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CONTENTS

ГОДИШНИК НА СОФИЙСКИЯ УНИВЕРСИТЕТ „СВ. КЛИМЕНТ ОХРИДСКИ“

БИОЛОГИЧЕСКИ ФАКУЛТЕТ
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CURRENT LIVING ALGAL CULTURE COLLECTIONS

KRISTIAN R. IVANOV*

Sofia University “St. Kliment Ohridski”, Faculty of Biology, Department of Botany, 8 Dragan Tsankov Blvd., BG-1164, Sofia, Bulgaria

Abstract. The paper provides a summary on 72 living algal collections, most of which are registered in the World Federation of Culture Collections (WFCC), the European Culture Collection Organisation (ECCO), or in the World Data Centre for Microorganisms (WDCM). The Collections are briefly described and represented in alphabetical order according to their distribution in Asia (17), Australia (2), Europe (41), North (11) and South America (5).

Keywords: algae, biodiversity, bioresources, culture collections, list of collections, survey,

INTRODUCTION

Living algal collections represent a reservoir of different algal species and strains kept in small amounts in liquid cultures or on agar media. Their purpose is to preserve biodiversity by keeping algae in controlled centers, away from environmental changes. In this way, in case of environmental changes that threaten the certain species, collections can be used to restore the biodiversity. Another main purpose of collections is to ensure scientific research, educational, industrial and other purposes with the species deposited in them because the great biotechnological potential of algae and their use in human life have been shown during the last decades

* *corresponding author*: K. R. Ivanov - Sofia University “St. Kliment Ohridski”, Faculty of Biology, Department of Botany, 8 Dragan Tsankov Blvd., BG-1164, Sofia, Bulgaria; kristianri@uni-sofia.bg

(Friedl et al. 2004). The future of the collections is focused on increasing their number worldwide and finding longer-term ways to preserve pure algal cultures. One such method is freezing with liquid nitrogen, a method that is currently mainly used for the storage of cell and tissue cultures but is increasingly used for plants and algae. Such collections are significant for biodiversity conservation, but also for development of scientific research and industry (Friedl et al. 2004). However, they are rarely covered in publications (Yuorieva et al. 2023).

Up to date a unified register for culture collections worldwide does not exist. Therefore, they are registered in different organizations like WFCC – World Federation of Culture Collections, ECCO – European Culture Collection Organisation and WDCM – World Data Centre for Microorganisms. The present paper provides summarized available data on living algal collections in the world.

Collections are represented in an alphabetical order according to their distribution in Asia, Australia, Europe, North and South America (**Figure 1**).

Fig. 1. The location of the living algal collections (dots) in the world.

Culture collections located in Asia:

1. **ALEC** - Algal Excellent Center (https://alec-tech.com/)
This is a repositorium of an agency in Thailand with more than 25 years of experience in research, development, technology transfer and services related to freshwater microalgae in the industrial sector according to the recognized potential of microalgae, which have an increasingly important role in various aspects. ALEC contains more than 1000 strains collected from different places all over Thailand. The center aims to conserve and sustainably use aquatic resources, but also to

research, develop and take things to the private sector. The center offers various services, provision of algal strains, species classification, microcystin assays, cultivation, consultation and more. ALEC is registered in WDCM with number 1218.

2. **CCCBMA-BT/WUSL -** Culture Collection of Cyanobacteria and Microalgae Department of Biotechnology Wayamba University of Sri Lanka
The collection is positioned in the Department of Biotechnology, Faculty of Agriculture & Plantation Management at Wayamba University of Sri Lanka in Makandura, Sri Lanka. It has more than 20 strains of the phyla: Cyanoprokaryota, Chlorophyta and Ochrophyta (Bacillariophyceae) isolated from numerous aquatic and terrestrial habitats in seven districts of Sri Lanka (Balsooriya 2019). The collection is registered at WFCC with number 1277.

3. **Collection of Marine Microalgae at the A. V. Zhirmunsky Institute of Marine Biology**
The collection is at A. V. Zhirmunsky Institute of Marine Biology of Russian Academy of Sciencen (NSCMB), based in Vladivostok, Russian Federation, under the Far Eastern Branch of Russian Academy of Sciences. The collection was opened in 1985 and became the first collection of marine microalgae in Russia. It started with 5 species. Over the years, throughout exchanging strains with other collections and isolations from the marine samples the number of species increased 23, from different systematic groups (Aizdaicher 2008).

4. **DBUP** – Algal Culture Collection of the University of Philippines (https://mnh.uplb.edu.ph/microbial-algal-culture-collection/)
This collection is positioned in the Museum of the Natural History of the University of Philippines Los Banos (UPLB) in Laguna, Philippines. The work on it began in the 1980s and for now has 50 strains of microalgae. The collection is registered in WFCC with number 444.

5. **EGE-MACC** – Ege University Microalgae Culture Collection (https://egemacc.ege.edu.tr/)
The collection is at Ege University, Turkey. It was created in 2004. Currently, it has about 100 strains of algae derived from different regions of Turkey (Atici 2020). The role of the collection is to preserve the algal resources of the country.

6. **FACHB - Freshwater Algae Culture Collection Chinese Academy of Sciences** (http://english.ihb.cas.cn/research/platforms/npmp/202012/t20201202_255451.html)
FACHB is the main algal collection in China (Song et al. 2014) and is located at the Institute of Hydrobiology of the Chinese Academy of Sciences in Wuhan,

Hubei, China. The collection was established in the 1960s. It contains more than 2,000 strains belonging to 120 genera and 8 divisions, most of which are isolated from numerous freshwaters and less are marine and brackish species, or soil algae (Song et al. 1999). Some of the strains are obtained through exchange with Algal Culture Collection of University of Texas (UTEX). FACHB is registered in WDCM with number 873.

7. **GAZI-MACC** - Gazi University Microalgae Culture Collection (https://gazi.edu.tr)
This collection is positioned in Gazi University in Ankara, Turkey. It provides biological resources for academic studies and research at biotechnology. At the same time it serves a role as a genetic conservation centre of the national algal species (Atici 2020).

8. **IBRC** – Iranian Biological Resources Center (https://www.acm-mrc.asia/index.html)
IBRC was opened in 2008 in Tehran, Iran under the direction of Academic Center for Education, Culture and Research. It has the role of a center at national and international level in areas such as collection, identification, quality control, classification, preservation, cultivation and distribution of various biological species, not only algae. The collection is registered in WDCM with number 950.

9. **IRK–A** – Algae Culture Collection of SIPPB SB RAS (http://www.sifibr.irk.ru/en/)
The collection is part of the Siberian Institute of Plant Physiology and Biochemistry of the Siberian Branch of the Russian Academy of Sciences (SIPPB SB RAS) in Irkutsk, Russia. It started working in 2003 to study the algae in the terrestrial ecosystems. For now, the collection has 165 strains: Cyanoprokaryota – 13, Chlorophyta – 130, Streptophyta – 9, and Ochrophyta – 13. The strains are isolated from different parts of the country.

10. **KCTC** - Korean Collection for Type Cultures (https://kctc.kribb.re.kr/en)
This collection is positioned in the Research Institute of Bioscience and Biotechnology in Jeonbuk, South Korea. It has start working in 1985 and has more than 200 strains. The collection provides live resources for scientific, academic and industrial studies. KCTC is registered in WFCC with number 597.

11. **KMMCC** – Korea Marine Microalgae Culture Center
The collection is located at Pukyung National University in Busan, South Korea. It was established in 1995. Currently the collection has more than 2200 strains of about 700 species from 233 genera. About 80% of the strains are marine, 16% are freshwater and 4% are brackish. Many of the strains are cryopreserved (Hur

2008, Hur et al. 2015).

12. **KU-MACC** – Kobe University Macroalgal Culture Collection (https://ku-macc.nbrp.jp/)
This collection is at Kobe University, Hyogo, Japan. It has been established in 2003 and holds more than 1100 strains of more than 300 species of macroalgae (Rhodophyta, Ulvophyceae, Charophyceae, Schizocladiophyceae, Phaeophyceae). The strains are kept as vegetative thalli and as preserved by means of cryopreservation (Kawai et al. 2020).

13. **NAIMCC** - National Agriculturally Important Microbial Culture Collection (https://nbaim.icar.gov.in/)
NAIMCC, established in 2004, is a part of the ICAR - Indian Institute of Seed Science and Technology, Mau, India. It is focused on microorganisms that are important for agricultural crops throughout the country. More than 7,000 strains of fungi, bacteria, actinomycetes and cyanobacteria (known also as blue-green algae, or cyanoprokaryotes), are deposited in it. The aim of the collection is to maintain, conserve and characterize various species collected from all over the country from different habitats and to use them in different research programs to establish the relationships between different species, which will affect agricultural communities. NAIMCC also provides strains for different genomic studies and bioinformatics. The collection is registered in WDCM with number 1060.

14. **NIES** - Microbial Culture Collection at the National Institute for Environmental Studies (https://mcc.nies.go.jp/index_en.html)
NIES is located in the National Institute for Environmental Studies in Tsukuba, Japan. It was founded in 1983 with a focus on organisms that cause environmental problems, such as the eutrophication of water bodies. Currently, there are more than 2000 strains, out of which about 300 are from endangered algae (Charales and red freshwater algae). The collection contains strains of cyanobacteria and eukaryotic algae. They are all maintained and available for educational and research purposes (Kasai et al. 2009). The collection accepts all strains of rare species, but also strains of common species and of species with proved application. NIES is registered in WDCM with number 591.

15. **NRMC** - National Repository for Microalgae and Cyanobacteria (https://www.bdu.ac.in/centers/NRMC-F/index.php)
NRMC was established in 2015 in Bharathidasan University suited in Tiruchirappalli, India. It aims to conduct an extensive survey of freshwater basins in southern India in order to reveal the spatial and temporal diversity of microalgae and cyanobacteria, as well as to clarify the ecological factors that influence them. Along with this, NRMC is focused on the biotechnological application of the cultivated algae, such

as biofuel production, extraction of proteins, pigments and production of various biologically active substances. The idea of the collection is to become a world center for different algal strains and to be offered to different organizations for educational, research and industrial purposes. NRMC is registered in WDCM with number 976.

16. **TISTR Culture Collection -** Thailand Institute of Scientific and Technological Research Culture Collection (https://www.tistr.or.th/tistr_culture/index.php)
The TISTR Culture Collection known also as Bangkok MIRCEN (World Network of Microbiological Resources Centres) is located in Klong Luang, Pathum Thani, Thailand. It was created in 1976. This collection has more than 5000 strains of bacteria, fungi, yeasts and about 430 strains of microalgae. It is oriented mainly to the biotechnology potential of the cultures and provides safe deposits, identification, isolation, preservation and documentation of the strains, but also training services.

17. **UMACC** – University of Malaya Algae Culture Collection
UMACC is located in The University of Malaya, Malaysia. It has been created in 1987 and has more than 200 strains derived from different habitats (marine, brackish, freshwater and aero-terrestrial) from Malaysia and from the polar region (Phang et al. 2004). The strains are used to study antiviral and anti-inflammatory effects. The collection is registered in WFCC with number 1059.

Culture collections located in Australia:

1. **ANACC -** Australian National Algae Culture Collection (https://www.csiro.au/en/about/facilities-collections/Collections/ANACC)
The Australian National Algae Culture Collection, housed at Hobart, Tasmania, holds more than 1000 strains of more than 300 microalgal marine and freshwater species. Although majority of them are isolated from Australian waters, there are also strains been sourced from tropical Australia to Antarctica. The collection is focused on the preservation of Australian biodiversity, but also to provide high-quality starter algal cultures to industry, educational institutions and research centers.

2. **CICCM -** The Cawthron Institute Culture Collection of Micro-Algae (https://www.cawthron.org.nz/ciccm/)
This collection is a part of Cawthron Institute in Nelson, New Zealand. It has more than 300 strains from 14 classes of freshwater and marine microalgae, some of which are toxic and unique for New Zealand. Big part of the strains are cryopreserved. The goal of the CICCM is to support research worldwide and to ensure the safety of the countries seafood.

Culture collections located in Europe:

1. **ACOI -** Coimbra Collection of Algae (http://acoi.ci.uc.pt/)
ACOI is located at the Department of Life Sciences, University of Coimbra, Portugal and contains about 3000 cultures assigned to species and about 1000 cultures assigned to a genus level, which are not yet added to the database. A gallery with images of algal species has also been created. Collection aims to start cryopreserving the algal strains for safer storage in long term plan (Santos & Santos 2004). ACOI is registered in the WFCC under number 906.

2. **ACKU** – Algal Culture Collection of Kyiv University (https://biomed.knu.ua/)
This collection is created in the early 1970s in the department of Lower Plants at the Kyiv National Taras Shevchenko University, Ukraine. The strains are isolated from soils from different part of the country and from some other countries like Luxemburg, Belgium, Russia, Check Republic and even from Antarctica. The collection is registered in WDCM with number 994.

3. **ACSSI** – Algal Collection of Soil Science Institute
This collection is situated at the Institute of Physical Chemical and Biological Problems in Soil Science of the Russian Academy of Sciences, in Pushkino, Moscow Region, Russia. It has more than 400 strains of soil algae, isolated from different soil types of the Russian territory. The goal of the collection is not only to maintain the algal strains and to be an important biological resource but also to study their biotechnological potential (Temraleeva 2016). The collection is registered in WFCC with number 1132.

4. **ACUF** – Algal collection at the University "Federico II" (https://www.acuf.net/)
This collection is located at the Department of Biology of the University “Federico I” of Naples, Italy. It has more than 600 strains of microalgae from the phyla Cyanoprokaryota, Rhodophyta, Chlorophyta and Ochrophyta (Bacillariophyceae), out of which over 250 strains are extremophilic algae.

5. **ACUS -** Algal Collection of Sofia University
ACUS is situated in the Faculty of Biology of Sofia University, Bulgaria. It was established in 2006 (Uzunov et al. 2012). Aero-terrestrial algal monocultures, as well as species from thermal springs and freshwater habitats have been deposited in it (Stoyneva 2012). They are represented by 197 strains of algae collected from different parts of the country kept on agar (Uzunov et al. 2012). Recently, in the framework of the project SUMMIT (Sofia University Marking Momentum for Innovation and Technological Transfer) 70-123-11/27.02.2023 the collection has been equipped additionally with three growth chambers and with a large photobioreactor for algal cultivation. ACUS is registered in the GSIM, WDCM and WFCC

under number 965.

6. **Algal collection of the Laboratory of Experimental Algology of the Bulgarian Academy of Sciences** (http://www.bio21.bas.bg/ippg/en/?page_id=218)
This collection is situated in Sofia in the Institute of Plant Physiology and Genetics (IPPG) of the Bulgarian Academy of Sciences (BAS) (Ivanova et al. 2020). Its main objectives are isolation, characterization of the physiology and biochemistry of the algal strains and their application in pharmaceutical and agricultural industries.

7. **Algobank Caen** (https://algobank.unicaen.fr/en/accueil/)
This is the microalgal culture collection of the Caen Basse-Normandie University in France. The collection provides long-term preservation of more than 300 brackish, freshwater and marine strains. Those biological resources can be provided to different institutions for education and industrial research.

8. **BACA** - Bank of Algae and Cyanobacteria of the Azores (https://baca.uac.pt)
BACA, established in 2018, is located at the University of the Azores in Ponta Delgada, Portugal. It contains strains of microalgae and cyanobacteria that were isolated earlier, in 2013, from freshwater, marine, brackish, thermal and terrestrial habitats from the nine islands of the Azores archipelago. Several strains of blue-green algae, kept in BACA, have unique phylogenetic features, thus contributing to the biodiversity the collection possesses and enhancing its biotechnological potential (Luz et al. 2019). The collection is registered in WDCM with number 1242.

9. **BCAC** - Bashkortostan Collection of Algae and Cyanobacteria (https://ufacity.info/press/news/128615.html)
BCAC, created in 1978, is located at the Bashkir State Pedagogical University named after M. Akmulla (M. Akmulla BGPU) in Ufa, Bashkortostan republic, western Russia. By far it is the largest in Russia with more than 1,200 strains of eukaryotic and blue-green algae isolated from the territory of Russia, Ukraine, USA, France, Germany and Antarctica, each strain being unique and can be used for both fundamental and applied research. Research is currently focused on molecular studies and taxonomy of terrestrial algae and cyanoprokaryotes, their biology and ecology, algal physiology and biochemistry and their identification.

10. **BCCM/DCG** - Diatom Collection Ghent https://bccm.belspo.be/about-DCG
BCCM - Belgian Co-Ordinated Collections of Micro-Organisms houses DCG (Diatom Collection Ghent), at Ghent University in Belgium. Currently the collection has more than 200 stains of diatoms, but it houses also some other microalgae that are interesting from a scientific point of view or that have the prospect of being integrated into production (Vanormelingen et al. 2012). The collection is oriented

towards taxonomy, and strains are characterised by morphological and DNA analyses, with additional studies on the evolution, ecology and life cycle of diatoms. For preservation, cryogenic methods are also used. Deposited strains are available for scientific and research purposes, but also for private companies with applied purposes. This colllection is registered in WDCM with number 1039.

11. **BCCM/ULC -** Culture Collection of (sub)polar cyanobacteria (https://bccm.belspo.be/about-ULC)
BCCM/ULC is located at the University of Liège in Belgium since 2011 and is one of the largest repositories of polar and subpolar blue-green algae, addressing their phenotypic and genotypic diversity, evolution, biogeography and ecophysiology, toxicity and physiological response to stressors. In addition to strains from polar zones, there are also strains from Belgian lakes that cause blooms and release toxins. Molecular approaches are used in species identification. The collection has a polar focus but seeks to deposit terrestrial, freshwater and marine strains from temperate, tropical and subtropical regions as well. To date more than 500 strains have been deposited in BCCM/ULC. This collection is registered in WDCM with number 982.

12. **BEA** – Banco Español de Algas (Spanish Bank of Algae) (https://marinebiotechnology.org/en/)
BEA is part of the University of Las Palmas de Gran Canaria, Spain. It has more than 1900 clonal strains, some of them are from tropical and subtropical regions. The collection provides isolation, characterization, conservation, development of cultivation techniques and supply, also it offers a great potential for development and application of algae and cyanobacteria from a technological and scientific point of view. BEA is part of ECCO and WFCC with number 837.

13. **BMCC** – Basque Microalgae Culture Collection (https://www.ehu.eus/en/web/bmcc/collection)
The collection is positioned in the Department of Plant Biology and Ecology, Faculty of Science and Technology of the University of the Basque Country, Leioa, Spain. The collection has more than 600 strains of phytoplankton algae from the following phyla: Cyanoprokaryota, Chlorophyta, Pyrrhophyta, Haptophyta and Ochrophyta. The collection is part of the REDESMI – the Spanish Network of Microorganisms, ECCO and is registered in 2020 in the WFCC with number 1232.

14. **CALU -** Collection of Algae of Leningrad University (https://researchpark.spbu.ru/en/home-eng-3)
This collection, maintained at the Saint-Petersburg State University, is one of the largest in Russia. The collection is not dedicated to algae alone but consists of bacteria strains as well (Pinevich et al 2004). About 1,000 strains are kept in liquid or

solid media and are used for research and educational purposes. CALU is registered in WDCM with number 461.

15. **CAUP** - Culture Collection of Algae of Charles University in Prague (https://botany.natur.cuni.cz/algo/caup.html)
CAUP was founded in 1961 and is located at Charles University in Prague, Czech Republik. This collection contains 246 strains of algae and cyanobacteria. The purpose of the collection is to maintain a small set of organisms, and to serve as a platform for education and taxonomic research in the university department (Puncocharova 1990). The planned development of the collection is its digitization. Since 1981, it is a member of the WFCC and is also a member of the FCCM (The Federation of Czech and Slovak Culture Collections of Microorganisms). CAUP is registered also in the WDCM with number 486.

16. **CCAC** - Central Collection of Algal Cultures (https://www.uni-due.de/biology/ccac/)
CCAC, founded in 2001, is located at the University of Duisburg-Essen (UDE), Germany and is one of the largest in the world. It was based on the Culture Collection of Algae at the University of Cologn with more than 7,500 algal strains from all over the world have been deposited in it (Surek & Melkonian 2004). Most strains are from freshwater and terrestrial habitats (85%), the remaining 15% are from marine and brackish habitats. Algal strains are stored in growth chambers. The collection accepts strains from all over the world. The collection is registered in WDCM and WFCC under number 807 and is registered also in ECCO.

17. **CCALA** - Culture Collection of Autotrophic Organisms (https://ccala.butbn.cas.cz/)
CCALA is one of the oldest algal collections, having started work as early as 1913 at the Karlov University in Prague, Czech Republik. In 1961, an algal collection in the city of Trebon, Czech Republic, was founded. In 1979, both collections were united and formed the current CCALA collection located in the Institute of Botany of the Czech Academy of Sciences. It aims to collect algal cultures from different habitats (polar regions, hot springs, soils, etc.). It supports algal strains from more than 50 countries, including cultures from the Arctic and Antarctica. Deposited cultures are available for teaching, research and commercial purposes. CCALA is registered in the WFCC with number 905.

18. **CCAM** – Culture Collection of Algae Marburg (https://www.uni-marburg.de/de/sammlungen/gesamtuebersicht/naturwiss-sammlungen/algenkultursammlung)
CCAM is located at the Department of Biology in Philipps University Marburg, Germany. The collection maintains algal strains from 75 taxa, most of which are diatoms. The strains are used for different research and educational purposes.

19. **CCAP -** Culture Collection of Algae and Protozoa (https://www.ccap.ac.uk/)
CCAP is in Scotland, United Kingdom, located within Scottish Association for Marine Science (SAMS) campus. It is a biological resource center that provides access to taxonomically defined live cultures of marine and freshwater algae from more than 3,000 strains for research, education and industry purposes (Gachon 2008). This collection is registered in WDCM under number 522.

20. **CCBA** – Culture Collection of Baltic Algae (https://ccba.ug.edu.pl/pages/en/home.php)
The collection is created in the mid-1980s and is a part of the Department of Oceanography and Geography of the University of Gdańsk, Poland. CCBA has about 300 strains of microalgae and some seaweeds mainly from the Baltic Sea.

21. **CCryo** – Culture Collection of Cryophilic Algae (https://www.izi-bb.fraunhofer.de/de/Forschung_Entwicklung/extremophilenforschung/sammlung-kryophiler-organismen.html)
CCryo, established in 1999, currently is located in Fraunhofer IZI-BB in Potsdam, Germany. It is focused on cryophilic algae collected during 7 expeditions in Norway and in the Antarctic. It contains 518 strains of 178 species from 101 genera (Leya 2020). About 90% of the strains are cryophilic, collected from various cold habitats (snow, permafrost, etc.) (Leya 2020). These strains are used for taxonomic and phylogenetic studies, as well as for physiological and genomic studies, showing the possibility of cryophilic algae to produce commercially potential substances. The purpose of the collection is to preserve and provide algal strains as needed to various institutions related to education, industry, private sector and others. CCryo is registered in WDCM with number 940.

22. **CCVIEO** – Culture Collection, focused on harmful algal blooms (HAB) species (https://vgohab.com/en/)
The collection is at Vigo Oceanographic Center of the Spanish Institute of Oceanography. It has about 300 strains from more than 80 species marine microalgae that cause harmful algal blooms. The strains are used to study their toxicity, genetics, pigments, life cycle, etc.

23. **CoSMi** - Collection of Sea Microorganisms (https://www.ogs.it/en/collection-marine-microorganisms-cosmi)
CoSMi is a part of the Italian Microbial Resource Research Infrastructure (MIRRI) and is situated in Milano, Italy. It is a biological resource center with a purpose to isolate, cultivate and identify marine microorganisms by combining morphological and molecular methods. To date, it contains more than 100 strains of living microalgae (diatoms, flagellates from different groups such as dinoflagellates, coccolithophorids, etc.). They are available for research, industrial and educational

purposes. CoSMi is registered with the WFCC under number 1209.

24. **Culture Collection of Cyanobacteria and Microalgae at the French National Museum of Natural History**
The collection is established in the late 1920s at the French National Museum of Natural History (MNHN) in Paris. It consists of more than 1,300 strains isolated from freshwater, benthic and terrestrial habitats in France. A lot of blue-green strains are blooming and are an ecological concern. The collection contributes to taxonomy, genetics and biodiversity research (Hamlaoui et al. 2022).

25. **GUMACC** - Göteborg University Marine Culture Collection (https://www.gu.se/en/marina-vetenskaper/about-us/algal-bank-gumacc)
The collection is located at the Department of Marine Ecology, Göteborg University, Sweden. It has 81 strains of marine microalgae that are cultivated in house since 1955 and provided mainly to the university staff for education and research. Since 1992 HPLC-analysis has been used to determine the taxonomical affiliation of the algal strains.

26. **HAMBI Culture Collection** (https://www.helsinki.fi/en/infrastructures/biodiversity-collections/infrastructures/microbial-domain-biological-resource-centre-hambi)
HAMBI Culture Collection is located at the University of Helsinki in Finland and is part of the Biodiversity Collections Research Infrastructure (HUBCRI) in the Helsinki Institute of Life Science. It is a resource of living microorganisms from various groups, such as archaea, bacteria, cyanobacteria, fungi, phages and yeasts. A large part of them is collected from the country. There are currently about 1,000 strains of blue-green algae. This collection is registered in the WFCC under number 779.

27. **IBASU-A** – The Microalgae Culture Collection of the M.G. Kholodny Institute of Botany, NAS of Ukraine
The collection is in Kyiv, at the Kholodny Institute of Botany at National Academy of Science of Ukraine. It started functioning in the 1950s and consists of more than 400 strains of halophitic and freshwated algae from the Chlorophyta division (Borisova et al. 2003; Friedl et al. 2004; Borysova et al. 2020). The collection is registered in WFCC with number 1282.

28. **IBSS** – Collections of algae in the Institute of Biology of the Southern Seas of Russian Academy of Sciences (https://ibss-ras.ru/about-ibss/structure-ibss/tsentry-kollektivnogo-polzovaniya/collection-of-hydrobionts-of-world-ocean/collections-of-plants/?sphrase_id=3633121)
These are seven collections included in the system of the aquatic plant collections

of the larger collection of marine hydrobints in the A. O. Kovalevsky Institute of Biology of the Southern Seas of Russian Academy of Sciences. The first is the Collection of anhydrobiotic algal cultures, created in 2005. Currently, it has more than 500 strains from four divisions: Cyanoprokaryota, Rhodophyta, Chlorophyta and Ochrophyta (Bacillariophyceae). This type of storage is making the preservation reliable and is suited for creating genetic banks (Kharchuk 2020). The second is the Collection of living cultures of marine planktonic microalgae, located in the Department of Ecological Physiology of Algae of the Institute of Biology of the Southern Seas and is one of the largest collections in post-Soviet era. It contains unicellular algae. The third is the Collection of microalgae and cyanobacteria cultures, which has been created in the year 2000 and with its more than 30 strains supports scientific and biotechnological research of microalgae and cyanobacteria. The fourth collection of the Institute is focused on carotenogenic microalgae and its data are available from the site "https://micro.depo.msu.ru/module/collection-public?openparams=%5bopen-id=91967043"https://micro.depo.msu.ru/module/collectionpublic?openparams=[open-id=91967043. There are also collections of living dinoflagellates and cryptophytes which contain more than 20 species and belong to the Aquaculture and pharmacology section of the Institute.

29. **IPPAS** - Culture collection of microalgae and cyanobacteria of the K. A. Timiryazev Institute of Plant Physiology of the Russian Academy of Sciences (IPPRAS) (https://en.cellreg.org/Collection-IPPAS.php)
Of the 27 Russian algae culture collections, IPPAS, established in 1958, is one of the oldest and the most diverse collection of biotechnologically important and model strains (Yuorieva et al. 2023). The collection is open for collaborations, and aims to provide targeted services and assistance to scientists working on the physiology and biochemistry of photosynthetic microorganisms or biotechnological applications of microalgae and cyanobacteria (Yuorieva et al. 2023). In addition to application of in vitro and cryopreservation techniques, there is cultivation of algae in bioreactors in liquid media from laboratory (5–20 L) to semi-industrial (150–630 L) scale. Currently, the collection holds 430 strains, including 243 strains of eukaryotic microalgae from Chlorophyta, Rhodophyta, Ochrophyta, and Euglenophyta and 187 strains of cyanobacteria belonging to 91 genera and 106 species. Since 1989, IPPAS has been a member of the ECCO and it is registered in WDCM under number 596.

30. **KPABG** – The Collection of Cyanoprokaryotes of the Polar-Alpine Botanic Garden-Institute (https://isling.org/cyano)
The collection is situated at the Polar-Alpine Botanic Garden-Institute named after N. A. Avrorina in Murmansk region of Russia. It has more than 2,000 strains. It's registered in WFCC with number 1281.

31. **MZCH** - Microalgae and Zygnematophyceae Collection Hamburg (https://www.biologie.uni-hamburg.de/en/einrichtungen/wissenschaftliche-sammlungen/algensammlung.html)
MZCH is a part of the University of Hamburg, Germany. It houses more than 600 strains of green algae from the class Zygnematophyceae (phylum Streptophyta), and more than 700 strains of green microalgae from the phylum Chlorophyta (Schwartzenberg 2013). The collection serves as a resource center as it allows experimental work with rare and in many cases endangered species and can provide in vitro cultures for educational purposes. MZCH collection is registered in the WFCC without a number.

32. **Nature Research Centre Collection of Pure Cultures of Algae and Cyanobacteria**
The collection is deposited in the Nature Research Centre in Vilnius, Lithuania. It is unique for the country and nowadays has more than 500 strains of 140 species, mainly blue-green (44%) and green (32%) freshwater and brackish algae. The strains are used for educational, biotechnological and research purposes (Koreivienė et al. 2016).

33. **NORCCA** - The Norwegian Culture Collection of Algae (https://norcca.scrol.net/)
NORCCA, located at the University of Oslo, Norway, is the largest in the Scandinavian peninsula. It was founded in 2016 after the merging of three collections (the NIVA Culture Collection of Algae (NIVA-CCA) (Friedl et al. 2004), the University of Oslo Culture Collection of Algae (UIO-CCA) and the Scandinavian Culture Collection of Algae and Protozoa (SCCAP) at the University of Copenhagen (UoC), Denmark) (Friedl et al. 2004). NORCCA contains more than 2,000 strains of cyanobacteria, micro- and macroalgae isolated from various freshwater, coastal and oceanic habitats of northern Europe. In addition to northern European strain, there are strains from all over the world. Algal representatives are quite diverse, covering 10 divisions. About half of the strains are from freshwater or terrestrial habitats and half are from marine or brackish waters. About 100 strains are macroalgae. The strains are used for research, educational, innovative and commercial purposes. NORCCA is registered in ECCO.

34. **PACC** - Plovdiv Algal Culture Collection (https://bio.uni-plovdiv.bg/en/botany-and-teaching-methods-in-biology/)
The living algae collection of the the Department of Botany of Plovdiv University "St. Paisii Hilendarski" was established in 1963 in. Over the years, the collection grew, and in 1980, it received various algal cultures from 11 foreign collections such as MPI, SAG, CCALA, IBI, CAUP, ALCP and others (Belkinova 2002). It contains 774 strains of 249 species from 103 genera.

35. **PCC** - Pasteur Cultures of Cyanobacteria collection (https://www.pasteur.fr/en/public-health/crbip/distribution/pcc)
PCC began its development at the University of California in the USA but was moved to France in 1971 to the Institute Pasteur in Paris. It contains more than 750 pure cultures of blue-green algal strains from different habitats. These strains are available for research purposes only.

36. **RCC** - Roscoff Culture Collection (https://roscoff-culture-collection.org/)
RCC is situated in Station Biologique De Roscoff which is run by Sorbonne University and the CNRS, France. It was created in the late 1970s. Nowadays RCC contains about 6,300 strains of marine planktonic algae, focusing on picocyanobacteria (*Prochlorococcus* and *Synechococcus*), picoeukaryotes (*Bolidomonas*, *Ostreococcus*, *Pelagomonas*), diatoms and Haptophyta (coccolithophorids). The strains are collected from all around the world. The future of the collection is to use molecular methods in order to clarify the classification of the picoplankton species (Vaulot et al. 2004). RCC is registered in WDCM with number 829 and in ECCO.

37. **SAG** - Sammlung von Algenkulturen at University of Göttingen (https://www.uni-goettingen.de/de/184982.html)
SAG is located at the University of Göttingen, Germany and is one of the richest in species and is long considered as important center for living biological resources of microalgae. There are about 2,300 strains in it, represented by almost all divisions and classes of eukaryotic algae and prokaryotic blue-green algae from more than 500 orders and 1,400 species. The strains were characterized using molecular methods. Pure algal cultures are cryopreserved (Friedl 2012). Collection supports scientific, biotechnological and educational centers worldwide, due to the many services it offers ex situ conservation, isolation and identification of species, provision of algal cultures to various institutions for educational, research and industrial purposes. SAG is registered in the WFCC under number 192.

38. **SYKOA** - Strain collection of microalgae and cyanobacteria from northern and arctic regions in the Institute of Biology of Komi Scientific Centre (https://ib.komisc.ru/sykoa/eng/)
SYKOA, situated in the Institute of Biology of Komi Scientific Centre of the Ural Branch of the Russian Academy of Sciences (IB Komi SC UB RAS), started working in 2010. To date, it contains more than 400 strains of algae collected from various terrestrial and freshwater habitats in the northeastern part of the European part of Russia. Green and blue-green species form the larger part of the collection, whereas Eustigmatophyceae and Xanthophyceae are represented by five species. Also, the collection contains rare species and strains with unclear systematic affiliation. The main goal of the collection is to preserve the species diversity of

microalgae from the Arctic and northern regions of European Russia and to collect samples for further use in floristic, systematic, evolutionary, molecular, genetic and ecological studies. SYKOA is registered in WDCM with number 1125.

39. **TAU-MAC -** Thessaloniki Aristotle University Microalgae and Cyanobacteria Collection (https://cyanobacteria.myspecies.info/)
At present, the algal collection of the Thessaloniki Aristotle University, Greece TAU-MAC contains 49 blue-green algal strains, representing 22 taxa, 16 genera and seven families (Chroococcaceae, Microcystaceae, Hapalosiphonaceae, Nostocaceae, Rivulariaceae, Phormidiaceae, Pseudanabaenaceae, Synechococcaceae), belonging to four orders: Chroococcales (18), Nostocales (15), Oscillatoriales (12) and Synechococcales (4) isolated manly from lakes of Greece, but also from different marine habitats of the country and aims to contribute to the knowledge of their biological diversity. TAU-MAC is registered in WDCM with number 1156.

40. **TCC** – Thonon Culture Collection (https://carrtel-collection.hub.inrae.fr/)
This collection is located at the Parachet Hydrobiology Station in Somme, France. It was created in the late 1950s. Currently, the collection has more than 500 freshwater algal strains. It is a part of The Biological Resource Centre for the Environment which is a network of collections whose objective is to improve the visibility of the biological resources maintained in centers from different parts of France (Mougin et al. 2018). The collection is registered in WDCM with number 1030.

41. **VKM -** All Russian Collection of Microorganisms (https://www.vkm.ru/index.htm)
VKM operates as a Department of the G. K. Skryabin Institute of Biochemistry and Physiology of Microorganisms at the Pushchino Biological Research Center of the Russian Academy of Science. It is one of the largest Russian collections and contains more than 23,000 strains of bacteria, archaebacteria, fungi, yeasts and algae (50 strains). The collection offers services related to the procurement of cultures, their deposit and storage, study and determination. VKM is registered in WDCM with number 342.

Culture collections located in North America:

1. **APCC** – Antarctic Protist Culture Collection (https://www.whoi.edu/science/B/protists/
The Antarctic culture collection is in the Woods Hole Oceanographic Institution, Massachusetts, USA and consists of about 80 strains of algae from the Antarctic marine water and other protists. The samples were obtained from water, ice and slush in the Ross Sea, Antarctica. The cultures are used in different physiological and molecular experiments that lead to better understanding of the Antarctic microbial community and its structure and function.

2. **ARC** – Algal Resources Collection (https://www.algalresourcescollection.com/)
The collection was created in the Florida Marine Research Institute in 1987 and was focused on toxic dinoflagellates from the region. In 1999, it was transferred to the Center for Marine Science at the University of North Carolina at Wilmington (UNCW), USA. In 2013, ARC was moved to the newly constructed MARBIONC (Marine Biotechnology in North Carolina) building at UNCW's Crest Research Park. Currently, its focus has expanded on the maintenance of toxic algal species from different taxonomic groups. The strains are used for commercial, educational, industrial and pharmaceutical research.

3. **ATCC** – American Type Culture Collection (https://www.atcc.org/)
ATCC is established in 1925 and is in Gaithersburg, Maryland, USA. The collection offers a great variety of different culture collections like algae, viruses, fungi, yeasts and many others. The algal cultures are represented by more than 150 strains from different habitats. They are used for academic/scientific research in the means of different uses like animal feed, textile pigments, fertilizers, biofuels and many more.

4. **CCCM** – Canadian Center for the Culture of Microorganisms (https://cccm.botany.ubc.ca/)
The CCCM is a part of the Department of Botany at the University of British Columbia, Canada. It contains two other collections Northeast Pacific Culture Collection (NEPCC) which has marine algae and Freshwater Algal Culture Collection (FWAC). Both collections have approximately 300 strains. About 75% of the strains are local, the other 25 % are from tropical and temperate regions. The maintained strains are used for research, education and commercial.

5. **CCMEE** – Culture Collection of Microorganisms from Extreme Environments
CCMEE is at The University of Oregon, USA. It's a unique collection focused on extremophile organisms (Shaw et al 2020). The algal strains are dominated by cyanoprokaryotes and red algae, collected from a range of extreme habitats. The goal of the collection is to stabilize the cultures for general research.

6. **CODIMAR** – Marine Dinoflagellates Collection (https://www.cibnor.gob.mx/investigacion/colecciones-biologicas/codimar)
This collection is suited in Northwest Biological Research Center in La Paz, Mexico. Its role is to gather dinoflagellates that cause harmful algal blooms and to develop a taxonomic and a biological bank. Currently the collection has 149 strains from the orders Peridiniales, Gonyaulacales, Gymnodiniales and Prorocentrales of the division Pyrrhophyta and class Raphidophyceae from the division Ochrophyta. The CODIMAR cultures can be provided to different scientific communities for research and educational purposes.

7. **CPCC** - the Canadian Phycological Culture Collection (https://uwaterloo.ca/canadian-phycological-culture-centre/about/history)
The Canadian Phycological Culture Collection (CPCC) was created in 1987 and was formerly known as University of Toronto Culture Collection of Algae and Cyanobacteria (UTCC) (Friedl et al. 2004 and references therein). It has more than 500 strains and is the the only full-service Canadian collection of cultivated algae. It has provided cultures to institutions in more than 30 countries.

8. **CRC** collection at *Chlamydomonas* Resource Center (https://www.chlamycollection.org/)
CRC, established in 1978 as *Chlamydomonas* Genetics Center at Duke University (Friedl et al. 2004 and references therein), is at the University of Minnesota, USA. There are over 3,900 strains of the genus *Chlamydomonas*. The main goal of the collection is to maintain and distribute strains of *Chlamydomonas* for different research like flagellar assembly and motility, metabolic pathways, genetics research and many others.

9. **NCMA** collection at Provasoli-Guillard National Center for Marine Algae and Microbiota (https://ncma.bigelow.org/; https://ncma.bigelow.org/who-we-are)
The NCMA originated from private culture collections established by Dr. Luigi Provazoli of Yale University and Dr. Robert R. L. Guillard of the Woods Hole Oceanographic Institution and was originally called the Culture Collection of Marine Phytoplankton (CCMP). It was founded in 1980 in the Woods Hole Oceanographic Institution, Massachusetts. Later, moved to Bigelow Laboratory for Ocean Sciences, Maine and in 1985, the name was changed from a "Collection" to a "Center" to reflect its wider services and in October 2011 the CCMP was renamed the Provasoli-Guillard National Center for Marine Algae and Microbiota (NCMA). Currently, it contains about 783 species represented by more than 2000 strains. NCMA is registered in WDCM with number 2.

10. **UTCC/CPCC** – Canadian Phycological Culture Centre (https://uwaterloo.ca/canadian-phycological-culture-centre/)
UTCC/CPCC is housed at the University of Waterloo, Canada. It was opened in 1987. Now, more than 300 algal strains collected from different habitats have been deposited in it. These strains are used for research purposes, oriented towards biofuel production, bioremediation, ecotoxicology, physiology and ecology (Acreman 2004). This collection is the only one in the country that offers algal cultures, nutrient media, isolation and organizing seminars for educational purposes. UTCC/CPCC is registered in WDCM with number 605.

11. **UTEX** – Culture Collection of Algae (https://utex.org/)
UTEX, located at the University of Texas at Austin, USA, was founded in 1976.

It originally had about 400 algal strains provided by the University of Cambridge, UK (Starr 1993). Currently, it contains more than 3,000 algal strains from more than 1,500 species and 450 genera. The collection is a repository of biological resources and a distribution center for algal cultures. Its mission is to promote and provide algae for scientific research, educational and practical applications. UTEX is registered in WDCM with number 606.

Culture collections located in South America:

1. **BCCUSP** – Brazilian Cyanobacteria Collection of the University of Sao Paulo
This collection belongs to the University of Sao Paulo, Brazil. It has more than 300 strains of blue-green and green algae. The strains are used for research of toxins, genetic analysis, physiology, biotechnological application, etc. (Lourenco et al. 2004). BCCUSP is registered in WFCC with number 844.

2. **CCAPE** - Culture Collection of Cyanobacteria and Algae of Pernambuco (https://collectory.sibbr.gov.br/collectory/public/show/co393)
This collection is located in the Laboratory of Phycology (LABFIC) of the Universidade Federal Rural de Pernambuco, Brazil and contains mainly strains of green algae (Chlorophyta). It has 90 strains of species that has commercial and biotechnological interest (Oliviera et al. 2018). The collection is registered in WFCC with number 1311.

3. **CCMA-UFSCar** – Culture Collection of Freshwater Microalgae
This collection is located the Federal University of Sao Carlos, Brazil. CCMA-UFSCar started working in 1977. Currently is the largest algal collection in Brazil, containing more than 700 strains of freshwater algae mostly of the Sao Paulo region. The strains are cryopreserved and they are used for different research projects (Tessarolli 2017). The collection is registered in WFCC with number 835.

4. **LAM** – Laboratory of Seaweed (https://lam.ib.usp.br/ingindex.html)
The collection is a part of the Institute of Bioscience of University of Sao Paulo, Brazil (Friedl et al. 2004). It startet working in the 1950s with the goal was to study and catalogue the Brazilian macroalgae that form the coastal algal diversity. In the 70s of the 20th century the collection started to maintain living seaweeds and to cultivate them in vitro, and study their physiology, phycocoloids and to proceed different molecular studies.

5. **The Marine Algae Collection of the Coast of Piaui** (https://collectory.sibbr.gov.br/collectory/public/show/co728?lang=en_GB)
This collection is at the State University of Piauí, Brazil (Friedl et al. 2004). It includes algae belonging to the Archaeplastida clade from the divisions Chloro-

phyta, Rhodophyta and Glaucophyta which play a fundamental ecological role in coastal ecosystems, contributing to primary production, habitat formation and the maintenance of marine biodiversity.

In conclusion, according to our best knowledge, there are 76 living algal collections (**Figure 1**), most of which are located in Europe and Asia (**Figure 2**).

