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Journal homepage: asn.shu.bg**Editor's choice****Biodiversity, Distribution and Ecology of Testate Amoebae (Arcellinida and Euglyphida) from the Bulgarian Black Sea Coast and the related brackish lakes. A Synthesis.****Vassil Golemansky¹, Rositsa Davidova²**

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Abstract: *The studies on the marine and freshwater testate amoebae of the Bulgarian Black Sea littoral and some related coastal brackish lakes are briefly reviewed. So far, a total of 184 species and subspecies of testate amoebae from 18 families and 45 genera from orders of Arcellinida and Euglyphida were published in national and international journals. The underground waters of the Bulgarian marine sand supralittoral are better studied than the related continental lakes. A total of about 45 species of marine interstitial testate amoebae are known, so far, and a big part of them were for the first time described from the Black Sea littoral. It's interesting to note the presence in the marine underground waters of the Black Sea littoral of many freshwater testate amoebae also, considered by us as eurybionts. That is due of the low and variable salinity of the littoral Black Sea waters during the year. The presence of some psammobiotic testate amoebae as Psammonobiotus lineare and Corythionella georgiana in some related brackish lakes is also of ecological interest. The general conclusion of the present synthesis is that the testate amoebae fauna of the Bulgarian Black Sea coast and the related with it many brackish and freshwater littoral lakes is few known yet and need more active researches.*

Keywords: Black Sea littoral, Testate amoebae, Species composition, Biotopic distribution

Vassil Grigorov Golemansky was born on December 26, 1933. He graduated high school in the town of Teteven (1951) and the Faculty of Biology of Sofia University (1957) with a qualification "Biologist-zoologist". From 1957 to 1966 he was assistant of zoology at the Faculty of Biology of Sofia University, and from 1966 he was at the Institute of Zoology - BAS, where he worked until his retirement in 2008. He worked as a scientific secretary of the Institute of Zoology (1967- 1972), Deputy Director of the Institute of Zoology (1972-1982), Deputy Director of the Unified Biology Center - BAS (1982-1988), Director of the Institute of Zoology (1989-2003). Professor at the Bulgarian Academy of Sciences since 1982 and at the Faculty of Biology of the Sofia University since 1986. He has lectured of students of the Faculty of Biology of the Sofia University (Zoology of Invertebrates and Protozoology), Shumen University (Zoology of Invertebrates), Plovdiv University (Protoparasitology) and others. Elected for Doctor of Biological Sciences (1980), Corresponding Member of BAS (1997), Academician (2003). Doctor Honoris Causa of Shumen University (2016). Author of 203 scientific publications, over 15 books, textbooks for universities (4), over 360 popular articles, etc.

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Introduction

Brief geomorphological characteristic of the Bulgarian Black Sea Coast and related lakes.

The Black Sea is a large intercontinental sea with a total surface about 413,500 km², disposed in the semiarid climatic zone between 46°32' N and 40°55' N and 27°27' E and 41°32' E [1]. Characteristic peculiarity of the Black Sea is the low salinity of the sea: 22-22.5‰ of the deeper layer and 15-18‰ of the surface layer of water [2].

The Bulgarian Black Sea Coast, disposed at the southwestern part of the sea is about 380 km long. On the Bulgarian littoral there are many lakes, marshes and river floods with a reach aquatic vegetation. The bigger littoral lakes on the Bulgarian Black Sea Coast, directly related with the sea, are 9 and they were object of more detailed hydrobiological research so far: Durankulak lake, Ezerets lake, Shabla lake, Varna lake, Beloslav lake, Pomoriisko lake, Atanasovsko lake, Burgas lake and Mandra lake (Fig. 1). There are some little local lakes in the southern Bulgarian coast, as Diavolsko lake, Stomoplo lake etc., but they aren't especially studied for testaceans so far. Depending on their hydrological and hydrochemical status the related lakes are brackish and hypersaline. Two of them belong to the category of the hyperhaline basins with a salinity about and more of 40‰ (Pomoriisko and Atanasovsko lakes). The other littoral lakes belong to the category of meso- and oligohaline basins, which salinity vary from 0.5‰ to 18‰. The salinity of all Bulgarian littoral lakes isn't constant during the year and is different during the four annual seasons. According some Bulgarian authors, they can be categorized also as poikilohaline basins [2-4]. All littoral lakes are overgrown by a reach aquatic vegetation, presented of *Phragmites communis*, *Typha ssp.*, *Carex ssp.*, *Myriophyllum sp.*, *Potamogeton ssp.*, etc. In some lakes, like Ezerets and Shabla lakes, a littoral stratum of sand is developed also.

