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OVERVIEW OF STUDIES ON AQUATIC ZOOSPORIC PARASITES AND THEIR HOSTS IN BULGARIA

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Abstract. The overview presents historic data on studies of aquatic zoosporic parasites in Bulgaria, their spread and hosts with a special focus on new genera, species and varieties described from the country. Conditionally, in this review carnivorous fungi are also discussed due to the opinion of some authors about their dual, predacious and parasitic mode of life. The review is based on 45 publications issued in the period 1912-2023 and provides data on 35 taxa from the phyla Chytridiomycota (23), Entomophtoromycota (1), Zoopagomycota (1), Oomycota (9) and Plasmodiophoromycota (1). They were found on hosts belonging to the groups of Algae (at least 16 species from Cyanoprokaryota, Ochrophyta, Chlorophyta and Streptophyta), Pseudofungi, Plants (pollen) and Invertebrates (Rotatoria). Most data were obtained from field freshwater samples collected in the towns Sofia and Plovdiv and their vicinities, in glacial lakes and peat bogs of Pirin Mt, while less data concern some peat bogs of Vitosha Mt, peat bogs and a swamp on Rila Mt, lakes in Rodopi Mts, swamps in the vicinity of Sofia, a swamp on Lyulin Mt, a swamp in the vicinity of the town Chirpan, an unidentified swamp near to the River Danube, fishponds near the town Plovdiv and a microreservoir on Vitosha Mt near the town Pernik. A single species was identified from mass algal cultures and waste waters. Four new genera, eleven species and one variety were described from the country by Bulgarian professors S. Konsuloff, N. Arnaudow and A. Valkanov. For three designated species we outline the nomen nudum status, the diagnosis of a monotypic genus *Hydatinophagus* is translated in English and some considerations on currently proposed taxonomical changes are provided. The necessity of future studies broadened in terms of regions, altitudes, habitats, localities and hosts in order to reveal

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the real biodiversity and spread of aquatic zoosporic parasites in Bulgaria, has been outlined.

Key words: algae, algal cultures, Chytridiomycota, carnivorous fungi, cyanobacteria, Entomophtoromycota, Fungi, Hydatinophagus, Oomycota, Plasmodiophoromycota, predacious fungi, Pseudofungi, rotifers, Zoopagomycota

INTRODUCTION

Nowadays there is no doubt about the inevitable diversity of fungal kingdom with historic data tracing back for centuries. In Bulgaria, situated on the Balkan Peninsula that has been recognized as a hot spot of biodiversity (Griffiths et al. 2004), studies on different groups of fungi and pseudofungi started more than a century ago (Denchev & Bakalova 2002). By contrast to their land counterparts, aquatic fungal parasites have gained less attention and number of publications considering these organisms is considerably lower. However, in present time, there is a worldwide rising interest to zoosporic parasites in natural systems and in aquacultures, but also in living algal cultures of scientific collections, due to the significant economic harm they may cause (*e.g.,* Lafferty et al. 2008; Rasconi et al. 2012, 2014, 2022; Carney & Lane 2014; Kagami et al. 2014; Gavrilović et al. 2022 among the many others), but also because of their increasingly recognized importance in natural ecosystems in regulation of algal blooms (*e.g.,* Gleason et al. 2015) and in providing alternative pathways in food webs, known as mycoloops (*e.g.,* Kagami et al. 2014). This paper presents an overview of historic data on diversity, hosts and spread of aquatic zoosporic parasites in Bulgaria with special focus on genera and species described from the country. The review provides discussion supported by translations from original texts of Bulgarian authors regarding some carnivorous fungi due to their later misinterpretation as parasites.

MATERIAL AND METHODS

Information for this review was obtained from the original publications, kept in the National library "Sts. Cyrillus and Methodius" of Bulgaria, in the libraries of Sofia University "St Kliment Ohridski" and of the different institutes of Bulgarian Academy of Sciences, as well as in the personal archives of the authors. It fully considers also the summarizing reviews by Petkoff (1939), Denchev & Bakalova (2002), Denchev (2007) and Stoyneva & Temniskova-Topalova (2007), as well as the world-wide used handbooks of Sparrow (1960) and Batko (1975). The current taxonomic status of the mentioned fungi and pseudofungi, if available, is provided according to the on-line databases Index Fungorum and MycoBank and relevant publications therein. In this way, the review covers 41 scientific papers and four books issued in the period 1921-2023.

Data are organized according to the habitats (natural or artificial wetlands,

mass cultures and waste waters) for different taxonomic groups, starting with the newly described genera, species and varieties in each phylum, and continuing with other species recorded from the country. The localities are pointed according to the texts in the original publications. Genera and species are presented in alphabetical order in each phylum.

I. Zoosporic parasites found in natural or artificial wetlands of Bulgaria, or in laboratory cultures obtained from field material

Phylum Chytridiomycota

New genera:

1) *Arnaudovia* Valkanov 1963 – this monotypic genus has been exclusively described as carnivorous with peculiar hyponeustonic mode of life (Valkanov 1963a), and therefore its transformations by Karling (1977) and Doweld (2014), which include it in the genus *Polyphagus* is debatable. Doubtless, the name given by Valkanov (1963) is invalid according to Art. 39.1 of the Melbourne Code (McNeil 2012) due to presence of German description and lack of designation of the holotype. However, in our opinion, the name has to be conserved due to the very peculiar ecology of the genus which belonged to the specific group of the neuston (*e.g.*, Valkanov 1968) and high, world-wide accepted expertise of prof. Alexander Valkanov (1904-1964). Index Fungorum Identification Number F550465;

2) *Dangeardiana* Valkanov 1964 – in Index Fungorum it is included as *Dangeardiana* Valkanov ex A. Batko 1970 with the explanation that this taxon, originally published by Valkanov (1964) with diagnosis only in German, which makes it is a nomen invalidum according to the Art. 43 of the ICBN, has been extended by Batko (1970) with a Latin diagnosis. However, Batko (1970) stressed that he provides a translation of the diagnosis of the genus and its monotypic species, and kept the generic and author name as they existed in the original publication. Considering that later, in his hydromycological handbook, Batko (1975) himself continued to use the generic name only with Valkanov as author, we believe that the taxonomic change proposed in Index Fungorum should be abandoned. Index Fungorum Registration Identifier 90935.