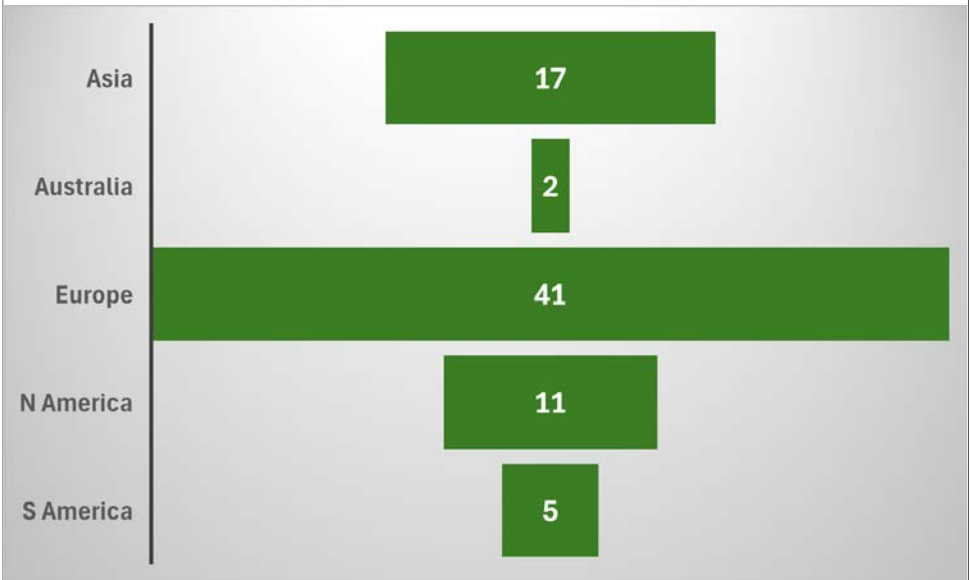

Fig. 2. Number of living algal collections in different geographic regions.

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CONFLICT OF INTERESTS

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

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CHECKLIST OF LITHOPHYTIC ALGAE IN BULGARIA (1905-2024)

MIROSLAV I. ANDROV*

[1]*Department of Botany, Faculty of Biology, Sofia University “St. Kliment Ohridski”, 8 Dragan Tsankov Blvd., 1164 Sofia, Bulgaria*

Abstract. The present paper provides a specified Checklist of lithophytic algae recorded in Bulgaria during the last 120 years, with indication of their epilithic or endolithic type. Altogether 369 taxa from seven phyla were reported in scientific papers from Bulgarian and foreign scientists: Cyanoprokaryota (208), Chlorophyta (83) and Streptophyta (22), Ochrophyta (52), Rhodophyta (2), Pyrrophyta (1) and Euglenophyta (1). Most of these algae were found from epilithic samples (342), and only 27 were endolithes. The number of taxa, identified after application of cultivation procedures and following of different stages of their development, is significantly lower (147) than the number of algae identified from field material by direct microscopy (222). However, it has to be stressed that during the last two decades all reports of lithophytes were based on a preliminary cultivation. The paper underlines the importance of living algal cultures, including those of lithophytes, for wide spectrum of biotechnological applications and stresses the need for future studies of this ecological group in Bulgaria.

Keywords: aero-terreserstrial algae, algal cultivation, cyanobacteria, cyanoprokaryotes, endolithes, epilithes, green algae

* *corresponding author*: M. I. Androv, Department of Botany, Faculty of Biology, Sofia University “St. Kliment Ohridski”, 8 Dragan Tsankov Blvd., 1164 Sofia, Bulgaria; androv@.uni-sofia.bg

INTRODUCTION

Aero-terrestrial algae are a diverse group of both pro- and eukaryotic algae, that permanently live outside of water environment and, as their name suggests, inhabit a wide range of aero-terrestrial substrates. One peculiar subgroup stands out from the rest, because even though all algae require some degree of moisture to survive, most lithophytes are known to thrive in almost completely dry environments, inhabiting both the surface and the inner parts of rocks and stones. The lithophytic algae, found on or inside rocky substrates, are an integral part of almost every ecosystem on Earth, serving as primary producers, but also playing an important role in rock weathering. Furthermore, the process of rock demineralization itself is crucial for the formation of the soil layer, giving it its unique properties such as chemical composition, density, water retention capability - all important factors for soil fertility. Even though this rather small, but quite diverse group of algae is involved in planetary scale processes, important for the wellbeing and survival of the human race, it is often overlooked and remains unexplored. This is due to the fact that these specific algae, as well as the aero-terrestrial group as a whole, require obligatory cultivation in a laboratory for their precise taxonomic identification. Ettl & Gärtner (1995, 2014) thoroughly explained that the reason behind the necessity of growing the collected material in artificial conditions is due to the need of tracking the complete life cycle - both young and adult stages, as well as the types of reproduction.

Lithophytes are split into several subgroups, divided on the basis of the specific inhabited part of the rocks or stone formations. According to different authors (for details see Ettl & Gärtner 1995, 2014) the three main distinguishable groups are epilithes, growing upon the surface of rocks, chasmolithes growing inside stone cracks and crevices, and endolithes, growing in the inner parts of the rock, usually inhabiting the outermost layers in transparent rocks, oftentimes around 1 cm in depth. The isolation process of the latter subgroup presents a significant challenge due to the need of disintegration (*i.e.* breaking) the substrate itself, since they cannot be examined directly inside the intact rock. Another challenge lies in emulating and maintaining the artificial conditions in the laboratory, required for the introduction of the isolated species into cultures and their subsequent long-term cultivation. Even though these unusual organisms, thriving in small pocketed and sometimes quite hostile habitats, as well as their unique adaptation mechanisms for survival, have captivated the attention of scientists in the past, the challenges surrounding their isolation and cultivation, make them one of the more difficult to work with subgroups of the aero-terrestrial fraction and thus one of the least studied and documented as a whole.

The first observations of lithophytic algae in Bulgaria date back to the beginning of the previous century, when Petkoff (1905) reported algal species, which grew on the surface of wet rocks, found in the Rila Mountains. His other early works, concerning the distribution of aero-terrestrial phycoflora, as well as those published by D. Vodenicharov and S. Draganov in the 60s, 70s and 80s, are compiled in a very detailed two parts review published by Uzunov et al. (2007, 2008). The review provides a complete history of the phycological studies of the aero-terrestrial algae in Bulgaria, as well as a checklist for all of the reported species belonging to this ecological group, lithophytes included. According to the list, provided by the authors, the total count of lithophytic algae is 169 species, 11 varieties and 2 forms. Among them 146 species, 10 varieties and 2 forms belong to algae that grow on rocks, and 23 species and 1 variety were recorded from stones. No clear distinction between the different types of lithophytes was made in the article, where it is briefly mentioned that the majority of the collected and examined species are epilithic and only a small percentage of the material could be regarded as being endolithic. The information provided by Uzunov et al. (2007,2008) serves as a basis of the present work, summarizing all the recorded data concerning the lithophytic algal flora described in the country during the last century. Much later, detailed review of the studies of aero-terrestrial algae along the Black Sea coast with some additions to the works of Uzunov et al. (2007, 2008) and emphasis on threatened and newly described species was published by Gärtner et al. (2018) and compared with the total algal biodiversity in the country estimated by Stoyneva (2014) as comprising of about 5500 species, varieties and forms from 650 genera of 8 divisions. In this review, eight publications were pointed as oriented towards epilithic algae (Petkoff 1905, 1919, Komárek 1956, Vodeničarov 1962, Draganov 1964, Starmach 1964, Vodenicharov et al. 1971, Draganov et al. 1984), and one work (Draganov et al. 1984) was shown to provide data on endolithic algae from the region.

In all these reviews there is no information about the identification procedures and about application or not of cultivation of algae for their determination. Therefore, below in the text, attention will be given to two papers, referred in Uzunov et al. (2007, 2008) since they are the first reports on Bulgarian lithophytic algae outside dark or artificially illuminated caves, obtained after cultivation on agar, encriched by Bold-Basal Medium (BBM). The first of them is Gärtner & Stoyneva's (2003) work, where the authors examine samples collected from the surface of some granite and limestone monuments in the country using direct observations and cultured material. This leads to the identification of three algal species from the green evolutionary lineage and to a record of free-living *Trebouxia arboricola* Puym., in particular. The second work belong to the same authors and presents the collected material from the rock surfaces in the Erma Gorge, near the town of Trun and from the open cave Prohodna near Karlukovo village (Stoyneva & Gärtner 2006). After the introduction in cultures, followed by identification using direct light microscopy, the authors report the presence of four lithophytic species, confirming the presence of the free-living non-lichenized form of *Trebouxia arboricola*.

Later on, Stoyneva et al. (2012) reported the finding of 48 lithophytic species collected from the Belogradchishki Skali complex, using the innovative technique for direct collection of aero-terrestrial algae developed a year earlier by Gärtner et al. (2010). Gärtner et al. (2012) published the first ultrastructural study of a lithophytic strain of *Vischeria stellata*, isolated from Belogradchik rocks. In a subsequent work Mancheva (2013) added 20 more species collected from the same locality. With that addition, the total of the species collected from the Belogradchishki Skali complex is 68 - 53 of them being epilithic and the other 15 belonging to the endolithic group, many of which are newfound for the country.

The most recent data, concerning the distribution of stone and rock dwelling algae, comes from the work of Stoyneva-Gärtner et al. (2024a), which focuses on the biodiversity of lithophytes inhabiting the surface of ancient megalithic structures located in South-Eastern Bulgaria. Using the aforementioned technique for direct isolation (Gärtner et al. 2010), samples were obtained from nine different megaliths, located in the region of Haskovo. After the subsequent long-term cultivation and taxonomical identification of the collected material, the study reports the presence of 90 species, belonging to the lithophytic subgroup. All poly- and monocultural samples from Erma Gorge, Prohodna cave, Belogradchik and Haskovo regions are cultivated, identified and later on deposited in the living Algal Collection of the Universitiy of Sofia (ACUS). The collection itself specializes in the storage and safekeeping of aero-terrestrial strains (Stoyneva et al., 2012), with special attention to lithophytes and soil algae, and is registered in the World Data Centre for Microorganisms (WDCM) in 2010 (Uzunov et al. 2012).

The present paper is a natural continuation to the previous phycological studies of aero-terrestrial algae in Bulgaria, focusing specifically on the lithophytic algae. It offers the first specified Checklist of lithophytic algae found in Bulgaria. The checklist aims to summarize the biodiversity of lithophytes reported from the country, both collected in the past and cultivated in recent years. Finally, this paper serves as a stepping stone and a guide for future investigations of the biodiversity and distribution of this unique, but quite neglected ecological group of algae.

MATERIALS AND METHODS

The provided Checklist of the species, varieties and forms of lithophytic algae found in the country is based upon the information available in the forementioned published literature. The nomenclature follows mainly Ettl & Gärtner (1995, 2014) and the current status of each taxon is checked and updated accordingly with the help of various internet databases such as AlgaeBase (Guiry & Guiry 2025), Catalogue of Life (CoL), World Register of Marince Species (WoRMS) and others. Next to the currently accepted taxonomic name of the entities, in brackets are given the synonyms found in literature.

RESULTS AND DISCUSSION

To date, a total of 369 lithophytic algae, belonging to seven phyla have been identified in Bulgaria. Most of them belong to the blue-green evolutionary lineage, or Cyanoprokaryota, (208), followed by the green algae from Chloro- (83) and Streptophyta (22), and the yellow-brown algae, or Ochrophyta (52). Finally, from the red evolutionary lineage, or Rhodophyta, there are two reported species, and single species have been documented from each of the phyla Pyrrophyta and Euglenophyta. The identified and reported algal taxa are listed below (**Table 1**), following an alphabetical order in the frames of each phylum.

Table 1. Checklist of lithophytic algae found in Bulgaria. Based upon the available data in the published literature, for each of the enlisted species, it is pointed out if the taxonomical identification was made by direct microscopy of the collected material, without subsequent cultivation (-) or the identification is based upon cultivation and examination of the complete lifecycle of the species, available in the sample (+). Species identified directly by light microscope in earlier literature and after cultivation in newer publications are marked by -/+. Furthermore, depending on their original habitat, the species are split into two major groups – epiliths (Ep), growing on the surface of the substrate and endoliths (En), growing inside the substrate itself. There are some peculiar species that exhibit both epilthic and endolithic development marked as (Ep/En). Lastly, a point of reference to the corresponding article is given for every single one of the reported lithophytic algae.

№	Taxon	Type	Cultivation	Reference
	CYANOPROKARYOTA			
1	*Anabaena licheniformis* Bory 1822	Ep	-	Uzunov et al. (2008)
2	*Anabaena* sp. ster. 1 (? *Trichormus* sp.)	Ep	+	Stoyneva-Gärtner et al. (2024a)
3	*Anabaena* sp. ster. 2 (? *Isocystis* sp.)	Ep	+	Stoyneva-Gärtner et al. (2024a)
4	*Anabaena* sp. ster. 3	Ep	+	Stoyneva-Gärtner et al. (2024a)
5	*Anagnostidinema acutissimum* (Kufferath) Strunecký, Bohunická, J. R. Johansen et J. Komárek 2017 (Syn. *Oscillatoria acutissima* Kufferath 1914)	Ep	-	Starmach (1964)
6	*Anathece endophytica* (W. et G. S. West) Komárek, Kaštovský et Jezberová 2011 (Syn. *Aphanothece saxicola* f. *endophytica* (West et G. S. West) Elenkin 1938)	Ep	-	Starmach (1964)
7	*Anathece minutissima* (West) Komárek, Kaštovský et Jezberová 2011 (Syn. *Aphanothece saxicola* f. *minutissima* (West) Elenkin)	Ep	-	Starmach (1964)

№	Taxon	Type	Culti-vation	Reference
8	*Aphanocapsa concharum* Hansgirg 1890	Ep	-	Uzunov et al. (2008)
9	*Aphanocapsa fuscolutea* Hansgirg 1893	Ep	+	Stoyneva-Gärtner et al. (2024a)
10	*Aphanocapsa grevillei* (Berkeley) Rabenhorst 1865	Ep	-	Uzunov et al. (2008)
11	*Aphanocapsa litoralis* Hansgirg 1892 (Syn. *Microcystis litoralis* (Hansgirg) Forti 1907)	Ep	-	Uzunov et al. (2008)
12	*Aphanocapsa marina* Hansgirg 1890	Ep	-	Uzunov et al. (2008)
13	*Aphanocapsa muscicola* (Meneghini) Wille 1919 (Syn. *Microcystis muscicola* (Meneghini) Elenkin 1936)	Ep	-	Starmach (1964)
14	*Aphanocapsa parasitica* (Kützing) Komárek et Anagnostidis 1995 (Syn. *Microcystis parasitica* Kützing 1843)	Ep	-	Starmach (1964), Vodenicharov et al., (1971)
15	*Aphanocapsa* cf. *rivularis* (Carmichael) Rabenhorst 1865	Ep	+	Stoyneva-Gärtner et al. (2024a)
16	*Aphanocapsa* sp. 1	Ep	+	Stoyneva-Gärtner et al. (2024a)
17	*Aphanocapsa* sp. 2	En	+	Stoyneva-Gärtner et al. (2024a)
18	*Aphanothece castagnei* (Kützing) Rabenhorst 1865 (Syn. *Gloeothece heufleri* Grunow ex Rabenhorst 1865)	Ep	-	Uzunov et al. (2008)
19	*Aphanothece elabens* (Meneghini) Elenkin 1936	Ep	-	Starmach (1964)
20	*Aphanothece longior* Naumann 1921	Ep	-	Starmach (1964)
21	*Aphanothece* cf. *saxicola* Nägeli 1849	Ep	+	Stoyneva-Gärtner et al. (2024a)
22	*Aphanothece stagnina* (Sprengel) A. Braun 1863	Ep	-	Vodenicharov et al. (1971)
23	*Aphanothece* sp. 1	Ep	+	Stoyneva-Gärtner et al. (2024a)
24	*Aphanothece* sp. 2	Ep	+	Stoyneva-Gärtner et al. (2024a)
25	*Brachytrichia quoyi* Bornet et Flahault 1886	Ep	-	Uzunov et al. (2008)
26	*Calothrix aeruginea* Thuret ex Bornet et Flahault 1886	Ep	-	Uzunov et al. (2008)
27	*Calothrix braunii* Bornet et Flahault 1886	Ep	-	Uzunov et al. (2008)

№	Taxon	Type	Culti-vation	Reference
28	*Calothrix confervicola* C.Agardh ex Bornet et Flahault 1886 (Syn. *Calothrix crustacea* Thuret)	Ep	-	Komárek 1956; Uzunov et al. (2008)
29	*Calothrix parietina* Thuret ex Bornet et Flahault 1886	Ep	-	Uzunov et al. (2008)
30	*Calothrix pulvinata* C.Agardh ex Bornet et Flahault 1886	Ep	-	Uzunov et al. (2008)
31	*Calothrix scopulorum* C.Agardh ex Bornet et Flahault 1886	Ep	-	Uzunov et al. (2008)
32	*Calothrix* sp. juv. (ad *Calothrix fusca* Bornet et Flahault)	Ep	+	Stoyneva-Gärtner et al. (2024a)
33	*Calothrix* spp.	Ep	-	Uzunov et al. (2008)
34	*Chamaesiphon polonicus* (Rostafinski) Hansgirg 1893	Ep	-	Uzunov et al. (2008)
35	*Chondrocystis dermochroa* (Nägeli ex Kützing) Komárek et Anagnostidis 1995 (Syn. *Gloeocapsa dermochroa* Nägeli ex Kützing 1849)	Ep	-	Starmach (1964)
36	*Chroococcidiopsis* sp.	En	+	Mancheva (2013)
37	*Chroococcidium* sp.	En	+	Mancheva (2013)
38	*Chroococcopsis* sp.	En	+	Mancheva (2013)
39	*Chroococcus cohaerens* (Brébisson) Nägeli 1849	Ep	-	Uzunov et al. (2008)
40	*Chroococcus globosus* (Elenkin) Hindák 1978 (Syn. *Aphanothece globosa* Elenkin 1914)	Ep	-	Starmach (1964)
41	*Chroococcus helveticus* Nägeli 1849	Ep	-	Uzunov et al. (2008)
42	*Chroococcus membraninus* (Meneghini) Nägeli 1849	Ep	-	Uzunov et al. (2008)
43	*Chroococcus minor* (Kützing) Nägeli 1849	Ep	-	Uzunov et al. (2008)
44	*Chroococcus minutus* (Kützing) Nägeli 1849	Ep	-	Komárek 1956, Uzunov et al. (2008)
45	*Chroococcus obliteratus* Richter 1885 (Syn. *Chroococcus minutus* f. *obliteratus* (Richter) Hansgirg)	Ep	-	Uzunov et al. (2008)
46	*Chroococcus turgidus* (Kützing) Nägeli 1849	Ep	-	Komárek (1956), Uzunov et al. (2008)
47	*Chroococcus varius* A. Braun 1876	Ep	-	Uzunov et al. (2008)
48	*Chroococcus* sp.	Ep	+	Mancheva (2013)

№	Taxon	Type	Culti-vation	Reference
49	*Clastidium setigerum* O. Kirchner 1880	Ep	-	Uzunov et al. (2008)
50	*Coleodesmium* sp.	Ep	+	Mancheva (2013)
51	*Cyanothece major* (Schröter) Komárek 1976 (Syn. *Synechococcus major* J. Schröter 1884)	Ep	-	Uzunov et al. (2008)
52	*Cyanobacterium cedrorum* (Sauvageau) Komárek, J. Kopecký et Cepák (Syn. *Synechococcus cedrorum* Sauvageau 1892)	Ep	-	Starmach (1964)
53	*Cylindrospermum majus* Kützing ex Bornet et Flahault 1888	Ep	-	Uzunov et al. (2008)
54	*Dermocarpa* sp.	Ep	-	Uzunov et al. (2008)
55	*Desmonostoc muscorum* (Bornet et Flahault) Hrouzek et Ventura 2013 (Syn. *Nostoc muscorum* C. Agardh ex Bornet et Flahault 1888)	Ep	-	Uzunov et al. (2008)
56	*Dichothrix gypsophila* Bornet et Flahault 1886	Ep	-	Uzunov et al. (2008)
57	*Dichothrix orsiniana* Bornet et Flahault 1886	Ep	-	Uzunov et al. (2008)
58	*Entophysalis granulosa* Kützing 1843	Ep	-	Draganov et al. (1984)
59	*Entophysalis major* Ercegovic 1932	Ep	-	Uzunov et al. (2008)
60	*Gloeobacter violaceus* Rippka, J. B.Waterbury et Cohen-Bazire 1974 (Syn. *Aphanothece caldariorum* P. G. Richter 1880)	Ep/ En	-/+	Starmach (1964),
61	*Gloeocapsa alpina* Nägeli 1865 (Syn. *Gloeocapsa fusco-lutea* Kirchner 1878)	Ep	-	Uzunov et al. (2008)
62	*Gloeocapsa atrata* Kützing, nom. illeg. 1843	Ep	-	Uzunov et al. (2008)
63	*Gloeocapsa sanguinea* (C. Agardh) Kützing 1843 (Syn. *Gloeocapsa itzigsohnii* Bornet 1882)	Ep	-	Uzunov et al. (2008)
64	*Gloeocapsa* sp.	Ep	-	Uzunov et al. (2008)
65	*Gloeocapsopsis crepidinum* (Thuret) Geitler ex Komárek 1993	Ep	-	Komarek (1956), Uzunov et al. (2008)
66	*Gloeocapsopsis magma* (Brébisson) Komárek et Anagnostidis ex Komárek 1993	Ep	-	Uzunov et al. (2008)
67	*Gloeothece confluens* Nägeli 1849	Ep	+	Stoyneva-Gärtner et al. (2024a)
68	*Gloeothece palea* (Kützing) Nägeli 1849	Ep	-	Starmach (1964)
69	*Gloeothece rupestris* (Lyngbye) Bornet 1880	Ep	-	Uzunov et al. (2008)

№	Taxon	Type	Culti-vation	Reference
70	*Hassallia byssoidea* Hassall ex Bornet et Flahault 1886 (Syn. *Tolypothrix byssoidea* Kirchner 1898)	Ep	-	Uzunov et al. (2008)
71	*Heteroleibleinia infixa* (Frémy) Anagnostidis et Komárek 1988	Ep	-	Uzunov et al. (2008)
72	*Homoeothrix juliana* (Gomont) Kirchner 1898	Ep	-	Vodenicharov et al. (1971)
73	*Homoeothrix margalefii* Komárek et Kalina, nom. illeg. 1965	Ep	-	Uzunov et al. (2008)
74	*Hydrococcus rivularis* Kützing 1833	Ep	-	Vodenicharov et al. (1971)
75	*Hyella* cf. *balani* Lehmann 1903	En	-	Uzunov et al. (2008)
76	*Isactis plana* Thuret ex Bornet et Flahault 1886	Ep	-	Komarek (1956), Uzunov et al. (2008)
77	*Jaaginema geminatum* (Schwabe ex Gomont) Anagnostidis et Komárek 1988	Ep	-	Uzunov et al. (2008)
78	*Jaaginema kuetzingianum* (Nägeli ex Gomont) Anagnostidis et Komárek 1988	Ep	-	Uzunov et al. (2008)
79	*Jaaginema longiarticulata* Anagnostidis et Komárek 1988: 396 (as '*longiarticulatum*')	Ep	-	Uzunov et al. (2008)
80	*Jaaginema neglectum* (Lemmermann) Anagnostidis et Komárek 1988 (Syn. *Oscillatoria neglecta* Lemmermann 1910)	Ep	-	Starmach (1964)
81	*Jaaginema pseudogeminatum* (G. Schmid) Anagnostidis et Komárek 1988 (Syn. *Oscillatoria pseudogeminata* G. Schmid 1914)	Ep	-	Starmach (1964)
82	*Kamptonema laetevirens* (H. M. Crouan et P. L. Crouan ex Gomont) Strunecký, Komárek et J. Smarda 2014 (Syn. *Phormidium laetevirens* (P. Crouan et H. Crouan ex Gomont) Anagnostidis et Komárek 1988)	Ep	-	Uzunov et al. (2008)
83	*Kyrtuthrix dalmatica* Ercegovic 1929	En	-	Uzunov et al. (2008)
84	*Leibleinia epiphytica* (Hieronymus) Compère 1985 (Syn. *Lyngbya epiphytica* Hieronymus 1898)	Ep	-	Uzunov et al. (2008)
85	*Leptolyngbya* 'Albertano-Kovacik green' 1992	Ep	+	Stoyneva-Gärtner et al. (2024a)
86	*Leptolyngbya amplivaginata* (Goor) Molinari et Guiry 2021 (Syn. *Lyngbya amplivaginata* Goor 1918)	Ep	-	Starmach (1964)

№	Taxon	Type	Culti-vation	Reference
87	*Leptolyngbya calotrichoides* (Gomont) Anagnostidis et Komárek 1988	Ep	-	Uzunov et al. (2008)
88	*Leptolyngbya compacta* Komárek 2001	Ep	+	Stoyneva-Gärtner et al. (2024a)
89	*Leptolyngbya* cf. *gloeophila* (Borzì) Anagnostidis et Komárek 1988	Ep	+	Stoyneva-Gärtner et al. (2024a)
90	*Leptolyngbya foveolarum* (Gomont) Anagnostidis et Komárek 1988	Ep	-/+	Uzunov et al. (2008), Stoyneva-Gärtner et al. (2024a)
91	*Leptolyngbya gracilis* (Lindstedt) Anagnostidis et Komárek 1988	Ep	-	Uzunov et al. (2008)
92	*Leptolyngbya gracillima* (Hansgirg) Anagnostidis et Komárek 1988	Ep	-	Uzunov et al. (2008)
93	*Leptolyngbya lagerheimii* (Gomont) Anagnostidis et Komárek 1988 (Syn. *Lyngbya lagerheimii* Gomont 1892)	Ep	-	Starmach (1964)
94	*Leptolyngbya nostocorum* (Bornet ex Gomont) Anagnostidis et Komárek 1988 (Syn. *Plectonema nostocorum* Bornet ex Gomont 1892)	Ep	-	Starmach (1964)
95	*Leptolyngbya perelegans* (Lemmermann) Anagnostidis et Komárek 1988 (Syn. *Lyngbya perelegans* Lemmermann 1899)	Ep	-	Starmach (1964)
96	*Leptolyngbya terebrans* (Bornet et Flahault ex Gomont) Anagnostidis et Komárek 1988	Ep/En	-/+	Uzunov et al. (2008), Mancheva (2013)
97	*Leptolyngbya* cf. *subtilissima* (Hansgirg) Komárek 2001	Ep	+	Stoyneva-Gärtner et al. (2024a)
98	*Leptolyngbya* sp. 1 (ad *Leptolyngbya compacta* Komárek)	Ep	+	Stoyneva-Gärtner et al. (2024a)
99	*Leptolyngbya* sp. 2	Ep	+	Stoyneva-Gärtner et al. (2024a)
100	*Leptolyngbya* sp. 3	Ep	+	Stoyneva-Gärtner et al. (2024a)
101	*Leptolyngbya* sp. 4 (? *Leiblenia* sp.)	Ep	+	Stoyneva-Gärtner et al. (2024a)
102	*Limnothrix redekei* (Goor) Meffert 1988 (Syn. *Oscillatoria redekei* Goor 1918)	Ep	-	Starmach (1964)
103	*Lyngbya confervoides* C. Agardh ex Gomont 1892	Ep	-	Uzunov et al. (2008)

№	Taxon	Type	Culti-vation	Reference
104	*Lyngbya lutea* Gomont 1892 (Syn. *Porphyrosiphon luteus* (Gomont) Anagnostidis et Komárek 1988)	Ep	-	Uzunov et al. (2008)
105	*Lyngbya martensiana* Meneghini ex Gomont 1892	Ep	-	Uzunov et al. (2008)
106	*Lyngbya semiplena* J.Agardh ex Gomont 1892	Ep	-	Komarek (1956), Uzunov et al. (2008)
107	*Lyngbya* spp.	Ep	-	Uzunov et al. (2008)
108	*Microchaete grisea* Thuret ex Bornet et Flahault 1886	Ep	-	Uzunov et al. (2008)
109	*Microcoleus amoenus* (Gomont) Strunecky, Komárek et J. R. Johansen 2013 (Syn. *Phormidium amoenum* Kützing 1988)	Ep	-	Uzunov et al. (2008)
110	*Microcoleus autumnalis* (Gomont) Strunecky, Komárek et J. R. Johansen 2013 (Syn. *Phormidium autumnale* Gomont 1892)	Ep	-	Uzunov et al. (2008)
111	*Microcoleus paludosus* Gomont 1892	Ep	-	Uzunov et al. (2008)
112	*Microcoleus vaginatus* Gomont 1892	Ep	-/+	Uzunov et al. (2008)
113	*Microcystis pulverea* (H. C. Wood) Forti 1907	Ep	+	Uzunov (2008)
114	*Microcystis smithii* Komárek et Anagnostidis 1995 (Syn. *Aphanocapsa pulchra* (Kützing) Rabenhorst 1865)	Ep	-	Draganov et al. (1984)
115	*Nostoc calcicola* Brébisson ex Bornet et Flahault 1886	Ep	-	Uzunov et al. (2008)
116	*Nostoc commune* Vaucher ex Bornet et Flahault 1888	Ep	-/+	Stoyneva & Gärtner (2006), Uzunov et al. (2008)
117	*Nostoc ellipsosporum* Rabenhorst ex Bornet et Flahault 1886	Ep	-	Uzunov et al. (2008)
118	*Nostoc humifusum* Carmichael ex Bornet et Flahault 1886	Ep	-	Uzunov et al. (2008)
119	*Nostoc linckia* Bornet ex Bornet et Flahault 1886	Ep	-/+	Uzunov et al. (2008), Stoyneva-Gärtner et al. (2024a)
120	*Nostoc microscopicum* Carmichael ex Bornet et Flahault 1886	Ep	+	Uzunov et al. (2008)
121	*Nostoc minutum* Desmazières ex Bornet et Flahault 1886	Ep	+	Stoyneva-Gärtner et al. (2024a)

№	Taxon	Type	Culti-vation	Reference
122	*Nostoc sphaericum* Vaucher ex Bornet et Flahault 1886	Ep	-	Uzunov et al. (2008)
123	*Oscillatoria corallinae* Gomont 1890	Ep	-	Komarek (1956), Uzunov et al. (2008)
124	*Oscillatoria funiformis* (Vouk) Komárek 2001	Ep	-	Komarek (1956), Uzunov et al. (2008)
125	*Oscillatoria longiarticulata* P. Crouan et H. Crouan ex Gomont 1892	Ep	-	Draganov et al. (1984)
126	*Oscillatoria princeps* Vaucher ex Gomont 1892	Ep	-	Uzunov et al. (2008)
127	*Oscillatoria sancta* f. *aequinoctialis* (Gomont) Elenkin 1949	Ep	-	Uzunov et al. (2008)
128	*Oscillatoria sancta* f. *caldariorum* Elenkin 1949	Ep	-	Uzunov et al. (2008)
129	*Oscillatoria simplicissima* Gomont 1892	Ep	-	Uzunov et al. (2008)
130	*Petalonema alatum* (Borzì ex Bornet et Flahault) Wolle 1887	Ep	-	Uzunov et al. (2008)
131	*Phormidesmis mollis* (Gomont) Turicchia, Ventura, Komárková et Komárek 2009 (Syn. *Phormidium molle* Gomont 1892)	Ep	-	Uzunov et al. (2008)
132	*Phormidiochaete crustacea* (Borzì ex Bornet et Flahault) Bohunická et Johansen 2011 (Syn. *Leptochaete crustacea* Borzì ex Bornet et Flahault 1886)	Ep	-	Vodenicharov et al. (1971)
133	*Phormidium ambiguum* Gomont 1892	Ep	+	Mancheva (2013)
134	*Phormidium breve* (Kützing ex Gomont) Anagnostidis et Komárek 1988	Ep	-	Uzunov et al. (2008)
135	*Phormidium corium* Gomont 1892	Ep	-	Uzunov et al. (2008)
136	*Phormidium diguetii* (Gomont) Anagnostidis et Komárek 1988 (Syn. *Lyngbya diguetii* Gomont 1895)	Ep	-	Starmach (1964)
137	*Phormidium hormoides* Setchell et N. L. Gardner 1918	Ep	-	Uzunov et al. (2008)
138	*Phormidium irriguum* (Kützing ex Gomont) Anagnostidis et Komárek 1988	Ep	-	Uzunov et al. (2008)
139	*Phormidium nigroviride* (Thwaites ex Gomont) Anagnostidis et Komárek 1988 (Syn. *Oscillatoria nigrovirides* Thwaites ex Gomont 1892)	Ep	-	Uzunov et al. (2008)
140	*Phormidium papyraceum* Gomont 1892	Ep	-	Uzunov et al. (2008)

№	Taxon	Type	Culti-vation	Reference
141	*Phormidium subfuscum* Kützing ex Gomont 1892	Ep	-	Uzunov et al. (2008)
142	*Phormidium subfuscum* var. *inaequale* Nägeli ex Forti 1907	Ep	-	Uzunov et al. (2008)
143	*Phormidium terebriforme* (C. Agardh ex Gomont) Anagnostidis et Komárek 1988	Ep	-	Uzunov et al. (2008)
144	*Phormidium* sp. 1	Ep	+	Mancheva (2013)
145	*Phormidium* sp. 2	En	+	Mancheva (2013)
146	*Phormidium* spp.	Ep	-	Uzunov et al. (2008)
147	*Planktolyngbya bipunctata* (Lemmermann) Anagnostidis et Komárek 1988 (Syn. *Lyngbya bipunctata* Lemmermann 1899)	Ep	-	Starmach (1964)
148	*Planktothrix agardhii* (Gomont) Anagnostidis et Komárek 1988	Ep	-	Uzunov et al. (2008)
149	*Pleurocapsa fuliginosa* Hauck 1885	Ep	-	Uzunov et al. (2008)
150	*Pleurocapsa minor* Hansgirg 1891 (Syn. *Scopulonema minus* (Hansgirg) Geitler 1942)	Ep	-	Vodenicharov et al. (1971)
151	*Pleurocapsa minuta* Geitler 1931	Ep	-	Uzunov et al. (2008)
152	*Pleurocapsa* sp.	Ep	-	Uzunov et al. (2008)
153	*Porphyrosiphon versicolor* (Gomont) Anagnostidis et Komárek 1988 (Syn. *Lyngbya versicolor* Gomont 1892)	Ep	-	Starmach (1964)
154	*Pseudophormidium battersii* (Gomont) Anagnostidis 2001	Ep	-	Uzunov et al. (2008)
155	*Pseudophormidium golenkinianum* (Gomont) Anagnostidis 2001	Ep	-	Komárek (1956), Uzunov et al. (2008)
156	*Pseudophormidium hollerbachianum* (Elenkin) Anagnostidis 2001	Ep	+	Stoyneva-Gärtner et al. (2024a)
157	*Pseudophormidium phormidioides* (Gomont) Anagnostidis et Komárek 1988	Ep	+	Mancheva (2013), Stoyneva-Gärtner et al. (2024a)
158	*Rivularia atra* Roth ex Bornet et Flahault 1886	Ep	-	Uzunov et al. (2008)
159	*Rivularia bullata* Berkeley ex Bornet et Flahault 1886	Ep	-	Komárek (1956), Uzunov et al. (2008)
160	*Rivularia haematites* C.Agardh ex Bornet et Flahault 1886	Ep	-	Uzunov et al. (2008)
161	*Rivularia rufescens* Nägeli ex Bornet et Flahault 1886	Ep	-	Uzunov et al. (2008)

№	Taxon	Type	Culti-vation	Reference
162	*Rhabdoderma lineare* Schmidle et Lauterborn 1900	Ep	-	Starmach (1964)
163	*Romeria minima* (Lemmermann) Komárek 2001 (Syn. *Rhabdoderma minimum* Lemmermann 1908)	Ep	-	Starmach (1964)
164	*Schizothrix cresswellii* Harvey ex Gomont 1892	Ep	-	Komárek (1956), Uzunov et al. (2008)
165	*Schizothrix coriacea* Gomont 1892	Ep	-	Starmach (1964)
166	*Schizothrix fasciculata* Gomont 1892	Ep	-	Starmach (1964)
167	*Schizothrix heufleri* Grunow ex Gomont 1892	Ep	-	Uzunov et al. (2008)
168	*Schizothrix lardacea* Gomont 1892	Ep	+	Uzunov et al. (2008), Mancheva (2013)
169	*Schizothrix lateritia* Gomont 1892	Ep	-	Starmach (1964)
170	*Schizothrix tenuis* Woronichin 1923	Ep	-	Starmach (1964)
171	*Schizothrix vaginata* Gomont 1892	Ep	-	Uzunov et al. (2008)
172	*Scytonema hoffmannii* C. Agardh ex Bornet et Flahault 1886	Ep	-	Uzunov et al. (2008)
173	*Scytonema mirabile* Bornet 1889	Ep	-	Uzunov et al. (2008)
174	*Scytonema myochrous* C. Agardh ex Bornet et Flahault 1886	Ep	-	Uzunov et al. (2008)
175	*Scytonema* sp.	Ep	+	Stoyneva-Gärtner et al. (2024a)
176	*Scytonematopsis crustacea* (Thuret ex Bornet et Flahault) Kováčik et Komárek 1988 (Syn. *Calothrix crustacea* Thuret ex Bornet et Flahault 1886)	Ep	-	Komarek (1956), Uzunov et al. (2008)
177	*Spelaeopogon* sp.	Ep	+	Mancheva (2013)
178	*Sphaeronema* sp.	Ep	-	Draganov et al. (1984)
179	*Spirulina adriatica* Hansgirg 1890	Ep	-	Uzunov et al. (2008)
180	*Spirulina meneghiniana* Zanardini ex Gomont 1892	Ep	-	Uzunov et al. (2008)
181	*Spirulina subsalsa* Oersted ex Gomont 1892	Ep	-	Petkoff (1905, 1919), Komárek (1956), Starmach (1969), Draganov et al. (1984)
182	*Spirulina* sp.	Ep	-	Uzunov et al. (2008)

№	Taxon	Type	Cultivation	Reference
183	*Stanieria minima* (Geitler) S. M. F. Silva et R. N. Pienaar 2000 (Syn. *Cyanocystis minima* (Geitler) Komárek et Anagnostidis 1986)	Ep	-	Uzunov et al. (2008)
184	*Stanieria sphaerica* (Setchell et N. L. Gardner) Anagnostidis et Pantazidou 1991	Ep	-	Uzunov et al. (2008)
185	*Stigonema hormoides* Bornet et Flahault 1886	Ep	-	Uzunov et al. (2008)
186	*Stigonema* cf. *hormoides* Bornet et Fhault 1886	Ep	+	Stoyneva-Gärtner et al. (2024a)
187	*Stigonema informe* Kützing ex Bornet et Flahault 1886	Ep	-	Uzunov et al. (2008)
188	*Stigonema mamillosum* C. Agardh ex Bornet et Flahault 1887	Ep	-	Uzunov et al. (2008)
189	*Stigonema mirabile* Beck 1929	Ep	-	Uzunov et al. (2008)
190	*Stigonema ocellatum* Thuret ex Bornet et Flahault 1886	Ep	-	Vodenicharov et al. (1971)
191	*Stigonema panniforme* Bornet et Flahault 1887	Ep	-	Uzunov et al. (2008)
192	*Stigonema turfaceum* Cooke ex Bornet et Flahault 1887	Ep	-	Uzunov et al. (2008)
193	*Symploca dubia* Gomont 1892	Ep	-	Starmach (1964)
194	*Symploca* cf. *dubia* Gomont 1892 (?*Leptolyngbya* sp.)	Ep	+	Stoyneva-Gärtner et al. (2024a)
195	*Symploca erecta* Pevalek 1916	Ep	+	Mancheva (2013)
196	*Symploca flotowiana* Kützing ex Gomont 1892	Ep	+	Mancheva (2013)
197	*Symploca fuscescens* Rabenhorst ex Forti 1907	Ep	+	Starmach (1964), Mancheva (2013)
198	*Symploca laeteviridis* Gomont 1892	Ep	-	Uzunov et al. (2008)
199	*Symploca meneghiniana* Kützing ex Gomont 1892	Ep	-	Vodenicharov et al. (1971)
200	*Symploca muscorum* Gomont 1892	Ep	-	Uzunov et al. (2008)
201	*Tolypothrix tenuis* Kützing ex Bornet et Flahault 1886	Ep	-	Uzunov et al. (2008)
202	*Trichocoleus delicatulus* (West et G. S. West) Anagnostidis 2001 (Syn. *Microcoleus delicatulus* West et G. S. West 1896)	Ep	-	Starmach (1964)
203	*Trichocoleus tenerrimus* (Gomont) Anagnostidis 2001 (Syn. *Microcoleus tenerrimus* Gomont 1892)	Ep	-	Uzunov et al. (2008)

№	Taxon	Type	Culti-vation	Reference
204	*Xenococcus pallidus* (Hansgirg) Komárek et Anagnostidis 1995	Ep	-	Uzunov et al. (2008)
205	*Xenococcus schousboei* Thuret 1880	Ep	-	Uzunov et al. (2008)
206	*Xenococcus* sp.	Ep	-	Uzunov et al. (2008)
207	*Xenotholos kerneri* (Hansgirg) M. Gold-Morgan, G. Montejano et J. Komárek 1994 (Syn. *Xenococcus kerneri* Hansgirg 1887)	Ep	-	Vodenicharov et al. (1971)
208	*Yonedaella* sp.	Ep	-	Uzunov et al. (2008)
	RHODOPHYTA			
1	*Chroodactylon ornatum* (C. Agardh) Basson 1979 (Syn. *Asterocytis smaragdina* (Reinsch) Forti 1907)	Ep	-	Uzunov et al. (2008)
2	*Porphyridium purpureum* (Bory) K. M. Drew et R. Ross 1965 (Syn. *Porphyridium cruentum* (S. F. Gray) Nägeli 1849)	Ep	-	Uzunov et al. (2008)
	CHLOROPHYTA			
1	*Apatococcus lobatus* (Chodat) Petersen 1928	Ep	+	Gärtner & Stoyneva (2003), Stoyneva & Gärtner (2006), Uzunov et al. (2008), Mancheva (2013), Stoyneva-Gärtner et al. (2024a)
2	*Borodinella polytetras* V. V. Miller 1927	Ep	-	Uzunov et al. (2008)
3	*Chaetomorpha aerea* (Dillwyn) Kützing 1849	Ep	-	Uzunov et al. (2008)
4	*Chaetomorpha linum* (O. F. Müller) Kützing 1845	Ep	-	Vodenicharov et al. (1971)
5	*Chlorella miniata* (Kützing) Oltmanns 1904	Ep	+	Uzunov et al. (2008), Mancheva (2013)
6	*Chlorella vulgaris* Beijerinck 1890	Ep	+	Mancheva (2013), Stoyneva-Gärtner et al. (2024a)
7	*Chlorella* sp.	Ep	+	Stoyneva-Gärtner et al. (2024a)
8	*Chlorella* sp. 1	Ep	+	Mancheva (2013)
9	*Chlorella* sp. 2	En	+	Mancheva (2013)
10	*Chlorella* sp. 3	Ep	+	Mancheva (2013)