Review of the investigation on the marine and brackish testate amoebae

Because of historical reasons the investigations of the animal biodiversity of the Bulgarian Black Sea Coast and the related lakes started after the liberation of the country at the end of 1878 year. The first studies of the Bulgarian zoologist Prof. Dr G. Chichkoff [5, 6] show a reach invertebrates fauna of about 249 species, 8 species of which were unknown so far for the Black Sea. In the list of the observed by Chichkov invertebrates lack any testate amoeba species.

Some years later Valkanov [7-9] have published a new series of contributions to the biodiversity of different animal groups, including some testate amoebae, inhabiting the Bulgarian Black Sea littoral and some related lakes also and prove its reach and diverse fauna. About twenty years later Valkanov [10], in a brief communication on the invertebrates of the Bulgarian Black Sea, reported for the observation of some new marine and freshwater animals, observed in the underground waters of some marine and lake sand beaches with low salinity. One of them was an unknown testate amoeba *Corythion acutum*, described by Wailes [11] from the underground waters of the sandy beach Camp Island (British Columbia, Canada). Really, it was the first psammobiotic testate amoeba, inhabiting a typical marine habitat with a high salinity. But Wailes did not suppose at that time that he had discovered a new specific marine testate amoeba and conclude: “*Although found on the sea shore, as described below, it is in all probably a freshwater or moss-inhabiting species*”. Wailes found in the same habitat some other freshwater testate amoebae as *Cyphoderia ampulla* and *Euglypha loevis* and suggested all they were imported in the marine sand supralittoral from the mosses by the continental fresh water. Many years later, Golemansky [12] in a study of the testate amoebae fauna of the big Ohrid lake in North Macedonia observed other unknown psammobiotic testate amoeba, inhabiting the underground water in a sand beach of the lake, named *Psammonobiotus communis*. The same *P. communis* was observed later in the supralittoral psammal of many other studied seas and oceans, as well as in some Great Canadian lakes [13, 14, 15, 16, 17, 18 etc].

Studies of Golemansky [13, 19, 20, 21, 22] and Valkanov [23] on the testate amoebae of the underground waters of the Bulgarian Black Sea Coast revealed the presence in this habitat of a reach specific taxocenosis of testate amoebae, presented by many unknown so far new genera and species. Later this testacean taxocenosis was observed in some other marine sand beaches over the world by Golemansky [13, 14, 15, 24], Chardez [25, 26], Sudzuki [17, 27] and others and it was proved it's cosmopolitan distribution.

The first attempt to an overview on the fauna of the Bulgarian Black Sea Coast and the related brackish and freshwater basins, including the testate amoebae, was published by Valkanov [28], and in two supplements to it by Valkanov and Marinov [29] and Marinov and Golemansky [30]. As a result, a

Fig. 1. Map of the Bulgarian Black Sea littoral and the related brackish lakes (With changes from Rhojdestvensky [2]).

total of 2258 species were established of which 675 species of Protists, mainly Algae and Protozoan's. A big part of them - about 68 species were testate amoebae.

The last about 20 years some new specialized investigations on the testate amoebae of the Bulgarian Black Sea sand supralittoral and the related basins were effectuated. Some of them contributed

19

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to the study of the biodiversity and the ecology of the marine interstitial testate amoebae of the marine sand supralittoral [31, 32, 33, 34, 35, 36 etc.]. Some new investigations on the testate amoebae of some related brackish lakes on the Bulgarian Black Sea Coast were realized too: on lakes Shabla and Eserets by Todorov and Golemansky [37] and on Durankulak lake by Davidova and Vasilev [38, 39].

The aim of the present publication is to summarize the known so far information on the biodiversity and the ecology of the testate amoebae fauna of the Bulgarian Black Sea Coast and the related brackish lakes, published in a number of different articles and books in Bulgarian and foreign languages. Many of them were published in Bulgarian from national journals and books, difficult to be found and read now by the young Bulgarian and foreign researchers, interested on the group. We hope our present synthesis will be a useful fundament and can stimulate the future researchers on the biodiversity and the ecology of marine, brackish and freshwater testate amoebae not in Bulgaria only, but in other marine countries around the Black Sea too.