New species, or varieties:

1) *Arnaudovia hyponeustonica* Valkanov 1963 – discovered in the landslide swamp Dragichevsko Blato on the slopes of Lyulin Mt near the village Dragichevo (Valkanov 1963a). It catches mainly flagellate algae from the genera *Phacotus* (division Chlorophyta), *Trachelomonas* and *Strombomonas* (division Euglenophyta), or also *Chlamydomonas* (division Chlorophyta), and rarer the euglenophytes *Euglena*, *Phacus*, *Lepocinclis*, *Peranema* and *Petalomonas* (Batko 1975). The species has been described as hyponeustonic carnivore by its author, and therefore its taxonomic transformations by Karling (1977) to *Polyphagus*

hyponeustonicus (Valkanov) Karling, as well as the current taxonomic renaming as another *Polyphagus* species, *Polyphagus arnaudovii* Doweld, sp. nov. by Doweld (2014) is hardly acceptable, especially in the lack of molecular-genetic data on the original material. *Polyphagus arnaudovii* Doweld is included in Index Fungorum as the currently accepted name with Index Fungorum Identification Number F550465. However, in the Mycobank, the name *Arnaudovia neustonica* Valkanov 1963 is accepted as legitime, and the taxon is included with number 326459;

2) *Bertramia diglenae* Konsuloff 1916 – discovered as a parasite of the rotifer *Cephalodella catellina* (Müller) 1786 (Syn. *Diglena catellina* Ehrenberg, 1830) in March and April in swamps from the vicinity of Sofia, with a note that the observed infection was very weak (Konsuloff 1916). Although a detailed description illustrated with five figures has been provided in the cited paper, the designation is not supplied by a diagnosis, or with a reference to the diagnosis, which shows that is not validly published according to the rules designation of a new taxon published without a description or diagnosis or reference to a description of diagnosis according to articles 32, 36, 41, 42, and 44 of the Code of algae, fungi and plants (McNeill et al. 2012; Turland et al. 2018). This makes it nomen nudum.

3) *Bertramia euchlanis* Konsuloff 1914 – discovered in the rotatoria *Euchlanis dilatata* Ehrenberg, 1832 collected from swamps in the vicinity of Sofia in March 1911 when the rotifer was most abundant (Konsuloff 1914). Although a detailed description illustrated with 16 figures has been provided in the cited paper, the designation is not supplied by a diagnosis, or with a reference to the diagnosis, which shows that is not validly published according to the rules for designation of a new taxon in the articles 32, 36, 41, 42, and 44 of the Code of algae, fungi and plants (McNeill et al. 2012; Turland et al. 2018). This makes it *nomen nudum*.

4) *Dangeardiana eudorinae* Valkanov 1964 - discovered in the oospores of the green flagellate alga *Eudorina elegans* Ehrenberg (Chlorophyta) collected from the vicinity of Sofia in July 1959 (Valkanov 1964). In Index Fungorum this species is included with the name *Dangeardiana eudorinae* Valkanov ex A. Batko 1970. The taxon has been originally published by Valkanov (1964) with diagnosis only in German, which makes it a nomen invalidum according to the Art. 43 of the International Code for Botanical Nomenclature (ICBN) and therefore it was extended by Batko (1970) with a Latin diagnosis. However, Batko (1970) only translated the diagnosis of the species and kept the original name and Valkanov's authorship, without proposing a new combination. Moreover, later, in his comprehensive hydromycological manual, Batko (1975) included the species with its original name and single author Valkanov. Therefore, in our opinion, the proposal for extension of the name made in Index Fungorum should be abandoned. Index Fungorum Registration Identifier 454277. In Mycobank the legitime name *D. eudorinae* Valkanov ex A. Batko is with number 312681 (but with *D. eudorinae* Valkanov 1964 as a current name) and the name *D. eudorinae* Valkanov, with status

"invalid" and with the same current name is with number 454277;

5) *Monocystis minima* Konsuloff 1916 – found as a parasite of the rotifer *Euchlanis dilatata* and *Mytilina mucronata* (Müller) 1773 (Syn. *Salpina mucronata* Ehrenberg) (Konsuloff 1916). Although a detailed description illustrated with 8 figures has been provided in the cited paper, the designation is not supplied by a diagnosis, or with a reference to the diagnosis, which shows that is not validly published according to the articles 32, 36, 41, 42, and 44 of the Code of algae, fungi and plants (McNeill et al. 2012; Turland et al. 2018). This makes it *nomen nudum*.

6) *Polyphagus asymmetricus* Valkanov ex Doweld 2014 – originally published by Valkanov (1963b) as hyponeustonic fungus on the yellow-green coccal alga *Botrydiopsis* sp. ("consuming *Botrydiopsis*") from the division Ochrophyta, class Xanthophyceae, collected from the fishponds near Plovdiv (Index Fungorum Registration Identifier 337468). According to Arts 39, 1 and 40, 1 (International Code of Nomenclature for algae, fungi and plants, adopted by the Eighteenth International Botanical Congress Melbourne, Australia, July 2011) considered as nomen invalidum due to lack of Latin diagnosis and holotype designation and currently published with additions in Index Fungorum by Doweld, sp. nov. IF550466 with indication of the iconotype: figures 1-11 on tab. 44 in Valkanov (1963), Arch. Protistenk. 106: 568, as a holotype (Doweld 2014). Index Fungorum Registration Identifier 550466. In Mycobank, *P. asymmetricus* Valkanov 1964 is included as a legitime name under number 337468 and *P. asymmetricus* Valkanov ex Doweld 2014 is shown also as a legitime name with number 550466;

7) *Rhizophydium epithemiae* (as "*Rhizophidium epithemiae*")– observed as a parasite of the coccal pennate diatom *Epithemia adnata* (Kützing) Brébisson (Syn. *Epithemia zebra* (Ehrenberg) Kützing; phylum Ochrophyta, class Bacillariophyceae) collected in Sofia (Valkanov 1931a). Although prof. Valkanov himself did not designate it as a new species by "n. sp." in the original publication and provided only a German description with a figure, this species is included in Index Fungorum (Index Fungorum Registration Identifier 252006) and in Mycobank (number 252006), as well as in Batko (1975) as *Rhizophydium epithemiae* Valkanov 1931. Sparrow (1960) also included this species with author Valkanov in his manual on aquatic phycomycetes with a question mark before the name. Based on this work, Danz & Quandt (2023) mentioned this parasite of *Epithemia adnata* as known from Bulgaria;

