacific (○ – empty tests, ● – living animals).

Taxa	Studied localities							
	Living	Patagonia, Punta Arenas				Terra	Valparaiso	
	ston Island	1	2	3	4	5	del Fuego 6	7 8
<i>Centropyxiella arenaria</i> Valk.	-	-	-	-	-	-	○	- -
<i>Centropyxiella golemanskyi</i> Chardez	○	-	-	-	-	-	-	- -
<i>Chardezia caudata</i> Gol.	-	-	-	-	-	-	-	○ -
<i>Corythionella acolla</i> Gol.	●	-	-	-	-	-	○	- -
<i>C. minima</i> Gol.	●	-	-	●	●	-	○	- -
<i>Cryptodiffugia lanceolata</i> Gol.	-	-	-	-	-	-	○	- -
<i>Cyclopyxis kahli</i> Defl.	-	-	-	-	-	-	○	- -
<i>Micramphora pontica</i> Valk.	●	-	-	●	-	-	-	○ -
<i>M. tokyoensis</i> Sudzuki	-	-	-	-	-	-	-	○ ○
<i>Microchlamys patella</i> Clap. & Lachm.	○	-	-	-	-	-	-	- -
<i>Micropsammella retorta</i> Gol.	-	-	-	-	-	-	-	○ -
<i>Ogdeniella elegans</i> (Gol.) Gol.	-	-	-	○	-	-	○	- -
<i>O. taschevi</i> Gol.	-	-	-	-	-	-	-	○ -
<i>Psammonobiotus communis</i> Gol.	●	○	○	●	●	-	○	- -
<i>P. linearis</i> Gol.	-	-	-	-	-	-	○	- -
<i>P. minutus</i> Gol.	-	-	-	-	-	-	-	○ ○
<i>Pseudocorythion acutum</i> (Wailes) Valk.	●	-	-	-	-	-	○	- -
<i>Ps. wailesi</i> Gol.	-	-	-	-	-	-	●	● ●
<i>Pseudodiffugia c.f. fascicularis</i> Pen.	○	-	-	-	-	-	-	- -
<i>Trinema enchelys</i> (Ehr.) Leidy	-	-	-	-	-	-	○	- -
Total taxa: 20	8	1	1	4	2	11	7	3



Figures 7-12. Scanning electron photographs of some common interstitial testate amoebae, found in subantarctic region of Chile and on Livingston Island. **7** – *Centropyxiella arenaria*; **8** – *Ogdeniella elegans*; **9** – *Psammonobiotus linearis*; **10** – *Pseudocorythion acutum*; **11** – *Corythionella minima*; **12** – *Chardezia caudata*.

Material and methods. The material from the sea supralittoral around BAS at Livingston Island and from Southwestern Chile the Pacific) was collected by Dr N. Chipev, member of the Bulgarian Antarctic expeditions (1994-1998). The samples from Chile were collected from the sand supralittoral of Strait of Magellan (Punta Arenas) and Gulf of Lapataja (Tierra del Fuego), a total of 7 stations. The temperature of the marine interstitial water of the studied stations varied from 9.1°C to 10.2°C, the water pH from 7.5 – 7.9, respectively and the salinity – from 33.2 ‰ to 35.0 ‰. The samples from Livingstone Island were collected in December, 1997, more precisely from 3 stations of the South Gulf of the island near the BAS:

Plaja Bulgara, Sea lion and Burd stone. The water temperature varied from 1 – 4 °C and the salinity from 30-35 ‰. The studied samples were taken by the method of Chappuis (1942). The living samples in 100 ml bottles with sand (1/3 from the volume) and marine water (2/3 from the volume), were transported to our laboratory and studied by light microscopes NU-2 and Amplival (Zeiss). For scanning electron microscopy specimens of some observed testate amoebae were washed several times in distilled water, the shells coated with gold in a vacuum coating unit. The photomicrographs were obtained using a JEOL JSM-5510, operating at 10 kV.