Over the past two to three decades, following recent developments in modern molecular biology, the classification of all living organisms and many major animal groups underwent substantial revisions. This also refers to unicellular animals (Protozoa) and testacean amoebae, united until recently in the class of Rhizopoda. Since the present publication is not a specialized taxonomic study, for considerations of convenience in presenting the published data on the testacean amoebae in Bulgaria, we use the widely accepted classification of the group by Meisterfeld [40, 41]. We also retain the names of previously established genera, species and varieties, as reported by the authors of various publications, which contain additional data about them. We hope that this can be useful for future research on testate amoebae, especially when there are more detailed morphological and taxonomic descriptions of these taxa by the authors who first identified and described them.

Results and Discussion

Studies of the testate amoebae of the Bulgarian Black Sea sand supralittoral and in the the related brakish- and freshwater basins, have identified a total of 184 species and subspecies belonging to 45 genera and 18 families of two main orders: Arcellinida and Euglyphida. Their list in alphabetical order is presented in Table 1.

The analysis of the table shows that the majority of the testate amoebae found to date, 130 species and subspecies, inhabit the coastal freshwater and brackish lakes, and are predominantly of the order of Arcellinida amoebae. In the underground waters of the Bulgarian Black Sea Coast with higher salinity, the

number of identified testate amoebae is significantly lower - 53 species and subspecies, predominantly of the Euglyphida order (Fig. 2).

Although the Bulgarian Black Sea coast is comparatively rich in coastal lakes and ponds, the knowledge of their unicellular fauna and especially its testate amoebae inhabitants are comparatively scarce. Only three of the larger seaside lakes - Durankulak, Ezerets and Shabla have been better explored

Fig. 2. Sea interstitial testate amoebae, found in the Bulgarian Black Sea coast: 1 - *Pseudocorythion acutum* Valkanov, 1970; 2 - *Pomoriella valkanovi* Golemansky, 1970; 3 - *Chardezia caudata* Golemansky, 1970; 4 - *Rhumbleriella filosa* Golemansky, 1970; 5 - *Alepiella tricornuta* Golemansky, 1970; 6 - *Corythionella pontica* Golemansky, 1970. (Photos by assoc. prof. Milcho Todorov)

Table 1. Checklist of the testate amoebas from the Bulgarian Black Sea coast and the related brackish lakes.

Taxa	Black Sea Coast	Brackish lakes					References
		Duranku lak Lake	Ezerets Lake	Shabla Lake	Varna Lake	Belosla v Lake	
	salinity 4-18‰	salinity to 4‰	salinity 0.5-2‰	salinity 0.5-2‰/	salinity 5-14‰	salinity 0.1-9‰	salinity to 1963 0.1-12‰
	supralittoral psammophil	benthal	benthal	hygrohalophil	benthal	benthal	benthal
Order Arcellinida							

24

C. delicatula Penard, 1902		+							[38]
C. discoïdes (Penard, 1890) Deflandre, 1929			+						[37]
C. ecomis (Ehrenberg, 1841) Leidy, 1879		+	+		+				[37, 38]
C. elongata (Penard, 1890) Thomas, 1959		+							[38]
C. hirsuta Deflandre, 1929		+							[38]
C. laevigata Penard, 1890		+							[38]
C. marsupiformis (Wallich, 1864) Deflandre, 1929			+						[37]
C. minuta Deflandre, 1929		+							[38]
C. plagiostoma Bonnet & Thomas, 1955			+		+				[37]
C. platystoma (Penard, 1890) Deflandre, 1929		+	+		+				[37, 38]
C. sylvatica (Deflandre, 1929) Bonnet & Thomas, 1955		+	+		+				[37, 38]
Cryptodiffugia brevicolla Golemansky, 1979	+								[30]
C. compressa Penard, 1902		+							[38]
C. lanceolata Golemansky, 1970	+								[19, 43, 45, 30]
C. paludosa Golemansky, 1981	+								[12, 30]
Cucurbitella mespiliformis Penard, 1902			+		+				[37]
Cyclopyxis eurystoma Deflandre, 1929		+	+		+				[37, 38]
C. kahli Deflandre, 1929		+	+		+				[37, 38]

26

D. elegans Penard, 1890		+	+	+			[37, 38]
D. foissneri Chardez, 1987			+	+			[37]
D. gassowskii Ogden, 1983			+				[37]
D. glans Penard, 1902		+		+			[37, 38]
D. globularis (Wallich, 1864) Leidy, 1877		+	+				[37, 38]
D. globulosa Dujardin, 1837		+	+	+			[37, 38]
D. gramen Penard, 1902		+	+	+		+	[28, 37, 38]
D. labiosa Wailes, 1919			+	+			[37]
D. lacustris (Penard, 1899) Ogden, 1983		+	+	+			[37, 38]
D. lanceolata Penard, 1890		+	+	+			[37, 38]
D. levanderi Playfair, 1918		+					[38]
D. linearis (Penard, 1890) G.-L. et Thomas, 1958			+	+			[37]
D. lithophila (Penard, 1902) G.-L. et Thomas, 1958		+	+	+			[37, 38]
D. lobostoma Leidy, 1879		+	+	+		+	[28, 37, 38]
D. longum Chardez, 1987				+			[37]
D. lucida Penard, 1890	+	+					[43, 45, 30, 38]
D. mammilaris Penard, 1893			+	+			[37]
D. manicata Penard, 1902		+	+	+			[37, 38]