8) *Rhizophydium pyriforme* Valkanov [as '*pyriformis*'] 1931 – parasite in the developed oospores of the yellow-green siphonal alga *Vaucheria* sp. (division Ochrophyta, class Xanthophyceae), collected from a well in the village of "Kazichane" (Valkanov 1931a). This village with current official name Kazichene is situated in the closest vicinity of the Bulgarian capital town Sofia. Included in the manual of aquatic phycomycetes with a question mark before the name (Sparrow 1960). Index Fungorum Registration Identifier 481659. Number

481659 in Mycobank;

9) *Rhizophidium pollinis* var. *pirinicum* n. var. Valkanow 1932 – discovered on the pollen collected from the glacial lake Vasillashko Ezero, Pirin Mt (Valkanow 1932). According to the author, the materials are well-distinguished from the species by the numerous small spines on the wall and might even represent a new species. Currently, *Rhizophidium pollinis* is considered as a synonym of *Globomyces pollinis-pini* (A. Braun) Letcher, but the variety has not been a subject of a taxonomic revision and is not mentioned in Index Fungorum and in Mycobank.

Recorded species:

1) *Bertramia asperospora* Fritsch – found in the months February and March as a parasite in the rotifer *Eosphora najas* Ehrenberg, 1830, but also in the rotifers *Cephalodella catellina* (Müller) 1786 (Syn. *Diglena catellina* Ehrenberg, 1830), *Callidina parasitica* Giglioli, 1863 and "*Colurus* sp." in different swamps from the vicinities of Sofia (Konsuloff 1912). Index Fungorum Registration Identifier 648307;

2) "*Botellus* sp. (?)" – found as a parasite of the rotifers *Hydatina senta* and *Brachionus calyciflorus* subsp. *calyciflorus* Pallas, 1766 (Syn. *Brachionus pala* Ehrenberg, 1838) and designated it in this way due to lack of different developmental stages (Konuloff 1916). The exact location was not identified, but since the other materials of Konsuloff (1912, 1914) from rotifer parasites were collected from "different swamps in the vicinity of Sofia" it is possible to suggest the same location for this parasite.

3) *Entophlictis bulligera* (Zopf.) Fisch. – found as a parasite in the green filamentous alga *Oedogonium* sp. (division Chlorophyta) collected "in the town Plovdiv" (Valkanov 1931a). As "*Entophlictis bulbigera* (Zopf.) Fisch." (? Err. typogr.) in Index Fungorum with a Index Fungorum Registration Identifier 535083. The taxon was not found in Mycobank;

4) *Entophlictis confervae-glomerata* (Cienk.) Sparrow– found as a parasite in the yellow-green alga *Vaucheria* sp. in field samples collected in the vicinities of the towns Sofia and Plovdiv (Valkanov 1931a). The material of Valkanov (1931a), published as "*Entophlictis rhizina* (Scenk.) v. Minden" was included by Sparrow (1960) in his manual on aquatic phycomycetes in *E. confervae-glomeratae*. Index Fungorum Registration Identifier 286363. The taxon was not found in Mycobank;

5) *Globomyces pollinis-pini* (A. Braun) Letcher (as "*Rhizophidium pollinis* (A. Braun) Zopf.") – first found in the natural material from pine pollen in the high-alpine peat bogs on the mountains Vitosha and Rila, and cultivated for months in the laboratory (Valkanov 1931a). Later outlined as being widely distributed in masses on the pollen in all sampled glacial lakes in Rila and Pirin Mts, where it "sometimes causes real epidemy on the pollen grains" (Valkanow 1932, p. 210). According to Sparrow (1960) this species is saprophytic and parasitic on floating pine pollen. Index Fungorum Registration Identifier 511788. Number 511788 in Mycobank;

6) *Harpochytrium hedenii* Wille – observed as a parasite in the filamentous green alga *Oedogonium* sp. (phylum Chlorophyta) collected in a swamp near the River Danube (Valkanov 1931a). Index Fungorum Registration Identifier 238016. Number 238016 in Mycobank;

7) *Olpidium* sp. – found in the green filamentous alga *Zygnema* sp. (division Streptophyta) collected from the "glacial lakes of Pirin Mt" (Valkanow 1932). The taxonomic affiliation of this genus is strongly disputable (*e.g.*, Barr 1990, Lay et al. 2018, Wijayawardene 2018) and due to the unavailability of the original material, we refer to this zoosporic parasite with its name according to the original publication. Index Fungorum Registration Identifier 816320;

8) *Phlyctochytrium hydrodictyi* (A. Braun) Schroeter (as "*Phlyctochytrium hydrodictii* (A. Braun) Schroeter") – recorded in the green alga *Hydrodictyon reticulatum* collected in the town Sofia (Valkanov 1931a). Index Fungorum Registration Identifier 431949 with a basionym *Chytridium hydrodictyi* A. Braun. In Mycobank the number is 159314 and it is included as a synonym of the currently accepted taxonomic name *Chytridium hydrodictyi* A. Braun;

9) *Polyphagus euglenae* (Bail.) Nowak (as "*Polyphagus euglenae* Now.") – found in the cultivated cysts of the flagellate algae *Euglena viridis* (O. F. Müller) Ehrenberg (division Euglenophyta) collected in the towns Sofia and Plovdiv (Valkanov 1931a). In the materials from Plovdiv, similar parasite was observed in the cysts of *Chlamydomonas* sp. (division Chlorophyta), but its belonging to *P. euglenae* was not proved (Valkanov 1931a). Index Fungorum Registration Identifier 208842 and number 208842 in Mycobank;

10) *Polyphagus fomini* Milovtz. – recorded from the fishponds near Plovdiv (Valkanov 1963b). Index Fungorum Registration Identifier 585378. In Mycobank the number is 575378;

11) *Rhizophydium mammilatum* (A. Braun) Fischer (as "*Rhizophidium mammilatum* (A. Braun) Fischer") – recorded in the yellow-green alga *Vaucheria* (as "*Conferva* sp.") from the town of Sofia (Valkanov 1931a). Included in Index Fungorum with an Index Fungorum Registration Identifier 439011 with synonym *Chytridium mammilatum* A. Braun and as *Chytridium mammillatum* A. Braun as current accepted name in MycoBank under number 177701;