Results. The studies on the marine interstitial testate amoebae from Antarctic region of Chile (Golemansky and Todorov, 1996) and Livingston Island (Golemansky and Todorov, 1999) show that this specific taxocenose, inhabiting the subantarctic region of the Pacific, is relatively abundant and diverse, presented by 20 species (Table 3). Most of the observed species were strict marine interstitial inhabitants of 7 psammobiotic genera: *Psammonobiotus* (3 species), *Pseudocorythion* (2), *Corythionella* (2), *Micramphora* (2), *Ogdeniella* (2), *Centropyxiella* (2), *Micropsammela* (1) and, *Chardezia* (1) (Fig. 2). In the studied stations 3 eurybiotic species were found also: *Trinema encheilis*, *Cyclopyxis kahli*, *Crypdomitella lanceolata* and *Microchlamys patella*.

The studies on the marine interstitial testate amoebae of the sand supralittoral of Livingston Island proved the presence of 8 species of testate amoebae from 7 genera only. The next 6 species were obligatory psammobiotic inhabitant, with cosmopolitan repartition in the World ocean: *Pseudocorythion acutum*, *Psammonobiotus communis*, *Corythionella acolla*, *C. minima*, *Micramphora pontica* and *Centropyxiella golemanskyi*. Two eurybiotic testate amoebae were identified also, namely *Microchlamys patella* and *Pseudodiffugia c.f. fascicularis* (Golemansky and Todorov, 1999).

Discussion. The results of the present studies prove the presence of a marine interstitial testate amoebae fauna in the cold subantarctic and Antarctic regions, too. But the number and the diversity of this specific testacean association were limited in comparison with those of the temperate seas and oceans. In the Mediterranean Sea, for example the marine interstitial testate amoebae were presented by 54 species and in the Baltic Sea – by 38, respectively (Golemansky, 1980, 1998a,b). We suppose that the extreme temperature conditions in the studied Antarctic regions are the main limiting factor for the biodiversity and abundance of the marine interstitial testate amoebae, too. Another limiting factor may be also the composition and the structure of the moderate sand beaches there, formed mainly by coarse heterogeneous sand, often mixed with gravel and stones.

Acknowledgements

Special thanks to my colleague Doc. Dr. M. Todorov from Bulgarian Academy of Sciences for his active collaboration on the study of the Rhizopods from the Antarctic. I would like to thank also my colleagues: Prof. R. Mecheva, Prof. N. Chipev, Dr. I. Pandurski, Dr. K. Dimitrov, Dr. E. Trakiiska, Dr. I. Yankov and Dr. A. Kovachev from the Bulgarian Academy of Sciences for the precise collection of the examined samples from the penguins of Livingston and St George Islands, as well for the soil, freshwater and marine samples from South Shetland Islands and Southern Chile. The technical assistance of Mrs. A. Zhecheva from the Institute of Zoology is highly appreciated too. Thanks also to the Bulgarian National Science Fund for the support of our studies by Grants B- 502/ 1995 and B- 801/1998.

References

- Barbosa, A. & Palacios, M.J. (2009) Health of Antarctic Birds: A review of their parasites, pathogens and diseases. *Polar Biology*, 32, 1095–1115.
- Beyens, L., Chardez, D., De Baere, D. & Verbruggen, C. (1995) The aquatic testate amoebae fauna of the Stromness Bay area, South Georgia. *Antarctic Science*, 7 (1), 3–8.
- Bonnet, L. (1981) Thécamoebiens (Rhizopoda, Testacea). *Publ. Comité Français Rech. Ant., C. N. F. R. A.*, 48, 23–32.
- Bonnet, L. & Thomas, R. (1958) Une technique d'isolement des Técamoebiens (Rhizopoda, Testacea) du sol et ses résultats. *Comptes rendus de l'Académie des sciences*, 247, 1901–1903.
- Chappuis, P.-A. (1942) Eine neue Methode zur Untersuchung der Grundwasserfauna. *Acta scientiarum mathematicarum et naturalium Universitatis Kolozsvár*, 6, 1–18.