№	Taxon	Type	Culti-vation	Reference
11	*Chloroidium ellipsoideum* (Gerneck) Darienko et al. 2010	Ep/En	+	Mancheva (2013), Stoyneva-Gärtner et al. (2024a)
12	*Chloroidium* cf. *ellipsoideum* (Gerneck) Darienko et al. 2010	Ep	+	Mancheva (2013)
13	*Choricystis parasitica* (K. Brandt) Pröschold et Darienko 2011 (Syn. *Choricystis minor* (Skuja) Fott 1976)	Ep	+	Mancheva (2013), Stoyneva-Gärtner et al. (2024a)
14	*Chromochloris zofingiensis* (Dönz) Fucíková et Lewis 2012 (Syn. *Muriella zofingiensis* (Dönz) Hindák 1982)	Ep	+	Stoyneva-Gärtner et al. (2024a)
15	*Cladophora glomerata* var. *genuina* f. *marina* Hanck (Syn. *Cladophora glomerata* f. *marina* (Kützing) Hauck 1884)	Ep	-	Uzunov et al. (2008)
16	*Cladophora vagabunda* (Linnaeus) Hoek 1963	Ep	-	Uzunov et al. (2008)
17	*Coccomyxa confluens* (Kützing) Fott 1974	Ep	+	Mancheva (2013)
18	*Coccomyxa subglobosa* Pascher 1915	Ep	+	Stoyneva-Gärtner et al. (2024a)
19	*Coccomyxa* sp.	Ep	+	Gärtner & Stoyneva (2003)
20	*Coccomyxa* sp. 1	Ep	+	Mancheva (2013)
21	*Coccomyxa* sp. 2	En	+	Mancheva (2013)
22	*Coelastrella terrestris* (Reisigl) Hegewald et Hanagata 2002	Ep	+	Stoyneva-Gärtner et al. (2024a)
23	*Coelastrella* sp.	Ep	+	Mancheva (2013)
24	*Coenobotrys gloeobotrydiformis* (Reisigl) Kostikov et al. 2002 (Syn. *Coccomyxa gloeobotrydiformis* Reisigl 1969)	Ep/En	+	Mancheva (2013), Stoyneva-Gärtner et al. (2024a)
25	*Desmococcus olivaceus* (Persoon ex Acharius) Laundon 1985 (Syn. *Protococcus viridis* C. Agardh 1824; *Pleurococcus vulgaris* Nägeli 1849)	Ep	+	Uzunov et al. (2008), Mancheva (2013), Stoyneva-Gärtner et al. (2024a)
26	*Desmococcus* cf. *olivaceus* (Persoon ex Acharius) Laundon 1985	Ep	+	Mancheva (2013)
27	*Desmococcus* sp. 1	Ep	+	Mancheva (2013)
28	*Desmococcus* sp. 2	En	+	Mancheva (2013)
29	*Deuterostichococcus tetrallantoideus* (Kol) Pröschold et Darienko 2020	Ep	+	Stoyneva-Gärtner et al. (2024a)

№	Taxon	Type	Culti-vation	Reference
30	*Edaphochlorella mirabilis* (Andreeva) Darienko et Pröschold 2016 (Syn. *Chlorella mirabilis* Andreeva 1973)	Ep/ En	+	Stoyneva-Gärtner et al. (2024a)
31	*Elliptochloris bilobata* Tschermak-Woess 1980	Ep/ En	+	Stoyneva-Gärtner et al. (2024a)
32	*Elliptochloris reniformis* Darienko et Pröschold 2016	En	+	Mancheva (2013)
33	*Elliptochloris subsphaerica* (Reisigl) Ettl et Gärtner 1995	Ep	+	Stoyneva-Gärtner et al. (2024a)
34	*Eubrownia aggregata* (R. M. Brown et Bold) Shin Watanabe et Lewis 2017	Ep	+	Stoyneva-Gärtner et al. (2024a)
35	*Gloeothece rupestris* (Lyngbye) Bornet 1880 (Syn. *Gloeocystis rupestris* (Lyngbye) Rabenhorst 1863)	Ep	-	Uzunov et al. (2008)
36	*Haematococcus lacustris* (Girod-Chantrans) Rostafinski 1875 (Syn. *Haematococcus pluvialis* Flotow 1844)	Ep	-	Vodenicharov et al. (1971)
37	*Hormidiospora* sp.	En	+	Mancheva (2013)
38	*Hormotila mucigena* Borzì 1883	Ep	-	Uzunov et al. (2008)
39	*Jaagichlorella luteoviridis* (Chodat) Darienko et Pröschold 2019 (Syn. *Chlorella luteoviridis* Chodat 1913)	Ep	+	Mancheva (2013)
40	*Keratococcus rhaphidioides* (Hansgirg) Pascher 1915 (Syn. *Dactylococcus rhaphidioides* Hansgirg 1887)	Ep	-	Vodenicharov et al. (1971)
41	*Lobosphaera undulata* (Shin Watanabe) Ettl et Gärtner 1995	Ep	+	Stoyneva-Gärtner et al. (2024a)
42	*Lobosphaeropsis lobophora* (Andreeva) Ettl et Gärtner 1995	Ep	+	Mancheva (2013), Stoyneva-Gärtner et al. (2024a)
43	*Mesotaenium pyrenoidosum* (P. A. Broady) Petlovany 2015 (Syn. *Fottea pyrenoidosa* P. A. Broady 1976)	Ep	+	Mancheva (2013)
44	*Monoraphidium nanum* (Ettl) Hindák 1980	Ep	+	Stoyneva-Gärtner et al. (2024a)
45	*Muriella decolor* Vischer 1936	Ep	+	Stoyneva-Gärtner et al. (2024a)
46	*Muriella terrestris* J. B. Petersen 1932	Ep	+	Stoyneva-Gärtner et al. (2024a)
47	*Muriella* sp.	En	+	Mancheva (2013)

№	Taxon	Type	Culti-vation	Reference
48	*Mychonastes homosphaera* (Skuja) Kalina et Puncochárová 1987	Ep/En	+	Mancheva (2013), Stoyneva-Gärtner et al. (2024a)
49	*Neglectella solitaria* (Wittrock) Stenclová et Kaštovský 2017 (Syn. *Oocystis solitaria* Wittrock 1879)	Ep	-	Starmach (1964)
50	*Neocystis brevis* (Vischer) Kostikov et Hoffmann 2002	Ep	+	Stoyneva-Gärtner et al. (2024a)
51	*Oocystis rupestris* Kirchner 1880	Ep	-	Uzunov et al. (2008)
52	*Palmella mucosa* Kützing 1843	Ep	-	Uzunov et al. (2008)
53	*Parachlorella kessleri* (Fott etNováková) Krienitz et al. 2004	Ep	+	Stoyneva-Gärtner et al. (2024a)
54	*Pleurastrum minutum* (Starr) Sciuto et al. 2023	Ep	+	Stoyneva-Gärtner et al. (2024a)
55	*Prasiola crispa* (Lightfoot) Kützing 1843	Ep/En	+	Uzunov et al. (2008), Mancheva (2013)
56	*Prasiola* sp.	Ep	+	Mancheva (2013)
57	*Printzina lagenifera* (Hildebrand) Thompson et Wujek 1992 (Syn. *Trentepohlia lagenifera* (Hildebrand) Wille 1878)	Ep	+	Mancheva (2013), Stoyneva-Gärtner et al. (2024a)
58	*Pseudodictyochloris multinucleata* (Broady) Ettl et Gärtner 1987	Ep	+	Stoyneva-Gärtner et al. (2024a)
59	*Pseudostichococcus monallantoides* var. *exiguus* (Gerneck) Pröschold et Darienko 2020 (Syn. *Stichococcus exiguus* Gerneck 1907)	Ep	+	Mancheva (2013), Stoyneva-Gärtner et al. (2024a)
60	*Radiococcus bilobatus* (Broady) Kostikov et al. 2002	Ep	+	Stoyneva-Gärtner et al. (2024a)
61	*Rhizoclonium riparium* (Roth) Harvey 1849 (Syn. *Rhizoclonium implexum* (Dillwyn) Kützing 1845)	Ep	-	Vodenicharov et al. (1971)
62	*Scenedesmus obtusus* Meyen 1829 (Syn. *Scenedesmus ovalternus* Chodat)	Ep	-	Uzunov et al. (2008)
63	*Scotiella tuberculata* Bourrelly 1952	Ep	+	Stoyneva-Gärtner et al. (2024a)
64	*Sphaerococcomyxa olivacea* (Petersen) Kostikov et al. 2002	Ep	+	Stoyneva-Gärtner et al. (2024a)
65	*Stephanosphaera pluvialis* Cohn 1852	Ep	-	Vodenicharov et al. (1971)

№	Taxon	Type	Culti-vation	Reference
66	*Stichococcus bacillaris* Nägeli 1849 (Syn. *Stichococcus bacillaris* var. *major* (Nägeli) Rabenhorst 1868)	Ep/En	+	Uzunov (2008), Mancheva (2013), Stoyneva-Gärtner et al. (2024a)
67	*Stichococcus chlorelloides* Grintzesco et S. Péterfi 1932	Ep	+	Stoyneva-Gärtner et al. (2024a)
68	*Stichococcus minutus* Grintzesco et S. Péterfi 1932	Ep/En	+	Mancheva (2013), Stoyneva-Gärtner et al. (2024a)
69	*Stichococcus mirabilis* Lagerheim 1893	Ep/En	+	Stoyneva-Gärtner et al. (2024a)
70	*Stichococcus* sp.	En	+	Mancheva (2013)
71	*Tetracystis pulchra* R. M. Brown et Bold 1964	Ep	+	Stoyneva-Gärtner et al. (2024a)
72	*Trebouxia arboricola* Puymaly 1924	Ep	+	Gärtner & Stoyneva (2003), Stoyneva & Gärtner (2006), Uzunov et al. (2008)
73	*Trebouxia* sp. (ad *Asterochloris excentrica* (Archibald) Skaloud et Peksa)	Ep	+	Stoyneva-Gärtner et al. (2024a)
74	*Trentepohlia abietina* (Flotow ex Kützing) Hansgirg 1886	Ep	+	Mancheva (2013)
75	*Trentepohlia arborum* (Agardh) Hariot 1889	Ep	+	Stoyneva-Gärtner et al. (2024a)
76	*Trentepohlia aurea* (Linnaeus) C. Martius 1817	Ep	+	Uzunov et al. (2008), Stoyneva & Gärtner (2006)
77	*Trentepohlia jolithus* (Linnaeus) Wallroth 1833	Ep	+	Uzunov et al. (2008), Stoyneva-Gärtner et al. (2024a)
78	*Trentepohlia* cf. *jucunda* (Cesati) Hariot 1889	En	+	Stoyneva-Gärtner et al. (2024a)
79	*Trentepohlia umbrina* (Kützing) Bornet 1873	Ep	+	Mancheva (2013)
80	*Trentepohlia* cf. *umbrina* (Kützing) Bornet 1874	Ep	+	Mancheva (2013)
81	*Trentepohlia* sp.	Ep	+	Mancheva (2013)
82	*Ulothrix tenerrima* (Kützing) Kützing 1843 (Syn. *Ulothrix variabilis* Kützing 1849)	Ep	+	Mancheva (2013)

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83	*Uvulifera mucosa* (Broady et Ingerfeld) Molinari 2016	Ep	+	Stoyneva-Gärtner et al. (2024a)
	STREPTOPHYTA			
1	*Cosmarium crenatum* var. *nanum* Wittrock 1883	Ep	-	Uzunov et al. (2008)
2	*Cosmarium curtum* var. *exiguum* f. *polymorpha* Petkoff	Ep	-	Uzunov et al. (2008)
3	*Cosmarium curtum* var. *exiguum* Hansgirg	Ep	-	Uzunov et al. (2008)
4	*Cosmarium laeve* Rabenhorst 1868	Ep	-	Starmach (1964)
5	*Cosmarium notabile* Brébisson 1856	Ep	-	Uzunov et al. (2008)
6	*Cosmarium tumens* Nordstedt 1872	Ep	-	Uzunov et al. (2008)
7	*Deuterostichococcus marinus* (Deason) Pröschold et Darienko 2020 (Syn. *Klebsormidium marinum* (Deason) P. C. Silva, K. M. Mattox et W. H. Blackwell 1972)	Ep	+	Mancheva (2013)
8	*Disphinctium* sp.	Ep	-	Uzunov et al. (2008)
9	*Euastrum binale* Ehrenberg ex Ralfs 1848	Ep	-	Uzunov et al. (2008)
10	*Klebsormidium crenulatum* (Kützing) Lokhorst 1985	Ep	+	Stoyneva-Gärtner et al. (2024a)
11	*Klebsormidium dissectum* (F. Gay) H. Ettl et G. Gärtner 1995	Ep	+	Mancheva (2013), Stoyneva-Gärtner et al. (2024a)
12	*Klebsormidium flaccidum* (Kützing) P. C. Silva, Mattox et W. H. Blackwell 1972 (Syn. *Hormiscia flaccida* var. *varia* (Kütz.) De Wild, *Hormiscia subtilis* var. *albicans* (Kütz.) Hansgirg)	Ep	+	Uzunov et al. (2008), Mancheva (2013), Stoyneva-Gärtner et al. (2024a)
13	*Klebsormidium* cf. *flaccidum* (Kützing) P. C. Silva, Mattox et W. H. Blackwell 1972	Ep	+	Stoyneva-Gärtner et al. (2024a)
14	*Klebsormidium klebsii* (G. M. Smith) P. C. Silva, K. R. Mattox et W.H. Blackwell 1972	Ep	+	Mancheva (2013), Stoyneva-Gärtner et al. (2024a)
15	*Klebsormidium pseudostichococcus* (Heering) H. Ettl et G. Gärtner 1995	Ep	-	Uzunov et al. (2008)
16	*Klebsormidium subtile* (Kützing) Mikhailyuk, Glaser, Holzinger et Karsten 2015	Ep	-	Uzunov et al. (2008)
17	*Mesotaenium chlamydosporum* De Bary 1858	Ep	-	Starmach (1964)

№	Taxon	Type	Culti-vation	Reference
18	*Mesotaenium macrococcum* (Kützing) J. Roy et Bisset 1894	Ep	+	Uzunov et al. (2008), Mancheva (2013)
19	*Mougeotia nummuloides* (Hassall) De Toni 1889	Ep	-	Uzunov et al. (2008)
20	*Roya obtusa* (Brébisson) West et G. S.West 1896	Ep	-	Uzunov et al. (2008)
21	*Spirogyra varians* (Hassall) Kützing 1849	Ep	-	Uzunov et al. (2008)
22	*Zygnema* sp.	Ep	-	Uzunov et al. (2008)
	OCHROPHYTA			
	Bacillariophyceae			
1	*Achnanthidium minutissimum* (Kützing) Czarnecki 1994 (Syn. *Achnanthes minutissima* Kützing 1833)	Ep	-	Starmach (1964)
2	*Amphora pediculus* (Kützing) Grunow 1875 (Syn. *Amphora ovalis* var. *pediculus* (Kützing) Van Heurck 1885)	Ep	-	Starmach (1964)
3	*Craticula cuspidata* (Kützing) D. G. Mann 1990 (Syn. *Navicula cuspidata* Kützing 1844)	Ep	-	Starmach (1964)
4	*Cymbella affinis* Kützing 1844	Ep	-	Starmach (1964)
5	*Cymbella cymbiformis* C. Agardh 1830	Ep	-	Starmach (1964)
6	*Denticula elegans* Kützing 1844	Ep	-	Starmach (1964)
7	*Diatoma vulgaris* Bory 1824	Ep	-	Starmach (1964)
8	*Epithemia gibba* (Ehrenberg) Kützing 1844 (Syn. *Rhopalodia gibba* (Ehrenberg) O.Müller 1895)	Ep	-	Starmach (1964)
9	*Frustulia amosseana* Lange-Bertalot 2000 (Syn. *Frustulia vulgaris* var. *capitata* Krasske 1923)	Ep	-	Starmach (1964)
10	*Frustulia vulgaris* (Thwaites) De Toni 1891	Ep	-	Starmach (1964)
11	*Gomphonema gracile* Ehrenberg 1838	Ep	-	Starmach (1964)
12	*Halamphora coffeiformis* (C. Agardh) Mereschkowsky 1903 (Syn. *Amphora coffeiformis* (C. Agardh) Kützing 1844)	Ep	-	Starmach (1964)
13	*Hantzschia amphioxys* (Ehrenberg) Grunow 1880	En	+	Mancheva (2013)
14	*Mastogloia elliptica* (C. Agardh) Cleve 1893	Ep	-	Starmach (1964)

№	Taxon	Type	Culti-vation	Reference
15	*Mastogloia smithii* Thwaites ex W. Smith 1856	Ep	-	Starmach (1964)
16	*Navicula cryptocephala* Kützing 1844	Ep	-	Starmach (1964)
17	*Navicula gregaria* Donkin 1861	Ep	-	Starmach (1964)
18	*Navicula* sp.	Ep	+	Mancheva (2013)
19	*Navicula* sp. s.l.	Ep	+	Stoyneva-Gärtner et al. (2024a)
20	*Neidium dubium* (Ehrenberg) Cleve 1894	Ep	-	Starmach (1964)
21	*Nitzschia communis* Rabenhorst 1860	Ep	-	Starmach (1964)
22	*Nitzschia palea* (Kützing) W. Smith 1856	Ep	-	Starmach (1964)
23	*Nitzschia vitrea* G.Norman 1861	Ep	-	Starmach (1964)
24	*Pinnularia decrescens* (Grunow) Krammer 2000 (Syn. *Pinnularia subsolaris* (Grunow) Cleve 1895)	Ep	-	Starmach (1964)
25	*Pinnularia biceps* f. *minutissima* (Hustedt) A. Cleve 1955 (Syn. *Pinnularia interrupta* f. *minutissima* Hustedt 1930)	Ep	-	Starmach (1964)
26	*Pinnularia* sp.	Ep	+	Stoyneva-Gärtner et al. (2024a)
27	*Stauroneis* sp.	Ep	+	Mancheva (2013)
28	*Sellaphora pupula* (Kützing) Mereschk-ovsky 1902 (Syn. *Navicula pupula* Kützing 1844)	Ep	-	Starmach (1964)
29	*Toxarium undulatum* var. *ponticum* Petkoff 1919	Ep	-	Uzunov et al. (2008)
30	*Ulnaria ulna* (Nitzsch) Compère 2001 (Syn. *Synedra ulna* (Nitzsch) Ehrenberg 1832)	Ep	-	Starmach (1964)
	Eustigmatophyceae			
31	*Chloridella minuta* Gayral et Mazancourt 1958	Ep	+	Stoyneva-Gärtner et al. (2024a)
32	*Chlorobotrys gloeothece* Pascher 1938	Ep	+	Stoyneva-Gärtner et al. (2024a)
33	*Gloeobotrys piriformis* Reisigl 1964	Ep	+	Stoyneva-Gärtner et al. (2024a)
34	*Gloeobotrys terrestris* Reisigl 1964	Ep	+	Stoyneva-Gärtner et al. (2024a)

№	Taxon	Type	Culti-vation	Reference
35	*Vischeria magna* (J. B. Petersen) Kryvenda, Rybalka, Wolf et Friedl 2018 (Syn. *Eustigmatos magnus* (J. B. Petersen) D. J. Hibberd 1981)	Ep	+	Mancheva (2013), Stoyneva-Gärtner et al. (2024a)
36	*Vischeria polyphem* (Pitschmann) Kryvenda, Rybalka, Wolf et Friedl 2018 (Syn. *Eustigmatos polyphem* (Pitschmann) D. J. Hibberd 1981)	Ep	+	Mancheva (2013)
37	*Vischeria stellata* (Chodat) Pascher 1938	Ep	+	Gärtner et al. (2012) Mancheva (2013), Stoyneva-Gärtner et al. (2024a)
38	*Vischeria* cf. *stellata* (Chodat) Pascher 1938	Ep	+	Mancheva (2013)
39	*Vischeria vischeri* (Hibberd) Kryvenda, Rybalka, Wolf et Friedl 2018 (Syn. *Eustigmatos vischeri* D.J.Hibberd 1981)	Ep	+	Mancheva (2013)
	Xanthophyceae			
40	*Botrydiopsis* sp.	Ep	+	Stoyneva-Gärtner et al. (2024a)
41	*Botryochloris simplex* Pascher 1938	Ep	+	Mancheva (2013)
42	*Bumilleriopsis* cf. *brevis* (Gerneck) Printz 1914	Ep	+	Mancheva (2013)
43	*Chlorellidium astigmatum* Schwarz 1979	Ep	+	Stoyneva-Gärtner et al. (2024a)
44	*Ellipsoidion perminimum* Pascher 1938	Ep	+	Stoyneva-Gärtner et al. (2024a)
45	*Heterococcus* sp.	Ep	+	Mancheva (2013)
46	*Monodus* cf. *coccomyxa* Pascher 1938	Ep	+	Mancheva (2013)
47	*Monodus guttula* Pascher 1938	Ep	+	Stoyneva-Gärtner et al. (2024a)
48	*Pleurochloris commutata* Pascher 1925	Ep	+	Stoyneva-Gärtner et al. (2024a)
49	*Tribonema minus* (Wille) Hazen 1902	Ep	+	Stoyneva-Gärtner et al. (2024a)
50	*Vaucheria racemosa* (Vaucher) De Candolle 1805	Ep	-	Uzunov et al. (2008)
51	*Vaucheria geminata* (Vaucher) De Candolle 1805	Ep	-	Uzunov et al. (2008)

№	Taxon	Type	Culti-vation	Reference
52	*Xanthonema* sp.	Ep	+	Mancheva (2013)
	PYRROPHYTA			
1	*Gloeodinium montanum* G. A. Klebs 1912	Ep	-	Uzunov et al. (2008)
	EUGLENOPHYTA			
1	*Trachelomonas irregularis* var. *minor* Svirenko 1914	Ep	-	Starmach (1964)

As it could be seen from this table, most of the reported species are real epilithes (342) and only a small number (27) are endolithes, with 16 of them being real endolithes and the other 11 being able to grow both epi- and endolithically. Most of the endolithes belong to the green evolutionary lineage, namely Chlorophyta – 17, where eight of them are endolithes and the other nine exhibit mixed (Ep/En) development, followed by the blue-green lineage (Cyanoprokaryota) with nine endolithes, two of which were found to grow both epi- and endolithically. Lastly, there are the Ochrophytes with a single pure endolithic specimen, belonging to the class of Bacillariophyceae. Of the total 369 reported species, 147 are identified upon introducing them in cultures and examining their complete life cycle, including young and adult stages. The other 222 taxa are described by using direct microscopy of the collected field material, without growing them in artificial conditions. Predominantly these are the species reported during the past century. It has to be underlined that some of the species, reported from samples collected from moist rocks, are not typical lithophytes, but classical aquatic inhabitants and their finding should be considered as occasional (*e.g.*, *Mougeotia*, *Spirogyra*, *Trachelomonas* and most diatoms).

In the different phyla, listed in **Table 1** and summarized on **Figure 1**, the cultivated to non-cultivated ratio is as follows – for Cyanoprokaryota (208) the cultivated species are 47 and the non-cultivated species are 161. In the green evolutionary lineage – Chlorophyta (83) the cultivated species are 68, the non-cultivated – 15, and in Streptophyta (22) – seven of the listed species are cultivated (predominantly belonging to the genus of *Klebsormidium*) and the other 15 species have not been introduced into cultures. For the yellow-brown evolutionary lineage, Ochrophyta (52) – 25 of the reported species has undergone long-term cultivation and the remaining 27 have been reported from direct microscopic observations. The red evolutionary lineage – Rhodophyta is represented by 2 species, which haven't been cultivated, and finally the pyrro- and euglenophytes are represented by a single species each, which also haven't been introduced into culture.

The presented data clearly show that most species that have undergone cultivation in laboratory setting belong to the green evolutionary lineage (Chlorophyta - 68 out

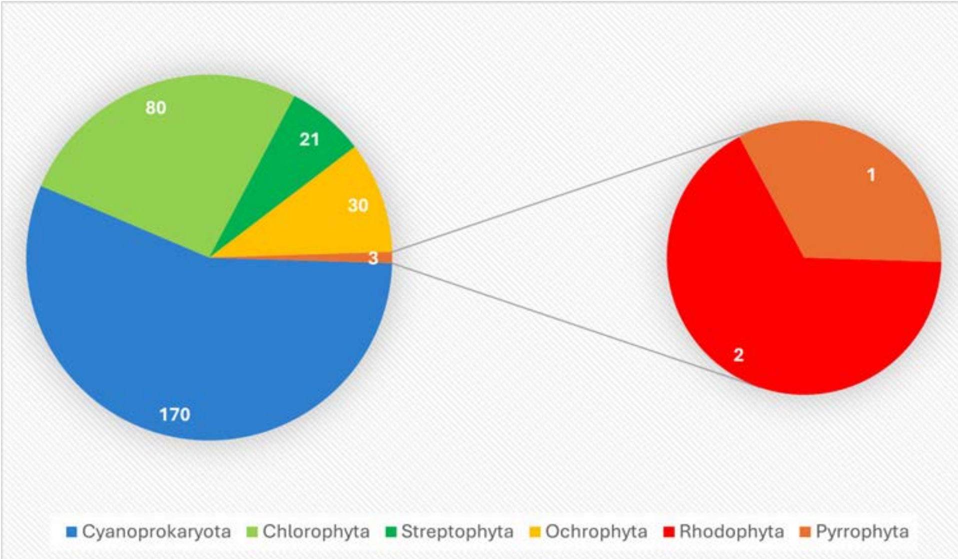

Fig. 1. Species distribution in seven of the main algal phyla. Total number of identified species is shown on each corresponding slice.

of 83, and Streptophyta - 7 out of 22), followed by Ochrophyta (25 of 52). The less common cultivated species are the cyanoprokaryotes (47 out of 208), most of which (161) are identified by direct microscopy methods, without introduction into culture (**Figure 2**). Finally, the red algae, the pyrrophytes and euglenophytes haven't been cultivated in artificial conditions, making them the only three phyla the species of which have been identified using direct microscopy only.

CONCLUSION

A rich biodiversity of lithophytes has been documented from different rock and stone substrates in Bulgaria: 369 algal taxa from seven phyla, most of which belong to the blue-green algae, or Cyanoprokaryota, (208), followed by the green algae from Chloro- (83) and Streptophyta (22), and the yellow-brown algae, or Ochrophyta (52), whereas from the red algae, or Rhodophyta, only two species have been reported, and from each of the phyla Pyrrhophyta and Euglenophyta only one species was recorded. Most of these algae were found from epilithic samples (342), and only 27 were endolithes. The number of algae, identified after application of cultivation procedures and following of different stages of their development, is significantly lower (147) than the number of algae identified from field material by direct microscopy (222). However, it has to be stressed that during the last two decades all reports of lithophytes were based on a preliminary cultivation.

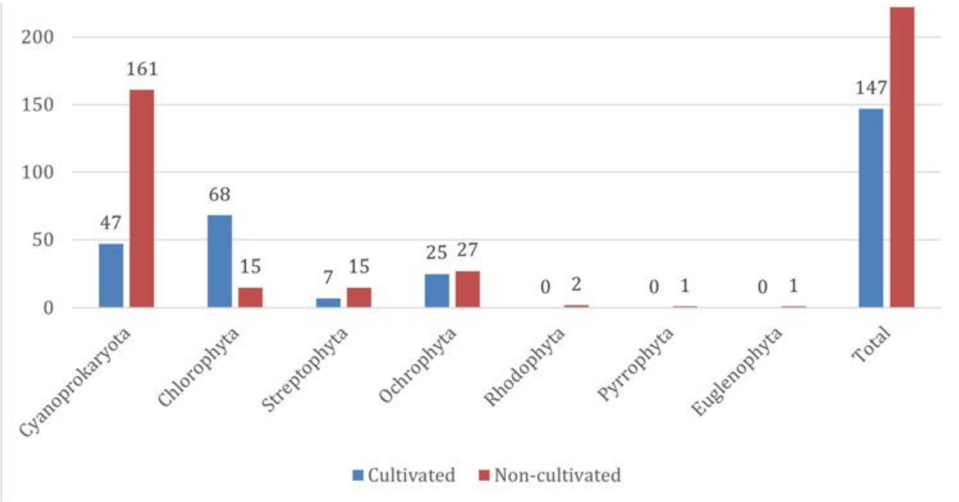

Fig. 2. Comparison between numbers of algae identified after cultivation procedure ("Cultivated") and algae identified from field material by direct light microscopy ("Non-cultivated") in each of the major algal phyla.

The application of cultivation is an important step when working with aero-terrestrial algae and lithophytes in particular. The ability to examine every single stage of their lifecycle is crucial for the precise taxonomical identification of the collected material. Observing both young and adult stages of species development, their unique morphological, reproductive, ecological and biochemical characteristics, provides key insights on the adaptational mechanisms of this peculiar group of algae and their ability to survive in harsh and sometimes even extreme habitats.

The cultivation strategy plays an important role not only for taxonomical studies, but also for screening and selection of promising biotechnological strains. Applying this cultivation strategy to purified monocultures could not only serve as a living archive of biodiversity (Stoyneva 2014), but could yield a substantial amount of information regarding the pigment composition, stored substances and specific biomolecules accumulated inside the cell – all important features for future industrial and biotechnological implementations (Stoyneva-Gärtner et al. 2019a). Among such strains are five strains of the eustigmatophycean genus *Vischeria*, which shows great potential for accumulation of various carotenoids (Stoykova et al. 2019, Stoyneva-Gärtner et al. 2019b) and is a promising source for lipids (Stoyneva-Gärtner et al. 2022). Another example includes strain of the filamentous streptophyte *Klebsormidium*, which is known for its great biotechnological potential (Stoyneva-Gärtner et al. 2019c) and currently has been shown as a promising agent of water purification (Valchev et al.

2019, 2021, 2023). Furthermore, the constant demand for clean, ecological and biocompatible cosmetics, harmless both to humans and the environment we live in, has reached its peak in the recent years. As shown by Stoyneva-Gärtner et al. (2020, 2024b), this bio trend calls for researching the inclusion of algae and algal byproducts in the various cosmetics people use in their everyday lives, where lithophytic algae could play a crucial role. These are only a few examples of the lithophytes, collected in Bulgaria, available from the living algal collection ACUS but it is possible to suppose that future studies will demonstrate their untapped biotechnological potential and more species will be find after conducting of more field trips in different parts of the country.

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CONFLICT OF INTERESTS

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

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

MAYA P. STOYNEVA-GÄRTNER* AND BLAGOY A. UZUNOV

Department of Botany, Faculty of Biology, Sofia University 'St Kliment Ohridski', 8 Dragan Tsankov Blvd., 1164 Sofia, Bulgaria

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

**corresponding author*: M. Stoyneva-Gärtner - Department of Botany, Faculty of Biology, Sofia University “St. Kliment Ohridski”, 8 Dragan Tsankov Blvd., 1164 Sofia, Bulgaria; mstoyneva@uni-sofia.bg.

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)

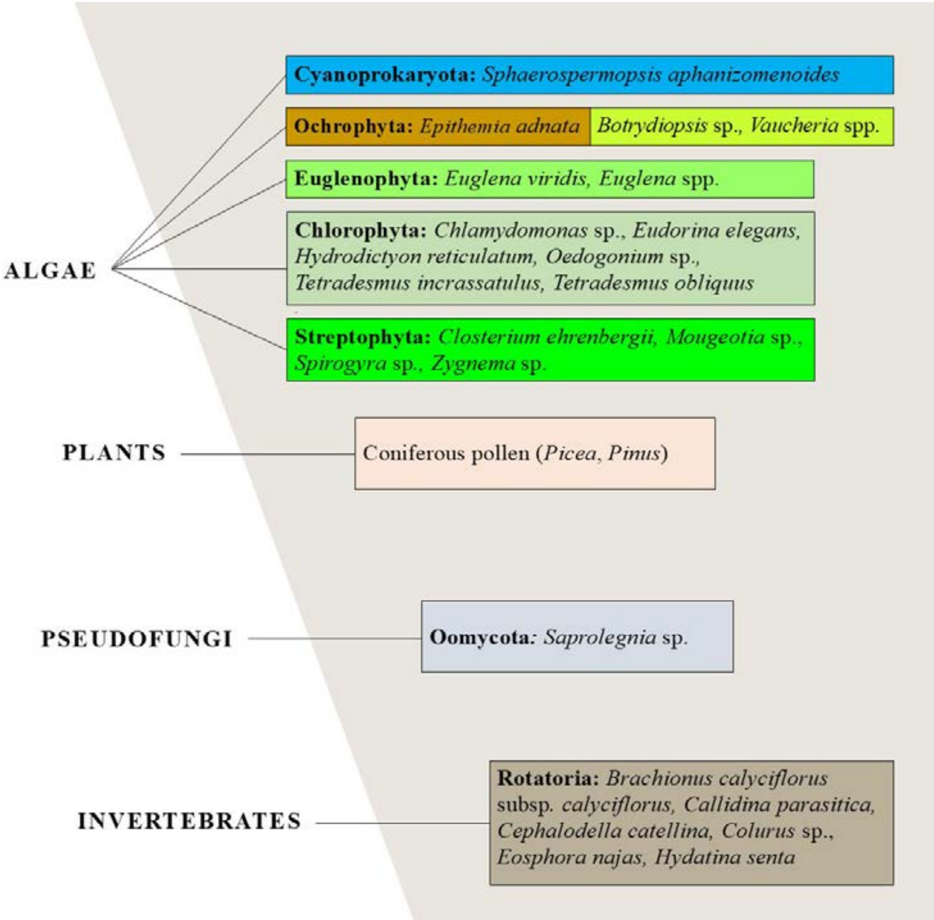
Prof. Nikola Arnaudov (1887-1961)

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.

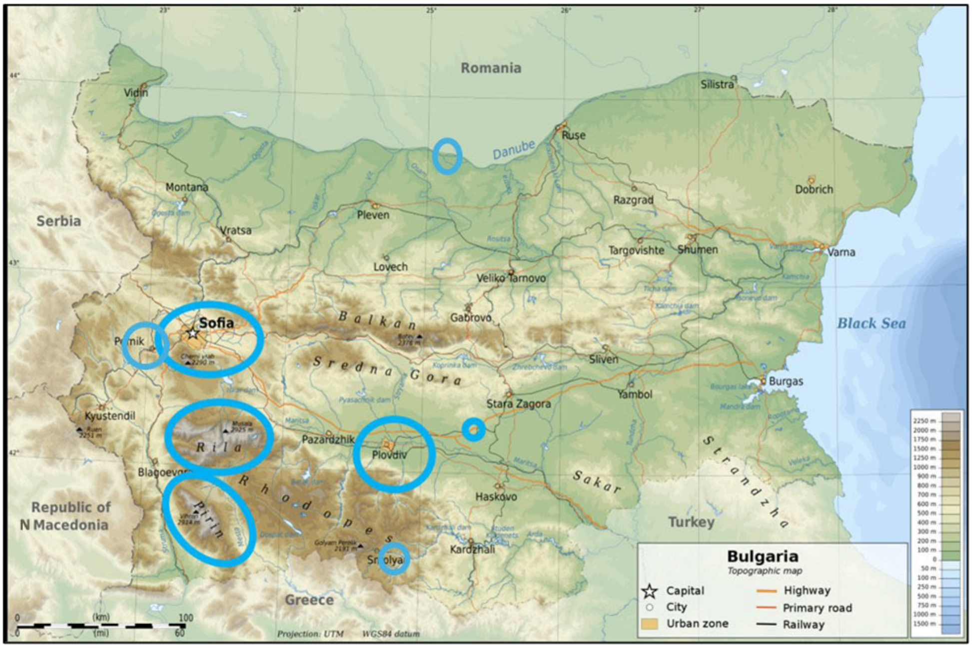


Fig. 2. Regions of Bulgaria with the localities, in which zoosporic parasites have been found by different authors (for details see the text of the paper), shown by blue circles (map modified after https://en.m.wikipedia.org/wiki/File:Bulgaria-geographic_map-en.svg). Note: The locality "swamp near the 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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BIODIVERSITY OF MACROMYCETES IN THE VICINITY OF CHELOPECH TOWN, STARA PLANINA MTS (2020-2021, 2023). I. SPECIES COMPOSITION, ECOTROPHIC GROUPS AND CONSERVATIONALLY IMPORTANT SPECIES

BLAGOY A. UZUNOV*, KRISTIAN R. IVANOV, MIROSLAV I. ANDROV, BOYAN T. MICHEV & MAYA P. STOYNEVA-GÄRTNER

[1] *Sofia University "St. Kliment Ohridski", Faculty of Biology, Department of Botany, 8 Dragan Tsankov Blvd., BG-1164, Sofia, Bulgaria*
[2] *Bulgarian Academy of Sciences, Institite of biodiversity and ecosystem services, Gagarin str. 2, BG-1113, Sofia, Bulgaria*

Abstract. The paper presents first results obtained on the biodiversity in terms of species composition and ecotrophic groups of macromycetes from Sredna Stara Planina Mts in the vicinity of Chelopech town. The studied area (20 km^2) was visited in the late spring/early summer periods of 2020 and 2023, as well as in late autumn/early winter periods of the years 2021 and 2023, covering 23 days. The field work was conducted in eight representative polygons of two main areas with application of transect method. Altogether 219 taxa, mostly Basidiomycota (208), were recorded with differences in their distribution in terms of area and seasons. The variation in these differences allows us to suppose the strong influence of local climate conditions on the results obtained. Regarding ecotrophic groups, most of the macromycetes were saprotrophs (147) which develop on different substrata, followed by mycorrhizal fungi (67) and very less, only five, were parasites. Among the recorded species, six were of conservational importance according to the Red List of Bulgarian macromycetes and contemporary Red Data Book of R Bulgaria. Comparison of the results obtained with updated list of Bulgarian macromycetes demonstrate the great biodiversity of the studied area, which comprises 83% of the biodiversity of macromycetes in Sredna Stara Planina Mts, 35%

**corresponding author*: B. A. Uzunov - Sofia University "St. Kliment Ohridski", Faculty of Biology, Department of Botany, 8 Dragan Tsankov Blvd., BG-1164, Sofia, Bulgaria; buzunov@uni-sofia.bg

of their diversity in the entire Stara Planina Mts, 14% of the total macromycetous biodiversity of Bulgaria and is compatible, even exceeding, the fungal diversity in other mountains of the country. Six species were of conservational importance according to the Red Data Book of R Bulgaria (1) and Red List of Bulgarian macromycetes (6). The paper presents a new locality for the species *Gomphidius roseus* monitored in the Bulgarian National Biodiversity Monitoring System (part Monitoring of Fungi) and currently obtained data indicate the favourable trends of its development in the country.

Key words: Ascomycota, Basidiomycota, biodiversity, critically endangered species, Dikarya, endangered species, fungi, mycorrhizal fungi, parasitic fungi, saprotrophic fungi, vulnerable species

INTRODUCTION

The importance of Fungi for the biosphere is practically undisputable since being devoid of chlorophyll and the possibility of self-feeding from solar energy, they are ecological consumers that break down organic compounds into simple inorganic substances with the help of a variety of enzymes. The role of Fungi in forest ecosystems is particularly important because they are the only organisms on the planet capable of breaking down the lignin of trees. On the other hand, Fungi help increase soil fertility, and some of them, known as mycorrhizal fungi, live together, in symbiosis, with woody, shrubby or herbaceous plants and are essential for their nutrition and development.

Macromycetes are well known in people's daily life with their practical division into edible/poisonous, medicinal and parasitic fungi. But in purely ecological terms, their usual division is different and more detailed, being based on lifestyle, habitat and typical substrate. In the frame of the both projects conducted on macromycetes in the vicinities of the town Chelopech in Sredna Stara Planina Mts, which provide the bases for the present paper, both approaches (practical and ecological) are reflected along with the taxonomic assessment in order to achieve the best knowledge on both the biodiversity of the studied macromycetes and their ecosystem services with the possibilities of their practical use. This paper presents the first part of the work in regard of species composition, ecotrophic groups and conservationally important species in the studied area.

MATERIAL AND METHODS

Investigated region

The region of investigation covers the south slopes of Stara Planina Mts in the part considered as Middle Stara Planina. It is situated between 530 and 1170 m a.s.l., being a part of the Zlatishko-Pirdopska kettle in the closest vicinity of Chelopech town. The typical climate is temperate continental with spring maximum and winter precipitation minimum. In particular, the southern mountain slopes are in the rain shadow area with average precipitation values of about 550 mm. In this area, the cinnamon forest soils predominate.

The investigated region occupies a territory of about 20 km^2 and besides natural mountain habitats and natural wetlands, including parts of the streams of the river Topolnitsa and its right tributary Vozdol, contains also arable lands and urban areas. For the purposes of the study, it was operationally divided in two parts: I - upper, northern part with territory of ca. 12 km^2, and II – lower, southern part of ca. 7.5 km^2.

Period of investigation

Field work was conducted during two seasons of the years 2020-2021 and 2023 – spring (May-June 2021, 2023) and autumn (October-December 2020, 2023) with 23 daily visits of the region. In the period 2020-2021 there were totally 14 visits, 7 in each season, on the following dates: 15.10.2020, 22.10.2020, 29.10.2020, 25.11.2020, 26.11.2020, 8.12.2020, 11.12.2020 and 12.05.2021, 14.05.2021, 17.05.2021, 26.05.2021, 29.05.2021, 04.06.2021, and 11.06.2021. In 2023, nine daily visits, four in spring and five in autumn, were conducted on the following dates: 25.05.2023, 19.06.2023, 20.06.2023, 27.06.2023, 13.10.2023, 14.10.2023, 27.10.2023, 8.12.2023 and 9.12.2023. All these dates were selected regarding the most favourable metereological conditions with an attempt to make visits on the close dates for different years.

For easier reading, in the text below, the autumn and spring periods of 2020-2021 will be named as "first period of investigation", and the spring-autumn period of 2023 will be noted as "second period of investigation".

During the field work initially, the entire area was visited to select representative working polygons, which would contain various types of habitats and include places suitable for the development of macromycetes, excluding the main urban areas, arable lands and water surfaces. Looking for suitable places for macromycete development, we tried to embrace all ecological groups of macromycets from different types of natural habitats: open and shadowed areas in deciduous forests (birch, beech, mixed), coniferous forests and plantations, mixed coniferous and deciduous forests, pastures and meadows, screes, riverside habitats, etc. The total area of these polygons was 1.2 км^2, and each of them had the following area: 1 - 394 592 m^2; 2 - 83 266 m^2; 3 - 156 217m^2; 4 - 44 825 m^2; 5 - 198 124 m^2; 6 - 16 543 m^2; 7 - 172 265 m^2 and 8 - 133 915 m^2.

Special attention was also paid to forest areas with a more active anthropogenic presence and activities such as roads and recreation places, as well as to some of the tourist trails located in the area and the designed eco-path "Chervenata Puteka" (which in Bulgarian language means The Red Pathway). As a result, 8 representative polygons were selected. Six of these polygons were situated in the first, northern part, and two were situated in the second, southern part of the area (**Figure 1**). Special attention was also paid to areas with a more active anthropogenic presence and activities of the enterprise (roads for the movement of equipment, built facilities, places for recreation, etc.), as well as to some of the tourist trails located in the area and the designed eco-path “Cherveniyat Kamuk” (which in Bulgarian language means The Red Stone). There, the work was conducted according to the transect method using a different number of sub-transects depending on the specific conditions of the terrain. All transects were repeatedly visited during both seasons, and each transect was covered 2-4 times in the frame of a single season.