12) "*Rhizophidium* v. *mindeni*" - parasite in the oogonia of the green alga *Oedogonium* sp. (division Chlorophyta) collected in the town Plovdiv (Valkanov 1931a). According to Sparrow (1960, p. 304) the fungus, found by Valkanov (1931a) is "probably referable to *Chytridium chaetophilum* or *Rhizophydium chaetiferum*". According to the same author (op. cit., p. 509) the identity with *C. chaetophilum* Scherfell could be supposed in a hardly possible way due to the finding of empty sporangia without rhizoids. This taxon is not included in Index Fungorum and in MycoBank;

13) *Rhizosiphon anabaenae* (Rodhe & Skuja) Canter - reported only from the mountain microreservoir Studena near the town Pernik as rarely developed on some

of the trichomes of the planktonic filamentous blue-green alga *Sphaerospermopsis aphanizomenoides* (Forti) Zapomelová, Jezberová, Hrouzek, Hisem, Reháková et Komárková in the summer of 2021 (Stoyneva-Gärtner et al. 2023). Index Fungorum Registration Identifier 336425. Number 338509 in Mycobank;

14) *Urceomyces sphaerocarpus* (Zopf) Letcher (Syn. *Rhizophidium sphaerocarpum* (Zopf.) Fischer – as "*Rhizophidium sphaerocarpum* (Zopf.) Fischer") – from the green filamentous alga *Mougeotia* sp. (division Streptophyta) collected in the town Plovdiv (Valkanov 1931a). Sparrow 1960) excluded the material of Valkanov (1931a) from this species due to well-developed and strongly branched rhizoids. Index Fungorum Registration Identifier 356847 and number 540858 in Mycobank.

Phyllum Entomophtoropmycota
Recorded species:

1) *Ancylistes closterii* Pfitzer – found as a parasite in the coccal green alga *Closterium ehrenbergii* (division Streptophyta) in a sample from the vicinity of Sofia (Valkanov 1931a). Arnaudow (1936) underlined the presence of infective hyphae in this fungus. Index Fungorum Registration Identifier 212068 and 212068 number in Mycobank.

Phyllum Zoopagomycota
Recorded species:

1) *Zoophagus insidians* Sommerst. – found in the swamp Dragichevsko Blato on Lyulin Mts near the town Pernik by Arnaudow (1921, 1925, 1936), and Arnaudow & Damowa (1948). Valkanov (1931a, p. 366) found it "twice in the vicinity of Sofia" and "cultivated it for months in the laboratory". In the same paper, Valkanov underlined the carnivorous mode of life of this fungus. In the same year, Valkanov (1931b) published additional comments on this fungus, outlining its capturing hyphae, again stressing on its carnivorous nature. Predation on rotifers with detailed description of their capturing, observed in long-lasting cultures, was described in detail by Arnaudow & Damowa (1948, p. 45), who noted the similarity of the rotifers captured by *Zoophagus* and *Sommerstorffia* (from the genera "*Monostyla, Cathypna, Ratulus, Lepadella, Diarella, Colurus* and *Salpina*"), but mentioned also capture of individuals from Oligochaeta. Their text to Fig. 5 on p. 152 explained that "*Zoophagus* used *Cladophora* as a support for the mycelium". In the description and discussion of this species the data and comments of Arnaudow (1921, 1925) usually have been taken into account in addition to the original description by Sommerstorf (Sparrow 1960). This especially concerns the finding of dioecism (heterothallism) with description of sexual organs and the detailed description of the system of well-developed hyphae with numerous short, upright peglike branches with mucilage secretion on the tips for capturing of rotifers (Arnaudow 1921, 1925). *Zoophagus insidians* is included in this review

conditionally since in his manual of aquatic phycomycetes Sparrow (1960, p. 1027) noted it as a "predacious parasite of freshwater rotifers". In the compendium on the studies of the cryptogams in Bulgaria, Petkoff (1939) especially underlined the contributive research on the morphology, reproduction and ecology of this predator conducted in Bulgaria by Prof. Nikola Arnaudow, a recognized specialist in carnivorous fungi. Index Fungorum Registration Identifier 209401 and 209401 number in Mycobank.

Phylum Oomycota

New genera:

1) *Hydatinophagus* Valkanov 1931– This new genus was discovered in February 1925 in a canal in the pine forest of Sofia Park "Borisova Gradina" as a parasite of the rotifer *Hydatina senta* O. F. Müller, 1773 (Valkanov 1931b). The author provided diagnosis of this monotypic genus only in Bulgarian language (without designation of the holotype and type locality), which is translated in the German summary of the paper, but without using the word "diagnosis". In a subsequent paper, Valkanov (1932) underlined that the same parasite was first mentioned by him under the name "*Aphanomyces hydatinae* n. sp." with a note that detailed description follows (Valkanov 1931a). *Hydatinophagus* was briefly mentioned by Sparrow (1960) only once, based on Valkanov (1932), in relation to its close taxonomic position, together with *Sommerstorffia* and *Synchaetophagus,* with *Zoophagus*. Index Fungorum Registration Identifier 20269.

2) *Sommerstorffia* Arnaudov 1923 (Arnaudow 1923a, b) - Index Fungorum Registration Identifier 20530. Included here conditionally since the genus was described as predacious with capturing hyphae and although its carnivorous mode of life was again stressed by the author (Arnaudow 1936), later it was commonly considered to be "parasitic in bodies of rotifers" (Sparrow 1960, p. 844).