D. microclaviformis (Kourov, 1925) Ogden, 1983									[37]
D. microstoma (Thomas, 1954) Ogden, 1983									[37, 38]
D. minuta Rampi, 1950									[38]
D. minuta v.grandis (Rampi, 1950) G.-L. & Thomas, 1958									[38]
D. molesta Penard, 1902									[37]
D. nodosa (Leidy, 1879) Ogden & Zivkovic, 1983									[37]
D. oblonga Ehrenberg, 1831									[37, 38]
D. parva (Thomas, 1954) Ogden, 1983									[37, 38]
D. pauli Ogden, 1983									[38]
D. penardi Hopkinson, 1909									[37, 38]
D. petricola Cash & Hopkinson, 1909									[37, 38]
D. pristis Penard, 1902									[37, 38]
D. pulex Penard, 1902									[37, 38]
D. rotunda Ogden, 1983									[37]
D. rubescens Penard, 1891									[37]
D. sarissa Li Sun Tai, 1931									[38]
D. scalpellum Penard, 1902									[37]

29

<i>Lagenodifflugia epioxi</i> (Chardez, Gaspar, 1984) Ogden, 1987										[37]
<i>Microchlamys patella</i> (Clap. & Lach., 1885) Cockerell, 1911	+		+							[45, 30, 37, 38]
<i>Nebela dentistoma</i> Penard, 1890				+						[37]
<i>N. militaris</i> Penard, 1890										[37]
<i>Netzelia oviformis</i> (Cash & Hopkinson, 1909) Ogden, 1979				+						[37]
<i>N. tuberculata</i> (Wallich, 1864) Ogden, 1980				+						[37]
<i>Paraqquadricula irregularis</i> (Archer, 1877) Deflandre,1932			+							[38]
<i>Pentagonia shablensis</i> Todorov, Golemansky, 1998				+						[37]
<i>Phryganella acropodia</i> (Hertw., Less., 1874) Hopkinson, 1909				+						[37]
<i>P. hemisphaerica</i> Penard, 1902			+							[38]
<i>P. nidulus</i> Penard, 1902				+						[37]
<i>P. paradoxa</i> Penard, 1902			+							[38]
<i>Phryganella sp.</i>									+	[28]
<i>Plagiopyxis declivis</i> Thomas, 1955			+	+					+	[37, 38]

[illegible]

[illegible]

33

34

Fig. 3. Light microscopy images of interstitial testate amoebae found in coastal brackish lake: 1 - *Corythionella georgiana* Nicholls, 2005; 2 - *Psammonobiotus linearis* Golemansky, 1970. Scale bars – 10 μm . (Photos by assoc. prof. Rositsa Davidova)

in this respect, and 91, 79 and 81 species and subspecies of testate amoebae respectively have been identified in them. The remaining lakes and ponds have never been studied to date in terms of their testate fauna, regardless of its vital environmental role and importance. The biggest Bulgarian seaside lakes - those of Varna, Mandra and Pomorie, which are subject to the greatest anthropogenic pressure due to their proximity to big cities and industrial zones, are virtually unexplored with regard to their protozoan fauna. Atanasovsko Lake is of significant interest in terms of its testate fauna, and it is also important from the point of view of ornithology.

Studies on the testate amoebae in the abovementioned coastal lakes led to the discovery of a new species - *Pentagonia shablensis* Todorov and Golemansky, 1998, and added to the existing knowledge about the morphology, taxonomy and ecological conditions necessary for the existence of their taxa. It is of biological and ecological importance to identify in some of the coastal brackish lakes interstitial testate amoebae, which are vital elements of the taxocenoses of the cosmopolitan supralittoral interstitial testate amoebae of the seas and large inland lakes. Such are, for example, the species *Corythionella georgiana* Nicholls, 2005 and *Psammonobiotus linearis* Golemansky, 1970, found in Durankulak Lake [38, 39] (Fig. 35

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3). They belong to the subdominant group, and were found in the benthal of the lake with a significant frequency and number of specimens.

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