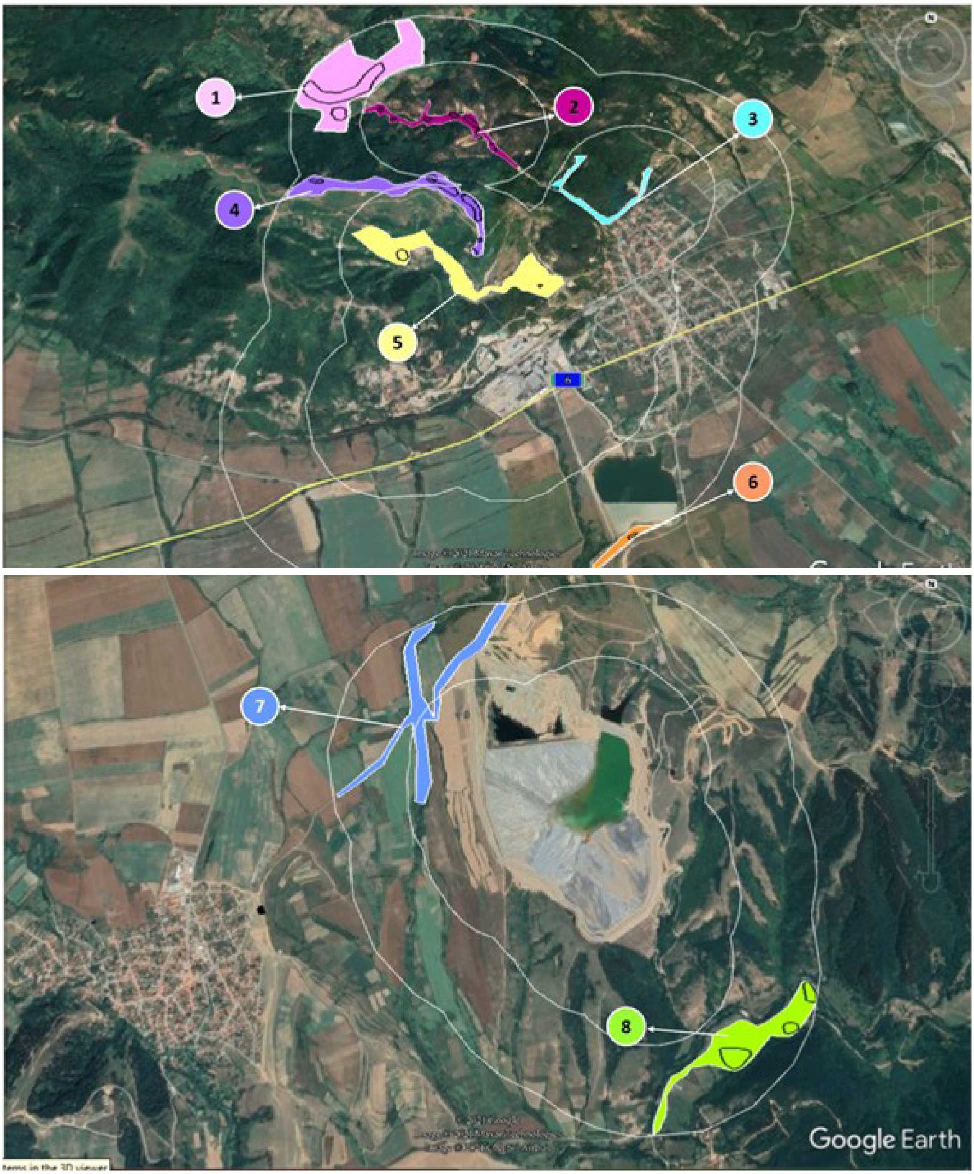

Fig. 1. Representative polygones in northern and southern part of the investigated region (modified after *Google Earth*).

Methods for collection, transportation, processing and identification of the macromycetes

The macromycetes, which are the subject of this investigation, usually are defined as large, multicellular and visible with naked eyes fungi, the fruiting bodies of which are larger than 5 mm. Taxonomically, macromycetes traditionally belong to two main phyla, Ascomycota and Basidiomycota, united in the subkingdom Dikarya (Hibbett et al. 2018).

According to the National Biodiversity Monitoring System (NBMS), and its part on the monitoring of fungi in particular (Denchev et al. 2013), when working with macromycetes, the reporting unit is the fruiting body. According to this methodology, the unit of observation is a point, and the reading is within a radius of 30 m around the point where fruiting bodies were observed for the first time in the presence of a suitable substrate for the development of the species. In accordance with this, when finding a fruiting body along a given transect, we investigated the local area within a radius of about 30 m (when the terrain conditions allow this).

The species were documented in situ using a camera and mobile phones with a positioning system for mapping purposes (**Figure 2**). After completing the transect determination, for the implementation of the subsequent laboratory work, the fruiting bodies were collected (1-5 specimens of each species according to the specific number in a given locality). They were cleaned on site using a special knife equipped with a brush (**Figure 2**) and transported on the same day to the laboratory in paper bags (large fruiting bodies) or in plastic tubes with a volume of 50 ml (small fruiting bodies) – **Figure 2**. The collected specimens were also subjected to drying using electric dehydrators (**Figure 2**) for their preservation and further inclusion in a herbarium. Their drying time varies according to the size and texture of the fruiting body and is between 4-15 hours.

The identification of macromycetes follows the standard modern European mycological literature (*e.g.,* Breitenbach & Kränzlin 1984, 1986, 1991, 1995, 2000, 2005; Hansen & Knudsen 1992, 1997, 2000; Laessoe & Petersen 2019) with actualization of the taxonomy according to the world-wide accepted on-line database Index Fungorum. It was made in a final version in laboratory conditions by applying a macroscopic examination of the fruiting bodies and microscopic methods for observing and determining the spore-bearing parts of the fruiting bodies, the size, type and sculpture of the spores, the type of cystids, etc., which are commonly used as diagnostic features.

In cases when only one fruiting body of a species has been found, but the species identification requires records of different developmental stages, or the fruiting body has been damaged (found in the form of parts left from knife cuts or broken by foragers that have passed before us the area, or as eaten by animals), the determination is made to the Genus level (indicated in standard nomenclature with “sp.”) or to the closest species by available characters (which is indicated in standard nomenclature with “cf.” before the species name).

After determination, the macromycete species are classified according to their ecotrophic requirements (*e.g.*, the substrate on which they occur) according to the available literature data (*e.g.,* Ollson et al. 2000, Harding 2008 for parasites) and according to our own field observations in the following three main ecological (ecotrophic) groups:

- saprotrophs (S)
- mycorrhizal fungi (M)
- parasites (P).

In cases of double mode of life, two ecological groups are shown (*e.g.,* S/P, or M/P).

The conservation status was evaluated according to the Red Data Book of R Bulgaria (Peev 2011) and Red List of Bulgarian macromycetes (Gyosheva et al. 2006). Fungi, included in the NBMS (part Monitoring of Fungi – Denchev et al. 2013), have been also taken into account and their status was evaluated according to the criteria and parametres enlisted in NBMS.

Fig. 2. Documentation, transportation and dehydration of the fruiting bodies.

RESULTS

Biodiversity of macromycetes

In total, 219 macromycetous species were found (**Table 1, Appendix 1**). They belonged to 187 genera, 57 families, 35 orders, 14 classes, 3 subphyla and 2 phyla – Ascomycota and Basidiomycota. The 171 species recorded in the period October 2020-June 2021 were from 83 genera, 40 families, 12 orders, 4 classes, 3 subphyla and 2 phyla. The 88 recorded species in the period June-December 2023 belonged to 57 genera, 32 families, 8 orders, 3 classes, 2 subphyla and 2 phyla (**Table 1)**.

Table 1. Taxonomic list of the macromycetes found in the Chelopech region of Stara Planina Mts and their distribution in both parts of the investigated region in different seasons and periods and in ecotrophic groups: I – northern, upper part; II – southern, lower part; Au – autumn, SP – spring, 20 – year 2020, 21 – year 2021, 23 – year 2023; EG – ecotrophic group: M – mycorrhizal fungus, S – saprotroph, and P – parasite. Inside the phyla subphyla, classes, orders, families, genera and species are organized in alphabetical order for easier reading.

Taxonomic categories / Macromycetes	**Au 20**		**Sp 21**		**Sp 23**		**Au 23**		**EG**
	I	**II**	**I**	**II**	**I**	**II**	**I**	**II**	**MSP**
Phylum Ascomycota									
Subphylum Ascomycotina									
Classis Ascomycetes									
Ordo Helotiales									
Familia Gelatinodiscaceae									
Genus *Ascocoryne* J. W. Groves & D. E. Wilson									
Ascocoryne sarcoides (Jacq.) J. W. Groves et D. E. Wilson	x								S
Subphylum Pezizomycotina									
Classis Pezizomycetes									
Ordo Pezizales									
Familia Helvellaceae									
Genus *Helvella* Fr.									
Helvella crispa (Scop.) Fr.						x			S
Helvella fibrosa (Wallr.) Korf						x			S
Helvella lacunosa Afzel.						x			S
Familia Pezizaceae									
Genus *Peziza* Fuckel									
Peziza saniosa J. F. Gmel.						x			S
Peziza varia (Hedw.) Alb. et Schwein.						x			S

Taxonomic categories / Macromycetes	Au 20		Sp 21		Sp 23		Au 23		EG
	I	II	I	II	I	II	I	II	MSP
Familia Pyronemataceae									
Genus *Humaria* Fuckel									
Humaria hemisphaerica (F. H. Wigg.) Fuckel						x			S
Ordo *Xylariales*									
Familia Hypoxylaceae									
Genus *Hypoxylon* Bull									
Hypoxylon fragiforme (Pers.) J. Kickx						x		x	S
Familia Xylariaceae									
Genus *Poronia* Willd.									
Poronia punctata (L.) Fr.					x	x			S
Genus *Xylaria* Hill ex Schrank									
Xylaria hypoxylon (L.) Grev.	x	x	x						S
Xylaria polymorpha (Pers.) Grev	x	x	x						S
Phylum Basidiomycota									
Subphylum Agaricomycotina									
Classis Agaricomycetes									
Ordo Agaricales									
Familia Agaricacae									
Genus *Agaricus* L.: Fr. emend Karst.									
Agaricus arvensis Schaeff.	x	x				x			S
Agaricus campestris L.	x	x	x			x		x	S
Agaricus cf. *sylvicola* (Vittad.) Peck	x								S
Agaricus sylvaticus Schaeff.	x	x							S
Agaricus cf. *xanthodermus* Genev.	x	x							S
Genus *Chlorophyllum* Massee									
Chlorophyllum rhacodes (Vittad.) Singer	x								M
Genus *Coprinus* Pers.									
Coprinus comatus (O. F. Müll.) Pers.	x							x	S
Genus *Lepiota* P. Browne									
Lepiota cristata (Bolton) P. Kumm	x	x					x		S
Lepiota erminea (Fr.) P. Kumm.						x			S
Lepiota ochraceodisca Bon	x								S
Lepiota cf. *subalba* Kühner ex P. D. Orton	x								S
Genus *Macrolepiota* Singer.									

Taxonomic categories / Macromycetes	Au 20		Sp 21		Sp 23		Au 23		EG
	I	**II**	**I**	**II**	**I**	**II**	**I**	**II**	**MSP**
Macrolepiota procera (Scop.) Singer	x	x				x	x		S
Macrolepiota mastoidea (Fr.) Singer	x								S
Familia Amanitaceae									
Genus *Amanita* Pers.									
Amanita excelsa (Fr.) Bertill.					x				M
Amanita muscaria (L.) Lam.	x								M
Amanita rubescens Pers.		x			x				M
Amanita pantherina (DC.) Krombh.	x					x			M
Amanita vaginata (Bull.) Lam.		x							M
Genus *Limacellopsis* Zhu L. Yang, Q. Cai et Y. Y. Cui									
Limacellopsis guttata (Pers.) Zhu L. Yang, Q. Cai et Y.Y. Cui	x								
Familia Bolbitiaceae									
Genus *Bolbitius* Fr.									
Bolbitius titubans (Bull.) Fr.		x							S
Genus *Conocybe* Fayod									
Conocybe pulchella (Velen.) Hauskn. & Svrček					x				S
Conocybe rickenii (Jul. Schäff.) Kühner	x								S
Familia Cortinariaceae									
Genus *Cortinarius* (Pers.) Gray									
Cortinarius cf. *alcalinophilus* Rob. Henry	x								M
Cortinarius anomalus (Fr.) Fr.	x								M
Cortinarius caperatus (Pers.) Fr.	x								M
Cortinarius cf. *decipiens* (Pers.) Fr.	x								M
Cortinarius helvelloides (Fr.) Fr.					x				M
Cortinarius lepidopus Cooke	x								M
Cortinarius purpureus (Bull.) Bidaud, Moënne-Locc. et Reumaux		x							M
Cortinarius sanguineus (Wulfen) Gray	x					x			M
Cortinarius semisanguineus (Fr.) Gillet	x								M
Cortinarius sp. 1	x								M
Cortinarius sp. 2	x	x							M
Familia Entolomataceae									
Genus *Clitopilus* (Fr. ex Rabenh.) P. Kumm.									
Clitopilus scyphoides (Fr.) Singer	x	x							S

Taxonomic categories / Macromycetes	Au 20		Sp 21		Sp 23		Au 23		EG
	I	II	I	II	I	II	I	II	MSP
Genus *Entoloma* P. Kumm.									
Entoloma cetratum (Fr.) M. M. Moser						x			S
Entoloma juncinum (Kühner et Romagn.) Noordel.	x					x			S
Entoloma vernum S. Lundell					x				S
Entoloma sp.		x							S
Familia Hydnangiaceae									
Genus *Laccaria* Berk. & Broome									
Laccaria laccata (Scop.) Cooke	x	x			x	x		x	M
Laccaria proxima (Boud.) Pat.	x								M
Familia Hygrophoraceae									
Genus *Arrhenia* Fr.									
Arrhenia spathulata (Fr.) Redhead								x	S
Genus *Hygrocybe* (Fr.) P. Kumm.									
Hygrocybe conica (Schaeff.) P. Kumm.						x			S
Hygrocybe miniata (Fr.) P. Kumm.						x			S
Hygrocybe sp.	x								S
Genus *Hygrophorus* Fr.									
Hygrophorus sp.	x								M
Hygrophorus hypothejus (Fr.) Fr.	x								M
Cf. *Hygrophorus unicolor* Gröger	x								M
Familia Hymenogastraceae									
Genus *Hebeloma* (Fr.) P. Kumm.									
Hebeloma crustuliniforme (Bull.) Quél.	x	x							M
Genus *Psilocybe* (Fr.) P. Kumm.									
Psilocybe liniformans Guzmán et Bas			x						S
Psilocybe tuberifera Fraiture	x								S
Psilocybe sp.	x								S
Familia Inocybaceae									
Genus *Inocybe* (Fr.) Fr.									
Inocybe praetervisa Quél.	x								M
Inocybe sp.	x								M
Genus *Inosperma* (Kühner) Matheny et Esteve-Rav									
Inosperma cervicolor (Pers.) Matheny et Esteve-Rav.	x								M

Taxonomic categories / Macromycetes	Au 20		Sp 21		Sp 23		Au 23		EG
	I	II	I	II	I	II	I	II	MSP
Genus *Mallocybe* (Kuyper) Matheny, Vizzini et Esteve-Rav									
Mallocybe terrigena (Fr.) Matheny, Vizzini et Esteve-Rav.			x	x					M
Genus *Pseudosperma* Matheny et Esteve-Rav.									
Pseudosperma rimosum (Bull.) Matheny et Esteve-Rav						x			M
Familia Lycoperdaceae									
Genus *Apioperdon* (Kreisel et D. Krüger) Vizzini									
Apioperdon pyriforme (Schaeff.) Vizzini					x	x	x	x	S
Genus *Bovista* Pers.									
Bovista cf. *aestivalis* (Bonord.) Demoulin	x								S
Bovista plumbea Pers.	x	x	x	x	x	x	x	x	S
Bovista nigriscens Pers.	x	x	x	x					S
Genus *Bovistella* Morgan									
Bovistella utriformis (Bull.) Demoulin et Rebriev	x	x	x	x	x	x	x	x	S
Genus *Lycoperdon* P. Micheli									
Lycoperdon echinatum Pers.	x	x							S
Lycoperdon perlatum Pers.	x	x				x			S
Lycoperdon pyriforme Willd.	x	x							S
Lycoperdon cf. *umbrinum* Pers.	x								S
Familia Marasmiaceae									
Genus *Crinipellis* Pat.									
Crinipellis scabella (Alb. et Schwein.) Murrill	x								
Genus *Marasmius* Fr.									
Marasmius oreades (Bolton) Fr.	x		x		x	x	x	x	S
Marasmius rotula (Scop.) Fr.				x					
Marasmius siccus (Schwein.) Fr.	x								S
Marasmius torquescens Quél.	x								S
Marasmius cf. *wynneae* Berk. et Broome	x								S
Marasmius sp.	x								S
Familia Mycenaceae									
Genus *Hemimycena* Singer									
Hemimycena cucullata (Pers.) Singer						x			S
Genus *Mycena* (Pers.) Roussel									

Taxonomic categories / Macromycetes	**Au 20**		**Sp 21**		**Sp 23**		**Au 23**		**EG**
	I	**II**	**I**	**II**	**I**	**II**	**I**	**II**	**MSP**
Mycena crocata (Schrad.) P. Kumm.	x	x							S
Mycena flavescens Velen.	x					x			S
Mycena galopus (Pers.) P. Kumm.	x					x			S
Mycena inclinata (Fr.) Quél.	x								S
Mycena pura (Pers.) P. Kumm.	x					x			S
Mycena rosea Gramberg	x					x			S
Mycena tenerrima (Berk.) Quél.		x							S
Mycena zephirus (Fr.) P. Kumm).	x								S
Familia Omphalotaceae									
Genus *Collybiopsis* (J. Schröt.) Earle									
Collybiopsis ramealis (Bull.) Millsp.						x			S
Genus *Gymnopus* (Pers.) Roussel									
Gymnopus dryophilus (Bull.) Murrill	x	x				x			S
Gymnopus foetidus (Sowerby) P.M. Kirk						x			S
Gymnopus fusipes (Bull.) Gray						x			S
Genus *Marasmiellus* Murrill									
Marasmiellus peronatus (Bolton) J. S. Oliveira	x								S
Marasmiellus ramealis (Bull.) Singer		x							S
Genus *Mycetinis* Earle									
Mycetinis alliaceus (Jacq.) Earle ex A.W. Wilson & Desjardin	x	x							S
Genus *Omphalotus* Fayoud									
Omphalotus olearius Singer		x							S
Genus Paragymnopus J. S. Oliveira									
Paragymnopus perforans (Hoffm.) J. S. Oliveira	x								S
Familia Pluteaceae									
Genus *Pluteus* Fr.									
Pluteus nanus (Pers.) P. Kumm.						x			S
Pluteus romellii (Britzelm.) Lapl.						x			S
Pluteus salicinus (Pers.) P. Kumm.		x							S
Pluteus semibulbosus (Lasch) Quél.	x								S
Familia Porotheleaceae									
Genus *Phloeomana* Redhead									
Phloeomana hiemalis (Osbeck) Redhead		x							S
Familia Psathyrellaceae									

Taxonomic categories / Macromycetes	Au 20		Sp 21		Sp 23		Au 23		EG
	I	II	I	II	I	II	I	II	MSP
Genus *Coprinopsis* P. Karst									
Coprinopsis atramentaria (Bull.) Redhead, Vilgalys et Moncalvo						x			S
Coprinopsis cinerea (Schaeff.) Redhead, Vilgalys et Moncalvo						x			S
Coprinopsis stercorea (Fr.) Redhead, Vilgalys et Moncalvo								x	S
Genus *Parasola* Redhead, Vilgalys & Hopple									
Parasola conopilea (Fr.) Örstadius et E. Larss.	x								S
Parasola plicatilis (Curtis) Redhead, Vilgalys et Hopple		x				x			S
Genus *Psathyrella* (Fr.) Quél									
Psathyrella candolleana (Fr.) Maire	x								S
Psathyrella corrugis (Pers.) Konrad et Maubl.	x								S
Psathyrella fusca (J. E. Lange) A. Pearson	x								S
Genus *Lacrymaria* Pat.									
Lacrymaria lacrymabunda (Bull.) Pat.	x								S
Cf. *Lacrymaria pyrotricha* (Holmsk.) Konrad et Maubl.	x								S
Familia Schizophyllaceae									
Genus *Schizophyllum* Fr.									
Schizophyllum commune Fr.	x	x	x	x	x	x	x	x	S
Familia Strophariaceae									
Genus *Agrocybe* Fayod									
Cf. *Agrocybe pediades* (Fr.) Fayod	x				x				S
Genus *Hypholoma* (Fr.) P. Kumm.									
Cf. *Hypholoma fasciculare* juv. (Huds.) P. Kumm.		x							S
Hypholoma capnoides (Fr.) P. Kumm.	x								S
Genus *Pholiota* (Fr.) P. Kumm.									
Pholiota aurivella (Batsch) P. Kumm.	x								S
Pholiota carbonaria (Fr.) Singer	x	x							S
Pholiota cf. *flammans* (Batsch) P. Kumm.	x								S
Pholiota lenta (Pers.) Singer	x								S
Genus *Protostropharia* Redhead, Moncalvo & Vilgalys									

Taxonomic categories / Macromycetes	**Au 20**		**Sp 21**		**Sp 23**		**Au 23**		**EG**
	I	**II**	**I**	**II**	**I**	**II**	**I**	**II**	**MSP**
Protostropharia semiglobata (Batch.) Redhead, Moncalvo et Vilgalys	x	x	x	x	x	x	x	x	S
Familia Tricholomataceae									
Genus *Delicatula* Fayod									
Delicatula integrella (Pers.) Fayod						x			S
Genus Infundibulicybe Harmaja									
Infundibulicybe geotropa (Bull.) Harmaja					x				S
Infundibulicybe gibba (Pers.) Harmaja						x			S
Genus *Lepista* (Fr.) W. G. Sm.									
Lepista nuda (Bull.) Cooke	x	x			x				S
Genus *Tricholoma* (Fr.) Staude									
Tricholoma lascivum (Fr.) Gillet	x								M
Tricholoma portentosum (Fr.) Quél.	x	x							M
Tricholoma terreum (Schaeff.) P. Kumm.	x								M
Tricholoma cf. *virgatum* (Fr.) P. Kumm.	x								M
Incertae sedis									
Genus *Clitocybe* (Fr.) Staude									
Clitocybe dealbata (Sowerby) P. Kumm.	x	x							S
Clitocybe odora (Bull.) P. Kumm.	x	x							S
Clitocybe sp.	x								
Genus *Collybia* (Fr.) Staude									
Collybia sp.	x	x							S
Genus *Cystoderma* Fayod									
Cystoderma superbum Huijsman	x								S
Cystoderma sp.	x								S
Genus *Gamundia* Raitelh.									
Gamundia striatula (Kühner) Raithelh.	x								S
Genus *Infundibulicybe* Harmaja									
Infundibulicybe geotropa (Bull.) Harmaja	x	x							S
Infundibulicybe gibba (Pers.) Harmaja	x	x							S
Genus *Lepista* (Fr.) W. G. Sm.									
Lepista nuda (Bull.) Cooke	x	x			x				S
Genus *Melanoleuca* Pat.									
Melanoleuca melaleuca (Pers.) Murrill	x				x				S
Genus *Panaeolus* (Fr.) Quél.									

Taxonomic categories / Macromycetes	**Au 20**		**Sp 21**		**Sp 23**		**Au 23**		**EG**
	I	**II**	**I**	**II**	**I**	**II**	**I**	**II**	**MSP**
Panaeolus papilionaceus (Bull.) Quél.	x	x			x				S
Panaeolus semiovatus (Sowerby) S. Lundell et Nannf.	x								S
Panaeolus subfirmus P. Karst.	x	x	x	x	x				S
Panaeolus sp.	x								S
Genus *Panaeolina* Maire									
Panaeolina foenisecii (Pers.) Maire	x				x				S
Genus *Phaeolepiota* Maire ex Konrad et Maubl									
Phaeolepiota aurea (Matt.) Maire	x								S
Ordo Auriculariales									
Familia Auriculariaceae									
Genus Auricularia Bull.									
Auricularia auriculae-judae Vail.	x								S/P
Genus *Exidia* Fr.									
Exidia glandulosa (Bull.) Fr.					x				S
Exidia nigricans (With.) P. Roberts	x								S
Ordo Boletales									
Familia Boletaceae									
Genus *Boletus* L.									
Boletus reticulatus Schaeff.		x							M
Genus *Chalciporus* Bataille									
Chalciporus sp.	x								M
Genus *Imleria* Vizzini									
Imleria badia (Fr.) Vizzini	x								M
Genus *Leccinum* Gray									
Leccinum melaneum (Smotl.) Pilát et Dermek						x			M
Leccinum scabrum (Bull.) Gray	x	x			x	x	x		M
Genus *Leccinellum* Bresinsky et Manfr. Binder									
Leccinellum pseudoscabrum (Kallenb.) Mikšik.	x								M
Genus *Xerocomellus* Šutara									
Xerocomellus chrysenteron (Bull.) Šutara					x				M
Genus *Xerocomus* Quél.									
Xerocomus ferrugineus (Schaeff.) Alessio					x				M
Familia Gomphidaceae									
Genus *Chroogomphus* (Singer) O. K. Mill.									

Taxonomic categories / Macromycetes	Au 20		Sp 21		Sp 23		Au 23		EG
	I	II	I	II	I	II	I	II	MSP
Chroogomphus rutilus (Schaeff.) O. K. Mill.	x								M
Familia Diplocystidiaceae									
Genus *Astraeus* Morgan									
Astraeus hygrometricus (Pers.) Morgan			x		x	x	x	x	S
Genus *Gomphidius* Fr.									
Gomphidius glutinosus (Schaeff.) Fr.	x								M/P
Gomphidius roseus Massee	x								P
Familia Gyroporaceae									
Genus *Gyroporus* Gray									
Gyroporus cyanescens (Bull.) Quél.	x								M
Genus *Hortiboletus* Simonini, Vizzini & Gelardi									
Hortiboletus bubalinus (Oolbekk. et Duin) L. Albert et Dima	x								M
Familia Paxillaceae									
Genus *Paxillus* Fr.									
Paxillus involutus (Batch.) Fr.		x							S
Paxillus rubicundulus P. D. Orton					x	x	x	x	S
Familia Sclerodermataceae									
Genus *Pisolithus* Alb. & Schwein.									
Pisolithus arhizus (Scop.) Rauschert					x				S
Genus *Scleroderma* Pers.									
Scleroderma citrinum Pers.							x		S
Familia Suilaceae									
Genus *Suillus* Gray									
Suillus bovinus (L.) Roussel.	x								M
Suillus cavipes (Klotzsch) A. H. Sm. & Thiers		x							M
Suillus granulatus (L.) Roussel	x	x			x				M
Suillus luteus (L.) Roussel	x								M
Suillus variegatus (Sw.) Richon & Roze	x								M
Suillus sp.	x								M
Familia Tapinellaceae									
Genus *Tapinella* E.-J. Gilbert									
Tapinella panuoides (Fr.) E.-J. Gilbert	x		x						S
Ordo Gantharellales									
Familia Hydnaceae									

Taxonomic categories / Macromycetes	Au 20		Sp 21		Sp 23		Au 23		EG
	I	II	I	II	I	II	I	II	MSP
Genus *Cantahrellus* Adans ex Fr.									
Cantharellus cibarius Fr.		x			x	x			S
Ordo Geastrales									
Familia Geastraceae									
Genus *Geastrum* Pers.									
Geastrum striatum DC	x								S
Ordo Hymenochaetales									
Familia Hymenochaetaceae									
Genus *Fomitiporia* Murrill									
Fomitiporia robusta (P. Karst.) Fiasson et Niemeläl		x		x					
Genus *Fuscosporia* Murrill									
Fuscoporia ferruginosa (Schrad.) Murrill.		x		x					S
Ordo Polyporales									
Familia Incrustoporiaceae									
Genus *Tyromyces* P. Karst.									
Tyromyces lacteus (Fr.) Murrill		x		x					S
Familia Polyporaceae									
Genus *Daedalea* Pers.									
Daedalea quercina (L.) Pers.							x		S
Genus *Daedaleopsis* J. Schröt.									
Daedaleopsis confragosa (Bolton) J. Schröt	x		x						S
Genus *Fomes* (Fr.) Fr.									
Fomes fomenatarius (L.) Fr.	x		x		x	x	x	x	S
Genus *Lentinus* Fr.									
Lentinus substrictus (Bolton) Zmitr. et Kovalenko						x			S
Genus *Neofavolus* Sotome & T. Hatt.									
Neofavolus suavissimus (Fr.) Seelan, Justo et Hibbett				x					S
Genus *Trametes* Fr.									
Trametes hirsuta (Wulfen) Lloyd	x	x	x	x	x	x	x	x	S
Trametes versicolor (L.) Lloyd							x	x	S
Ordo Russulales									
Familia Auriscalpiaceae									
Genus *Lentinellus* P. Karst.									
Lentinellus ursinus (Fr.) Kühner		x		x					S/P

Taxonomic categories / Macromycetes	Au 20		Sp 21		Sp 23		Au 23		EG
	I	II	I	II	I	II	I	II	MSP
Familia Russulaceae									
Genus *Lactarius* Pers.									
Lactarius deliciosus (L.) Gray	x	x							M
Lactarius cf. *fulvissimus* Romagn.	x								M
Lactarius pallidus Pers.						x			M
Lactarius pubescens Fr.		x					x		M
Lactarius rufus (Scop.) Fr.	x	x			x				M
Lactarius scrobiculatus (Scop.) Fr.	x								M
Lactarius cf. *subdulcis* (Pers.) Gray	x								M
Lactarius torminosus (Schaeff.) Gray	x								M
Lactarius cf. *zonarius* (Bull.) Fr.	x								M
Genus *Lactifluus* (Pers.) Roussel.									
Lactifluus piperatus (L.) Roussel		x				x			M
Genus *Russula* Pers.									
Russula adusta (Pers.) Fr.						x			M
Russula cyanoxantha (Schaeff.) Fr.						x			M
Russula densifolia Secr. ex Gillet						x			M
Russula heterophylla (Fr.) Fr.		x							
Russula ochroleuca Fr.	x					x			M
Russula olivacea Pers.	x	x							M
Russula sanguinea Fr.	x								M
Russula vesca Fr.		x			x				M
Russula veternosa Fr.	x	x							M
Russula sp.		x							M
Familia Stereaceae									
Genus *Stereum* Hill ex Pers.									
Stereum hirsutum (Willd.) Pers.					x	x	x	x	S
Stereum insignitum Quél.	x		x						S
Stereum rugosum Pers.						x		x	S
Stereum subtomentosum Pouzar	x		x						S
Ordo Thelephorales									
Familia Thelephoraceae									
Genus *Thelephora* Ehrh. ex Willd.									
Thelephora anthocephala (Bull.) Fr.						x			S
Thelephora hirsuta Will.	x	x	x	x					S

Taxonomic categories / Macromycetes	Au 20		Sp 21		Sp 23		Au 23		EG
	I	II	I	II	I	II	I	II	MSP
Thelephora terrestris Ehrh.	x				x				S
Classis Dacrymycetes									
Ordo Dacrymycetales									
Familia Dacrymycetaceae									
Genus *Calocera (*Fr.) Fr.									
Calocera cornea (Batch.) Fr.	x								S

The richest in species during the entire investigated period were Basidiomycota, represented by 171 taxa, and Agaricomycetes as their richest class (**Table 1**, **Figure 3**). Basiodiomycota were the main biodiversity constituents also during both periods of investigation: 1) In 2020-2021, 168 basiodiomycetes were found, which represent 98% of the total macromycetous biodiversity in the region with richest class Agaricomycetes; 2) In 2023, 80 basidiomycetes represent 91% of the total macromycetous biodiversity in the region and, once more, most of them belong to the class Agaricomycetes. By contrast, the total number of Ascomycota species was only 11, with three species found in the first investigation period, and eight in the second period, respectively (**Table 1**, **Figure 3**).

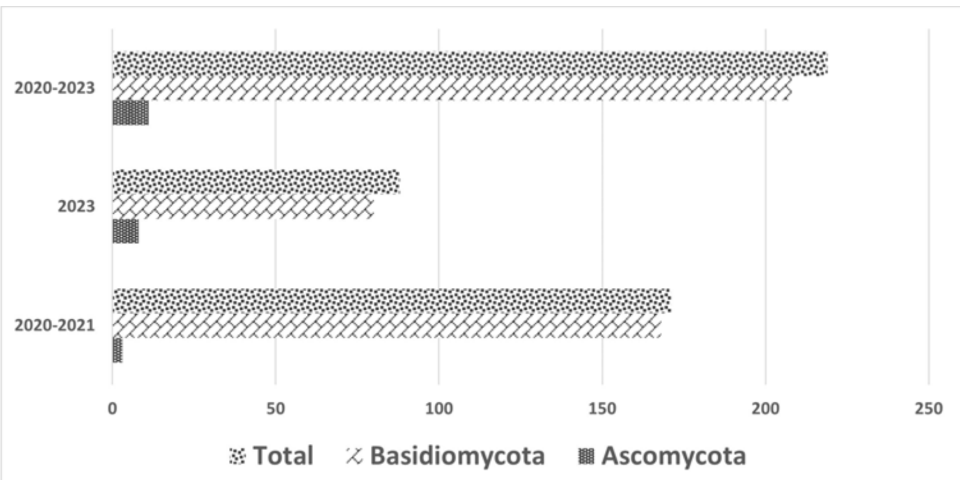

Fig. 3. Distribution of macromycete species in the phyla Ascomycota and Basidiomycota compared to the total number of macromycetes found in the study area during the entire investigation period (2020-2023) and during both main subperiods 2020-2021 (May-June 2020 and October-December 2021) and 2023 (May-June 2023 and October-December 2023).

According to the taxonomic wealth of the orders, the richest is Agaricales (141 species, or 65% from the total macromycetous biodiversity) followed by Russulales

(25, or 11%), Boletales (24, or 11%), Polyporales (7, or 3%) and Pezizales (6, or 3%). The wealth of the other orders is as follows: Thelephorales and Xylariales (each with 4 species), Auriculariales (3), Hymenocladales (2), Cantharellaes, Geastrales and Helotiales (each with a single species) – **Figure 4**.

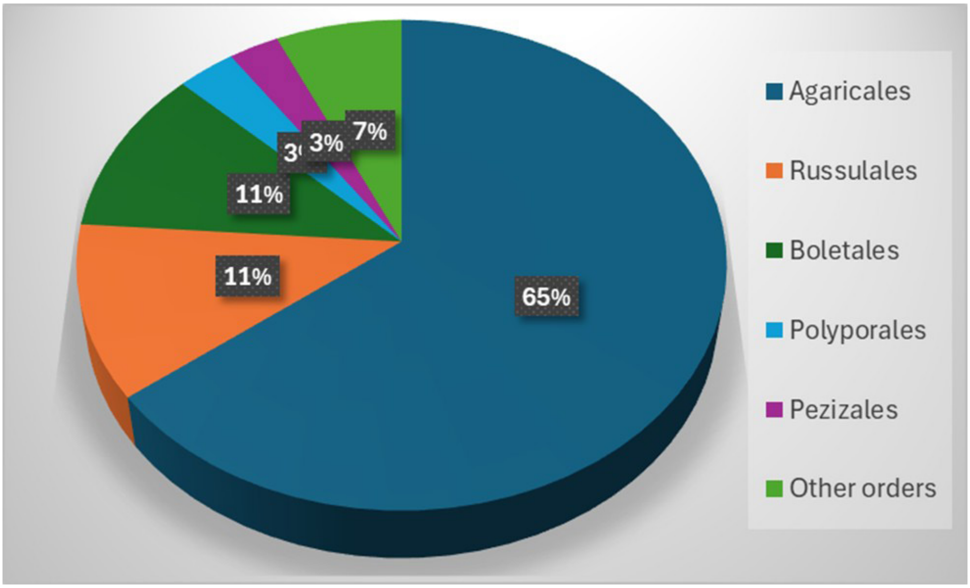

Fig. 4. Biodiversity (species wealth) of orders of macromycetes from the Chelopech region found during the entire period of investigation (2020-2023).

In the period 2020-2021, the taxonomic wealth of orders is similar: Agaricales (117 species, or 69% from the total biodiversity for this period), Russulales (20, or 12%), Boletales (19, or 11%), and Polyporales (4, or 2%). The other eight orders comprise 6% of the total biodiversity for the period, each of them being represented by 1-2 species: Xylariales, Hymenochaetales and Thelephorales (each with 2 species), Helotiales, Auriculariales, Cantharellales, Geastrales and Dacrymycetales (each with one species) – **Figure 5**.

In 2023, the order is also similar: Agaricales (52 species, or 59%), Russulales (11, 13%), Boletales (9, 10%), Pezizales (6, or 7%), and Polyporales (5, or 6%). The other three orders comprise 5% of the total biodiversity for this period, as follows: Xylariales and Thellephorales (each with 2 species, or 2%) and Cantharellales with a single species, or 1% (**Figure 6**).

Regarding the genera, the richest during the entire period of investigation were *Cortinarius* (11), *Russula* (10) and *Lactarius* (9), followed by *Mycena* (8), *Marasmius* (6), *Agaricus* and *Amanita* (each with five species), *Entoloma*, *Lepiota*, *Lycoperdon*, *Panaeolus*, *Pholiota*, *Pluteus*, *Stereum* and *Tricholoma* (each with four species) – **Table 1**. In 2020-2021, the biggest number of species had the genus *Cortinarius* (10), followed by *Lactarius*, *Mycena* and *Russula* (each with

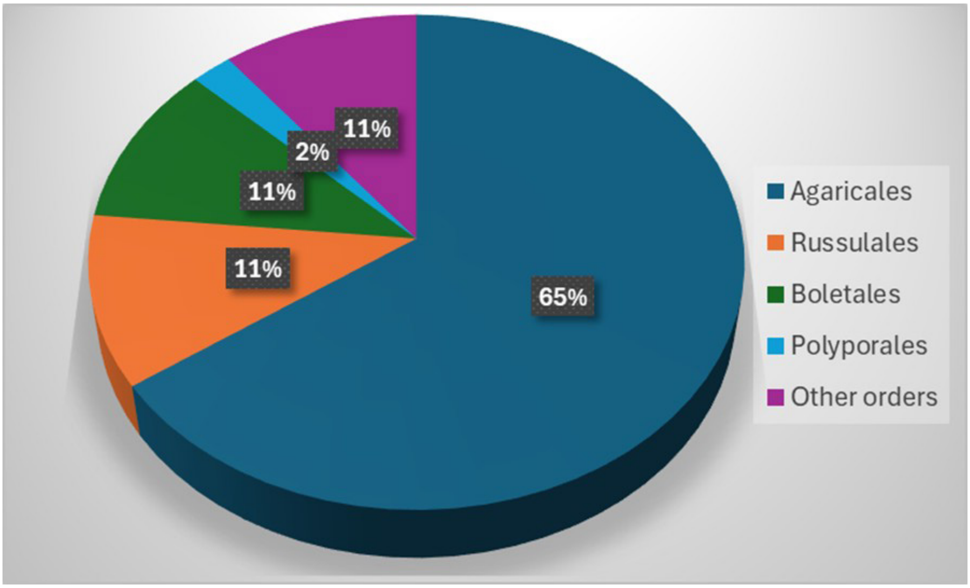

Fig. 5. Biodiversity (species wealth) of orders of macromycetes from the Chelopech region found during the first period of investigation (2020-2021).

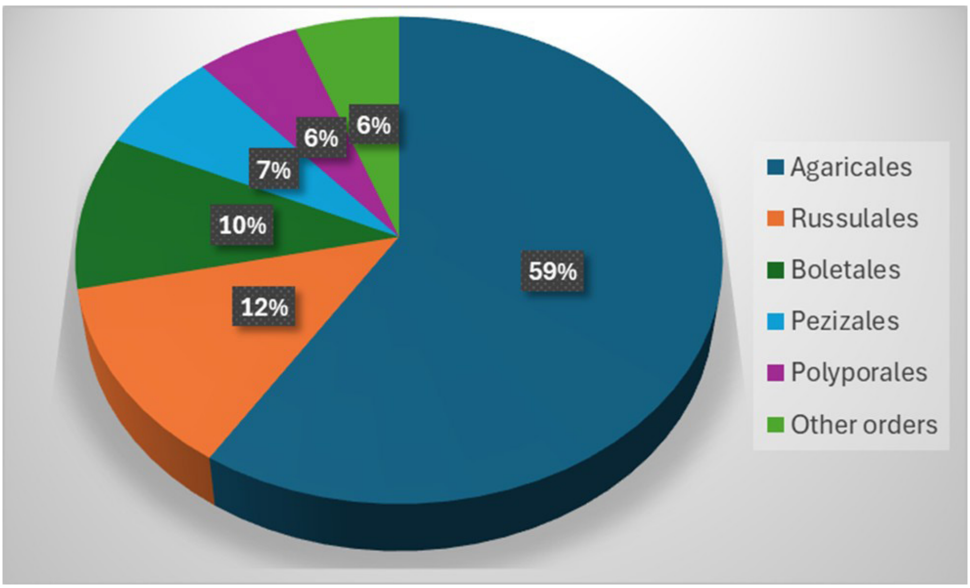

Fig. 6. Biodiversity (species wealth) of orders of macromycetes from the Chelopech region found during the first period of investigation (2023).

eight sspecies) – **Table 1**. Relatively rich were the genera *Marasmius* and *Suillus* (each with six species), *Agaricus* (5 species), followed by *Lycoperdon*, *Panaeolus*, *Pholiota* and *Tricholoma* (each with four species), and from 50 genera only a single species was found. In 2023, most of the found species were from the genus *Russula* (5), followed by *Mycena* (4) – **Table 1**. Each one of the genera *Amanita*, *Coprinopsis*, *Helvella* and *Lactarius* was represented by three species, followed by *Agaricus*, *Cortinarius*, *Hygrocybe*, *Infundibulocybe*, *Leccinum*, *Lepiota*, *Panaeolus*, *Peziza*, *Thellephora* and *Trametes* (each with two species), and 47 genera were represented by a single species.

During the entire period of investigation, in the upper, northern part of the studied area 161 macromycetes have been found (**Figure 7**). They represent 74% of the total biodiversity in the region, In the lower, southern part of the examined area this number was 117 (or 53% of the total biodiversity) and common, found in both parts, were 62 species, which constituted 28% of the total biodiversity. The floristic similarity between both parts, estimated according to Sorensens Similarity Index, was 45%.