New species:

1) *Hydatinophagus apsteinii* Valkanov 1931 (Syn. *Aphanomyces hydatinae* Valkanov 1931) – parasite of the rotifer *Hydatina senta* O. F. Müller, 1773 found for first time in February 1925 in a canal located in the pine forest of the Sofia Park "Borisova Gradina" (Valkanov 1931b). Since then, there this fungus was found regularly as abundant on the same rotifers (Valkanov 1931b). The author provided detailed description of the species and supplied it with in a combined generic and species diagnosis in Bulgarian language, without providing a holotype and type locality, but explaining the etymology of the species epithet. The same diagnosis is translated in the German summary of the paper, but without using the word "diagnosis". Valkanov (1931b) especially noted that the most important for the life cycle of this fungus and of the rotifer was the late winter period, February-March, when the snow was melting. Later, Valkanov (1932) stressed that the same parasite was published earlier by him under the name *Aphanomyces hydatinae* n. sp. (Valkanov 1931a, c). In Valkanov (1931a) it was written only

that this new species was found as a parasite in the rotifer in Sofia, but it was included with a question mark and a note that it will be described in detail in a subsequent publication. In this subsequent paper (Valkanov 1931c) detailed description regarding morphology and biology of the fungus was provided and was illustrated by 16 figures. Based on these two publications, the species was included in Sparrow (1960). Valkanov (1931b,c) mentioned also that the same fungus was observed earlier in the vicinities of Sofia by Bulgarian zoologist Prof. Stefan Konsuloff, who reported it in a short communication as *Pythium* sp. (Konsuloff 1908). However, we did not find such a text in the cited paper, which was published in 1916 and not in 1908. There, Konsuloff (1916) described in detail a parasite of *Hydatina senta* and *Brachionus calyciflorus* subsp. *calyciflorus* Pallas, 1766 (Syn. *Brachionus pala* Ehrenberg, 1838) and designated it as a "*Botellus* sp. (?)" due to lack of different developmental stages. Comparisons between *Aphanomyces* and *Zoophagus* are available in Arnaudow (1936), and their importance was underlined by Petkoff (1939) in his compendium on the studies of cryptogams in Bulgaria. Arnaudow (1936) documented peculiar, singular case of saprotrophic feeding of *Aphanomyces* on nearby lying dead rotifers, which were not captured by the fungus and according to the author, chemotactic attraction of the hyphae could be supposed. However, Arnaudow (1936) stressed the occasional character of such untypical behaviour of this parasitic fungus. According to the Editorial comment in Index Fungorum regarding *Aphanomyces hydatinae* the "generic name in this combination is not considered to apply to an organism within the fungal clade" (Index Fungorum Registration Identifier 252637). The number of *A. hydatinae* in Mycobank is 252637. The information provided above allows us to speculate that including *Aphanomyces hydatinae* as a separate taxon with a legitime status in both Index Fungorum and Mycobank is a misinterpretation and *A. hydatinae* should be taken as a synonym of *Hydatinophagus apsteinii*. Obviously, similar understanding had Batko (1975), who included in his hydromycological handbook *Hydatinophagus apsteinii* as a synonym of *Aphanomyces hydatinae*. Regarding *Hydatinophagus apsteinii*, the Editorial comment in Index Fungorum states that the "generic name in this combination is not considered to apply to an organism within the fungal clade" (Index Fungorum Registration Identifier 224101). The number in Mycobank is 224101.

Considering all data collected above and taking into account the opinion of the author – Alexander Valkanov, who changed his mind and transferred *Aphanomyces hydatinae* to *Hydatinophagus apsteinii* and supplied it with a Bulgarian diagnosis, we decided to translate its original Bulgarian diagnosis in English in order to legitimate the name of the monotypic genus *Hydatinophagus* and its species *Hydatinophagus apsteinii*, keeping the authorship of Valkanov (1931b) and to accept *Aphanomyces hydatinae* as its synonym.

***Hydatinophagus apsteinii* n.g., n. sp.**

Diagnosis: The development of the fungus starts in the stomach of the host. The

mycelium is widely branched, fulfilling the entire host body cavern: the ends of the hyphae extend out of the host coverage and turn into non-differentiated sporangia; spores arranged in a single raw in the sporangium; when coming outside they became arranged around the opening of the sporangium and encyst. A secondary zoospore generation follows. Oogonia terminal and intramatrical, antheriadia formed on both male and female hyphae, male hyphae being thinner. Oospore single per oogonium, with a smooth cell wall and contains a large, excentric lipoid droplet. A parasite in *Hydatina senta*.

Holotype: Iconotype: figs 1-11 in Annual of Sofia University, Physico-Mathematical Faculty, Book 3, Volume 27, pp. 216-222 (Valkanov 1931c).

Type locality: Canal in a pine forest in the Sofia Park "Borisova Gradina"

Etymology: The generic name is related with the parasitic mode of life and the host, whereas the species epithet is given in honour of Prof. K. Apstein.

2) *Hyphochytrium hydrodictyi* Valkanov 1929 (as "*Hypochytrium hydrodictii*") – described as an abundantly developed parasite in the young cells of the green coccal alga *Hydrodictyon reticulatum* (L.) Bory (division Chlorophyta) collected from Sofia (Valkanov 1929, 1931a). The zoospores of this species have been described to bear an anterior flagellum (Valkanov 1929), which, in the opinion of Sparrow (1960) made necessary its segregation from the true chytrids, where it was originally placed by the author. Currently, in Index Fungorum it is positioned in Oomycota, where according to the Editorial comments the "generic name in this combination is not considered to apply to an organism within the fungal clade" (Index Fungorum Registration Identifier 267497). The species is included in MycoBank with number 267497;

3) *Sommerstorffia spinosa* Arnaudow 1923 – found in the swamp Dragichevsko Blato, situated on Lyulin Mt near village Dragichevo in the vicinity of the town Pernik as developed together with *Zoophagus* and inevitably described as a predator fungus (Arnaudow 1923a, b). Later, the carnivporous mode of life was stressed again by Arnaudow (1936) and Arnaudow & Dimova (1948). Arnaudow & Damowa (1948, p. 45) followed in detail the capture of rotifers from the genera "*Monostyla, Cathypna, Ratulus, Lepadella, Diarella, Colurus* and *Salpina*" and in the text to Fig. 5 on p. 152 explicitly wrote that "*Zoophagus* used *Cladophora* as a support for the mycelium". Sparrow (1929) recorded *S. spinosa* and depicted "thallus epiphytic on an alga, bearing three capturing organs and a parasitized rotifer from which a zoosporangium emerges" (Sparrow 1960, fig. 66, pp. 843-844). According to the text on host substratum in Index Fungorum *S. spinosa* was found "on *Cladophora* sp." (Index Fungorum Registration Identifier 278613). However, in our opinion, considering the original data, this green siphonocladal alga from the division Chlorophyta cannot be taken as a host. *S. spinosa* is included with the number 278613 in Mycobank.