- Chardez, D. (1972) Étude sur les thécamoebiens des biotopes interstitiels, psammons littoraux et zones marginales souterraines des eaux douces. *Bulletin des recherches agronomiques de Gembloux*, N.S. 6, 257–268.
- Chardez, D. & Thomas, R. (1980) Técamoebiens du Mésopsammon des Plages de la Lacanau et Leporge-Ocean (Gironde, France). *Acta Protozoologica*, 19 (3), 277–285.
- Clarke, J.R. & Kerry K.R. (1993) Diseases and Parasites of Penguins. *Korean Journal of Polar Research*, 4 (2), 79–96.
- De Vries, M. (1937) Methods used in plant sociology and agricultural botanical grassland research. *Herbage Reviews*, 5, 76–82.
- Del Hoyo J., Eliot, A. & Sargatal, J. (1992) *Handbook of the birds of the world*. V.I. Linx edicionis, ICBP, Barcelona.
- Duignan, J.P. (2001) Diseses of Penguins. *Surveillance*, 28, 5–11.
- Duszynski, D.W., Couch, L. & Upton S.J. (2003) The Coccidia of Spheniciformes (penguins). Coccidia of the World (www.state.edu/parasitology/worldcoccidia/PENGUINS).
- Golemansky, V. (1969) Sur une biocenose thécamoebienne peu connue des eaux souterraines littorales des mers. Progress in Protozoology, III., Leningrad, *Nauka*, p. 194.
- Golemansky, V. (1970a) *Chardezia caudata* gen. n. sp. n. et *Rhumbleriella filosa* gen. n. sp. n. – deux thécamoebiens nouveaux du psammon littoral de la Mer Noire (Rhizopoda, Testacea). *Bulletin de l'Institut de Zoologie et Musée de Sofia*, 32, 121–125.
- Golemansky, V. (1970b) Rhizopodes nouveaux du psammon littoral de la Mer Noire (Note préliminaire). *Protistologica*, 6 (4), 365–371.
- Golemansky, V. (1970c) Thécamoebiens (Rhizopoda, Testacea) nouveaux des eaux souterraines littorales de la Mer Noire. *Acta Protozoologica*, 8 (2), 41–46.
- Golemansky, V. (1971) Taxonomische und zoogeographische Notizen über die thekamöbe Fauna (Rhizopoda, Testacea) der Küstengrundgewässer der sowjetischen Fernostküste (Japanisches Meer) und der Westküste Kanadas (Stiller Ozean). *Archiv für Protistenkunde*, 113, 235–249.
- Golemansky, V. (1976) Contribution à l'étude des Rhizopodes et des Hélizoires du psammal supralittoral de la Méditerranée. *Acta Protozoologica*, 15, 35–45.
- Golemansky, V. (1980) *La faune técamoebienne interstitielle du psammal supralittoral des mers*. Sofia, DrSc Thesis, 344 p. (in Bulgarian)
- Golemansky, V. (1998a) Interstitial testate amoebae (Rhizopoda: Arcellinida and Gromiida) from the Finish coast of the Baltic Sea and summary checklist of the interstitial testate amoebae in the Baltic Sea. *Acta Protozoologica*, 37, 133–137.
- Golemansky, V. (1998b) Interstitial testate amoebae (Rhizopoda: Testacea) from the Italian coast of the Mediterranean Sea. *Acta Protozoologica*, 37, 139–143.
- Golemansky, V. (2003) *Eimeria pygosceli* n. sp. (Coccidia: Eimeriidae) from the Penguins (Pygoscelidae) of the Livingston Island (the Antarctic). *Acta zoologica bulgarica*, 55 (2), 3–8.
- Golemansky, V. & Todorov, M. (1996) Interstitial Rhizopods (Rhizopoda: Testacea & Foraminiferida) from the Antarctic Region of Chile and Valparaiso in the Pacific. In: Golemansky, V. & Chipev, N. (eds.), *Bulgarian Antarctic Research. Life Sciences*. Pensoft Publ., Sofia, pp. 62–69.
- Golemansky, V. & Todorov, M. (1999) First report of the interstitial testate amoebae (Protozoa: Testacea) in the marine supralittoral of the Livingston Island (Antarctic). In: Golemansky, V. & Chipev, N. (eds.), *Bulgarian Antarctic Research. Life Sciences*. II. V. Pensoft Publ., Sofia, pp. 43–47.
- Golemansky, V. & Todorov M. (2004) Additional data and summarized check-list on the rhizopods (Rhizopoda: Amoebida & Testacea) from Livingston Island, South Shetlands, Antarctic. In: Golemansky, V. & N. Chipev (eds.), *Bulgarian Antarctic Research. Life Sciences*. IV. Pensoft Publ., Sofia-Moscow, pp. 83–93.
- Grospietsch, T. (1971) Beitrag zur Ökologie der Testaceen Rhizopoden von Marion Island. In: van Zuideren-Bakker, E.M., Winterbottom, J.M. & Dyer, R.A. (eds.), *Marion and Prince Edward Islands*. Balkema, Cape Town, pp. 411–419.
- Harrigan, K.E. (1991) Causes of mortality of Little Penguins *Eudyptula minor* in Victoria. *Emu*, 9 (5), 273–277.
- Heal, O.W. (1965) Observation on the testate amoebae (Protozoa: Rhizopoda) from Signy Island, South Orkney Islands. *British Antarctic Survey Bulletin*, 6, 43–47.