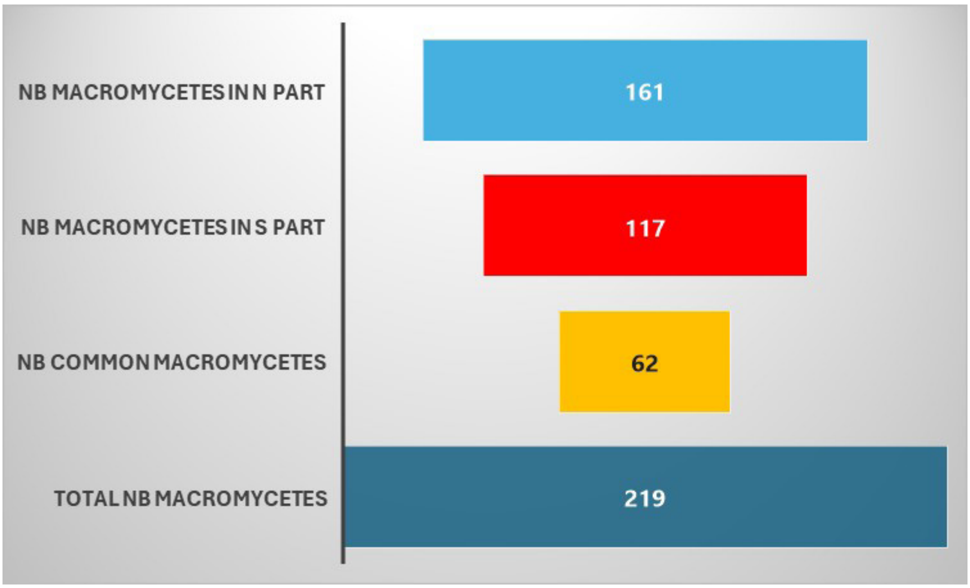

Fig. 7. Comparison of the total number (NB) of macromycetous species in the upper, northern (N) part of the investigated area, in the lower, southern (S) part of the investigated area, common species found in both areas and total number in the entire area in the period 2020-2023.

In the first investigated period, 2020-2021, in the upper, northern part of the area we found 142 species, while in the lower, southern part only 64 species were recorded (**Figure 8**). Common for both parts out of all 171 macromycetes were only 37 species (**Figure 8**), which represented 22% of all macromycetes, and the floristic similarity, expressed as Sorensens Similarity Index, was only 35%.

By contrast, in 2023 the biodiversity of macromycetes in the southern part was higher: 64 species against the 41 species in the northern part (**Figure 9**). Out of the total number of 88 macromycetes recorded during this period, common were only

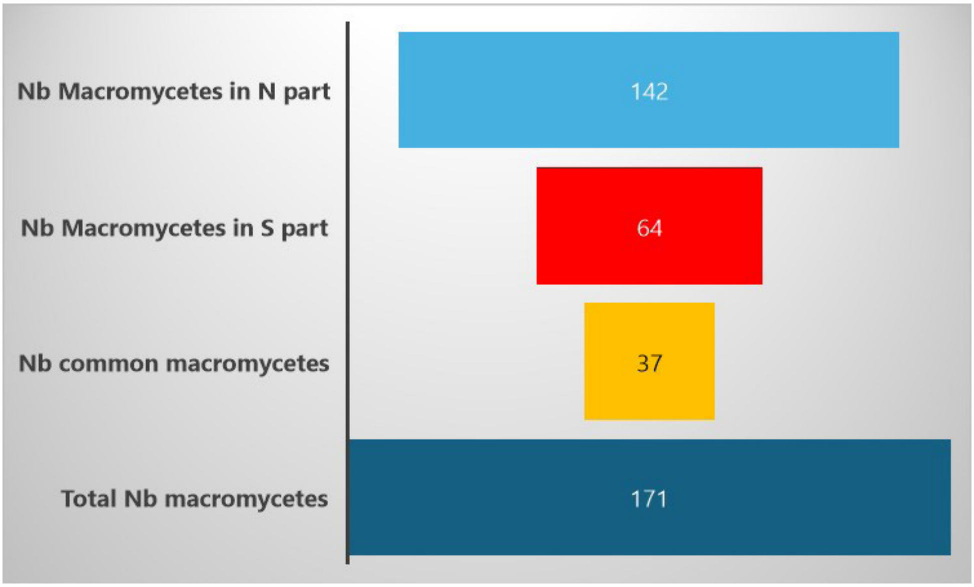

Fig. 8. Comparison of the total number (Nb) of macromycetous species in the upper, northern (N) part of the investigated area, in the lower, southern (S) part of the investigated area, common species found in both areas and total number in the entire area in the period May-June 2020 and October-December 2021.

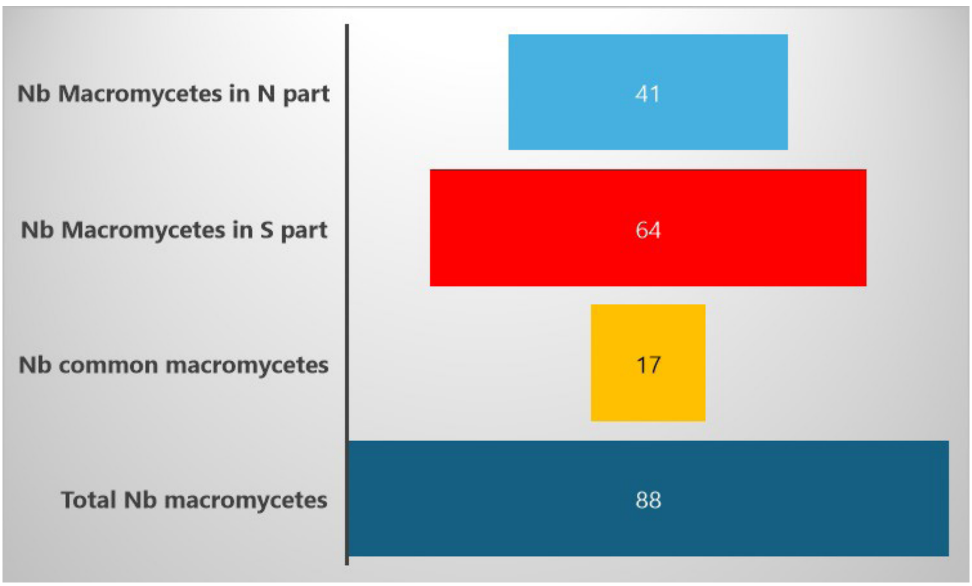

Fig. 9. Comparison of the total number (Nb) of macromycetous species in the upper, northern (N) part of the investigated area, in the lower, southern (S) part of the investigated area, common species found in both areas and total number in the entire area in the period May-June and October-December 2023.

17 species (**Figure 9**), which represented 19% of the biodiversity, and the Sorensen Similarity Index was 39%.

Biodiversity in spring and autumn was quite different: 186 macromycetes were found in the autumn/early winter periods of investigation, and almost twice as few, 94, were found in the spring/early summer periods. Common for both periods were 52 species. In 2020-2021, common for both seasons were 21 macromycetes, with 165 species found in the autumn and 27 in the spring due to the untypically cold and rainy weather in May-June 2020. Just the opposite, in 2023, the number in spring/summer period was much higher (80 species) in comparison with the autumn/winter period (25 species) due to the extremely dry autumn of 2023.

Regarding the ecological mode of life and trophicity, 147 species (67% of all recorded macromycetes) were saprotrophs, 67 (31%) were mycorrhizal formers and only five (2%) were parasites (**Table 1**, **Figure 10**).

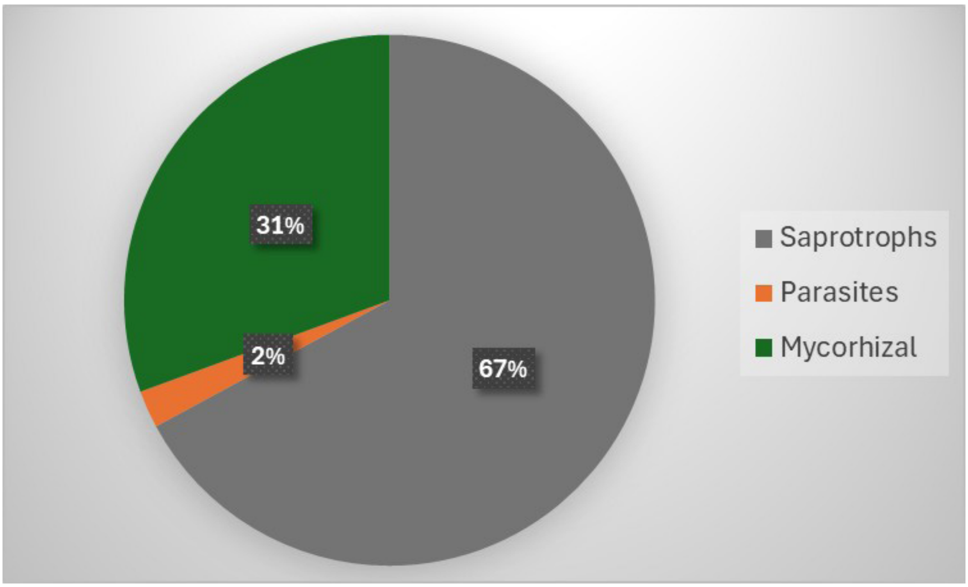

Fig. 10. Biodiversity in ecotrophic groups of macromycetes in the region of Chelopech (2020-2023).

In 2020-2021, all three groups were found in the investigated area (**Table 1**). The lowest was the number of parasitic fungi (5, or 3% of the diversity), some of which were considered ectomycorrhizal, and it has to be mentioned that for one of them, *Gomphidius glutinosus*, the host is yet unknown. Another fungus, *Gomphidius roseus* is considered as a parasite upon the mycelium of *Suillus bovinus* (Olsson et al. 2000). Although usually considered as a classical saprotroph, *Auricularia auriculae-judae* has been pointed also as a weak parasite (Harding 2008). The biggest is the number of saprotrophs (101, or 59%) which inhabit different substrata. For example, among them are saprotrophs on soils (*e.g.*, *Bovista nigriscens*), forest litter (*e.g.*, *Mycena flavescens*), dead wood (*e.g.*, *Ascocoryne sarcoides*), excrements (*e.g.*, *Protostropharia semiglobata*), fireplaces and fire stations (*e.g*., *Pholiota carbonaria*). Some of the saprotrophs found (*e.g.*, *Bolbitius titubans*) are characterized by broad spectra and occur on various substrata. All ascomycetous fungi were saprotrophs on dead wood (xylotrophs), and all 93 other saprotrophic species were basidiomycetous species. The mycorrhizal fungi were 65 species (38%), some of which form mycorrhiza with coniferous trees (*e.g.*, with *Pinus* - *Russula sanguinea*, or with *Picea* - *Tricholoma terreum*), with deciduous trees (*Betula*, *Carpinus*, *Corylus*, *Fagus*, *Fraxinus* – *Russula olivacea*, and *Tricholoma lascivum* with *Fagus*), with both coniferous and deciduous trees (*e.g*., *Laccaria proxima*).

During the field work in 2023 we did not find parasitic macromycetes. Then, again, the biggest was the number of saprotrophs (67 species, or 76% of the diversity), which occupied different substrata: soil (*e.g.*, *Agaricus campestris*), forest litter (*e.g.*, *Mycena rosella*), decaying wood (*e.g.*, *Daedalea quercina*, *Trametes hirsuta*), or excrements (*e.g.*, *Protostropharia semiglobata*, *Poronia punctrata*). Mycorhizal species were 21 species (24% of the diversity), some of which interracted with coniferous trees (*e.g.*, with *Pinus* – *Lactarius rufus*), with different deciduous trees (*Amanita pantherina*), or with both types of trees (*e.g.*, *Lactifluus piperatus*, *Russula vesca*).

Among the recorded species, there were six conservationally important species, threatened to a different degree in the context of the Red List of Bulgarian macromycetes (Gyosheva et al. 2006) and the Red Data Book of RBulgaria (Peev et al. 2011):

Lentinellus ursinus – Critically endangered (CR) according to the Red List and Red Book

Cortinarius caperatus – Endangered (EN) according to the Red List

Arrhenia spathulata - Vulnerable (VU) according to the Red List

Gomphidius roseus – Vulnerable (VU) according to the Red List

Peziza saniosa – Vulnerable (VU) according to the Red List

Poronia punctata – Vulnerable (VU) according to the Red List

The paper presents a new locality for the species *Gomphidius roseus*, included in NBMS (part Monitoring of Fungi), which is included there with three referent localities: two in Rila Mts (over the hut Treshtenik and in the National Park Rila) and in Middle part of Rodopi MTS (Denchev et al. 2013). This new finding in a non-disturb and non-polluted locality indicates the favourable trend in the status of this macromycete, which is not collected for food, according to Criterium 1, parameter 1,1, and Criterium 3, parameters 3.1-36 of NBMS.

DISCUSSION

The study demonstrates the great biodiversity of the macromycetes in the investigated area in the vicinity of Chelopech in the middle part of Stara Planina

Mts (Sredna Stara Planina): 219 species with prevalence of basidiomycetous representatives (171). Comparison with the actualized list of macromycetes found in Bulgaria during the last 100 years (**Table 2**) shows that this diversity represents:

Table 2. Comparison of the number of macromycete species in the 14 floristic regions and subregions of Bulgaria according to Denchev & Assyov (2013) with the number of the recorded by us species in the studied area of Chelopech (*), situated in Sredna Stara Planina Mts. In brackets the popular geographic Bulgarian names are transliterated.

Floristic region/subregion	Number of species
Black Sea Coast	364
North-Eastern Bulgaria	181
Danube Plain ("Dunavska Ravnina")	134
Forebalkan ("Predbalkan)	86
Stara Planina Mts	619
***Western Stara Planina ("Zapadna Stara Plnina") - 268 species	
***Middle Stara Planina ("Sredna Stara Planina") - 264 species	219* (Chelopech)
***Eastern Stara Planina ("Iztochna Stara Planina") - 364 species	
Sofia	258
Znepolski Region	405
Vitosha Mt	599
Western Border Mts ("Zapadni Granichni Planini")	39
Struma Valley	63
Belastsa Mt	24
Slavyanka Mt	3
Valley ot the River Mesta	14
Pirin Mts	342
Rila Mts	643
Sredna Gora Mts	411
***Western Sredna Gora ("Zapadna Sredna Gora") – 393 species	
***Eastern Sredna Gora ("Iztchna Sredna Gora") - 32 species	
Rodopi Mts	758
***Western part ("Zapadni Rodopi") - 456 species	
***Central part ("Centralni Rodopi") - 455 species	
***Eastern part ("Iztochni Rodopi") - 233 species	
Thracian Valley ("Trakiyska Nizina")	246
Tundzha Hilly Plain ("Tundzhanska Hulmista Rvnina")	67
Strandzha Mts	219

- 83% from the biodiversity of macromycetes in Sredna Stara Planina Mts (264 species);
- 35% from the biodiversity of macromycetes of the entire Stara Planina Mts (619 вида);
- 14% from the biodiversity of macromycetes in the whole territory of Bulgaria (1537 species).

Data in **Table 2** clearly show that the macromycetous diversity in the investigated region in the vicinity of Chelopech town, that occupies ca. 20 km^2, is compatible with their diversity in other mountains and larger regions of the country. Moreover, the diversity documented from Chelopech region of Stara Planina Mts strongly exceeds the numbers of species in many regions of the country. However, we have boldly to underline that such comparison is very relative since there are many "white spots" in the knowledge about the distribution of mushrooms in Bulgaria and there is a great unevenness in the studies of different regions during different periods. But the comparison with the summarized data for Bulgaria (Denchev & Assyov 2013) clearly demonstrates the perspectivity of future investigations of the mycota, monitoring and conservation of the fungi in the studied area.

The differences in the total number of species found in different areas, seasons and periods of investigation is logically explainable with the different local climatic conditions considering that in 2023 the precipation in the region was seven times lower than in 2020. Nevertheless, in this second period of investigation, 49 macromycetes (227 of the total diversity) were observed for first time in the studied area.

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AUTHORS CONTRIBUTION

The contribution of both PhD students K. Ivanov and M. Androv is equal and is based on their field work during the second period of investigation (2023).

CONFLICT OF INTERESTS

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

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Appendix 1

Arrhenia spathulata

Peziza saniosa

Agaricus campestris

Poronia punctata

Humaria hemisphaerica

ГОДИШНИК НА СОФИЙСКИЯ УНИВЕРСИТЕТ „СВ. КЛИМЕНТ ОХРИДСКИ“

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APPLICATION OF A SELF-ORGANIZING MAP (SOM) FOR GROUPING OF OAK ECOSYSTEMS BY DIFFERENT CHARACTERISTICS IN BULGARIA

MARIYANA LYUBENOVA[1*], ALBENA STANEVA[2], SERGEI VARBANOV[3] & ALEXANDRE CHIKALANOV[4]

[1] Sofia University “St. Kliment Ohridski”, Faculty of Biology, Department of Botany, 8 Dragan Tsankov Blvd., BG-1164, Sofia, Bulgaria
[2]NP Vitosha Directory, 17 Antim I Str., 1303 Sofia, Bulgaria
[3] Sofia University „St. Kliment Ohridski”, Information Tecknology, Tzarigradsko Shosse Bld., 2 bl., 2 fl., 1113 Sofia, Bulgaria
[4] University for Library Studies and Information Technologies, Faculty of Information Sciences, 119 Tzarigradsko Shosse Bld., 1784 Sofia, Bulgaria

Abstract. Neural networks (NNs) can use a lot of heterogeneous data that may lack repeatability and confirmability, as well as analogues in the other systems involved in the analysis, which is typical for the available ecosystem data. NN have hardly been used for the study of plant communities in Bulgaria. In this article, xerothermic oak communities in Bulgaria from up to 180 polygons were studied, and 122 characteristics of the ecotype and plant communities were included in the analysis. After conducting self-training of the SOM, different groups of polygons were obtained with respect to soils, exposure, altitude, climate and mixed characteristics. All the obtained groups correspond to the ecological characteristics of communities, which confirm the capabilities of SOM for grouping the plant communities for different purposes.

Key words: xerothermic oak forests, *Quercus frainetto* Ten., *Quercus cerris* L., Neural Network (NN)

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* *corresponding author*: M. Lyubenova – Sofia University “St. Kliment Ohridski”, Faculty of Biology, Department of Botany, 8 Dragan Tsankov Blvd., BG-1164, Sofia, Bulgaria; ryana_l@yahoo.com

INTRODUCTION

Neural networks (NNs) are a group of specific computational models for information processing. They are characterized by the use of multiple interconnected artificial neurons. They are often used in practice as nonlinear models for data analysis or a decision-making tool. The NNs are the systems of interconnected nodes that work similar to neurons in the human brain. Using algorithms, they can recognize hidden patterns and correlations in the raw data; grouping and classifying them and continuously learning and improving over time (Mouton et al. 2020). One of the founders of NNs and of the Self Organizing Map (SOM) is Teuvo Kohenen – professor at the Helsinki Academy and founder of a research center on NNs in 1995 (Kohonen 2001; Wehrenset al. 2007). Other famous developments in the field of NNs are: Algorithms, Applications and Programming Techniques , Genetic Algorithms in Search, Optimization, Machine Learning, etc. (*e.g.* Goldberg 1989; Freeman & Kohonen 1995).

In Bulgaria, the NNs have hardly been used to study plant communities. The only development to date was reported at the Scientific Conference on Ecology in 2004 on the topic: "Use of neural networks and genetic algorithms in assessing the condition of chestnut forests in Belasitsa" (Varbanov et al. 2005). This publication presents the first results of experimentally using the SOM neural network (Freeman & Kohonen 1995) to visualize and categorize measurements, as well as genetic algorithms (Goldberg 1989) to investigate the degree of influence of the various indicators on the condition of the chestnut forests in Belasitza (Varbanov et al. 2005).

NNs can use a lot of heterogeneous data, which have no analogues in the other systems included in the analysis, lack repeatability and verifiability, which is characteristic of the available data on ecosystems. SOM is one of the neural network models that has the following advantages:

- Convenience for visual representation of accepted measurements;
- Multidimensional categorization of quantitative and qualitative indicators;
- Contributing to a clearer understanding of the distribution and relationships between features from complex data;
- Identifying a small number of functions and showing the significance of the macro-biological system;
- Anticipating the reactions of the system to external influences;
- Ability to add data at a later stage;
- SOM is the self-learning neural network model that constructs a topological map that indicates the high-dimensional space of the data so as to preserve the relative closeness of the samples (Kohonen 2001);
- SOM was successfully applied to the analysis of chestnut forests in Belasitza mountain, Bulgaria.

The main disadvantage of neural networks is that most of them need training or self-training before they can be practically used, which requires considerable time, especially for large neural networks.

MATERIALS AND METHODS

According to the climatic zoning (Velev 2002), 72 of the studied sites refer to the continental-Mediterranean area. The average annual temperature is 13-14.0°C. The average January temperature is positive (1-2°C), and the average July temperature is 24-25°C. The annual temperature range does not exceed 23-24°C. The precipitation regime with an autumn-winter maximum and a summer minimum, is characteristic of the Mediterranean type of climate. The annual precipitation amounts are between 500-600 mm, and in the southern mountainous area they reach 700-1000 mm. The snow cover lasts for 1-2 days, and in the Struma and Mesta valleys it forms once every few years (**Table 1**). Sixty eight of the studied objects belong to the temperate continental area. The average annual amplitude is from 25 to 26°C. The average January temperature is from -2 to -3°C, and the average July from 25 to 26°C. In this area, the lowest winter temperatures in Bulgaria were recorded (-38.3°C in the town Trun), as well as very high summer temperatures (45°C in the town Boychinovtsi). The annual amount of precipitation is from 500 to 600 mm, and in the Danube Plain, the Pre-Balkan and the lowlands it reaches 800 mm, with a clear trend of increase towards the south and in height. The rainfall is minimal in February and maximal in June. Eighteen objects belong to the transitional-continental area. The annual temperature range is about 23°C. The average January temperature is -1°C and is characterized by some instability. It is possible to rise to 20°C and also fall to -25°C, -30°C. The average July temperature is 24-26°C, and the maximum summer temperatures reach 40°C. The mild winter, cool spring and hot summer are characteristic of the area. The annual amount of precipitation is 550 mm - 600 mm, and in the region of the Upper Thracian Lowland it is below 500 mm. The snow cover is unstable - there is no constant and continuous snow accumulation.

Table 1. Distribution of the studied objects by climatic regions (ordered by number of investigated objects).

Climatic regions	**Number of investigated objects**	**Average annual temperature [t °C]**	**Annual sum of precipitations [mm]**	**Dry period [days]**
Continental-Mediterranean area	72	11,3 – 12,5	533 - 650	60-150
Moderately continental area	68	8-11,5	578-1050	30-90
Transitional-continental area	18	10 - 12,9	554-644	80-120

According to the soil zoning of the country (Ninov 2002), the considered sites fall into the Carpathian-Danube soil area and the Mediterranean soil area, which are essential parts of the Subboreal and Subtropical soil sector of Europe. Soil zoning is four-tiered with separately provided soil sectors, districts, sub-districts and provinces (**Table 2**). The largest number of sites refer to the Sofia-Kraischte and Strandzha soil provinces (29 each), followed by the Central Thrace-Tundzha (28) and Provadiya (24) soil provinces.

Table 2. Distribution of the studied sites by soil-geographical zoning (ordered according to the numbers of the investigated objects).

Soil-geographic region Province	Number of investigated objects	Soil type - FAO
Sofia-Kraischte	29	Leptosols, Luvisols
Strandzha	29	Leptosols, Chromic luvisols
Middle Thracia-Tundzha	28	Chromic luvisols
Provadiya	24	Chernozems, Luvic phaenozems
Struma-Mesta	9	Leptosols, Chromic luvisols
Ludogorie	8	Luvisols, Phaenozems
Central Pre-Balkan	7	Luvisols, Planosols
Eastern Balkan	6	Luvisols
Eastern Rhodopes-Sakar	6	Chromic luvisols
Srednogorie	6	Chromic luvisols
Western Danube	6	Chernozems, Luvisols, Luvisols
Western Pre-Balkan	5	Luvisols, Planosols
Vitosha - Srednogorie	4	Luvisols
Osogovo-Belasitsa	2	Umbric Leptosol
Pre-Balkan	2	Leptosols
Western Rhodopes	2	Luvisols

The studied areas fall into all three vegetation regions of the country – the European Broadleaf Forest Region, Eurasian Steppe and Forest Steppe Region and Mediterranean Sclerophyllous Forest Region (Bondev 2002). The studied 158 sites fall into 19 districts according to the geobotanical zoning (Bondev 2002) (**Table 3**).

The analysis included 113 to 122 characteristics (described in detail below), from 93 to 180 polygons dominated by *Quercus frainetto* Ten. and *Quercus cerris* L. from different regions of Bulgaria using published literary sources and the National Monitoring System (Bondev et al. 1976, 1994, 1998; Kochev & Tsanova 1978; Bondev & Bogoev 1981; Bondev & Nikolov 1983; Angelov 1986; Meshinev & Nikolov 1986, 1987; Bondev & Georgiev 1987; Lyubenova & Bondev 1987, 1998a, b; Yurukova & Bondev 1990; Bondev & Lyubenova 1992; Lyubenova 1992, 1995a-c, 1996a, b, 2004; Grupce et al. 1993; Gateva 1994; Lalova 1994a, b; Melovski et al. 1994; Ljubenova 1995a-c, 1997, 1999; Ninov 1995; Lyubenova & Sazdov 1995; Lyubenova et al. 1996, 2008;

Ljubenova & Mirchev 1998; Popov 1999; EAA 2000; Lyubenova & Dimova 2000; Kurteva et al. 2002; Broshilova & Broshilov 2008). The data on the structural and functional characteristics of oak ecosystems, obtained through the destructive methods during the period of similar studies under the International Biological Program in Bulgaria (1970-2000), were used for the training of SOM.

Table 3. Distribution of the investigated sites by geobotanical zoning (ordered according to the numbers of the investigated objects).

District in the Geobotanical zoning	Number of investigated objects	Association
Strandzha Mt	25	*Quercus frainetto*
Eastern Balkan Mts	18	*Quercus cerris*
Ludogorie	12	*Quercus frainetto* - *Quercus cerris*
Novi Pazar	12	*Quercus frainetto*, *Quercus cerris*
East Rhodopes Mts	11	*Quercus frainetto*
West Coast of the Black Sea	10	*Quercus frainetto*
Pre–Balkan Mts	9	*Quercetum crataego-festucosum*
Sofia	9	*Quercus frainetto*
Upper Thracian Lowland	9	*Quercus frainetto*, *Quercus dalechampi*
Danube Hilly-Plain	8	*Quercus frainetto* - *Quercus cerris*
Ihtiman-Srednogorie	6	*Quercus cerris*, *Quercus dalechampi*
West Balkan Mts	6	*Quercus cerris*, *Quercus frainetto*, *Fagus sylvatica*
Sredna Gora Mts	5	*Quercus rubra*
Straldzha-Aitos	5	*Quercus cerris*, *Quercus frainetto*
Vitosha Mt	5	*Quercus dalechampi*, *Quercus cerris*
Upper Struma River	4	*Quercus frainetto*
Kotel-Preslav	1	*Quercus frainetto*
Rila Mt	1	*Quercus frainetto*

The preliminary data preparation includes:

1) Qualitative (non-numeric) values are transformed into quantitative, encoded with appropriate numerical values;

2) When values are missing for all characteristics of a given group for a certain area, they are filled with the average value of the respective characteristics of the remaining areas;

3) The values of each characteristic are normalized, *i.e.*, represented in the range from 0 to 1.

4) As a result of the performed preprocessing of the data, each area is represented

by numerical values for 122 characteristics;
5) Part of the data related to cardinal directions is encoded using trigonometric functions sin and cos - (**Table 4**)

During the network training, data for 122 characteristics (designated by f – feature) are presented in the form of vectors for each polygon (c) - a total of 93 to 180. The characteristics and their measurement units (provided in brackets) are as follows: f1- year of investigation; f2-f20 location (geobotanical district); f21-f22 - latitude and longitude (°); f23-f30 –average temperature (°C), precipitation average (mm), drought period (days), dry period (days), average altitude (m a.s.l.), exposure – sinus and cosinus, average incline (degrees [°].); f31-f40 - soil groups according to FAO; f41-f57 - soil forming rocks; f58 - soil pH; f59 - soil humus (tones ha^{-1}); f60-f69 – associations; f70-f79 – bonitas (I-I), average area (m^2); f72-f74 - origin – average age (years), Slope (0-9, av.), number of layers, average diameter at a breast height (DBH, cm), average height (H, m); f80-f85 - biomass (BM) stock (m^3), overground BM, tree layer BM, bush layer BM, herb layer BM and underground BM (tones ha^{-1}); f87-f91 – average annual overground production (AAOP) of tree layer, AAOP of bush layer, AAOP of herb layer, average annual O2 production (tones ha^{-1}); f92-f94 – average ammual litter-fall and average mulch (both in tones ha^{-1}) and litter-fall coefficient; f95-f97 – overground energy stocks in buch and herb layers and underground energy stocks (kJ ha^{-1}); f98-f100 - annual energy accumulation in tree, buch and herb layers (kJ ha^{-1}); f101-105 – content of calcium (Ca), potassium (K), magnesium (Mg), nitrogen (N) and phosphorus (P) in the phytomass (tones ha^{-1}) and f106- f113 - content of iron (Fe), manganese (Mn), copper (Cu), lead (Pb), zink (Zn), cadmium (Cd), cobalt (Co) and strontium (Sr) in the phytomass (tones ha^{-1}); f114-f122 – mulch and litter-fall and different fractions as leaves, branches – annual and perennial, bark, wood and acorns (tones ha^{-1}).

Table 4. Coding the data on the directions of the world.

-

Direction of the world	Sin	Cos
North	0	1
North-East	0,7	0,7
East	1	0
South-East	0,7	-0,7
South	0	-1
South-West	-0,7	-0,7
West	-1	0
North-West	-0,7	0,7

As a result of the performed preprocessing of the data, each area is represented by numerical values for all abovementioned 122 characteristics. When initializing the Neural Network (NN), the data have two main dimensions (feature (f) - 122 and case (c) - 180) and a rectangular shape. Then a NN is generated as a set of neurons organized in a rectangular grid with dimensions 10/10 (or, also 10x10), which means that it is composed of 100 neurons. Each neuron contains a vector of 122 numbers. In the training process, the vector of a given neuron iteratively changes based on the provided data and can eventually represent multiple input patterns or measurements. The part of the interpretation of the obtained results is done through the characteristics variance analysis of the areas in the formed groups. The variance of characteristic x is calculated using the following statistical formula:

$$D[x] = \sum \frac{(x - mean(x))^2}{length(x) - 1}$$

where x is the vector of characteristic values, mean (x) is their arithmetic mean, and length (x) is the number of elements in x.

RESULTS AND DISCUSSION

A two-dimensional rectangular Self-Organizing Map (SOM) has been generated with dimensions 10 x10. Each neuron is initialized with a vector of 122 relatively small (in the range from 0 to 0.1) random numbers. To observe changes in the process, the network is visualized by representing each neuron in the form of a "star" histogram based on its vector (**Figure 1**). The attached figure depicts the network after a certain period of self-training. Neurons in which the corresponding input patterns are mapped, are marked with a red diamond in the center of the star. **Figures 2** and **3** illustrate neurons in the process of self-training of the network.

The first major grouping confirms the ecological nature of the studied forest communities: *Quercus cerris* + *Quercus frainetto* communities on Luvisols; *Quercus cerris* + *Quercus frainetto* communities on Cambisols; *Quercus cerris* + *Quercus frainetto* communities on Planosols; *Quercus cerris* + *Quercus frainetto* communities on Vertisols; *Quercus cerris* + *Quercus frainetto* communities on Chernozems; *Quercus frainetto* dominated communities on Fluvisols; *Quercus frainetto* dominated communities on Leptosols; *Quercus frainetto* dominated communities on Alisols; *Quercus frainetto* dominated communities on Regosols and *Quercus cerris* dominated communities on Luvic phaeozems.

The second major grouping confirms the ecological nature of the studied forest communities: *Quercus frainetto* communities on SE slopes; *Quercus frainetto* communities on E slopes; *Quercus cerris* communities on NE slopes; *Quercus cerris* + *Quercus. frainetto* communities on different slopes and medium range of precipitations. The overall trend in the distribution of the communities is that *Quercus frainetto* communities occupy areas characterized by low altitude and average precipitation compared to *Quercus cerris* communities. In the mixed communities, a similar trend is not observed, which is likely due to human intervention in maintaining forest sustainability.

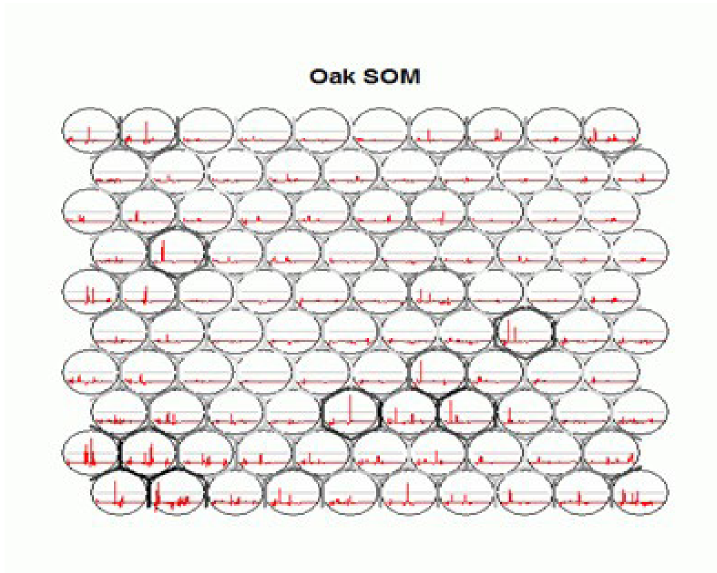


Fig. 1. Example of a SOM Polygon exploration based on 98 features.

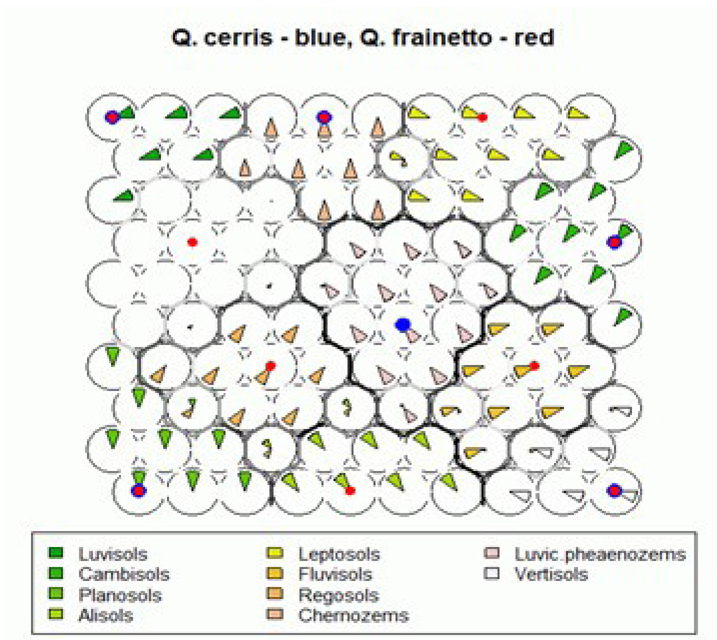


Fig. 2. SOM from the studied polygons based on the soil type data.

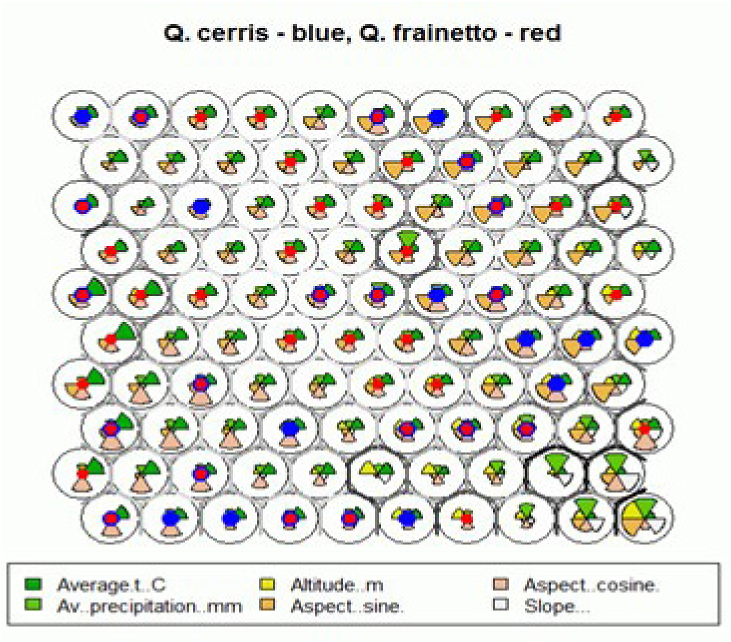


Fig. 3. SOM from the studied polygons based on the combined climate and topography data.

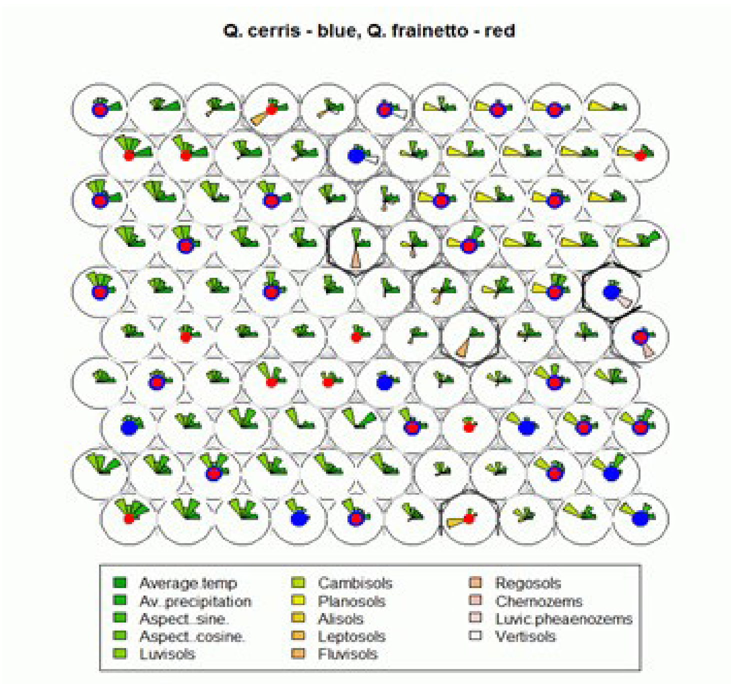


Fig. 4. SOM from the studied polygons based on the soil, climate and topographic data.

Table 5. Characteristics of 5th neuron, received by using abiotic characteristics. The abbreviations are as follows: **t** – town, **v** – village

Assoc.	Poly-gon	Place	Lati-tude	Long-titude	Soil-forming rock	Soil	Abiotic characteristics		Characteristics of the associations	
Quercus frainetto + Quercus cerris - Crataegus monoguna - Brachyodium pinatum *Quercus frainetto + Quercus cerris Crataegus monoguna - Brachyodium pinatum*, *Quercus frainetto*, *Quercus frainetto*, *Quercus frainetto*, *Quercus frainetto*, *Quercus cerris*, *Quercus cerris*, *Quercus cerris*, *Quercus cerris*	c18, c46, c74, c76, c77, c90, c93, c94, c95, c104	t. Montana Western Surnena Gora -Karlovo t. Charmanli, t. Bourgass, v. Tzarevo, v. Staro Orjahovo, t. Varna, v. Staro Orjahovo, t. Isperih, Palamara – t. Shumen	414818 422005 420415 425434 430845 430203 434243	255155 273014 275713 274606 274140 275008 264827	sandstone andesite sandstone clays and marls marls sand and lime-stone loess loess clay	*Luvisols*	Altitude [m a.s.l.]	59-500	Origin	natural - sprout
							Incline (slope) [degrees]	0–3^{o}	Average age	30-144
							Exposure	S, SE	Average slope	0.6–0.7
							Average temper-ature [^{o}C]	11.4	Number of layers	3
							Average precipi-tation [mm]	615–694	DBH [cm]	13-17.5
							Dry period [days]	88 - 75	Hav	7-19
									Overground BM	
							[tones ha^{-1}]	157.6		
									Underground BM,	
							[tones ha^{-1}]	47.6		
									Mulch [tones ha^{-1}]	16.65
									MOB [tones ha^{-1}]	12.95
									O_2 production, [tones ha^{-1}]	11.1
									Annual Litter	
							-fall [tones ha-1]	3.7		
									A*	3.5

Table 6. Characteristics of 30th neuron, received by using abiotic characteristics. Abbreviations used are as follows: **t** – town., **v** – village, nbh - **???**

Assoc.	Poly-gon	Place	Lati-tude	Long-titude	Soil-forming rock	Soil	Abiotic characteristics		Characteristics of the associations	
Quercus cerris *Quercus dale-champii* *Quercus cerris* *Quercus cerris+ Quercus frainetto* *Quercus fraineto* *Quercus virgiliana+ Quercus cerris+H. bulbosum*	c58 c68 c96 c169 c173 c177 c178	t. Svoge t. Ichtiman v. Rosino v. Dragichevo v. Debelt nbh.Pancharevo (t. Sofia) t. Nova Zagora	425960 422545 424353	230760 235601 243101	quartzite mica schist gneiss-granites	*Cambisols*	Altitude [m a.s.l.]	868-930	Origin	natural - sprout
							Incline, ^{0}	3-10	Average age	35-40
							Exposure	E, NE	Average slope	0.7
							Tav °C	10.1	Number of layers	3
							Pav, mm	533-630	DBH [cm]	11-16
							Dry period [days]	40	Hav	10-15
									Overground BM [tones ha^{-1}]	44.0
									Overground BM [tones ha^{-1}]	28.0
									Mulch [tones ha^{-1}]	12.3
									MOB [tones ha^{-1}]	7.9
									O_2 production, [tones ha^{-1}]	6.8
								4.1	Annual Litter-fall [tones ha^{-1}]	4.1
									A*	3.0

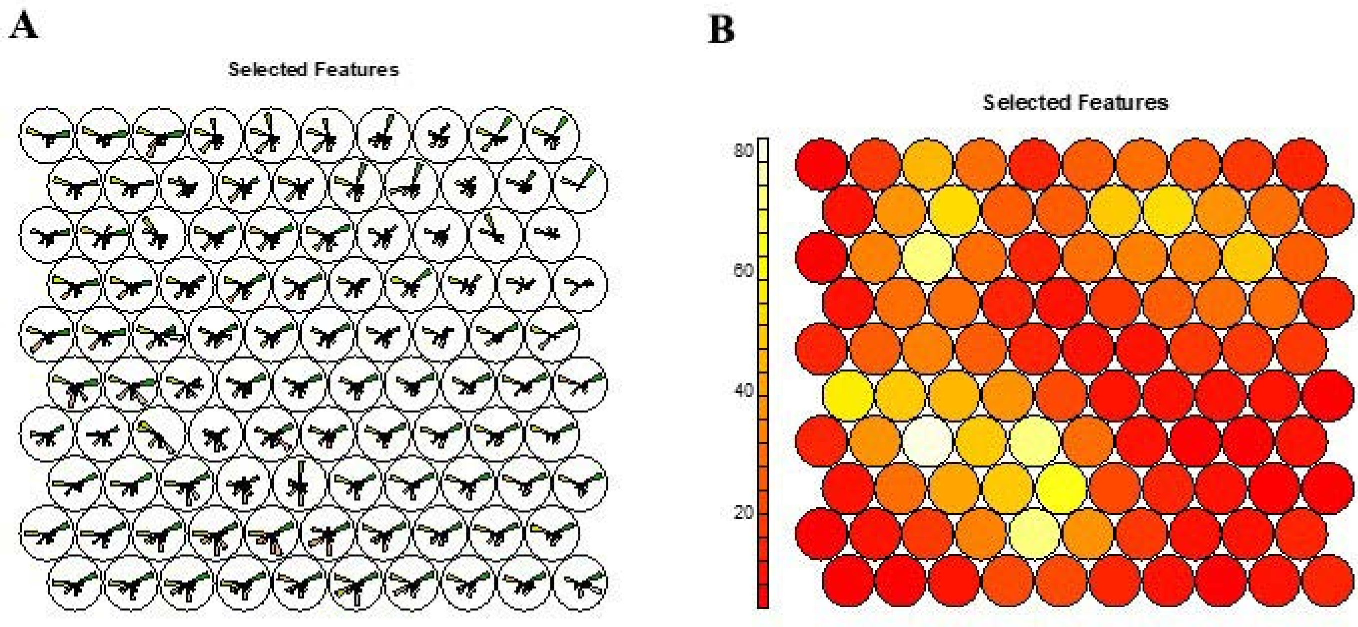
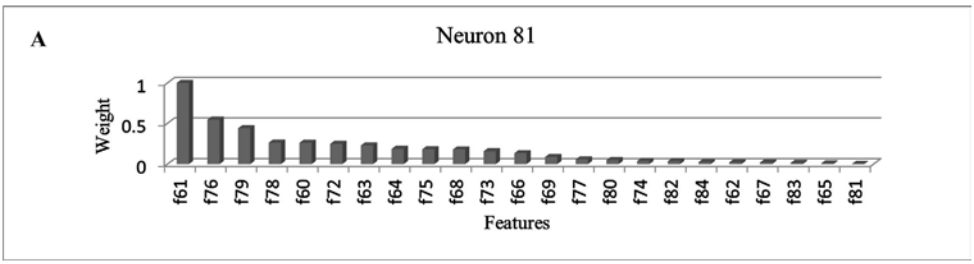


Fig. 5. Received mixed neurons based on available characteristics.