Recorded species:

1) *Ducellieria chodatii* (F. Ducell.) Teiling – found on the pine pollen grains

collected in a field sample from the glacial lake Popovo Ezero, Pirin Mt (Stoyneva et al. 2013). Before this reported as the green coccal alga *Coelastrum chodatii* Ducellier (division Chlorophyta) altogether four times for mountain water bodies of different types and size, surrounded by conifers: (1) a small swamp among *Picea abies* and *Pinus mugo* near the rivulet Urdina Reka and (2) peat bogs near the rivulet Marinovitsa in Rila Mts (Wodenitscharov 1960a, b, 1962), (3) mountain lakes Smolyansko Ezero and (4) Chairski Ezera in Rodopi Mts (Kiriakov 1981). On the bases of the first data the species was included in the Bulgarian algal flora as known from Rila Mts (Vodenicharov et al. 1971). According to the Editorial comment in Index Fungorum the "generic name in this combination is not considered to apply to an organism within the fungal clade" (Index Fungorum Registration Identifier 477272). The reclassification to a separate clade in Oomycota was currently demonstrated by Buaya & Thines (2023);

2) *Myzocytium proliferum* (Schenk.) J. Shroeter (as "*Mysocytium proliferum* Schenk.") – found in the green filamentous alga *Spirogyra* sp. (division Streptophyta) collected from Sofia (Valkanov 1931a). According to the Editorial comment in Index Fungorum the "generic name in this combination is not considered to apply to an organism within the fungal clade" (Index Fungorum Registration Identifier 428924). In Mycobank "*Myzocytium proliferum* (Schenk.) Schenk." is included with number 229095, *Mysocytium proliferum* var. *proliferum* without authors name is included with number 420450, and *Myzocytium proliferum* f. *proliferum* without authors name is under number 428924;

3) *Myzocytium rabenhorstii* (Zopf.) M. W. Dick (as Syn. *Lagenidium rabenhorstii* Zopf.) – recorded in the green filamentous algae *Mougeotia* sp. and *Spirogyra* sp. (both from the division Streptophyta) collected in the towns Sofia and Plovdiv (Valkanov 1931a). According to the Editorial comment in Index Fungorum the "generic name in this combination is not considered to apply to an organism within the fungal clade" (Index Fungorum Registration Identifier 483157). Number 483157 in Mycobank;

4) *Olpidiopsis saprolegniae* (A. Braun) Cornu (as Syn. *Pseudolpidium saprolegniae* (A. Braun) A. Fisch.) – reported as a parasite in *Saprolegnia* species from the towns Sofia and Plovdiv" (Valkanov 1931a). Although *Pseudolpidium saprolegniae* is considered synonymous to *Olpidiopsis saprolegniae*, Sparrow (1960) in his manual of aquatic phycomycetes enlisted separately the materials identified as *Pseudolpidium*, including the material described by Valkanov (1931a). According to the Editorial comment in Index Fungorum the "generic name in this combination is not considered to apply to an organism within the fungal clade" (Index Fungorum Registration Identifier 228583). In Mycobank, *Psedolpidium saprolegniae* is included with number 204292 and *Olpidiopsis saprolegniae* (A. Braun) Cornu – with number 228583.

5) *Pythium diclinum* Tokun (as Syn. *Pythium gracile* Schenk.) – observed in the green algae from the genera *Hydrodictyon* (Chlorophyta) and *Spirogyra*

(Streptophyta) collected in the towns Sofia and Plovdiv (Valkanov 1931a). According to the Editorial comment in Index Fungorum the "generic name in this combination is not considered to apply to an organism within the fungal clade" (Index Fungorum Registration Identifier 154248). Number 263154 in Mycobank;

6) *Sphaerita endogena* P. A. Dang. – found in the flagellate *Euglena viridis* and other *Euglena species* (phylum Euglenophyta) in cultures obtained from the natural material collected in the towns Sofia and Plovdiv (Valkanov 1931a). In the opinion of Sparrow (960), the material of Valkanov (1931a) and the other material from *Euglena* had to be referred to *Sphaerita dangeardii* Chatton et Brodsky (Index Fungorum, Index Fungorum Registration Identifier 477283), for which he proposed as synonym *Sphaerita endogena* Dang. p.p. (in *Euglena*). According to the Editorial comment in Index Fungorum the "generic name in this combination is not considered to apply to an organism within the fungal clade" (Index Fungorum Registration Identifier 238302). Number 238302 in Mycobank.

Phylum Plasmodiophoromycota

Recorded species:

1) *Woronina glomerata* (Cornu) A. Fisch. – found as parasite in the yellow-green siphonal alga *Vaucheria* sp. (phylum Ochrophyta, class Xanthophyceae) in field material obtained from the town Plovdiv and from the vicinity of the town Chirpan (Valkanov 1931a). According to the Editorial comment in Index Fungorum the "generic name in this combination is not considered to apply to an organism within the fungal clade" (Index Fungorum Registration Identifier 477270). The species is included in Mycobank with number 477270.

II. Zoosporic parasites in mass algal cultures in Bulgaria

Phylum Chytridiomycota

Recorded species:

1) *Rhizophydium scenedesmi* (Fott) Karling (Syn. *Phlyctidium scenedesmi* Fott) – in cultures of the green coccal algae *Tetradesmus obliquus* (Turpin) M. J. Wynne (Syn. *Scenedesmus acutus* Meyen) and *Tetradesmus inrassatulus* (Bohlin) M. J. Wynne (Syn. *Scenedesmus incrassatulus* Bohlin) (division Chlorophyta) (Puneva 1985, Puneva et al. 1998, Puneva & Toncheva-Panova 1995, Puneva 2006, Puneva & Christov 2004, 2006); Index Fungorum Registration Identifier 480416. Number 480758 in Mycobank.

III. Zoosporic parasites in industrial waters

Phylum Chytridiomycota

Recorded species:

1) *Rhizophydium scenedesmi* (Fott) Karling (Syn. *Phlyctidium scenedesmi* Fott) – on the green alga *Tetradesmus obliquus* (Syn. *Scenedesmus acutus*) in a waste waters from a paper factory (Monchev et al. 1988; Puneva & Monchev 1997);

Index Fungorum Registration Identifier 480416. Number 480758 in Mycobank.