- Mason, R. W., Hartley, W.J. & Dubey, J.P. (1991) Lethal toxoplasmosis in a Little Penguin (*Eudiptula minor*) from Tasmania. *Journal of Parasitol. Diseases*, 77 (2): 328–334.
- Obendorf, H.L. & McCool, K. (1980) Mortality in Little Penguins (*Eudiptula minor*) along the Coast of Victoria, Australia. *Journal of Wildlife Diseases*, 16 (2), 251–259.
- Rose, K. (2005) *Common diseases of Urban Wildlife. Burds. Part 1*. The Australian Registry of Wildlife Health, Merlburn.
- Smith, H.G. (1972) The terrestrial Protozoa of Elephant Island, South Shetland Islands. *British Antarctic Survey Bulletin*, 31, 55–62.
- Smith, H.G. (1973) The Signy Island terrestrial reference sites II: The Protozoa. *British Antarctic Survey Bulletin*, 33/34, 83–87.
- Smith, H.G. (1974) A comparative study of Protozoa inhabiting Drepanocladus moss carpet in the South Orkney Islands. *British Antarctic Survey Bulletin*, 38, 1–16.
- Smith, H.G. (1978) The distribution and ecology of terrestrial Protozoa of sub-Antarctic and maritime Antarctic Islands. *Scientific Reports British Antarctic Survey*, 95, 104 p.
- Smith, H. G. (1992) Distribution and ecology of the testate rhizopod fauna of the continental Antarctic zone. *Polar Biology*, 12, 629–634.
- Smith, H. G. & Wilkinson, D.M. (1987) Biogeography of testate rhizopods in the Southern Temperate and Antarctic zones. In: *Colloque sur les Ecosystèmes Subantarctique*, Paimpont, C. N. F. R. A., 58, 83–96.
- Sudzuki, M. (1979) Psammobiont Rhizopoda and Actinopoda from marine beaches of Japan. *Acta Protozoologica*, 18 (2), 293–304.
- Todorov, M. & Golemansky, V. (1996) Notes on testate amoebae (Protozoa: Rhizopoda) from Livingston Island, South Shetland Islands, Antarctic. In: Golemansky, V. & N. Chipev (eds.), *Bulgarian Antarctic Research. Life Sciences*. Pensoft Publ., Sofia-Moscow, pp. 70–81.
- Todorov, M. & Golemansky, V. (1999) Biotopic distribution of testate amoebae (Protozoa: Testacea) in continental habitats of the Livingston Island (Antarctic). In: Golemansky, V. & N. Chipev (eds.), *Bulgarian Antarctic Research. Life Sciences. II*. Pensoft Publ., Sofia-Moscow, pp. 48–56.