As a result of the self-training using soil, climatic, and topographic characteristics, a map of neurons grouping the objects has been obtained (**Figure 4**). **Table 5** and **6** extract and display all characteristics of the objects falling into neuron 5 and neuron 30. Objects grouped in these two neurons exhibit differences in their abiotic and biotic characteristics.

During further self-training of the SOM network, 23 sequentially selected commonly occurring abiotic and biotic features (designated as "features") were included for 180 cases (objects), as some cases lack certain functional characteristics. As a result, two maps of neurons were visualized, shown on Fig. 5. Darker gray borders indicate a greater difference between adjacent neurons (**Figure 5A**). Essentially the same information but visualized differently. In **Figure 5B**, the similarity of the obtained neurons is better observed, with the scale on the left indicating the coloring based on the difference between the neuron and its neighbors. In other words, red neurons are very similar to their neighbors. In the lower right quadrant of the map, there is a clustering of very similar cases. Since structural and functional characteristics of forest communities were used to obtain these groupings, and the cases that fall into one neuron are very similar to each other, it is logical to perceive the neurons as separate ecosystems.

In the following table (**Table 7**), each neuron is sequentially shown along with the cases that fall into it. The cases that fall into one neuron are very similar to each other. From **Table 7** it can be seen that 21 neurons contain only 1 case, 17 neurons contain 2 cases, 7 neurons contain 3 cases, 3 neurons c ontain 4 cases, 6 neurons



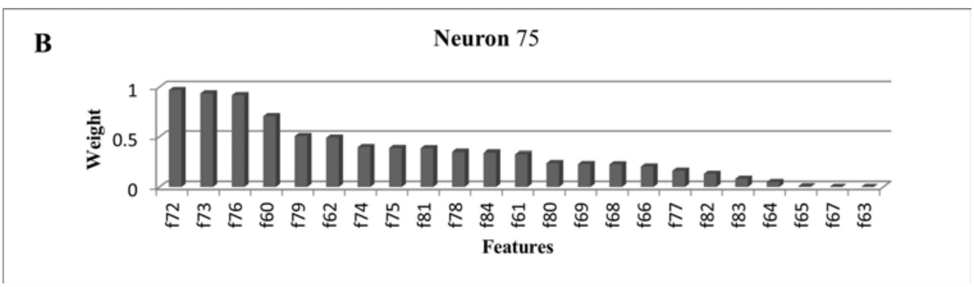


Fig. 6. Importance of case factors in neurons 81 (A) and 75 (B).

Table 7. Neurons and causes.

Neurons	4, 5, 6, 14, 15, 16, 23, 29, 33, 35, 41, 42, 46, 54, 55, 60, 72, 73, 76, 86, 93	1, 8, 10, 21, 25, 27, 28, 49,53, 64, 65, 90, 79, 87, 100, 99, 70	3, 7, 17, 18, 39, 50, 91	30, 48, 95	2, 11, 13, 47, 51, 71	56, 67	61, 12	19	75	81
№ of neurons	21	17	7	3	6	2	2	1	1	1
Causes	c145 c120 c118 c8 c48 c24 c41 c27 c33 c1 c6 c35	c133, c142, c122, c125, c39, c40, c139, c147, c28, c32, c5, c100, c18, c22, c114, c164, c4, c109, c7, c49, c43, c47, c55, c56, c178, c179, c111, c112, c57, c108, c19, c106, c165, c168	c34, c149, c156, c90, c123, c129, c117, c128, c130, c64, c115, c124, c113, c126, c131, c116, c119, c127, c65, c69, c169	c63, c78, c83, c99, c46, c50, c175, c180, c29, c30, c31, c42, c29, c30, c31, c42	c143, c144, c151, c159, c160, c134, c135, c138, c141, c152, c132, c137, c146, c150, c158, c10, c11, c12, c174, c177, c21, c45, c104, c105, c170, c9, c44, c171, c172, c173	c14, c23, c37, c38, c51, c54, c16, c52, c96, c97, c98, c166	c66, c68, c84, c86, c88, c94, c95, c136, c140, c148, c154, c155, c157, c161	c17, c61, c62, c71, c73, c74, c75, c76, c121	c36, c59, c60, c77, c79, c81, c82, c85, c87, c89	c53, c58, c67, c70, c72, c80, c91, c92, c93, c101, c102, c103, c163, c167
№ of causes	1	2	3	4	5	6	7	9	10	14

contain 5 cases, 2 neurons contain 6 cases, 2 neurons contain 7 cases, 1 neuron each contains 9 and 10 cases respectively, and only one neuron, namely 81, has the largest group of similar cases - 14.

Figure 6 presents the importance of individual characteristics in grouping the cases in neurons 81 and 75, characterized by the largest number of cases (14 and 10, respectively). For neuron 81, features with the highest importance are f61, f76, and f79, representing the characteristics such as association type, average slope, and tree layer height. The features f78, f60, f72, and f63 have almost equal weight - DBHav., forest origin, presence of *Quercus cerris* or *Quercus pubescens* in the stand. For the grouping of cases in neuron 75 important are the origin - f72, f73, f74, as well as the slope, the presence of *Quercus cerris*, age, and aboveground biomass - f76, f60, f75, and f81.

CONCLUSION

The NNs are artificial intelligence trained to process data in a way necessary for humans. In the current development, a specitic type of NN - SOM - was used. A corpus of published data from literary sources and reports from national monitoring, including 122 measurements from up to 180 polygons of xerothermic oak forests in various regions of Bulgaria, was processed. As a result of the analysis and self-training of SOM, 100 neurons were generated, marking 41 groups of structurally and functionally similar polygons. Based on them, satisfactory groups of forest phytocenoses were obtained. The results include a visual topological map of the data, as well as the identification of similar groups and can serve as a basis for the analysis and discovery of patterns in the similarity and difference of xerothermic oak forests from different regions of the country. The conducted analysis revealed that the applied by us characteristics (ecological factors) have different importance as classification units (determining the similarity between them and grouping them) or limiting factors (determining the functional state) for the functioning of the studied oak communities. These original results can serve as a basis for the ecosystem classification of xerothermic oak forests in Bulgaria and their subsequent modeling as structural and functional units of vegetation cover and valuing the provided ecosystem services.

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THE SECRETS OF THE EDELWEISS (*LEONTOPODIUM* R. BR. EX CASS.): A REVIEW ON THIS SYMBOL, SCIENTIFIC OBJECT AND A NATURAL TREASURE

ANTON A. POZUMENTSHTIKOV[1*], IRINA A. BOYCHEVA[2], GEORGI N. BONCHEV[2], MEGLENA L. KITANOVA[3], MIROSLAVA K. ZHIPONOVA[1]

[1] Sofia University "St. Kliment Ohridski", Faculty of Biology, Department of Plant Physiology, 8 Dragan Tsankov Blvd., BG-1164, Sofia, Bulgaria
[2] Department of Molecular Biology and Genetics, Institute of Plant Physiology and Genetics, Bulgarian Academy of Sciences, Acad. Georgi Bonchev str., Bl. 21, 1113 Sofia, Bulgaria
[3] Sofia University "St. Kliment Ohridski", Faculty of Biology, Department of Genetics, 8 Dragan Tsankov Blvd., BG-1164, Sofia, Bulgaria

Abstract. The edelweiss (*Leontopodium* R. Br. ex Cass), a symbol of endurance and beauty, captivates attention far beyond its delicate appearance. Found in the harsh alpine environments of Europe and Asia, this remarkable plant thrives where life faces its greatest challenges. Its cultural significance is rivaled only by its unique adaptations, making it a subject of fascination in both folklore and science. This literature review explores the multifaceted nature of the edelweiss, delving into its ecological role, biochemical properties, genetic diversity and potential applications. Why edelweiss? The answer lies in its rarity, its resilience in extreme conditions, and the unique phytochemicals it produces, which have demonstrated intriguing medicinal properties. By synthesizing existing research, this review seeks to provide a comprehensive understanding of the edelweiss, highlighting its ecological, cultural, and scientific importance. As research on alpine flora continues to expand, the edelweiss has emerged not only as a botanical emblem, but also as a source of untapped scientific potential.

* *corresponding author*: A. A. Pozumentshtikov – Sofia University "St. Kliment Ohridski", Faculty of Biology, Department of Botany, 8 Dragan Tsankov Blvd., BG-1164, Sofia, Bulgaria; pozumentsh@uni-sofia.bg

Key words: alpine flora, bioactivities, ecological adaptation, in vitro micropropagation, secondary metabolites, taxonomy

The plant name edelweiss comes from the German words "edel" (noble) and "weiss" (white), while the genus name *Leontopodium* derives from the Greek "leon" (lion) and "podion" (foot), referring to a lion's paw. Linnaeus initially classified the genus as *Gnaphalium* (from the Greek "gnafallon," meaning wool shavings) before Cassini renamed it in 1819 (Cassini 1819; Flann et al. 2010)

Morphology: The genus *Leontopodium* consists of perennial herbaceous plants typically covered with whitish hairs. The stems may be single or multiple, with alternating leaves. Basal leaves are on petioles, while stem leaves are sessile. The flowers, with a double perianth, are arranged in complex inflorescences at the top of the stem, surrounded by snow-white stem leaves. The funnel-shaped corolla consists of five fused petals, encircled by transparent, multi-branched filamentous structures – modified calyces retained as a pappus for the achenes. The flowers contain fused stamens and a pistil column with lobes, and linear stigmas. The fruits are smooth, fibrous, cylindrical-obovate seeds without endosperm. Pollination is entomophilous, with insects like flies (Muscidae family) attracted by the white stem leaves (Kuzmanov 2012; Metodiev 2021; Pavlova et al. 2023). The achene trichomes and the carpopodium exhibit taxonomic significance (Ma et al. 2022).

The Bulgarian edelweiss populations (*L. nivale* (Ten.) Hand.-Mazz) have a diverse rhizome, one or several erect stems, and oblong or linear leaves that are hairy on both sides. Its inflorescences consist of 5–12 globose capitula with white or yellowish tubular flowers. The outer flowers are female, while the inner ones are functionally male. The fruit seeds (achenes) are cylindrical or obovate, with numerous white hairs (pappus) (Kuzmanov 2012).

In Bulgaria, two subspecies are recognized: *L. nivale* subsp. *nivale* (syn. *L. alpinum* var. *pirinicum* Velen.), found in Pirin Mt (2170–2605 m a.s.l.), and *L. nivale* subsp. *alpinum* Greuter, found in the Balkan Mts (**Figure 1**). Subspecies *nivale* has shorter stems, spread trichomes, and white-woolly leaves, while subsp. *alpinum* has taller stems, closely adhering trichomes, and greenish upper surface of the leaves (Kuzmanov 2012; Kozuharova et al. 2018). It blooms from July to August and bears fruit from September to October (Bancheva 2015).

Ecomorphology: The hairs on the bracts, flowerheads, and with less density on the whole plant, serve to limit excessive water loss through transpiration, a vital adaptation for its survival in dry habitats, and protection against ultraviolet rays. Some scientists suggest that this defense occurs through absorption within the protective fibrous layer and energy dissipation, aided by diffraction effects, which is possible because of the fibrous nature of the plant's filaments. The plant's fibrous surface functions as a photonic device, controlling light interactions similarly to

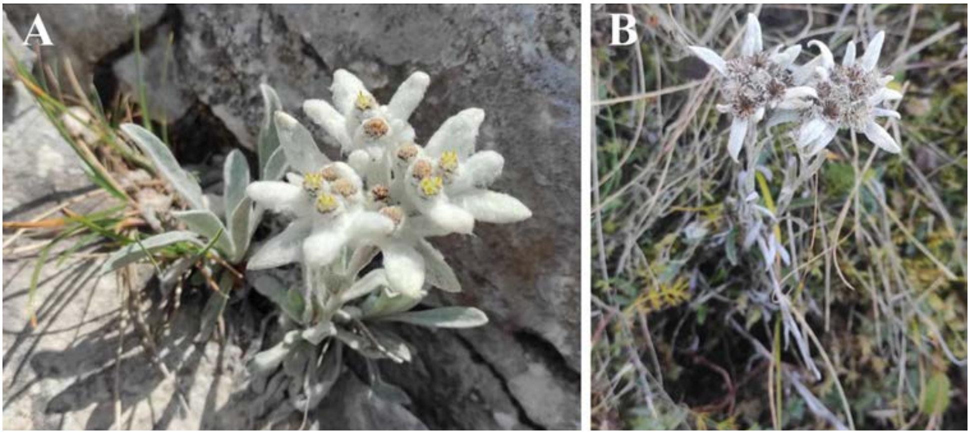

Fig. 1. Bulgarian species of edelweiss.**A** – *L. nivale* subsp. *nivale*, Pirin Mts., Kazanite area (August, flowering phase). **B** – L. *nivale* subsp. *alpinum*, Balkan Mts., Kozya Stena reserve (September, seeds, senescing). Source: A. A. Pozumentshtikov

engineered optical devices such as waveguides or diffraction gratings (Vigneron et al. 2005). These unique structure adaptations make the edelweiss highly resistant to harsh conditions, ensuring its survival in challenging mountainous environments.

Ecology, habitats and biogeography: The edelweiss comprises of endemic and stenobiont plants, thriving in high-altitude sunny rocky slopes, grassy steppe-like regions, and alpine pastures at elevations between 1800–3400 m a.s.l. It predominantly grows on limestone and humus-rich soils (Ischer et al. 2014). Its distribution extends from Asia (Hindu Kush, Pamir-Altai, Tian Shan, Sino-Tibetan Mountains, Qinghai-Tibet Plateau) to Europe (Pirin, Balkan Mts., Carpathians, Alps, Prokletija). Edelweiss plants inhabit hard-to-reach areas like rock crevices and stony glades in coniferous, subalpine, and alpine belts. Populations are sparse, consisting mainly of individual plants or small tufts, characterized by low reproductive potential and limited migratory abilities (Bancheva 2015).

In Bulgaria, the edelweiss plants grow at 1700–2800 m altitude in the marble karst of northern Pirin Mt (including areas around Vihren Peak, Kazanite, Bayuvi Dupki-Djindzhiritsa Reserve, and Kamenititsa), and the limestone Balkan Range (it is found in locations such as the Kozyata Stena Reserve, Korudere, Triglav, Mazalat, and Zli Vruh). Protected under the Biological Diversity Act, edelweiss habitats fall within the Pirin and Central Balkan National Parks, various reserves, and NATURA 2000 sites (Tzonev et al. 2009; Roussakova 2009). Major threats to the species include its habitat specificity, low abundance and density, fragmented distribution, weak regeneration, and destruction by tourists (Bancheva 2015).

Autecological studies conducted in the Swiss Alps indicate that moisture index, summer temperature, slope, and topographic location are key factors for edelweiss habitats. The plant thrives in areas with low average temperatures, high-altitudes, and steep-slopes, primarily on calcareous or limestone-rich rocks, while avoiding silicate rocks. It occupies grass-poor, open pasture communities, scree, and other growth-limiting environments, classifying it as a heliophyte and making it vulnerable to competition. Edelweiss plants prefer low-humidity environments with well-aerated soils and average summer temperatures below 10°C, consistent with its high-mountain distribution. Since they coexists with thermophilic species (*e.g.*, *Galium lucidum* All., *Astragalus monspessulanus* L. and *Phleum phleoides* (L.) H. Karst), this suggests that the current distribution may not fully represent the realized niche of the genus. Although it inhabits steep slopes, studies indicate that this factor is not of great importance and most likely this is also part of its niche, due to continuous tourist harvesting, but it may also be due to its preference for dry habitats with prolonged erosion, which can help reduce competition (Ischer et al. 2014).

In Bulgaria, *L. nivale* subsp. *nivale* is calciphilic, growing in neutral to slightly alkaline soils (pH ~7.5) with poor morphology and quality (Kozuharova et al. 2018). As a chasmophyte, it inhabits areas with 50–80% plant cover, often dominated by *Sesleria korabensis* (Kumm. & Jav.) Deyl and *Carex kitaibeliana* Degen ex Bech., along with other psychrophytic and cryophytic hekistothermal vegetation in the alpine treeless zone. It participates in unique plant communities in Pirin, such as *Leontopodio-Potentilletum stojanovii* (Mucina et al. 1990), and contributes to southern European alpine tundra alliances like *Leontopodio nivalis-Elynion myosuroidis* (Chytrý et al. 2015).

Ex situ studies by Kozuharova et al. (2018) reveal that *L. nivale* subsp. *nivale* dies off after four years when transplanted to lower altitudes near the town of Dobrinishte. This highlights the incompatibility of wild edelweiss with garden cultivation and underscores the threats posed by rising temperatures and climate change, including the loss of snow cover, which is essential for maintaining humidity and providing protection. These findings emphasize the vulnerability of this species to environmental changes critical to its survival and development.

Symbiotic associations – rhizosphere and endosphere: The rhizosphere, in direct contact with plant roots, is enriched by rhizodeposition, which attracts and shapes specific microbial communities. These communities are influenced by factors such as soil type, plant genotype, and root system architecture, all of which modify the rhizosphere environment (Hütsch et al. 2002; Bais et al. 2006; Minz et al. 2013; Ofek-Lalzar et al. 2014; Saleem et al. 2018). The endosphere, closely linked to the rhizosphere, houses microorganisms that are transmitted horizontally or vertically. This includes non-pathogenic endophytes that thrive in nutrient-rich, protected environments under strong selective pressures (Compant et al. 2010; Hardoim et al. 2015; Wang M. et al. 2016b). These environments facilitate interactions via secondary metabolites and processes such as antibiosis and biofilm formation (Compant et al. 2010; Abisado et al. 2018).

Actinobacteria, a type of Gram-positive bacteria, are widely recognized for their production of secondary metabolites, including antitumor, antiparasitic, insecticidal, antibacterial, antifungal, immunomodulatory agents, and herbicides. They are the primary source of most antibiotics in use today (Barka et al. 2016). Oberhofer et al. (2019) proposed that *L. nivale* subsp. *alpinum* might host novel actinobacteria. From rhizosphere soil, they isolated 77 actinobacterial strains, identifying genera such as *Actinokineospora*, *Kitasatospora*, *Asanoa*, *Microbacterium*, *Micromonospora*, *Micrococcus*, *Mycobacterium*, *Nocardia* and *Streptomyces* using the 16S rRNA molecular marker. Plant tissue analysis revealed diverse operational taxonomic units, with microbial diversity decreasing from roots to rhizomes, leaves, and inflorescences. The performed metagenomic study also highlighted a significant presence of unculturable actinobacteria, including taxa like Rubrobacteridae, Thermoleophilales, Acidimicrobiales, and unclassified taxons. These findings suggest the potential for isolating bioactive compounds for pharmaceutical applications (Oberhofer et al. 2019).

A strain of rhizobacterium, *Sphingomonas* sp. Cra20, was isolated from the roots of *L. leontopodioides* which grows at elevation 3800 m in Tianshan, China (Luo et al. 2020). This bacterium performs nitrogen fixation and produces siderophores. Studies on its effect on *Arabidopsis thaliana* demonstrated increased root fresh weight and lateral root development. Initially attributed to auxin synthesis, it was later discovered that the bacterium lacks auxin synthesis pathways; instead, the growth enhancement is due to volatile organic compounds it releases.

Pollination: Species differentiation within the genus *Leontopodium* is challenging due to apomixis, a form of asexual reproduction where seeds are produced without gamete fusion. This process complicates species identification as it limits genetic variation, making it more difficult to use genetic markers for classification. In *L. nivale* subsp. *alpinum*, a specific form of apomixis called diplospory has been identified. Diplospory involves the development of the embryo sac from the megaspore mother cell without undergoing meiosis, resulting in a diploid embryo sac. This diploid egg can develop into an embryo via parthenogenesis, bypassing fertilization (Erhardt 1993; Noyes 2007; Blöch et al. 2010). This subspecies reproduces both sexually and asexually, with sexual reproduction typically prevailing (Hörandl et al. 2011). Populations exhibit diverse reproductive systems, including hermaphroditic, gynomonoecious (both hermaphroditic and female flowers on one plant), and andromonoecious (hermaphroditic and male flowers on one plant) types. The proportions of these systems vary geographically, further complicating species differentiation and blurring distinctions between populations (Kozuharova et al. 2018).

Field studies have revealed that *L. alpinum* emits both a sweet and a sweat-like odor. The sweat-like scent is most likely due to the presence of compounds such as 3-methyl-2-pentenoic acid, butyric acid, and other fatty acids, while the sweet aroma is attributed to 2-phenylethanol and phenylacetic acid. Compounds like 3-hexanol and 3-hexenyl acetate contribute to a green, grassy note, while 5-dien-4-ol imparts a woody, herbaceous scent. Nectar analysis has identified glucose, fructose, 16 types of amino acids (including some non-proteinogenic ones), lipids, phenols, and proteins. Interestingly, nectar is primarily secreted by hermaphrodites and male flowers. The most common pollinators belong to the order Diptera, predominantly flies from the family Muscidae, along with insects from Hymenoptera, Coleoptera and Lepidoptera. Although the inflorescences of edelweiss may not appear specialized for attracting specific pollinators, the unique combination of its sweet yet sweat-like odor acts as a specific attractant for flies. Additionally, the white, hairy stem leaves serve as optical landmarks that further attract pollinators. An intriguing observation involves the butterfly *Erebia tyndarus* Esper, a pollinator known to land on sweaty human skin or clothing to drink sweat for its nitrogen and dissolved salts. The ubiquity of flies and the adaptation to fly pollination provide a significant ecological advantage in the harsh environments where the edelweiss thrives. This form of pollination is both ecologically functional and critical for the plant's survival under such conditions (Erhardt 1993).

Chromosome number and karyotype studies: The polyploidy assures increased genetic material that provides a diverse genetic base on which evolution can act, offering a broader range of adaptive options and facilitating evolutionary leaps (Payne & Wagner 2019; Van de Peer et al. 2020). The genus *Leontopodium* has been reported to encompass varying ploidy cytotypes. According to most chromosome number studies, two basic chromosome numbers are described: x=12 and x=13 (Meng et al. 2012; Russell et al. 2013). The relatively small and numerous chromosomes are the likely reason for discrepancies in the reported chromosome numbers of species within the genus (Russell et al. 2013) (**Figure 2**). The tribe Gnaphalieae, to which the genus belongs, exhibits a wide variety of karyotypes (Watanabe et al. 1999), however, in the study by Meng et al. (2012), the karyotypes of the *Leontopodium* species were found to be unimodal, indicating no significant differences in chromosome length. While variations in basic chromosome numbers are common in the Gnaphalieae tribe, further research is needed for a better understanding of these differences (Stille et al. 2014).

Using flow cytometry, Russel et al. (2013) reported a relatively small genome size ranging from 0.93 pg to 1.14 pg (1C=3000Mb), along with notable differences in chromosome structure. For instance, *L. artemisiifolium* (Léveillé) Beauv. was found to possess a pair of heteromorphic chromosomes, which vary in size relative to each other and the other chromosomes (based on five species from a single population). Typically, heteromorphic chromosomes are observed in dioecious

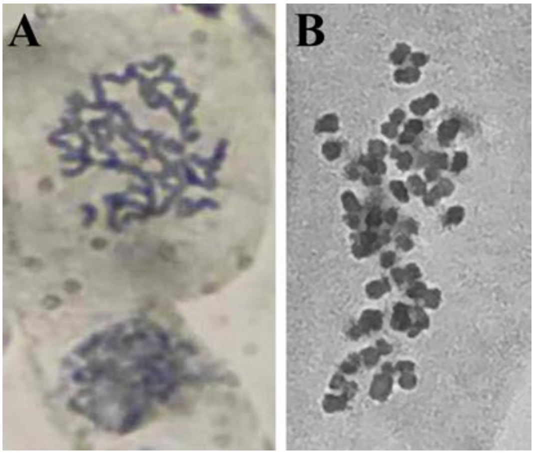

Fig. 2. Chromosomes of commercially available edelweiss assigned as *L. alpinum* (*Stella alpina*, by Hortus Sementi, https://www.agrogradina.bg/cvetq-edelvais-hort) stained with Gomori's hematoxylin, pretreated in: **A** – 0,01% colchicine for 5 min; **B** – 0,05% colchicine for 2 h at RT. Magnification 1000x (immersion oil). Source: A. A. Pozumentshtikov, M. Kitanova.

plants (CHARLESWORTH 2002). According to the authors, this polymorphism is most likely the result of homoploid hybridization or represents a transitional stage of chromosome rearrangements within an actively evolving taxon (RUSSELL ET AL. 2013). Although European species share the same basic chromosome number as some Asian species, further analyses are needed to determine whether the basic chromosome numbers x=12 and x=13 evolved independently multiple times or originated from a single evolutionary event.

Cytological variation at the diploid karyotype level, as demonstrated by RUSSELL ET AL. (2013), supports the hypothesis of rapid speciation within the genus, accompanied by changes in basic chromosome numbers as suggested by SAFER ET AL. (2011). According to SAFER ET AL. (2011), the low genetic variation observed within the genus indicates a rapid rate of diversification, which complicates resolving evolutionary relationships and understanding how chromosome numbers evolved. Further research is required to clarify whether speciation occurred after large-scale genomic events in diploid species or whether such events were drivers of speciation. Previous studies have suggested that frequent changes in chromosome number may precede noticeable morphological changes or differences in DNA sequences (RUSSEL ET AL. 2013), highlighting the significant role of polyploidy in the evolution of the genus. Interestingly, polyploid species are more common in regions such as Europe and Russia, which may indicate that polyploids possess superior dispersal abilities or greater tolerance for diverse environmental conditions, as observed in other genera (VAN DE PEER ET AL. 2020).

An earlier study by MENG ET AL. (2012) reported a basic chromosome number of x=14, though they also observed plants with a basic chromosome number of x=12. They noted that the Gnaphalieae tribe typically has chromosome numbers derived as multiples of 7, where n=14 is the most common (ANDERBERG 1994; WATANABE ET AL. 1999). The authors suggested that the basic chromosome numbers of x=12 and x=13 likely resulted from secondary reduction leading to dysploidy (WATANABE ET AL. 1999). Dysploidy is characteristic of many genera within the Asteraceae family, including *Leontopodium*, and is believed to have evolved as an adaptation to diverse habitats. In angiosperms, it is generally thought that symmetric karyotypes evolve first, with asymmetric ones developing later (Stebbins 1971; Stace 1989). However, changes in ploidy levels and karyotype asymmetry in the genus do not come together. This indicates that both polyploidy and dysploidy play critical roles in karyotype evolution and speciation, though environmental factors may also influence karyotype asymmetry (Meng et al. 2012).

According to Stille et al. (2014), the basic chromosome numbers studied in 16 *Leontopodium* species are x=6, 8, 9 and 11, with x=8 being the most common. They concluded that the basic chromosome numbers might be useful for systematic and phylogenetic analyses but identifying them is challenging due to the high intraspecific variability within the genus. Additionally, they suggested that the large differences in basic chromosome numbers within species likely have limited relevance for phylogenetic inferences, as these differences may not carry biologically significant information.

Tantray et al. (2021) conducted an insightful study examining the meiotic division of species from geographically isolated regions of the Himalayas. In *L. jacotianum* Beauv. (2n=4x=48), they identified meiotic abnormalities such as cytomixis and structural heterozygosity, which directly affected pollen viability. Their findings confirmed that the species is tetraploid, whereas previous studies have defined it as diploid (2n=24) or having a dysploid chromosome set (2n=28) (Khatoon & Ali 1988). The species is described as "evolutionarily active" due to its intraspecific variation in chromosome number. Cytomixis is believed to contribute to aneuploidy and polyploidy by producing hypoploid and hyperploid cells, which can lead to uneven gametes and impact fertility. Up to 5–7 pollen mother cells (PMCs) were involved in the chromatin transfer in *L. jacotianum*, making it one of the most affected plants. Notably, *L. jacotianum* exhibited the highest percentage of PMCs involved in cytomixis, with 32% of its PMCs displaying this phenomenon. This high rate of cytomixis significantly impacted the plant's pollen fertility, as chromosomal abnormalities caused by the process disrupted normal pollen development. These findings suggest that *L. jacotianum* may experience a higher rate of chromosomal aberrations, likely influencing its evolution and reproductive success (Tantray et al. 2021). In *L. jacotianum*, structural heterozygosity was observed to result from reciprocal translocations, which can lead to unbalanced chromosome segregation during cell division. This imbalance produces daughter cells with chromosome duplications or deletions. While cells with deletions are typically eliminated by natural selection, they can persist in polyploid individuals (Schubert & Lysak 2011; Tantray et al. 2021). Such structural changes are essential for the speciation and adaptation of plant species. In *L. jacotianum*, these changes form an integral part of its genetic system, aiding in evolutionary processes (Rieseberg 2001; Rieseberg & Willis 2007; Tantray et al. 2021).

It is possible that meiotic anomalies, like those observed in *L. jacotianum*, occur in other *Leontopodium* species and play a role in the evolution and adaptation of the genus, potentially influencing speciation.

Taxonomy and phylogeny of the genus *Leontopodium:* The genus *Leontopodium* R. Br. ex Cass. (Asteraceae) belongs to the subtribe Gnaphaliinae of the major tribe Gnaphalieae the latter ones including 178 genera and about 2,100 species (Smissen et al. 2020). *Leontopodium* comprises 58 species that are distributed across Asia and Europe (Bayer et al. 2007; Chen et al. 2011). Russell et al. (2013) reported the division of the genus into two sections: *Leontopodium*, which is more widely distributed across Eurasia, and *Nobilia* (Beauverd) Hand.-Mazz., which is found in the Himalayas, China and Japan. The infrageneric taxonomy system, species delimitation, and interspecies systematic relationships of *Leontopodium* remains controversial due to incomplete taxon sampling, incongruence among DNA markers and morphology and influenced by the high rate of hybridizations between species (Stille et al. 2014). In Europe two species occur, *L. alpinum* Cass. and *L. nivale* (Ten.) Huet ex Hand.-Mazz (Safer et al. 2011). China has the largest number of *Leontopodium* species in the world (37) and the West and Southwest regions of China (*e.g.,* Qinghai-Tibet Plateau) are the centers of speciation and diversification of this genus (ca. 20 species) (Chen et al. 2011; Chen & Bayer 2011; Meng et al. 2012). For example, two morphologically similar species, *L. caespitosum* (from the Hengduan Shan) and *L. microphyllum* (Taiwan), were basal, suggesting they are part of an ancestral stock within the genus (Blöch et al. 2010). The speciation and diversification of edelweiss in the Tubetan plateau is influenced by climatic and geomorphological patterns in this region coupled with repeated, partial re-colonisations of large high-altitude areas (i.e. during the Pleistocene glaciation cycles during the last cold snap (120,000 years ago). New species have been constantly described as *L. nyingchiense*, a new species from Xizang (Tibet), China (He et al. 2024)

The genus *Leontopodium* is part of the so-called FLAG clade named after the four large genera *Filago*, *Leontopodium*, *Antennaria*, and *Gamochaeta* (Galbany-Casals et al. 2010). Nie et al. (2016) and later Xu et al. (2023) have also confirmed the presence of this clade and that the genus *Leontopodium* is monophyletic. The latter study, based on chloroplast genomes and nuclear genes, addressed the complex phylogenetic relationships within this genus with observed phylogenetic ambiguity and incongruence between chloroplast and nuclear genes. *Leontopodium* species were divided into three main clades in the chloroplast genome phylogenetic tree and six main clades in the nuclear gene phylogenetic tree. Chloroplast trees had higher support values and were more effective for phylogenetic resolution also supporting the distinction of the sections *Nobilia* and *Leontopodium* as reported by Russell et al. (2013). Moreover, the authors found out that the characteristics of the leaf base, stem types, and carpopodium base were phylogenetically correlated and may have potential value in the taxonomic study of *Leontopodium*.

Blöch et al. (2010) explored phylogenetic relationships between 22 *Leontopodium* species from a broad geographic range and with a good representation from the Himalayan/Tibetan center of diversity based on chloroplast markers and nuclear genes. The recently described Southeast Tibetan monotypic *Sinoleontopodium* (*S. lingianum* Y. L. Chen Dickoré) was shown to belong to *Leontopodium*. In another study based on Amplified Fragment Length Polymorphism (AFLP) analysis of 38 populations of 16 different species, Safer et al. (2011) distinguished 10 distinct groups with clear boundaries within the genus, confirming that the species are indeed very closely related with a short evolutionary history.

Few studies explored the population divergence of Far East Asian *Leontopodium populations* (*e.g.*, *L. japonicum*), a species restricted to the temperate regions of China, Korea, and Japan. Jeon et al. (2015) and Lee et al. (2016) reported distinct genetic isolation of Korean populations (Korea *vs.* China and Japan). By comparison, a non-significant level of differentiation, but a high degree of genetic diversity, was detected between Chinese and Japanese populations. These data support the notion that, rather than migrating southward from more northern latitudes, current populations in Korea are distributed due to colonization via East China Sea land bridges, similar to movement by warm-temperate species. Furthermore, geographical isolation because of an oceanic barrier has probably led to allopatric speciation for Korean populations.

The chloroplast markers showed low rates of sequence divergence within this genus *Leontopodium* despite the presence of morphologically diverse species (Blöch et al. 2010). They have perhaps arisen due to rapid radiation and hybridisation triggered by multiple, severe climatic changes, and habitat fragmentation and rejoining. Ecological constraints might also have been responsible for the occurrence of similar morphological characters in unrelated lineages, resulting in the grouping of taxa with few morphological similarities.

According to Blöch et al. (2010) and Safer et al. (2011), *L. alpinum* and *L. nivale* comprise a genetically distinct group of the European section of *Leontopodium*. The genetic diversity (Diversity-Weighted (DW) values) of Asian populations (*e.g.,* the Yunnan group) is higher compared to European ones, which further supports the idea that this is an ancient and long-term isolated population from which the genus originated. Despite the wide geographic distance, European species show surprisingly little divergence from its Asian relatives. The question is still unresolved as to whether the European taxa comprise two distinct species, subspecies, or a series of varieties or unclassifiable forms. Currently, *L. alpinum* and *L. nivale* are rather recognized as separate species, however, a combined approach, including additional genetic analyses, ecological studies and morpho-taxonomic methods, should be used to confirm these hypotheses. Although the evolution of these two European species is under debate, they have most likely migrated from Asia (Sino-Himalayan region) via Middle Asia during the last cold snap (Pleistocene; 120,000 years ago) along with widespread herb-grass and Artemisia-grass steppe formations (Grichuk 1992). Safer et al. (2011) showed that the populations from Bulgaria (Pirin and Balkan Mts.) are with higher diversity among the European ones and is probably the most ancient and genetically distinct, suggesting that it may have served as a long-term refuge for the species during past climate changes.

Secondary metabolites and bioactivities: Numerous compounds of different classes have been identified in different edelweiss species (**Figure 3**).

Terpene derivatives	Phenolic compounds	Hydrocarbons, etc.
• Sesquiterpenes: hexahydrofarnesyl acetone and bisabolone • Tricyclic sesquiterpenes • Oxygenated sesquiterpenes n-pentadecanal and β-caryophyllene • Bisabolone-type sesquiterpenes • Monoterpenes: linalool and β-ionone	• Coumarin derivatives: oxyobliquin, meotoxyobliquin, hydroxyobliquin • Lignans (leoligin) • Phenylpropanoids • Flavonoids (tannins) • Lignins • Polyphenols (caffeic acids)	• Fatty acids - linoleic, linolenic and palmitic acids • Leontoaerialoside A, B, C, D and E • Leontopodic acid (substituted glucaric acid) • Polyacetylenes • Benzofurans (benzofuran glycoside) • Steroids • Alkanes

Fig. 3. Classes of secondary metabolites identified in edelweiss according to Bicchi et al. 1975; Schwaiger et al. 2002, 2004;Stuppner et al. 2002; Dobner et al. 2003b; Ganzera et al. 2012; Chen et al. 2018.

Antibacterial activity: Dobner et al. (2003a) investigated the antibacterial activity of *L. alpinum*. The authors tested crude extracts of aerial parts and roots obtained using dichloromethane, methanol, or 70% aqueous methanol as solvents. Dichloromethane extracts demonstrated significant inhibition against the strains: *Streptococcus pyogenes*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Escherichia coli*. Sesquiterpenes and other compounds selectively inhibited strains of *Enterococcus faecium*, *S. aureus*, *Streptococcus pneumoniae*, and *S. pyogenes*. Linoleic and linolenic acids were the most potent compounds, with a minimal inhibitory concentration (MIC) of 4 µg ml^{-1}, supporting the traditional use of these metabolites for gastrointestinal issues and dysentery.

Gao et al. (2017) tested the antibacterial activity of essential oil of *L. leontopodioides* (Will.) Beauv. that showed strong activity against the Gram-positive strains of *S. aureus* (MIC = 0.039 mg ml^{-1}) and *B. subtilis* (MIC = 0.313 mg ml^{-1}), while no inhibition was observed for the Gram-negative *E. coli* and P. *aeruginosa* (MIC > 5 mg ml^{-1}). Qian et al. (2018) tested the essential oil of *L. longifolium* against 8 microorganisms, including four bacterial strains of *S. aureus, E. coli*, *B. subtilis*, *P. aeruginosa*, and four fungi (*Candida albicans*, *Aspergillus flavus*, *Mucor mucedo*, *Phytophthora parasitica*). The most sensitive to the oil was

S. aureus, whereas *E. coli* showed limited inhibition, and no antifungal activity was established.

Anti-inflammatory activity: Dobner et al. (2004) reported that extracts, fractions, and pure constituents of *L. alpinum* applied topically significantly reduced croton oil-induced ear edema in mice. Dichloromethane extracts of aerial parts exhibited concentration-dependent anti-inflammatory effects. Bisabolane-type sesquiterpenes reduced leukocyte accumulation and inflammation by 46%, while the coumarin (obliquin), lignan (leoligin), and tricyclic sesquiterpenes reduced inflammation by 50%. Lariciresinols were identified as TNF-α inhibitors, and linoleic and linolenic acids demonstrated anti-inflammatory effects by inhibiting the cyclooxygenase/lipoxygenase pathways in the arachidonic acid metabolism. This inhibition prevents the synthesis of pro-inflammatory eicosanoids from arachidonic acid, including prostaglandins, thromboxanes, and leukotrienes. Additionally, the coumarin and the 7α-siliferol-5-ene sesquiterpene inhibited IL-8-induced leukocyte chemotaxis by up to 58% at a low concentration.

Antioxidant activity: The antioxidant activity of essential oils from *L. leontopodioides* and *L. longifolium* was evaluated using a DPPH radical scavenging assay in comparison with a synthetic antioxidant. The results indicated that the edelweiss essential oils required much higher concentrations for comparable effects (Gao et al. 2017; Qian et al. 2018).

Cytotoxic activity: Gao et al. (2017) investigated the cytotoxic effects of *L. leontopodioides* essential oil on HepG2 (liver cancer cell lines) and MCF-7 (breast adenocarcinoma cell lines) using the MTT assay. The oil, dissolved in DMSO, was tested alongside doxorubicin as a positive control. The essential oil inhibited cell growth in a dose- and time-dependent manner over 72 h. Although much less potent than doxorubicin, the oil exhibited notable activity. The authors attributed its effects to phenols, aldehydes, and alcohols, particularly β-caryophyllene, which may inhibit tumor cell motility, invasion, and aggregation.

The studies emphasized the importance of screening for beneficial substances from edelweiss and emphasized the need to evaluate individual compounds as well as their synergistic effects when tested in fractions.

Applications: There are many reported uses of edelweiss' biological potential (**Figure 4**).

Accordingly, numerous patents were generated using edelweiss as few of them are shown in **Table 1**.

The diseases traditionally treated with edelweiss are often related to bacterial infections and inflammations (Dobner et al. 2003a). In European folk medicine, extracts from one of the edelweiss species, *L. alpinum*, also known as "Herba

Leontopodii”, have been used to treat diarrhea, dysentery, angina, bronchitis, cancer, and other conditions in both humans and animals. For oral use, the herb was boiled in wine, and milk was then added. When used for breast cancer, it was boiled in water, and the resulting extract was applied as a compress.

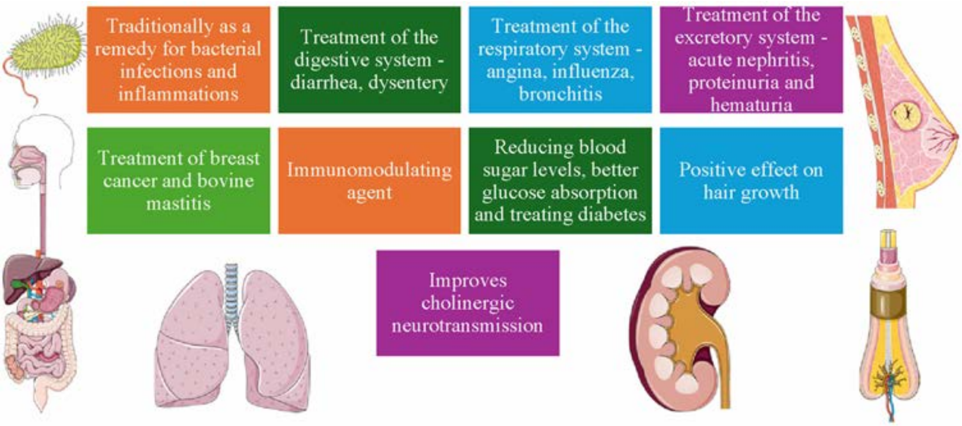

Fig. 4. Applications of edelweiss bioactivity. According to: He (1994); Wu (2002); Dobner et al. (2003a); Schwaiger et al. (2004); Hornick et al. (2008); Duwensee et al. (2011); Li et al. (2012); Yang et al. (2015); Scharinger et al. (2016); Wang et al. (2016b); Yu et al. (2016); Qi et al. (2017); Zhao et al. (2019); Campiche et al. (2022); Aguchem et al. (2024).