DISCUSSION

The historic publications on aquatic zoosporic parasites in Bulgaria could be divided in three main periods:

1) 1908-1971 – 24 papers containing data on 34 taxa belonging to five phyla (Chytridiomycota, Entomophtoromycota, Zoopagomycota, Oomycota and Plasmodiophoromycota) documented mainly from natural habitats or from laboratory cultures. In the period 1908-1916 four papers on rotatorian parasites were published by Prof. Stefan Konsuloff (1885-1954). Unfortunately, due to political reasons, many scientific publications of this zoologist have not been cited and are difficultly available even nowadays. Since 1921 the publications on zoosporic parasities were issued by the prominent Bulgarian professors Alexander Valkanov (1904-1971) (with different writing of his family in scientific publications as Valkanov and Valkanow) and Nikola Arnaudov (1887-1963) (as Arnaudow in the foreign scientific publications). Their papers have been discussed by Sparrow (1929, 1960), Batko (1970, 1975), Karling (1977), Doweld (2014), Danz & Quandt (2023), etc. and have been mentioned in the summaries by Denchev & Bakalova (2002), Denchev (2007) and Stoyneva & Temniskova-Topalova (2007);

Prof. Stefan Konsuloff (1885-1954)

Prof. Dr Alexander Valkanov (1904-1964)

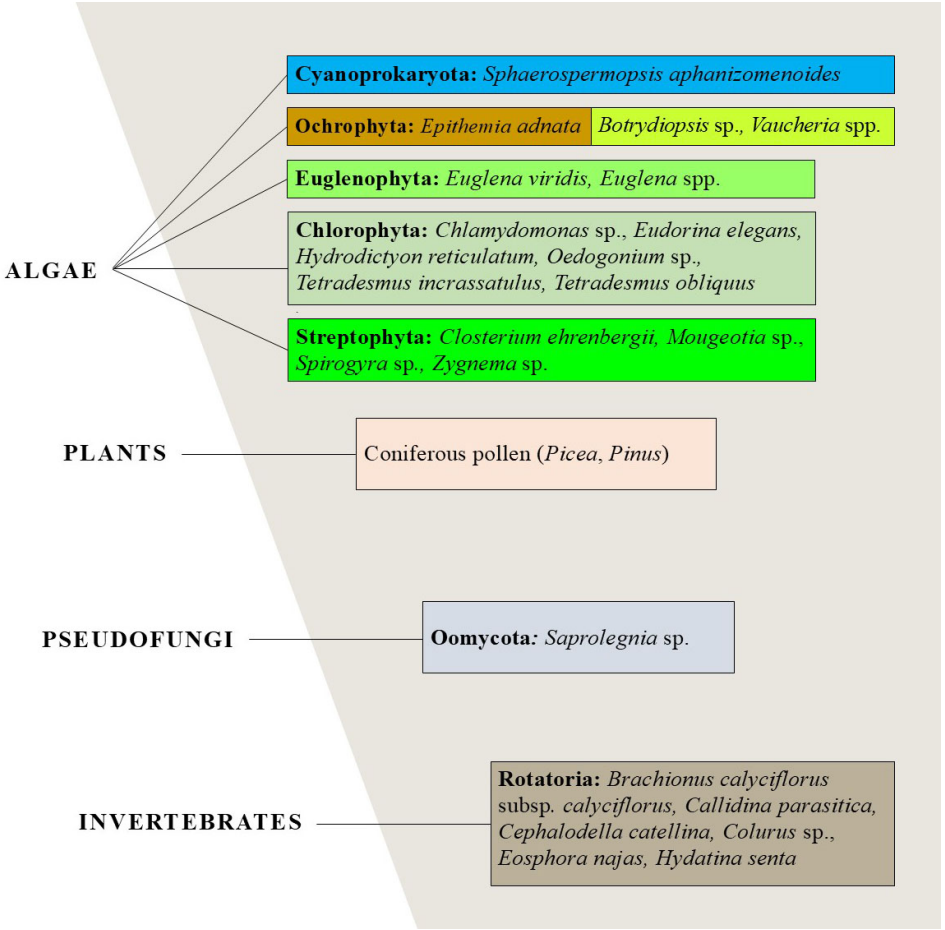
Prof. Nikola Arnaudov (1887-1961)

In the period 1960-1971 four papers with data on the spread in mountain wetlands of the oomycete *Ducellieria*, considered at that time as a green alga *Coelastrum*, were published by the algologists Prof. DrSc Dimitar Vodenicharov (1929-2011) (with different writing of his name in scientific journals as Vodenicharov and Wodenitscharov) and Prof. Dr Ivan Kiriakov (1943-2019);

2) 1985-2006 – nine papers on the chytid *Rhizophydium scenedesmi*, a parasite in mass algal cultures and waste waters, published by the Bulgarian algologist Assoc. prof. Dr Irina Puneva and her co-authors, all mentioned in the summary by Stoyneva & Temniskova-Topalova (2007).

3) 2013-2023 - current published data on one oomycete and one chytrid in Bulgarian wetlands are available from two papers by the authors of this overview (Stoyneva et al. 2013; Stoyneva-Gärtner et al. 2023, respectively). The first of them has been discussed in Buaya & Thines (2023), who confirmed the development of *Coelastrum*-like form in this parasite but revised its provisional placement in the Leptomitales by Dick (2001) and assigned it to its own order, Ducellierales (MycoBank MB 849453).

Although there are different opinions about the taxonomic affiliations of the reported aquatic zoosporic pathogens, according to classification in Index Fungorum, studies in Bulgaria covered the zoosporic parasites from the phyla Chytridiomycota, Entomophtoromycota, Zoopagomycota, Oomycota and Plasmodiophoromycota. Altogether, four new genera, eleven new species and one new variety have been described from field material obtained from different, but only freshwater Bulgarian wetlands. Up-to-now, in the country, only *Rhizophydim scenedesmi* was reported from mass algal cultures and waste-water material. Total number of algal hosts identified is at least 15 (**Figure 1**), most of which belong to the green evolutionary line – ten species from nine genera, out of which six are from Chlorophyta (*Chlamydomonas* sp., *Eudorina elegans*, *Hydrodictyon reticulatum*, *Oedogonium* spp., *Tetradesmus obliguus*, *Tetradesmus incrassatulus*) and four are from Streptophyta (*Closterium ehrenbergii*, *Mougeotia* spp., *Spirogyra* spp., *Zygnema* spp.). At least three hosts are from the yellow-brown evolutionary line, Ochrophyta, spread in classes Bacillariophyceae (*Epithemia adnata*) and Xanthophyceae (*Botrydiopsis* sp., *Vaucheria* spp.). From prokaryotic algae, only the cyanoprokaryote *Sphaerospermopsis aphanizomenoides* has been shown as a host, and from the mesokaryotic algae, hosts are different species from the genus *Euglena*, among which only *Euglena* viridis was identified. Here we do not consider the numerous euglenophytes and two chlorophytes captured by carnivorous fungi. According to the morphological types of the hosts, most infected were the coccal algae from different groups (*Botrydiopsis* sp., *Closterium ehrenbergii*, *Epithemia adnata*, *Hydrodictyon reticulatum*, *Tetradesmus obliguus*, *Tetradesmus incrassatulus*) followed by filamentous algae (*Mougeotia*, *Oedogonium*, *Sphaerospermopsis*, *Spirogyra*, *Zygnema*) and flagellates (*Eudorina elegans*, *Chlamydomonas* sp., *Euglena viridis*, *Euglena* spp.), with single hosts from the siphonal algae (*Vaucheria* spp.). It is noteworthy to mention, that zoosporic parasites live in algal vegetative cells, in reproductive cells (oogonia, oospores) and in resting stages (cysts). According to the ecological affiliations, hosts cover the ecological groups of neuston (hyponeuston), phytobenthos and phytoplankton. Considering pollen, data concern only coniferous pollen (*Picea*, *Pinus*), which is



widely spread in the alpine regions where the studied glacial lakes and peat bogs were located (**Figure 1**). The single host from the large kingdom of Fungi, in which

Fig. 1. Distribution of hosts of zoosporic parasites (for algae they are organized in the main taxonomic groups, shown with relevant colours (Cyanoprokaryota – blue-green, Ochrophyta, Bacillariophyceae – brown, Xanthophyceae – yellow-green, Euglenophyta – green; Streptophyta – bright green, and Chlorophyta – light green).

zoosporic parasite was found, was the pseudofungus *Saprolegnia* sp. (**Figure 1**). The invertebrate hosts include freshwater planktonic *Rotatoria* such as *Brachionus calyciflorus* subsp. *calyciflorus*, *Callidina parasitica, Cephalodella catellina*, *Colurus* sp., *Eosphora najas* and *Hydatina senta* (**Figure 1**).

Comparison of phyla shows that more parasites on more hosts were found among Chytridiomycota (23) than in Oomycota (9), while the phyla Entomophtoromycota, Zoopagomycota and Plasmodiophoromycota were represented by single species. Regarding ecology, it has to be noted that different opinions exist about the

predacious or parasitic mode of life, as well as about saprotrophic *vs* parasitic character of some of the discovered fungi and more investigations in this respect are needed. In relation to habitats, less studies cover the aquacultures and wastewaters, whereas most data concern field material and all recorded zoosporic parasites were from different freshwaters (pools, canal, swamps, peat bogs, lakes, fishponds, microreservoir) in the regions of towns Sofia, Plovdiv, Pernik, Chirpan and in the mountains Pirin, Rila, Rodopi, Vitosha and Lyulin, as well as near to the River Danube. According to our best knowledge, there are no data on Bulgarian Black Sea waters and on our coastal mesohaline, euhaline or hyperhaline wetlands. This brief analysis of the published studies shows that reported habitats and hosts cover a very small part of the country (**Figure 2**) and cannot show the real spread of aquatic zoosporic parasites in Bulgaria.

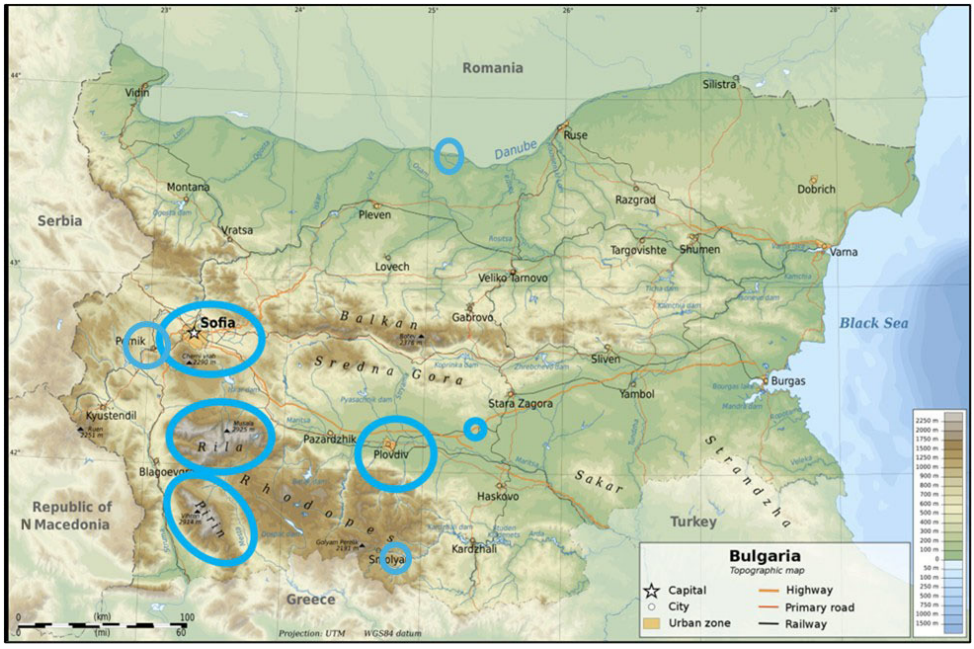


Fig. 2. Regions of Bulgaria with the localities, in which zoosporic parasites have been found by different authors (for details see the text of the paper), shown by blue circles (map modified after https://en.m.wikipedia.org/wiki/File:Bulgaria-geographic_map-en.svg). Note: The locality "swamp near the River Danube" in Valkanov (1931a) cannot be correctly identified and, therefore, is included provisionally in the middle of the river region.

Therefore, future purposive studies in different wetland types, as well as in more different localities of different altitudes and country regions are necessary to reveal the real biodiversity of these organisms and their hosts in Bulgaria.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests regarding the publication of this article.

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