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The aerial parts of *L. leontopodioides*, have been used in Chinese folk medicine to treat albuminuria, hematuria, and vaginitis (Li et al. 2012). In the Tibetan Plateau and Inner Mongolia regions of China, the aerial parts of several edelweiss species, such as *L. leontopodiodes*, *L. haplophylloides* Hand.-Mazz., *L. nanum* (Hooker & Thomson ex Clarke) Hand.-Mazz., *L. longifolium*, and *L. dedekensii* (Bureau & Franchet) Beauv., have been used in folk medicine to treat acute nephritis and proteinuria (Schweiger et al. 2004).

The species *L. leontopodioides* has been associated with the treatment of influenza, bronchitis, and acute and chronic nephritis. It has also been described as an immunomodulatory agent (Yu et al. 2016; Qi et al. 2017). He (1994) reported the successful treatment of 100 patients using this edelweiss species, with an average of 15 doses of the plant required for a cure. Wu (2002) showed that edelweiss effectively prevented and treated bovine mastitis, as the plant extracts

Table 1. *Leontopodium* sp. patents. Source: Google Patents: https://patents.google.com/.

Patent WO2007006492A1 (2007) Use of extracts and constituents of *Leontopodium* as enhancers of cholinergic function, WO Patent, Helmut Prast, Judith Rollinger, Stefan Schwaiger, Hermann Stuppner

Patent US20130053438A1 (2013) Pharmaceutical compositions comprising lignans and their derivatives for the medical management of angiogenesis and hypovascularity, US Patent, WO Patent, Medizinische Universitaet Wien, Universitaet Innsbruck

Patent DE102013210463A1 (2014) Hair restorer containing *Leontopodium* extract, DE Patent, Henkel AG and Co., KGaA

Patent US9498430B1 (2016) A bi-phasic, non-emulsion cosmetic composition for application to skin, CN Patent, Restorsea LLC

Patent US20220040070A1 (2022) Skin lightening compostions, US Patent, Kay Mary Inc.

Patent CN111202708B (2022) Cosmetic composition for improving the skin, CN Patent, Biological Fd&c Co., Ltd

Patent	Description	Current Assignee or Inventor
US20220040070A1 (2022)	Compositions and methods for use that include a combination of *L. alpinum* extract and other extracts	Kay Mary Inc.
CN111202708B (2022)	Invention relates to a cosmetic composition for improving skin, comprising a plant cell complex culture, and more particularly, to a cosmetic composition comprising: a *L. alpinum* cell culture or an extract thereof and other extracts.	Biological Fd&c Co. Ltd.
US9498430B1 (2016)	A bi-phasic, non-emulsion cosmetic composition for application to skin includes a hydrophobic liquid phase (essential oils) and a hydrophilic liquid phase.	Restorsea LLC
DE102013210463A1 (2014)	The application relates to the use of an active ingredient or mixture of active ingredients, obtainable from plants of the genus *Leontopodium*, for revitalizing hair.	Henkel AG and Co., KGaA
US20130053438A1 (2013)	The present invention relates to a pharmaceutical composition for stimulating angiogenesis and/or the treatment or prevention of hypovascularity and/or the prevention and/or treatment of an angiogenic disorder/disease, whereby the composition comprises specific compounds which may be obtained from *L. alpinum* Cass., (Edelweiss).	Medizinische Universitaet Wien, Universitaet Innsbruck
WO2007006492A1 (2007)	Use of a plant extract from at least one *Leontopodium* species for the preparation of a medicament for the treatment of diseases which can be modulated by the selective inhibition of the enzyme acetylcholinesterase and/or increase of acetylcholine concentration at the cholinergic synapse.	Helmut Prast, Judith Rollinger, Stefan Schwaiger, Hermann Stuppner

enhanced immunomodulation in the bovine mammary gland (Zhao et al. 2019). Additionally, studies have reported that an aqueous decoction of this species can prevent and treat diabetes, demonstrating a significant reduction in blood sugar levels and improved blood glucose levels in hyperglycemic mice (Yang et al. 2015).

Oxidative stress and reactive oxygen species (ROS) are key contributors to hair loss. *L. alpinum* var. *helvetia* shows promise in treating hair growth disorders. Hair growth occurs in three phases: anagen (growth), catagen (regression), and telogen (rest). In a study using an ethanol:water (60/40) extract, *L. alpinum* prolonged the anagen phase in ex vivo hair follicles, delaying progression to the catagen and telogen phases. The extract stimulated keratinocyte proliferation in the hair matrix and enhanced dermal papilla cell inductance, both of which are crucial for hair growth. In vivo, the application of the extract significantly increased hair density (+13,200 hairs across the scalp) and improved the anagen-to-catagen/telogen ratio, promoting active hair growth (Campiche et al. 2022).

Researchers investigated whether *L. alpinum* contains substances that enhance cholinergic neurotransmission. Intracerebroventricular injection of crude dichloromethane root extract increased extracellular acetylcholine (ACh) levels in rat brains by 79% at 1 mg ml^{-1} and inhibited acetylcholinesterase (AChE) in vitro. Fractionation revealed that sesquiterpene-rich fractions elevated ACh levels. Further testing identified compounds such as isocomene, β-isocomene, silphinene, and modhephene, with isocomene (2 μmol) demonstrating the strongest effect. A sesquiterpene acetate fraction containing silphiperfole acetate and 14-acetoxy-isocomene also boosted ACh levels. Individual testing revealed prolonged effects for 14-acetoxy-isocomene and a peak effect for silphiperfole acetate. Behavioral studies showed isocomene reversed scopolamine-induced amnesia in mice, enabling them to recall and recognize an object they had previously seen, thereby improving episodic memory without enhancing overall memory. This type of object recognition relies on episodic memory, which is often affected in dementia and Alzheimer's disease. Additionally, mice injected with 42 nmol of isocomene exhibited improved working and spatial memory in a T-maze, along with reduced neophobia, a behavior often associated with aging (Hornick et al. 2008).

Leoligin: Leoligin, a natural lignan derived from *L. nivale* ssp. *alpinum* roots, exhibits promising preventive and therapeutic potential in cardiovascular diseases, particularly atherosclerosis. Cholesterol efflux, a key elimination process, directs cholesterol from peripheral tissues to the liver for excretion. Leoligin effectively induced cholesterol efflux from THP-1 macrophages in a concentration-dependent manner, outperforming pioglitazone at 10 μM. It was particularly effective in the presence of apo A1 (apolipoprotein A1), a major component of HDL cholesterol and a potent cholesterol acceptor (Wang et al. 2016a).

The cholesteryl ester transfer protein (CETP), which plays a role in HDL metabolism, facilitates the exchange of triglyceride and cholesteryl esters between lipoproteins. In vitro studies demonstrated that leoligin activated CETP within nanomolar ranges (100 pM–1 nM). In vivo studies of CETP-expressing transgenic mice revealed increased CETP activity and reduced LDL cholesterol levels following leoligin treatment (administered in DMSO and diluted in 0.5% methylcellulose). However, CETP's role remains controversial, as it can be either proatherogenic or antiatherogenic depending on LDL generation and degradation pathways (Duwensee et al. 2011; Aguchem et al. 2024).

Apoe−/− mice, which are prone to atherosclerosis due to impaired lipoprotein clearance, showed reduced total cholesterol and LDL cholesterol levels after leoligin treatment (1 μM–100 μM). These mice, which do not express CETP, exhibited improvements in total cholesterol/HDL and LDL/HDL ratios, particularly at lower doses. A dose-dependent effect was observed at higher concentrations, specifically with improvements in the LDL/HDL ratio, while serum triglycerides remained unchanged. Leoligin also inhibited HMG-CoA reductase (HMGCR), the rate-limiting enzyme in cholesterol synthesis, in a concentration-dependent manner, with effects comparable to pravastatin at 5 μM and 50 μM. However, after 16 weeks, the effects diminished due to HMGCR upregulation, resulting in increased lipid and cholesterol deposition in the liver. Despite these changes, no protective effect against atherosclerotic plaques was observed. Unexplained weight loss and counter-regulation effects were noted in high-dose groups (Scharinger et al. 2016).

In vitro cultivation: Tissue cultures can be initiated using seeds or apical buds, or roots, after tissue sterilization (**Table 2**).

Seeds can be cultured on ½ Murashige and Skoog (MS) nutrient medium, while apical buds and roots can be grown for rooted in an agar-solidified (7 g l^{-1}) MS salt medium supplemented with the naphthaleneacetic acid (NAA) (0.1 mg l^{-1}), kinetin (0.05 mg l^{-1}), thiamine HCI (0.4 mg l^{-1}), mesoinositol (80 mg l^{-1}), casein hydrolysate (100 mg l^{-1}) and sucrose (30 g l^{-1}). The resulting cultures can be subcultured by separating rosettes (**Figure 5**). Following ex vitro adaptation is also possible using potting compost (Hook 1993). In vitro tissue cultures are particularly useful for conserving the natural and endangered populations of the plant.

Fig. 5. Cultivated edelweiss tissue culture (commercially available edelweiss assigned as *L. alpinum - Stella alpina* by Hortus Sementi, https://www.agrogradina.bg/cvetq-edelvais-hort). Source: A. A. Pozumentshtikov.

Table 2. Sterilization protocols for in vitro cultivation.

Sterilization protocol	Species	Reference
1. 70% ethanol for 30 s; 2. Rinse with distilled water; 3. 0.3% commercial NaClO on a shaker for 20 min; 4. Rinse several times with sterile distilled water.	*L. alpinum*	Cho et al. 2020
1. 0.1% HgCl2 for 10 min	*L. alpinum*	Ciocan et al. 2023
1. 70% ethanol for 3 min; 2. 10% commercial NaClO for 30 min; 3. Rinse several times with sterile distilled water.	*L. nivale*	Pace et al. 2009
1. Chlorhexidine 3 times (for 10 min) and rinse with distilled water each time; 2. 1% AgNO3 and rinsed three times with sterile distilled water.	*L. palibinianium*	Pianova (Berdasova) et al. 2021
1. 10% aq. solution of commercial NaClO for 20 min; 2. Rinse with sterile distilled water.	*L. alpinum*	Hook 1993

Callus culture: Callus culture involves growing unorganized, undifferentiated plant cells (callus) on a nutrient medium under sterile conditions. Callus formation is typically induced when plant tissues, such as leaves, stems, or roots, are wounded or treated with specific plant growth regulators like auxins and cytokinins. Several protocols have been reported for generating callus cultures (**Table 3**).

Table 3. Protocols for the induction of callus culture.

Growth regulator/component	Quantity	Source
6-Benzylaminopurine/6-BAP (cytokinin)	0.5–3 mg ml^{-1}	Cho et al. 2020
2,4-Dichlorophenoxyacetic acid/2,4-D (auxin)	0.3–1 mg ml^{-1}	
6-BAP	2 mg l^{-1}	Pace et al. 2009
2,4-D	1 mg l^{-1}	
Maintained in a medium containing:		
1-naphthaleneacetic acid/NAA (auxin)	0.1 mg l^{-1}	
6-BAP	0.4 mg l^{-1}	
MS		Kim et al. 2023
sucrose	3% (w/v)	
Gelatin	4% (w/v)	
6-BAP	0.5 mg l^{-1}	
2,4-D	0.3 mg l^{-1}	
Agar	9 g l^{-1}	Hook 1993
2,4-D	0.22 mg l^{-1}	
NAA	0.18 mg l^{-1}	
Glycine	2 mg l^{-1}	
Nicotinic acid	0.5 mg l^{-1}	
Pyridoxine HCl	0.5 mg l^{-1}	
Meosinositol	200 mg l^{-1}	
Thiamine HCl	0.5 mg l^{-1}	
Sucrose	30 g l^{-1}	

An extract from lyophilized callus culture of *L. alpinum*, obtained via heat extraction, has demonstrated strong antioxidant activity, especially under UVB exposure. At a 1% concentration, its antioxidant effect is comparable to that of N-acetyl cysteine (NAC) and surpasses vitamin C, with notable improvements in cell viability. This activity is attributed to the extract's ability to inhibit hydrogen peroxide and ROS. Additionally, the extract reduces inflammation and wrinkles while improving skin hydration. Clinical in vivo tests confirm that regular application to the face improves the appearance of wrinkles around the eyes, boosts skin elasticity, and increases both skin density and thickness compared to placebo groups. RNA sequencing in keratinocyte cells revealed that the extract upregulated genes encoding proteins critical for skin barrier development, programmed cell death, and keratinization. At the same time, it downregulated genes associated with stress responses, including those related to metal exposure, oxidation, injury, hypoxia, and viral infections, indicating no adverse effects on the skin (Cho et al. 2020).

Another research team identified several bioactive compounds in the callus extract of *L. alpinum*, including leontopodic acid A and B, syringin, chlorogenic acid, cynarin, isochlorogenic acid A and C, isoquercitrin (Meng et al. 2023). Their study demonstrated that the callus extract protected fibroblast models from blue light-induced damage by reducing ROS levels. At concentrations of 10–15 mg ml-1, the extract promoted collagen (COL-I) production and inhibited the secretion of matrix metalloproteinase-1 (MMP-1, also known as collagenase), skin opsin (OPN3), ROS levels, and calcium influx. The mechanism of action likely involves the inhibition of OPN3-dependent calcium signal transduction pathways, thereby reducing oxidative stress caused by ROS. By suppressing these pathways, the extract prevents the secretion of MMP-1 secretion, which is critical for collagen breakdown, offering protection against blue light-induced damage.

In conclusion, it is possible to state that complementary phyloecological studies would elucidate the influence of climatic and geographical factors on the distribution and evolutionary dynamics of more sensitive organisms such as edelweiss. The integration of these approaches will not only deepen our understanding of the evolutionary mechanisms underlying these organisms but also provide the scientific foundation needed to revise their taxonomic classifications. Such knowledge may be pivotal for conserving biodiversity and ensuring the long-term sustainability of ecosystems. This is especially important for species inhabiting microhabitats with highly restricted distributions, as they are among the most vulnerable to environmental changes or other competing more adaptable organisms. Understanding the ecological dynamics of these environments is therefore critical for developing effective conservation strategies aimed at mitigating the impacts of climate change and habitat loss. Such strategies will be vital for safeguarding vulnerable species and maintaining the integrity of ecosystems.

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CONFLICT OF INTERESTS

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

AUTHORS CONTRIBUTION

A.A.P. designed and wrote the manuscript. I.A.B., G.N.B., M.L.K. and M.K.Z. contributed to adding information and improving the manuscript.

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REPORT ON THE CAREER FORUM 2024: EVENT PLANNING, IMPACT AND REFLECTIONS ON PAST SUCCESSES AT THE FACULTY OF BIOLOGY

ZLATINA D. ZHELEVA*, YORDAN Y. GEORGIEV*, MARIA Y. GENDZHOVA, LYUBEN S. NOVOSELSKI, TEODORA P. KOLEVA, MIROSLAVA K. ZHIPONOVA, DENITSA R. TEOFANOVA, ILIYANA K. RASHEVA, DILYANA P. NIKOLOVA, VYARA S. MAMATARKOVA

Faculty of Biology, Sofia University "St. Kliment Ohridski", 8 Dragan Tsankov Blvd., 1164 Sofia, Bulgaria

Abstract. The Career Forum 2024 at the Faculty of Biology (BF) at Sofia University "St. Kliment Ohridski" (SU) was organized by the Faculty Student Council (FSC) with the support of the Career Center of BF and the Deans Council. The event took place on the vernal equinox on March 20/21. It introduced the Institutes of the Bulgarian Academy of Sciences (BAS), the Agricultural Academy (AA), and various business organizations, including Government institutions in the fields of biology, biotechnology, ecology and healthcare from across the country. Feedback from students and participating employers was overwhelmingly positive, reflecting the high quality of the event's organization and presentations. This report aims to highlight the details of the event's occurrence and its outcomes for all involved parties. Additionally, it seeks to review the history of similar events held at the BF over the years. Four major career-oriented events held between years 2016 and 2019, provided a solid foundation for improving the quality of impact on students and their future employers.

Keywords: biological sciences, biotechnologies, employers, methodology of biology education, professional realization, students

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* *corresponding authors*: Y. Y. Georgiev and Z. D. Zheleva, Faculty of Biology, Sofia University "St. Kliment Ohridski", 8 Dragan Tsankov Blvd., 1164 Sofia, Bulgaria; ygeorgiev@uni-sofia.bg, zdjeleva@gmail.com

The initiative of career-oriented events at the Faculty of Biology (BF) of Sofia University "St. Kliment Ohridski" (SU) began to take a more purposeful shape through the organization of Youth Scientific Conferences "Kliment's Days" (YSC) in November. Motivated and inspired faculty members in the organizing committees kept on developing programs for young researchers, encompassing key biological fields such as Biological Diversity, Ecology, Environmental Protection and Sustainable Development, Molecular Biology and Biotechnology; and Biology Education (YSC-2012). Over time, the conferences expanded to include sponsors and collaborators from various national or international companies actively participating in the sessions (BF Conferences 2024).

In 2016, the YSC was organized for the first time with additional interactive panels devoted to career orientation (YSC-2016). One of them was BioFest-2016, where members of the faculty departments, together with interested students, created interactive stands to demonstrate to school pupils why biology is fascinating (BioFest-2016) (**Figure 1**)

Fig. 1. The poster of BioFest-2016 and the organization in the main hall on the first floor of BF. "*If you already love BIOLOGY or are just curious, come to us and you will find out how vast it is. We will show you why BIOLOGY is the science FOR life. Because life is biology. We will tell you why BIOLOGY is the science OF life. Because for every biologist it is the science of his life. Come, we will share with you our passion, our secrets... and something else*". (BioFest-2016: http://conf2016.biofac.info/biofest).

The other important panel was the roundtable discussion titled “Career Development” which brought together BF students (bachelors, masters and PhD students) and employers from biology-related companies (Roundtable-2016). Representatives from Biovet, the Fund for Wild Flora and Fauna (FWFF), Fullbright, ProViotic and the University Botanic Gardens introduced students to their fields of work and career opportunities.

The next 2017 year, two YSC-associated Roundtables were set up – “Career Development” and “Trends in E-learning” (Roundtables-2017). In parallel, a Federation of European Biochemical Societies (FEBS) Workshop on Molecular Life Sciences Education was held (FEBS-2017) (**Figure 2**). Invited publishing entities, including Pensoft, Elsevier, Bulgarian Journal of Agricultural Science and “Bulgarian Science - Learn More” Journal, shared advice on writing scientific articles, conducting literature searches, and performing scientific communication. Additionally, the implementation of E-learning in primary and higher education was discussed.

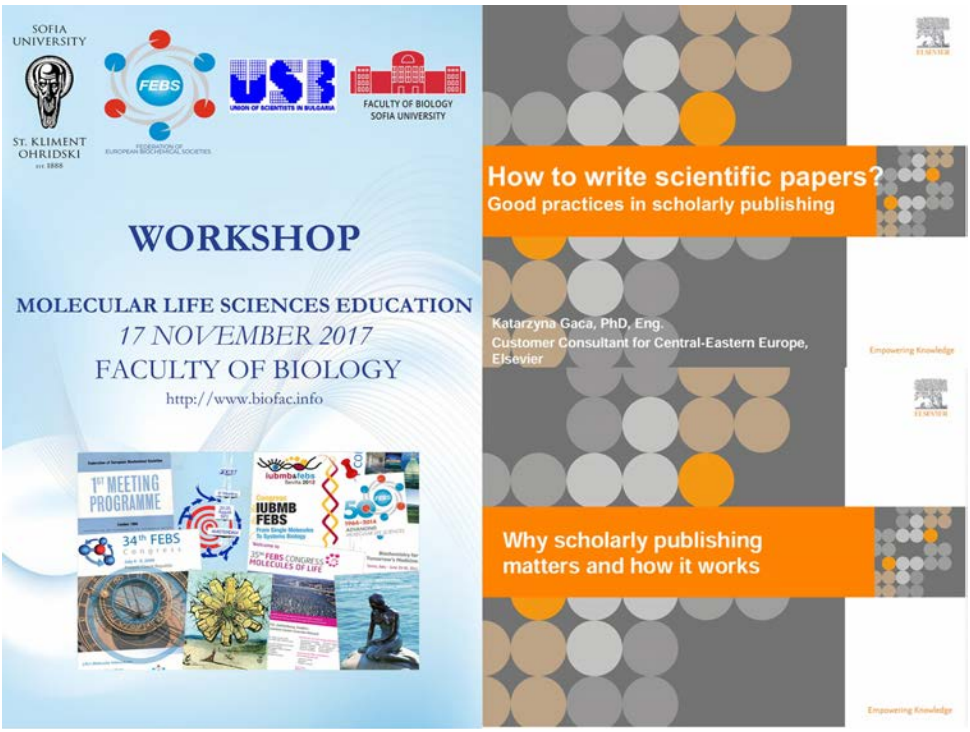


Fig. 2. The poster of FEBS Workshop on Molecular Life Sciences Education alongside the Youth International Scientific Conference and a part of the informative presentation about writing scientific papers from Elsevier in the Roundtable of Career Development 2017.

In 2018, the BF career event was organized during the spring, in May, although it was still held under the scope of the YSC (Roundtable-2018). A total of 13 companies participated, including Chief Executive Officers and staff from managerial, commercial, and production departments of biotechnological and pharmaceutical companies (Vetprom/Vpharma, Biovet, Merck, Aquachim, Veolia, Sofia Zoo, PPD, National Genetic Laboratory, Bioregeneration Biobank, Teach for Bulgaria, Center for Applied Studies and Innovation (CASI), Centre of Microbial and Plant Genetics Belgium, European Scientific Council). They presented their activities in a lecture panel, followed by discussions with the students at their individual booths (**Figure 3**). During the same time period, a spectacular and vibrant outdoor BioFest-2018 was organized by BF members to promote biology among school pupils and the broader public (BioFest-2018-AM-TV).

Fig. 3. Footage of the report from Alma Mater TV of the Roundtable of Career Development 2018. (Roundtable-2018-AM TV: https://youtu.be/PVs9eRIVwEw?si=a1WRRlf6ibGZnHAF)

The Day of Career Development in May 2019 marked the first fully independent career event (Day of Carrer Development-2019). On one side, the faculty departments' members focused on presenting the Master's programs through posters and brochures (**Figure 4**). The Career Center of Sofia University (SU Career Center) also took part with useful career guidelines. On the other side, 13 business companies participated in the event, including PPD, RIDACOM, Labimex, FOT, Sofia Zoo, Comac Medical, WWF, Bul Bio, Biovet, Vetprom, "Teach for Bulgaria", C3i Solutions, Green Balkans (**Figure 4**).

Fig. 4. Snapshots from the Opening of the Day of Career Development 2019, the booths of participating companies and departments of the BF in the courtyard and the lectures in the Aula.

The YSC organizers included BF, supported by the SU Fund of Scientific Research, the Scientific Research Department at SU, the Union of Scientists in Bulgaria, and the Students' Club "S.K.O.R.E.C." at BF, in addition to the contributions of staff members and engaged diploma students, the role of the Faculty Student Council (FSC) proved to be invaluable. The FSC actively participated in creating artwork, applying for funding, and promoting career initiatives. Their official involvement contributed to developing a more attractive, constructive and impactful environment at each event.

From 2016 to 2023, the YSCs and their corresponding Roundtables, aimed to target undergraduate (B.Sc.), graduate (M.Sc.) and PhD students across three professional fields: Biological Sciences, Biotechnologies, and Methodology of Biology Education. Employers were carefully selected in alignment with these professional fields and contacted through face-to-face meetings, conversations with alumni employed at the companies, phone calls, and e-mail correspondence. Each event provided a valuable opportunity for both foreign and Bulgarian scientists from diverse fields to network, fostering potential future collaborations and the exchange of experience. The organization of each event, which involved forming organization teams, inviting participants, designing the schedule, and preparing the venue, required between one and three months. Despite the lack of official statistics, approximately 50 students actively participated in each YSC on average.

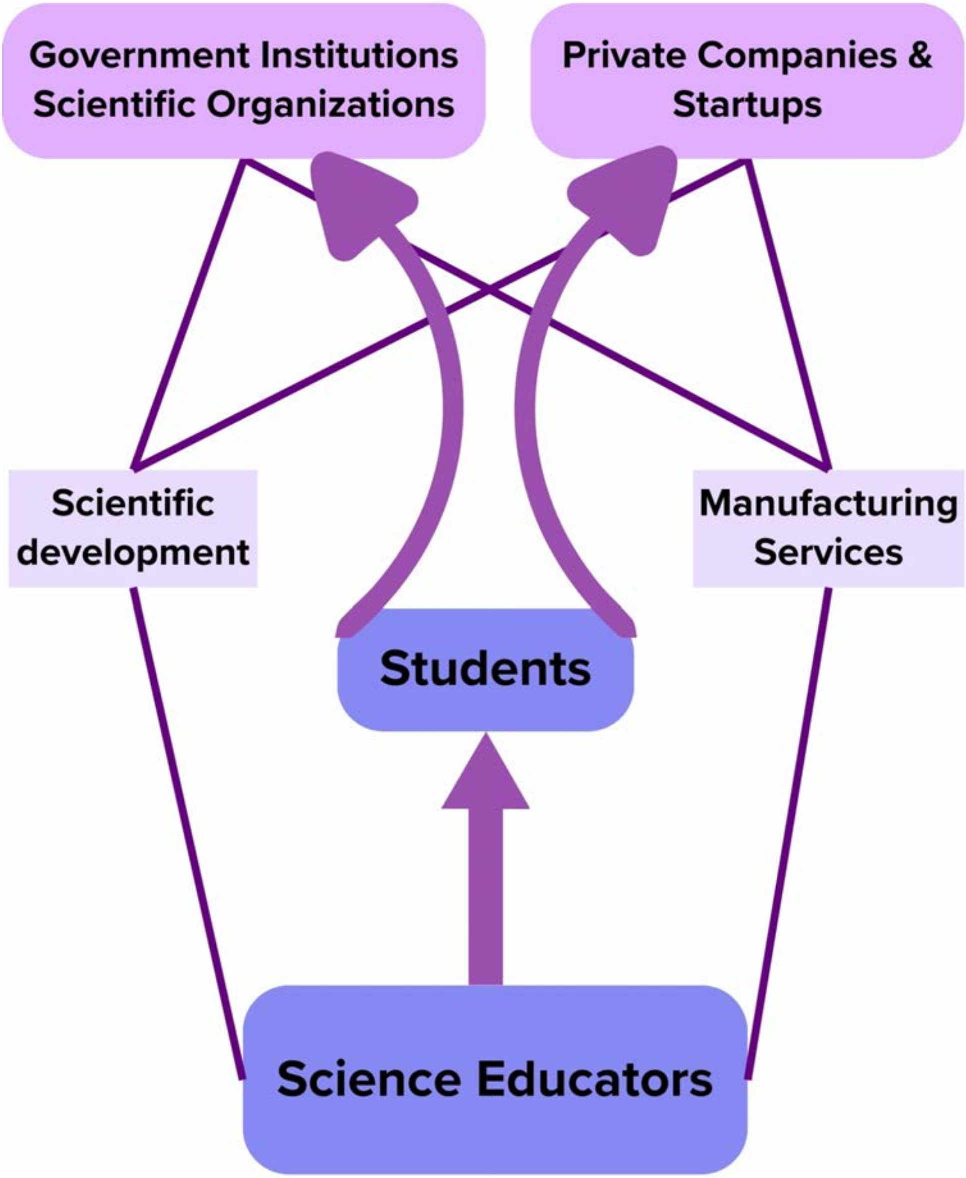


Fig. 5. The flow of specialized workforce and new knowledge in the context of career development and societal progress.

Estimates suggest that 50–90% of all students in the BF attended the career events, with M.Sc. students typically representing a higher proportion, likely due to their smaller cohort size. These events also attracted students from other faculties, such as Physics or Chemistry and Pharmacy, as well as from various scientific institutions.

The organizers' motivation was driven by the objective of encouraging students to achieve higher career realization aligned with their higher education. This was complemented by a shared goal of making science publicly accessible and understandable, while fostering the practice of sharing experiences and ideas between students and scientists. Pursuing these goals aims to enhance students' professional ambitions to work as molecular biologists, ecologists, biotechnologists, or science teachers, and to demonstrate them the tangible opportunities for their efforts to be rewarded within our country (**Figure 5**).

The Career Forum 2024 is the result of an initiative aimed at addressing a problem identified by students in their final year of Bachelor's studies. Many of them had a vague vision for their future career and were left to conduct their own search into available opportunities. It is reasonable to assume that the pandemic not only disturbed the usual structure of university classes and workplace activities but also significantly impacted the organization of events of any kind. This led to nearly four years of stagnation in career development and orientation at the BF. The involvement of former FSC students during this period was largely limited to the public relations campaigns for the events held in 2018 and 2019. These factors further motivated the current FSC students to take action. In 2024, they organized an event designed to showcase as many opportunities as possible for students to realize their potential in various scientific fields.

For the second time since 2019, a career-oriented event in BF was held independently. The Career Forum was the first event in this series to be organized predominantly by students. The organization of the event began approximately two months before the selected dates. A total of 49 companies (including private laboratories and biotechnology-related businesses), 19 institutions, and 10 institutes of Bulgarian Academy of Sciences (BAS) were contacted. Of these, 39 accepted the invitation to participate, representing exactly 50% of the inquiries. Additionally, a career consultant from the "Career Development" Sector of SU responded to the invitation and participated in the event. The full table, List of Employers, with contacts of all scientific organizations and companies can be accessed by the link to the online system of education of SU (Career and Professional Development BF-SU 2024).

All confirmed participants were asked to send their presentations via email prior to the event to ensure seamless and efficient transitions between each presenter. Requirements and guidelines regarding posters and booths were also provided in advance to allow participants to prepare adequately. To manage communication via email, phone calls, or direct interactions, students were assigned as coordinators, each responsible for a few participants. Additionally, a dedicated team was tasked with drafting and defending the funding project within the Diaphanum system, part of the Student Council of SU. This funding covered catering for both days of the event (**Figure 6**).

Fig. 6. Catering in front of the BF Aula from the opening until the end of the first panel and in the Exhibition Hall for the second panel.

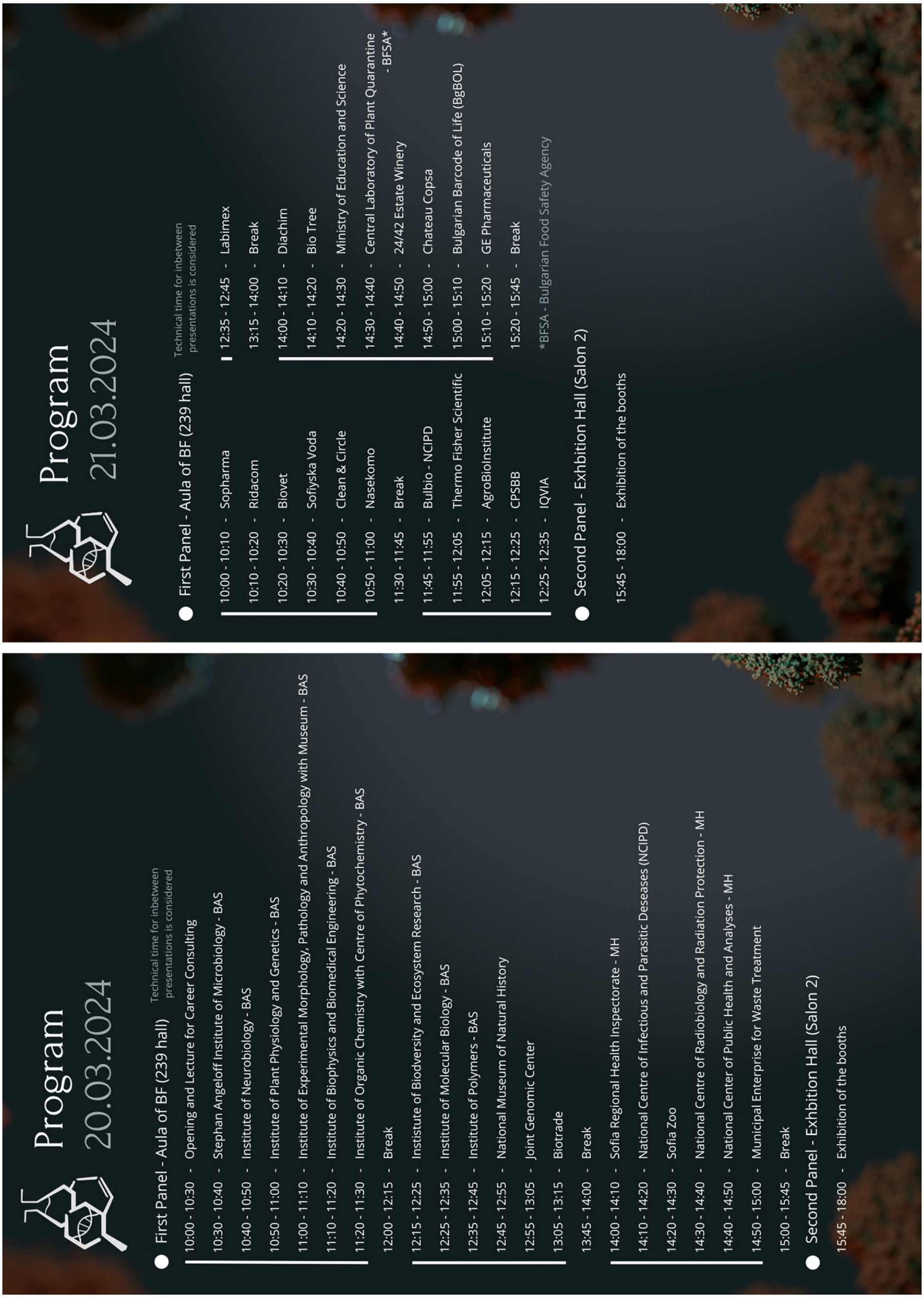


Fig. 7. The program of the two-day event.

The program of the Career Forum was structured in a way that the institutes of BAS were presented in consecutive blocks, followed by the institutions and companies. The scheduling took into account the availability of some scientific organizations after the conducted communication, ensuring that their presentations were arranged around the time frames when they were able to attend (**Figure 7**).

The operator of Alma Mater TV attended one of the event days to capture its essence. During the first panel, representatives from the organizing team were featured in the report through interviews. In the break before the second panel, interviews were conducted with presenters from scientific organizations and attending students (**Fugures 8** and **9**). Students from the organizing team also participated as reporters in the video and assisted the editors in its production (Career forum-2024-AM-TV).

The methodology of modern higher education requires active learning strategies. This is especially important for students in various organizations, as building competencies in event management, PR campaigns and communication, and teamwork necessitates learning from each other and through the process itself. Active students, in turn, inspire their educators by motivating them to share valuable practices and insights. This creates a positive ecosystem of collaboration and constructive dialogue, fostering better outcomes and transforming the mindsets of everyone involved.

Fig. 8. Footage from the Aula of BF during the presentations.

Taking the methodological approaches into account, a campaign to gather feedback was planned. Guest-participants were provided with two copies of questionnaires: one addressing the quality of education at the university and the other focusing on their requirements when hiring young research the scientific organizations, 33 gave feedback. The results were compiled into a collection of opinions regarding the professional fields in BF. The feedback from the participants about the event

Fig. 9. Footage from the booths in the Exhibition Hall of BF.

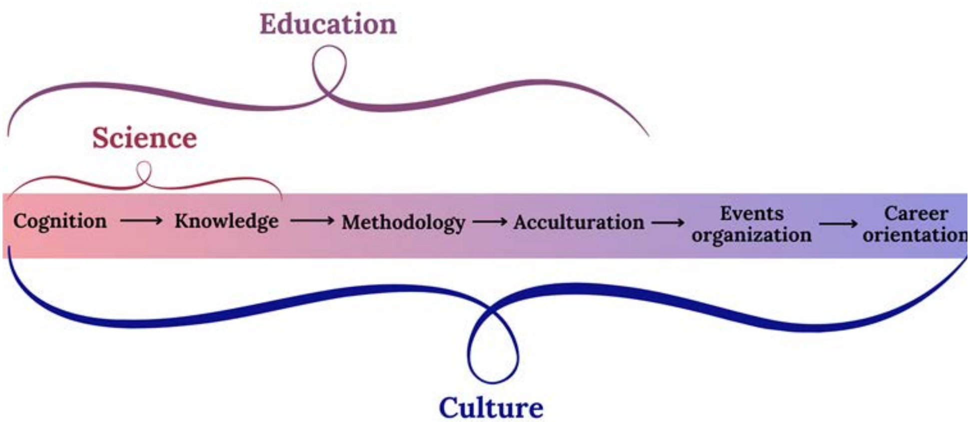
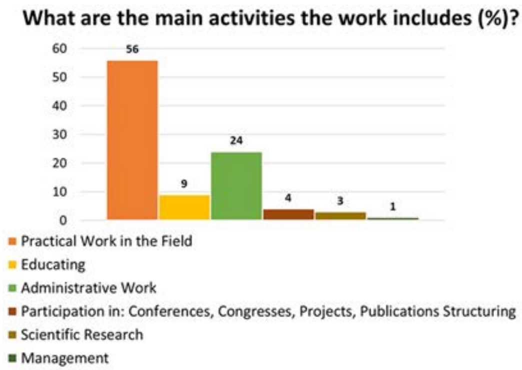


Fig. 10. Links between science, active education and career development.

and the level of preparation of BF students was generally positive. According to the responses, over half of the roles involve practical work in their respective fields, while a quarter pertains to administrative tasks. Nearly 9% is related to education, with the remaining 8% encompassing management, research, participation in conferences, and other activities. Notably, 57% of the answers pointed to the combination of theoretical knowledge and practical skills as the most crucial factor in students' education. In contrast, attributes such as teamwork, motivation, and affinity for administrative work were considered the least significant (**Figure 11**).



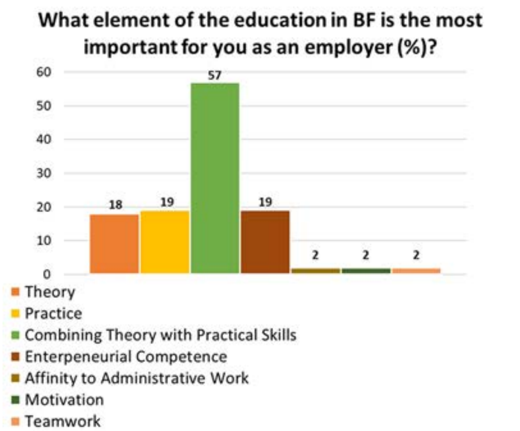


Fig. 11. Description of the general nature of the work in scientific organizations and the most valued qualities in students among all those listed.

On the other hand, when hiring, the surveyed employers considered creative problem-solving, teamwork skills and students' performance during interviews, as the most important factors overall (**Figure 12**).

Over 40% of the responses rated the quality of education and theoretical preparation of young scientists at the BF as excellent. Nearly 40% rated the practical preparation and individual task-solving skills as excellent, while 30% rated them as good. Motivation for professional development was rated as excellent in 31% of the answers and as good in 16%. More than half of the total respondents expressed positive feedback regarding all suggested types of collaborations with the university – with the highest interest shown in student internships (**Figure 13**). There were some answers to the open question: "What are your suggestions for developing the professional education field?":

- "The education needs to include giving knowledge and practical skills linked to the newest achievements in Biotechnology, for example working with cell cultures, GMO, recombinant proteins, etc. Interdisciplinary approach – incorporating not only the biological aspects, but also engineering, chemical and information technology."
- "Spending more time on discussing and critiquing scientific publications for acquiring theoretical and practical insight of the modern scientific research. Incorporating more practical terrain and laboratory exercises."
- "The Biotechnology program is developing well. In the Institute there is one Bachelor student who is well prepared theoretically and practically."

- “Higher criteria for assessment of the students. Better incorporation of the link between theory and practice in the education process.”
- “There are financial instruments for creating opportunities for students if good communication is maintained. Theoretical material should be reduced in favor of practice; emphasizing the positives and opportunities in our educational system; better informing the students and keeping a friendlier atmosphere.”

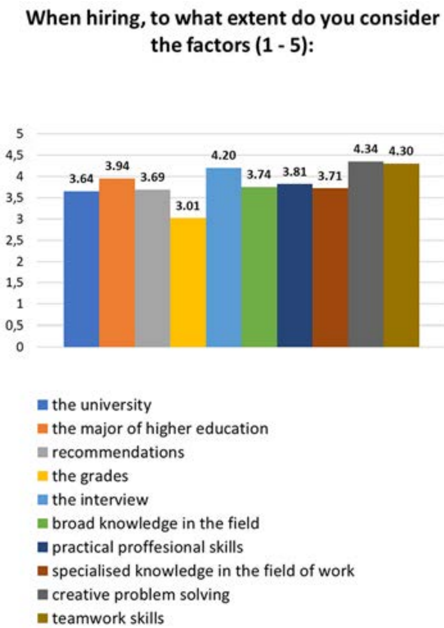


Fig. 12. Evaluation of the importance of each student quality in employment consideration (values represent an average score).

The feedback from the survey respondents among the students (76 replies) showed high interest and satisfaction. According to 100% of them, the event was useful and informative. For 92.11% of the students, all their questions were answered. Additionally, 98.68% expressed interest in attending a similar event next year. Of the responses, 61.84% were strictly positive, stating that the event helped orient them toward a specific organization. Next, 61.6% of the ratings about the general impression of the event were the highest score (5) and 28.8% were the second highest score (4). The organization of the forum was rated 5 by 76.3% of the respondents. Among the students who attended and rated the presentations in the first panel, 46.6% gave a score of 5, while 43.8% –gave a score of 4. The second panel, which included discussions and demonstrations, was rated 5 by 63.2% and 4 by 26.3% (**Figure 14**). There were also suggestions for other potential employers to be invited for the next Career Forum, including Comac Medical, ”Cibalab”, “Ramus”, “Kandilarov” labs, Inna Essentials, Damascena, Vodoraslo, WWF, Gosmile, Bulgarian Society for the Protection of Birds, other private schools and companies.

The following citations are the answers to the question “What else can you share from your impression of the event?”:

- “Thank you for organizing the event, it was really helpful as it showed me more opportunities for growth after my education, because I am not sure in which discipline I would like to get in yet. Looking forward to the second day of the forum!”
- “Compact effective organization and a big spectrum of companies in the different fields of Biology. Well done job!”

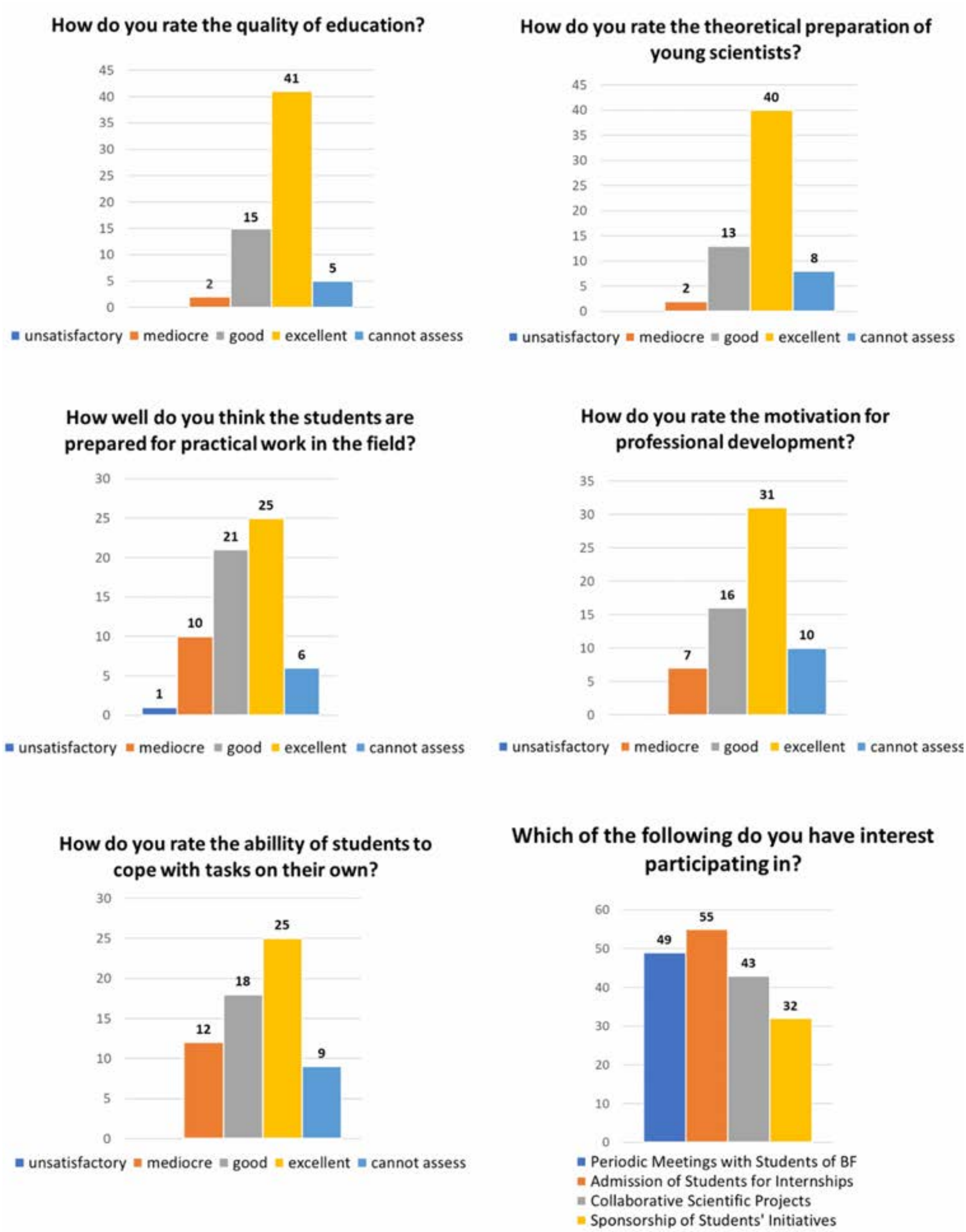


Fig. 13. Evaluation of different education aspects in BF and students' qualities according to the employers.

- "The students should be dismissed from lectures in the time period of the forum and the presentations should be more oriented towards internships for Bachelors."

- “Some of the companies need to be more specific about what their activities include and what qualities do they search for in a new candidate.”

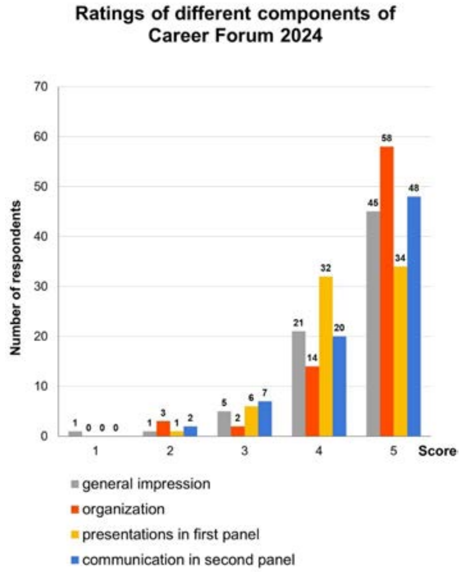


Fig. 14. Evaluation of main elements in the Career Forum by the attending students.

Such initiatives are highly beneficial and encouraging for students and all participants. Career forums are events that bridge the gap between higher education institutions and the labor market. In line with modern university trends toward student-centered education that guarantees the acquisition of both subject-specific and broad competencies, future career forums should present the full range of professional opportunities for our students: from academic research, to teaching science options and business start-ups and spin-offs. These forums should also encourage students to prsue their professions.

It is also of great importance to encourage students to actively participate in such conferences and career forums, allowing them to both connect with the scientific community and explore work placement opportunities. A well-executed advertising campaign would certainly enhance the positive outcomes of such career and scientific events. As more companies agree to participate, others would also be attracted, increasing the credibility and popularity of our events. Thorough management and detailed organization are essential for their success. The interest and desire for a future career forum demonstrate that the organizing parties have addressed the key aspects needed for a productive gathering of people in the science field. Involving active students in organizing the event contributes to its usefulness and significance, showing all students in the faculty the potential perspectives for their future careers. While it requires a great deal of effort to bring all these ideas to life, the results are certainly worth it.

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CONFLICT OF INTERESTS

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

AUTHOR CONTRIBUTIONS

Y.Y.G. and Z.D.Z. designed and wrote the manuscript. M.Y.G., L.S.N., T.P.K., M.K.Z., D.R.T., I.K.R., D.P.N., V.S.M. contributed to adding information and improving the manuscript.

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ГОДИШНИК НА СОФИЙСКИЯ УНИВЕРСИТЕТ „СВ. КЛИМЕНТ ОХРИДСКИ“

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REPORT ON THE SEVENTH INTERNATIONAL “FASCINATION OF PLANTS DAY” (FOPD) THAT BROUGHT TO LIGHT THE VOLUNTARY SPIRIT INTO THE FACULTY OF BIOLOGY

YORDAN Y. GEORGIEV[1*], ZLATINA D. ZHELEVA[1*], DESISLAVA I. MANTOVSKA[1], ANA-MARIA R. NEDELCHEVA[1], ANELIA V. IANTCHEVA[2] & MIROSLAVA K. ZHIPONOVA[1],

[1] Department of Plant Physiology, Faculty of Biology, Sofia University “St. Kliment Ohridski”, 8 Dragan Tsankov Blvd., 1164 Sofia, Bulgaria
[2] Agricultural Academy, Agrobioinstitute, 8 Dragan Tsankov Blvd., 1164 Sofia, Bulgaria

Abstract. The seventh international “Fascination of Plants Day” (FoPD) was held in May 2024 at the Faculty of Biology (BF) of Sofia University “St. Kliment Ohridski” (SU), in collaboration with the Agrobioinstitute (ABI) of the Agricultural Academy (AA). The event was a part of Sofia University’s “May Days of Culture” and aimed to highlight the significance of plant science and the fascinating world of plants. FoPD-2024 brought together academic members and student volunteers, which resulted in a unique combination of fresh perspectives, creativity, and high-quality presentations. Through interactive talks and demonstrations, curious facts were shared about topics such as vampire plants, urban tree species, and plant communication through light. Key discussions explored the role of plants in our lives, addressing questions like: What are plant "superfoods"? Why are legumes important? What exactly is organic farming, and is it practiced in our country? How do electromagnetic fields impact precision agriculture? How do microalgae serve as vital sources of useful substances? Answers were also provided to questions about plant diseases and their defense mechanisms. Visitors were introduced to a “laboratory oasis” that showcased the powers and health benefits of medicinal plants. STEM experiments

* *corresponding authors*: Y. Y. Georgiev and Z. D. Zheleva, Faculty of Biology, Sofia University “St. Kliment Ohridski”, 8 Dragan Tsankov Blvd., 1164 Sofia, Bulgaria; ygeorgiev@uni-sofia.bg, zdjeleva@gmail.com

involving plants were organized for the curious ones, offering hands-on learning experiences. Attendees were also delighted by the plant collections at the Faculty of Biology and the Institute of Decorative and Medicinal Plants, which featured a variety of greenhouse-grown plants suitable for decoration or as gifts. The S.K.O.R.E.C. student club demonstrated its volunteer initiatives focused on biodiversity knowledge and conservation. The organizers' creativity shone in a special plant workshop and a children's corner. Additionally, an entertaining quiz was held to test what visitors had learned during FoPD. This exciting event successfully celebrated the captivating world of plants and their invaluable role in our lives.

Keywords: academic members, Faculty Student Council, methodology, plant sciences

Fascination of Plants Day (FoPD) was initiated and supported by the European Plant Science Organisation (EPSO), whose mission is to enhance the impact and visibility of plant science (Zhiponova et al. 2020). The event was first launched in 2012 and through its biennial editions evolved into an international initiative and steadily gained worldwide recognition. In 2024, the seventh FoPD initiative united 65 countries, hosting 868 plant-based outreach activities for people of diverse backgrounds (FoPD Statistics-2024).

The Faculty of Biology (BF) at Sofia University "St. Kliment Ohridski" (SU) and the Agrobioinstitute (ABI) of the Agricultural Academy (AA) began collaboratively organizing plant-related events in 2015, marking Bulgaria's first participation in FoPD (Zhiponova et al. 2020). Since then, both institutions have consistently promoted plant science as part of FoPD initiatives (Zhiponova et al. 2020, 2022; FoPD-Success story-2022). FoPD-2024 was celebrated on May 17, 2024, with remarkable contributions from representatives of the Faculty Student Council (FSC) and academic members specializing in plant science (**Figures 1a, b**).

The coordination for the FoPD-2024 event began nearly two months in advance and followed a structured sequence: planning the program; drafting the announcement; distributing information to BF and ABI colleagues (staff and students), schoolteachers and media; applying for funding; and organizing logistics. Topics were collected from all interested representative groups within BF and ABI, who individually selected the focus of their presentations and invited students to participate in the demonstrations (**Table 1**). This approach allowed researchers to effectively disseminate information about ongoing projects while introducing young colleagues to the art and skills of popularizing science. As a result, a significant academic participation activity was recorded: approximately 53 bachelors, 7 magisters/masters, and 3 PhD students; 18 members of BF from six departments; four senior researchers from ABI; and one senior researcher from the Institute of Ornamental and Medicinal Plants (**Table 1**).

For the first time, FSC was exclusively involved in every stage of the FoPD-2024 event's organization. From the outset, representatives of ABI, BF, and FSC worked collaboratively on the event planning. Notably, the FSC contributed significantly by creating the visually appealing announcement and booth map (**Figure 2**), coordinating the Diaphanum funding project, decorating the Exhibition Hall (**Figures 1c, d**), and organizing the QUIZ content, as well as workshops for children alongside the representatives of ABI.

Fig. 1. FoPD-2024 organization. **a**) Opening the event. From left to right: the main coordinator of the event Prof. Anelia Yantcheva from ABI; the main organizer - Assoc. Prof. Miroslava Zhiponova from the Department of Plant Physiology in BF, Assoc. Prof. Lyuben Zagorchev - Dean of BF and Assoc. Prof. Lyubomir Kenderov - Vice-Dean of Economic Affairs of BF. **b**) FSC representatives in front of the wall for pictures: students in the organization Yordan Georgiev and Zlatina Zheleva; **c**) Setting up the booths of the event in the Exhibition Hall of BF; **d**) The arrangement of the wall for pictures by FSC.

The FSC also played a key role in promoting the event among BF students, while colleagues from the Department of Methodology of Biology Teaching at BF disseminated information to schools through the regional departments of education. For the general public, an audio invitation to attend FoPD-2024 was broadcast on the program Afternoon for the Curious (hosted by Ani Kostova interviewing Assoc. Prof. Miroslava Zhiponova) on Radio Hristo Botev (Radio show "Afternoon for the Curious" 2024).

Scientists and educators from ABI and BF departments, as well as many students with a passion for various aspects of plant science worked collaboratively to share their enthusiasm and knowledge with other students from BF and school pupils (**Figure 3**; **Table 1**).

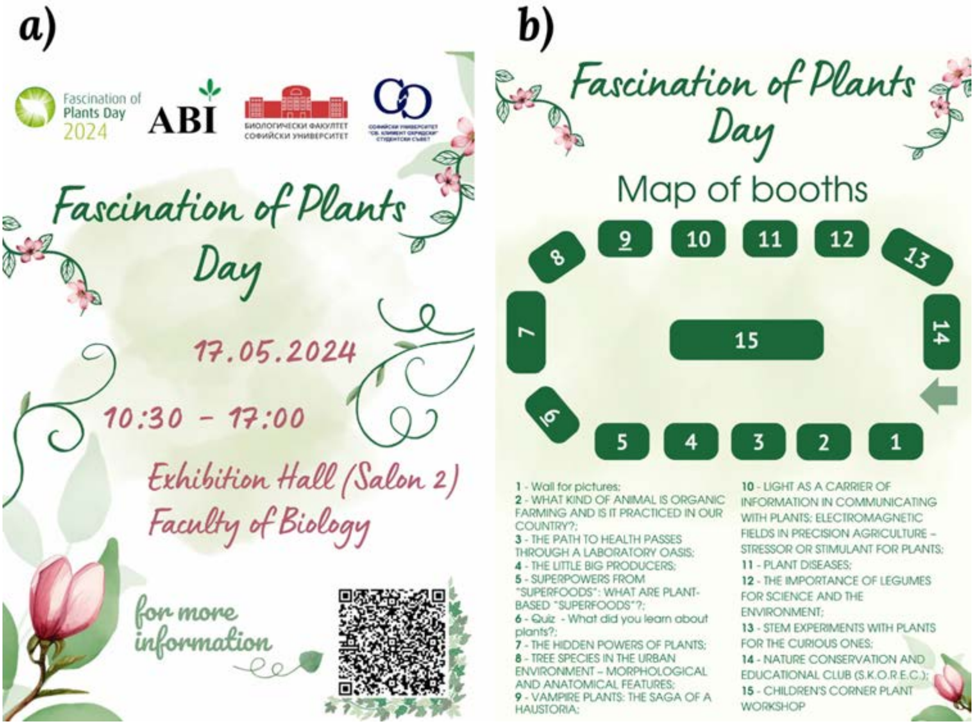

Fig. 2. FoPD-2024 announcement and map of the interactive boots (in Bulgarian).

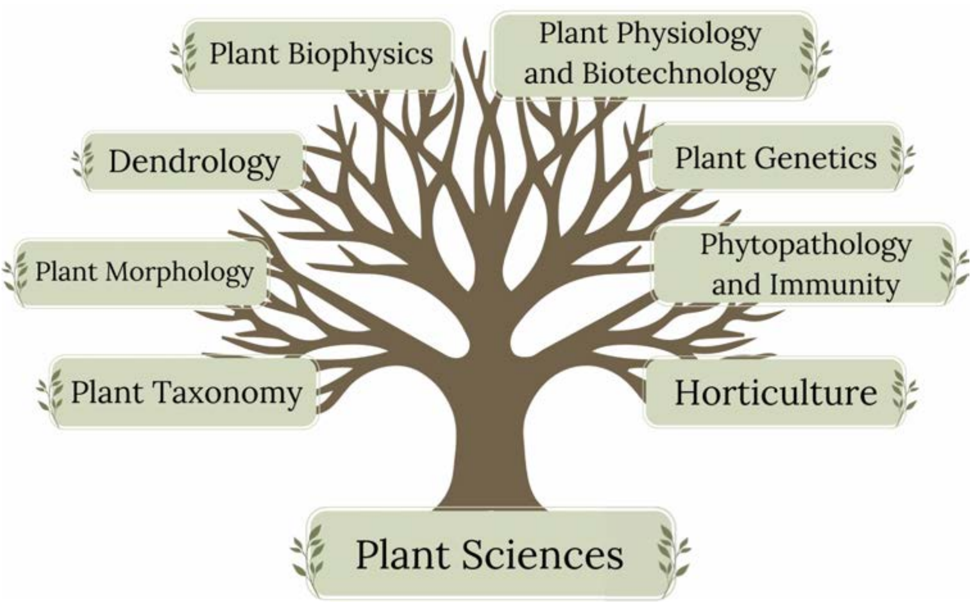

Fig. 3. Plant Sciences on the FoPD-2024 in BF, SU.

Table 1. FoPD Program (May 17, 2024) in BF, SU.

Participating Organization	Information – Topics, Participants and Funding	Description
Department of Biochemistry, BF (**Fig. 4c**)	"VAMPIRE PLANTS: THE SAGA OF A HAUSTORIA" BIANCA MARINOVA (MSc 2y Biochemistry) Supervisors: Assoc. Prof. Dr. DENITSA TEOFANOVA; Assoc. Prof. Dr. LYUBEN ZAGORCHEV Financing: European Union-NextGenerationEU, under the National Recovery and Resilience Plan of the Republic of Bulgaria, Project No BG-RRP-2.004-0008	Young and old will have the opportunity to get acquainted with some strange and lesser-known types of plants, namely the parasitic ones. With us you will learn more about parasitism in plants - what their "vampire teeth" (haustoria) are, what is cuckoo yarn and how the science around it is entangled and unraveled. Welcome to the world of vampire plants, they don't bite!
Department of Biophysics and Radiobiology, BF (**Fig. 4b**)	"LIGHT AS A CARRIER OF INFORMATION IN COMMUNICATING WITH PLANTS" Assist. Prof. Dr. MOMCHIL PAUNOV; Assist. Prof. Dr. BOYANA ANGELOVA; Assist. Prof. Dr. VLADIMIR ALEXANDROV; Assoc. Prof. Dr. MARGARITA KUZMANOVA; Prof. Dr. VASILIY GOLTSEV	"Fluorescence experts" will demonstrate the capabilities of chlorophyll fluorescence to obtain a large set of data on the functional state of photosynthesis. However, the large amount of information requires reliable analysis, which is possible only through the application of modern information methods, such as neural networks, allowing the recognition of various stress factors (drought, mineral deficiencies) and even species affiliation in plants.
Department of Biophysics and Radiobiology, BF (**Fig. 6c**)	"ELECTROMAGNETIC FIELDS IN PRECISION AGRICULTURE – STRESSOR OR STIMULANT FOR PLANTS" Assist. Prof. Dr. MOMCHIL PAUNOV; Assist. Prof. Dr. BOYANA ANGELOVA; Assist. Prof. Dr. VLADIMIR ALEXANDROV; Assoc. Prof. Dr. MARGARITA KUZMANOVA; Prof. Dr. VASILIY GOLTSEV Financing: Bulgarian National Science Fund-MES КП-06-Н67/4 12.12.2022	Wireless communication technologies are the basis of "precision agriculture". What is known so far about the influence of electromagnetic fields used in wireless communications on the physiological state of plants, and what are the results of current scientific experiments.

Participating Organization	Information – Topics, Participants and Funding	Description
Department of Botany, BF (**Fig. 4a**)	"TREE SPECIES IN THE URBAN ENVIRONMENT – MORPHOLOGICAL AND ANATOMICAL FEATURES" Rayna Nikolova (BSc 3y, Agrobiotechnologies); Supervisors: Assoc. Prof. Dr. Tsveta Ganeva; Assoc. Prof. Dr. Miroslava Stefanova	How to recognize some of the most common tree species in city parks? We will show you morphological features that serve as a distinction. We will introduce you to structures that are invisible to the human eye, but necessary for the survival of plants. We will give you a different perspective on the trees that surround us in everyday life.
Department of Methodology of Biology Teaching, BF (**Fig. 4d**)	"STEM EXPERIMENTS WITH PLANTS FOR THE CURIOUS ONES" Gabriela Dimitrova; Ioanna Angelova; Gabriela Yankova; Emily Markova; Victoria Alexandrova; Gabriel Goranov; Mikhail Dobrev; Krassimira Shopova; Stanislav Todorov; Iva Petrova; Margarita Eremieva; Borislav Kolev (BSc Binary specialities); Supervisor: Assoc. Prof. Dr. Kameliya Yotovska	STEM experiments and demonstrations with plants will be presented by student-to-be teachers, who are sure to enrich the knowledge about plants of young and old curious people. And maybe some future scientist and discoverer will fall in love with science in the outdoor laboratory.
Department of Plant Physiology, BF (**Fig. 6a**)	"WHAT IS ORGANIC FARMING AND IS IT PRACTICED IN OUR COUNTRY?" Dimitar Danchev (PhD student); Margarita Popova; Georgi Ivanov; Ivanka Malincheva (MSc 1y, Plant Biotechnologies); Financing: PhD project Research Fund of SU-№80-10-38/09.04.2024	Demonstration of plant crops, seeds and organic farming practices. We will tell you why organic farming is a necessary alternative for the future – for cleaner and sufficiently large production, as well as for the protection and restoration of the soil and the organisms in it.

Participating Organization	Information – Topics, Participants and Funding	Description
Department of Plant Physiology, BF (**Fig. 4e; Fig. 5b**)	"THE PATH TO HEALTH PASSES THROUGH A LABORATORY OASIS" Anna Zaharieva (MSc 1y, Plant Biotechnologies); Ana-Maria Nedelcheva; Desislava Raykova; Anton Pozumentschikov; Georgi Spakhiev; Pavel Pintev; Yordanka Bakalska; Desislava Goranova; Nadya Toleva (BSc 4y Molecular Biology) Supervisors: Assist. Prof. Dr. Desislava Mantovska; Assist. Prof. Dr. Mariya Rogova; Assoc. Prof. Dr. Zhenya Yordanova Financing: Bulgarian National Science Fund-MES: КП-06-Н56/9 12.11.2021 & КП-06-М71/1 05.12.2023; Research Fund of SU-№ 80-10-27/8.4.2024	Plant biotechnology is an important approach for preserving and studying medicinal plants, producing valuable compounds that plants, animals, and humans use for their health. We will show you an in vitro collection of Bulgarian medicinal plants and explain why and how we study them.
Department of Plant Physiology, BF (**Fig. 10**)	"DO YOU KNOW ABOUT THE PLANT TREASURY AT THE FACULTY OF BIOLOGY?" Yordan Georgiev (BSc 4y, Molecular Biology), Zlatina Zheleva (BSc 3y, Biology&Chemstry) Supervisor: biologist Aneliya Raycheva Financing: Operational Program "Research, Innovation and Digitalization for Smart Transformation", PRIDST 2021-2027, funded by EU and Bulgarian Government	The Faculty of Biology has secret gardens (greenhouses) where plants are grown, saved and propagated. These diverse species are studied or simply serve to aesthetically complement and enjoy the spaces we inhabit. Come and learn about the potential of greenhouses, where you can work yourself or buy a floral gift+surprise, made with care, skill and love.
Department of Plant Physiology, BF (**Fig. 5c & 8c**)	"THE LITTLE BIG PRODUCERS" Zornitsa Karcheva; Zhaneta Georgieva (PhD students) Financing: PhD project Research Fund of SU-№80-10-119/16.04.2024	The "Microalgae Girls" are ready to introduce guests with a smile to the endless treasury of the Earth's green "liquid gold" to tell about the incredible possibilities of microalgae. They will assure guests that in their own interesting way, algae have beauty, charm and bring hope – for new discoveries that will make the planet a better place!

Participating Organization	Information – Topics, Participants and Funding	Description
Department of Plant Physiology, BF (**Fig. 7**)	"THE HIDDEN POWERS OF PLANTS" Adriana Parisheva; Alex Hristov; Bilyana Piperkova; Borislava Stoycheva; Vasilena Dimitrova; Vasilena Stoichkova; Viktoria Marinova; Viktoria Terziyska; Virginia Spasova; Evgenia Sotirovska; Zornitsa Tileva; Iren Ilieva; Kalina Georgieva; Katerina Taraninova; Katrin Nikolova; Maria Kostova; Mila Ilieva; Monika Konstantinova; Nikolay Genov; Niya Atanasova; Rumyana Stefanova, Yordan Georgiev (BSc 4y Molecular Biology) Supervisors: Assist. Prof. Dr. Detelina Petrova; Assoc. Prof. Dr. Miroslava Zhiponova Financing: Bulgarian National Science Fund-MES КП-06-Н56/9 12.11.2021; NextGenerationEU, № SUMMIT BG-RRP-2.004-0008-C01	Students from the elective course in Plant Resistance and Phytoimmunity will present developed projects aimed at studying two interesting Bulgarian plants – catnip and mountain plantain.
Department of Plant Physiology, BF (**Fig. 5a**)	"SUPERPOWERS FROM "SUPERFOODS": WHAT ARE PLANT-BASED "SUPERFOODS"?" Kristina Stoyanova (BSc Biotechnology) Supervisor: Assist. Prof. Dr. Marieta Hristozkova	What are the scientific ideas behind the marketing term "superfoods," which are emerging as an increasingly important category of healthy eating products? Are they only associated with exotic places of origin, or can we grow them at home?
S.K.O.R.E.C., BF	NATURE CONSERVATION AND EDUCATIONAL CLUB in the Faculty of Biology, University of Sofia (https://www.facebook.com/groups/170251143796/about) Plamen Petrov, Vyara Ivanova, Yuzelim Komarevska, Petya Kairyakova, Detelina Hristova, Angelina Dandarova, Siyana Stoeva, Sofia Berkova, Anna Lukanova, Nikola Tariyski, Supervisor: Asst. Prof. Dr. Atanas Grozdanov	Student club for volunteer activities related to the study and conservation of biodiversity.

Participating Organization	Information – Topics, Participants and Funding	Description
FSC, BF (**Fig. 8a, b**)	QUIZ & PLANT WORKSHOP Yordan Georgiev (BSc 4y, Molecular Biology), Zlatina Zheleva (BSc 3y, Biology&Chemstry)	Students will organize a fun quiz and a plant workshop.
ABI	“PLANT DISEASES” Assist. Prof. Dr. Aneta Lyubenova	Visitors will receive information about diseases of plants and agricultural crops.
ABI (**Fig. 6b**)	“THE IMPORTANCE OF LEGUMES FOR SCIENCE AND THE ENVIRONMENT” Prof. Anelia Iantcheva, Assoc. Prof. Mariana Radkova Financing: 101081329 Legume Generation project	Visitors will learn about model and cultivated legumes, the process of nitrogen fixation, research related to the function of genes involved in this process, the morphology of nitrogen-fixing bacteria, and many more curious and scientific facts about legumes.
ABI (**Fig. 8b**)	CHILDREN'S CORNER Assist. Prof. Dr. Lilia Georgieva, Assist. Pavlina Vassileva	Children will be able to have fun and learn with drawing, coloring, and special activities with plants.
Institute of Ornamental and Medicinal Plants, AA (**Fig. 10**)	PLANT SALES EXHIBITION Assoc. Prof. Dr. Nadezhda Zapryanova	Presentation of variety of plant species that you can purchase.

Fig. 4. FoPD-2024 program. **a**) “Trees in the urban areas”; **b**) “Communication between plants through light”; **c**) “Plants vampires”; **d**) “Coloring chrysanthemums”; **e**) In vitro culturing and adaptation of plants.

Fig. 5. FoPD-2024 program. **a**) “Superfoods”; **b**) “Bulgarian medicinal plants in vitro collection”; **c**) “Microalgae”.

Fig. 6. FoPD-2024 program. **a**) “Organic farming”; **b**) “The importance of legumes”; **c**) “Effect of electromagnetic fields on plants”.

Fig. 7. FoPD-2024 program. “The hidden powers of plants”.

Through narratives and demonstrations fascinating facts were presented on topics such as “plants vampires”, trees in the urban areas, plants’ communication through light, and many others (**Figures 4-9**). Moreover, questions about the roles of plants in our lives, superfoods derived from plants, the concept of agriculture, and its development in our country were addressed using innovative educational methodologies. The full FoPD-2024 program, including all topics, is summarized in Table 1.

The online platform “Quizizz” proved to be an excellent tool for testing children’s and students’ understanding of the covered topics while also serving as a fun and engaging form of entertainment. The game consisted of 30 questions – some with multiple-choice answers, while others required filling in words, values, or full responses. Throughout the event, the quiz was played in six repetitions with

Fig. 8. FoPD-2024 program. Groups taking part in the quiz (**a**) and workshops (**b**). **c**) Art activities with microalgae.

Fig. 9. FoPD-2024 program. Handmade items used for quiz awards and decorations. Courtesy of Babka.handknitting https://www.facebook.com/Babka.handknitting/photos

different groups of closely aged students. Each round took around 30 minutes to be completed. The winner of each round received a prize – handmade keyholders made from organic materials and plants from the greenhouse, which brought great joy to the winners (**Figures 8a, 9**). Additionally, plant workshops and art initiatives encouraged creativity and fostered communication among the participants.

The Students Council of Sofia University "St. Kliment Ohridski" accounts for a significant portion of students' projects funding through the Diaphanum system. For the FoPD event, the project covered expenses related to planting pots, soil and decorations (**Figure 10**). The plants were planned for use in educational activities, demonstrations and as prizes for the quiz. The project was written by the students involved in organizing the event and was successfully presented and approved in its entirety. After acquiring the necessary materials, a group of students with an

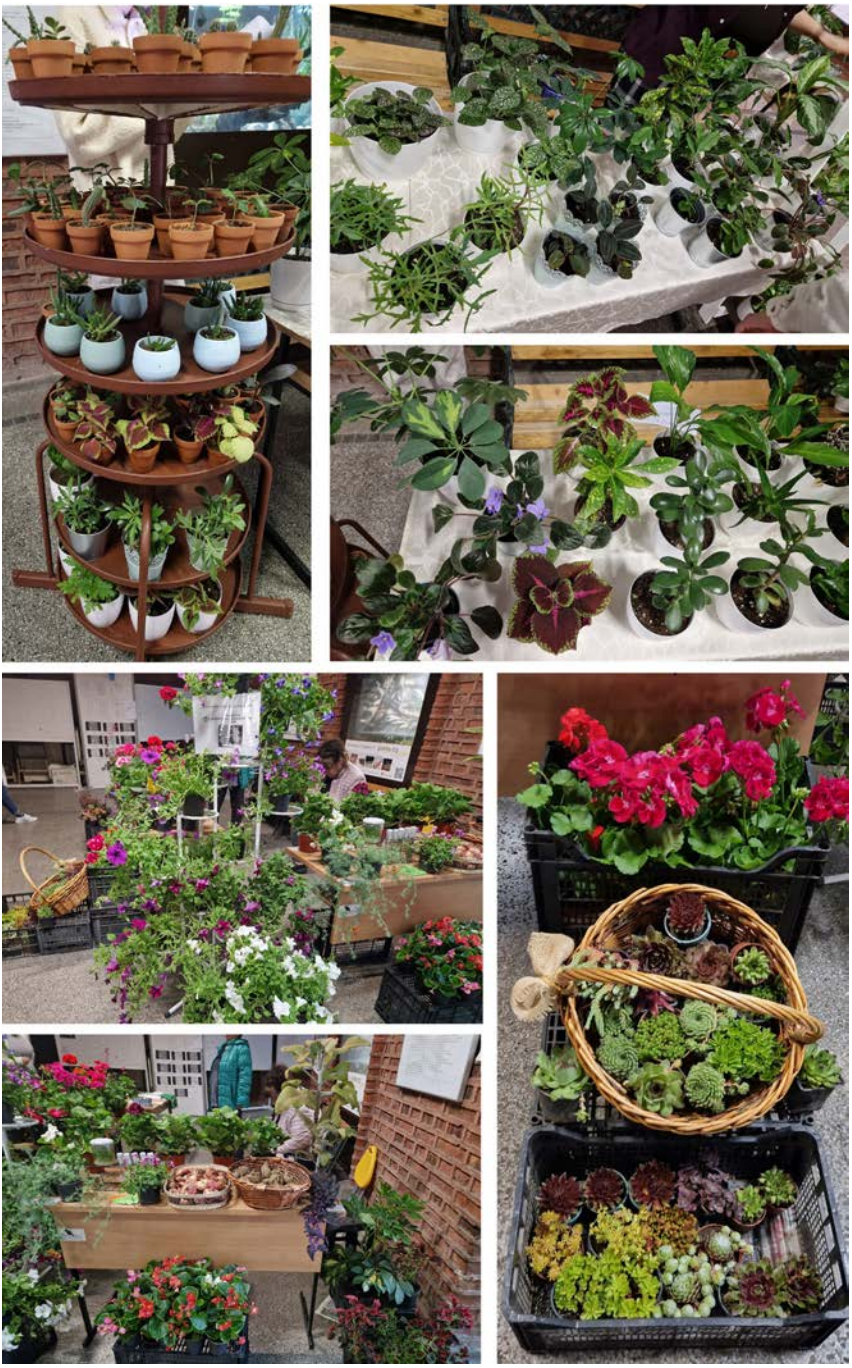

Fig. 10. FoPD-2024 program. "Plant treasures" in BF greenhouse and the Institute of Ornamental and Medicinal Plants, AA.

interest in botany and plant physiology volunteered to repot all the plants from the greenhouse. Members of the Student Council provided valuable support with documentation, deadlines, and advice on preparing reports. They also showed great interest in the event and ensured they visited on the day to see the results.

The major target groups of FoPD include students, school pupils, children within various age groups and people involved in any plant subjects with the aim of sparking their interest and educating them to respect nature. The Department of Biology Education integrated the preparation of future biology teachers with their participation in the event by creating a booth featuring demonstrations designed by students for their peers and other visitors. This exemplifies the multifaceted nature of the FoPD initiative.

Another form of student participation involved presenting their projects developed during elective courses, such as Plant Resistance and Phytoimmunity. Additionally, diploma students from various BF departments joined their supervisors to present interesting aspects of the projects they were involved in.

The event highlighted not only the beauty of plant sciences but also the process of active learning and the competence-based approach, which fosters a combination of skills, knowledge and perspectives essential for developing teaching and educational strategies throughout one's career (**Figure 11**). Students were prompted to apply teamwork, curiosity and interest in specific subjects, effectively sharing their enthusiasm and ideas with others in an impactful and inspiring way.

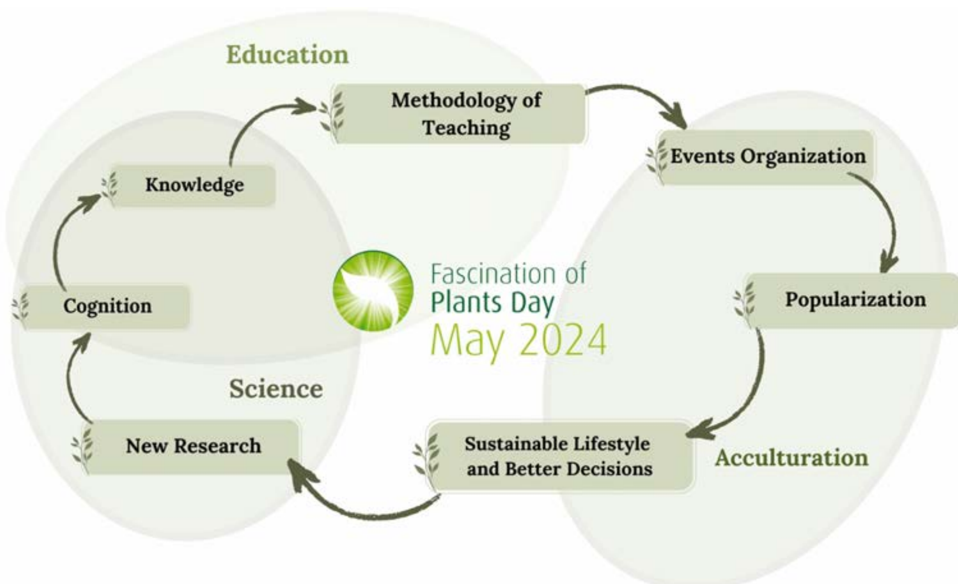

Fig. 11. Connections between transferring knowledge, methodology and events about plants.

FoPD-2024, like previous initiatives, successfully highlighted the fascination of plants among students and outside visitors. The researchers' enthusiasm was strongly supported by the volunteer spirit of interested students and the FSC, creating a unique atmosphere of learning, teaching, celebration and fulfillment. The event established a solid foundation for organizing similar initiatives in the future and brought together individuals with a shared passion for plant biology and science.

ACKNOWLEDGEMENTS

We are grateful to Student Council of Sofia University "St. Kliment Ohridski" who manage the system Diaphanum for funding of students' projects. We thank Babka.handknitting for providing gifts for the visitors. The FoPD-2024 event was supported financially by the projects funded by Sofia University, Ministry of Education and Science and European Union listed in Table 1.

CONFLICT OF INTERESTS

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

AUTHORS CONTRIBUTION

Y.Y.G., Z.D.Z. and M.K.Z. designed and wrote the manuscript, D.I.M., A-M.R.N., and A.V.I. contributed in adding information and improving the manuscript.

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Book 2 – Botany of the Annual of Sofia University is a **peer-reviewed periodical,** issued yearly in one volume, which is **published on-line with an open access and in a printed version** with two relevant IUSSNs.

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The **title** of the paper must be concise, but informative, describing the matter of the contribution as well as possible. If a Latin name of a species is used in the title, it is recommended to indicate the division, class, order, or family to which it belongs.

The **authors‘ given names** must be spelled in full, while a middle name should be abbreviated: full first name(s), middle initials and surname(s). The authors‘ **address(es)** should be stated on the first page of the paper below the title. The addresses should be as complete as possible (affiliation, street, postal code, town, country). In case of authors from different affiliations, a number (superscript) should be put in the end of the authors name and the same number with a normal font size should be placed before the address. The postal adress and the email of the corresponding author should be indicated as a footnote on the first page.

<u>Example</u>:

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The proper paper text must be preceeded by an English summary **(„Abstract“**), which should express the important new results precisely and should be limited to 300 words. Please, remember that the abstract will be seen and used by many more people than the full paper will! Subsequently up to 6 **key words** (or key word combinations) suitable for information-retrieval system are to be listed (in alphabetical order). The key words should not repeat those, which already are mentioned in the title. The disposition of the paper sections should be in agreement with common use. The „**Introduction**“ should outline the essential background for the work and the reasons why it was undertaken. It should clearly explain the purpose of the work and its relations to other studies in this field. Before the material and method description, optionally, due to author‘ decission, a description of the **studied site/s** could be included. Descriptions of **materials and methods** should provide sufficient information to permit repetition of the experimental work. This includes proper documentation of the sources of cultures, plants and fungi used in the work. Authors should consider depositing voucher nmaterial in an internationally reputable museum, collection or herbarium and the relevant numbers or codes should be provided in the text. All new gene or protein seguences should be submitted to major databases (DDBJ, EMB, GenBank) before the submission of manuscripts and the accession codes should be indicated in the manuscript. The geographic names should be <u>transliterated</u> from the common geographic names used in the certain country (*e.g*. Rodopi Mts instead of Rhodopes). The proper Bulgarian legislative documents for translation and transliteration are cited at the end of this Instruction. The origin of the material investigated, methods of preparation and the herbaria and collections in which the vouchers are deposited, should be indicated completely. In case of work with threatened species and protected areas it is recommended to provide the permission data. For the metric measurements **SI-units** are requested. They shouls not be followed by ful stops and slashes have to be replaced by minus index (*e.g.* mg l^{-1} should be used instead of mg/l). Please, use % instead of *per cent*.

Not commonly used abbreviations should be explained at the end of the chapter „**Material and methods**". The „**Results**" preferably have to be separated from the „**Discussion**". The discussion should deal with the interpretation of the results, not only recapitulate them. It should evaluate the results in relation to the reasons why the study was undertaken, place the results in the context of the other work, and point out their significance.

The beginning of a paragraph should be indicated by indenting the first line.

The scientific names of the taxa (genera, species and lower ones) must be quoted completely, denominating the name of the genus, species epitheton (if necessary subspecies, cultivar etc.) and the author, when mentioned for the first time in the text. Full scientific names, as a rule, should be mentioned in the summary also. The author names in the scientific names should not be formatted. The classification system used is up to the authors, but in case of different from commonly approached, should be properly indicated.

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Journals:

Ivanov I. P. 2013. Photosynthetic CO2-fixation pathways. – Ann. Rev. Plant Physiol. 21 (2): 141–263.

Ivanov I. P. & Petrov P. I. 2013. Photosynthetic CO2-fixation pathways. – Ann. Rev. Plant Physiol. 21 (2): 141–263.

Ivanov I. P., Petrov P. I. & Dimitrov V. N. 2013. Photosynthetic CO2-fixation pathways. – Ann. Rev. Plant Physiol. 21 (2): 141–263.

Alternatively, we accept full text citations of journal titles. However, the reference list must be consistent in this regard.

Books:

Dimitrov D. G. & Ivanov A. N. 2017. Biodiveristy of the seashores of Bulgaria. Springer, Heidelberg, 405 pp.

Ivanov W. H., Stoyanov H. M. & Petrov F. B. (Eds) 2000. Water ecosystems. Elsevier, New York, 265 pp.

Book chapters:

Petrov F. K. 2000. Grazing in water ecosystems. – In: Ivanov W. J., Stoyanov H. P. & Petrov F. B. (Eds), Water ecosystems, Elsevier, New York, 59–105.

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Petrov F. K. 2000. *Padina pavonica*. – In: Ivanov W. J., Stoyanov H. P. & Petrov F. B. (Eds), Water ecosystems, Elsevier, New York, p. 49.

Conference papers (or abstracts if they provide essential information):

Bogdanov D. M. 2017. Danube Delta. - In: Somov N. P. & Karakudis F. E. (Eds), Proceedings of the First European Symposium *Conservation and management of biodiversity in the European seashores*, Melnik, Bulgaria, 8-12 May 2017, 36-46.

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Or, alternatively, depending on the order of date and place in the original title of the Proceedings/Abstract books:

Bogdan D. M. 2017. Biosphere reserves and special legislation for environmental protection. - In: Venev N. (Ed-in-Chief), Book of Abstracts First European

Symposium *Conservation and management of biodiversity in the European seashores*, 8-12 May 2017, Primorsko, Bulgaria, p. 36.

Electronic publications should be cited with their author or title in the references with indication of the date of retrievement or of the last access of their full web address:

Geneva M. M. 2011. *Cortinarius caperatus*. – In: Penev D. (Ed.), Red Data Book of the Republic of Bulgaria. Vol. 1. Fungi. Retrieved from http://eclab. bas.bg/rdb/en/vol1/ on 14.11.2014.

Index Fungorum. Retrieved from http://www.indexfungorum.org/Names/Names.asp on 19.11.2017.

Or, alternatively

Index Fungorum. http://www.indexfungorum.org/Names/Names.asp (Last accessed on 19.11.2017).

In special cases, as an exception, the websites of electronic publications could be placed in the text.

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Journal:

Petkov N. H. 1915. La flore algologique du mont Pirin-planina. - Sbornik na Bulgarskata Akademiya na Naukite 20: 1–128 (In Bulgarian).

Petkov N. H. 1915. La flore algologique du mont Pirin-planina. - Sbornik na Bulgarskata Akademiya na Naukite 20: 1–128 (In Bulgarian, French and Russian summ.).

Book:

Valkanov D. E., Draganova P. M. & Tsvetkova B. B. 1978. Flora of Bulgaria. Algae. Izd. Narodna Prosveta, Sofia, 642 pp. (In Bulgarian)

Valkanov D. E., Draganova P. M. & Tsvetkova B. B. 1978. Flora of Bulgaria. Algae. Izd. Narodna Prosveta, Sofia, 642 pp. (In Bulgarian, English summ.)

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Footnotes should be avoided.

Transliteration should follow the Bulgarian legislative documents (State Gazette 19/13.03.2009, 77/01.10.2010, 77/09.10.2012, 68/02.08.2013 - http:// lex. bg/en/laws/ldoc/2135623667). **The geographic names should be fully transliterated** except the cases of titles of published works.

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