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# UNIVERSITY BOTANIC GARDENS – A HISTORICAL OVERVIEW

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**Abstract.** The Botanic Garden of the Sofia University „Saint Kliment Ohridski“ in Sofia, already boasts a 130-year history. The article provides a brief overview of the founding and development of the University Botanic Gardens and its scientific, educational, cultural, and social significance.

**Keywords:** botanic garden, Sofia University, Varna, Balchik

Botanic gardens appeared in Europe as gardens with medicinal plants. The oldest existing botanic garden is the Botanic Garden of Padua, in Italy. It was created in 1545 for growing medicinal plants to be used for the training of medical students. Botanic gardens were founded also in Bologna in 1568, Valencia in 1567, and in Northern and Western Europe - Montpellier in 1593, Leiden in 1587, Leipzig in 1597, Oxford in 1621, Paris in 1635, Berlin in 1646, in Amsterdam 1682, Vienna in 1754 etc. (Stern 1971, Kiehn 2008, Spencer & Cross 2017). During the Renaissance, the botanic gardens in Europe became officially accepted as institutions, often belonging to universities. Many of them maintain herbaria used for research work. Gradually, botanic gardens started to include plants without medicinal properties but considered interesting, beautiful, or exotic. There are now

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more than 400 botanic gardens in The European Union (Cheney et. al. 2000).

According to BGCI (Botanical Garden Conservation International), botanic gardens are institutions holding documented collections of living plants for the purpose of scientific research, conservation, display, and education (https://www.bgci.org/about/botanic-gardens-and-plant-conservation/).

The Botanic Gardens of the Sofia University „St. Kliment Ohridski“, represent three gardens in three locations – the cities of Sofia and Varna and the town Balchik. They were established at different time and each garden has its unique development and characteristics. The University Botanic Garden in Sofia already boasts a 130-year history.

In October 1888, the oldest university in Bulgaria was founded – the Sofia University "St. Kliment Ohridski". Open as a Higher Course of Pedagogy, after several months was renamed the Higher School, which was housed in the building of today's Faculty of Journalism and Mass Communications. The first three faculties were the faculty of History and Philology, the Faculty of Physics and Mathematics, and the Legal faculty. Botanical Institute at the Faculty of Physics and Mathematics was founded in 1891. The head of the institute was the first Bulgarian professor in botany Stefan Georgiev (1859 – 1900). The main direction of research work at the institute was floristics – Stefan Georgiev studies mainly the higher flora of the country, his assistant Stefan Petkov studies the green algae, and Sava Kazandzhiev studies lichens. Only a year after the foundation of the Botanical Institute (in 1892), under the leadership of Prof. Georgiev, the Sofia Botanic Garden has been established (Stanev 2010; Kitanov & Chakarov 2017).

In the first years after the establishment of the Botanic Garden, Prof. Georgiev did all the work himself. Whenever wasn't lecturing, he spent that time collecting live plants from nature for the garden and preparing plants for the herbarium. To Prof. Georgiev, the Botanic Garden and the herbarium were some of the most important links of the Botanical Institute, closely related to the teaching of botany and research work. A hothouse was built in the garden for which he ordered from Erfurt in Germany the delivery of orchids, cacti, poinsettias, and other exotic plants. On January 1, 1896, the gardener's position at the School of High Education was taken by Karl Neff, son of the Swiss gardener Daniel Neff and with his help, the University Botanic Garden was completed. Prof Georgiev made great efforts to expand the botanic garden by joining the abandoned municipal park at the Doctor monument in Sofia. His idea was to build an arboretum in this place with different native and foreign plant species. Due to some problems in maintenance after Georgiev's death, the management of the Botanic garden at the Doctor monument was handed back to the Municipality of Sofia (Stanev 2017; Kitanov & Chakarov 2017).

At various times the Botanic Garden was under the direction of famous botanists as academician Prof. Nikolai Stojanov (1883 - 1968), who was also head of the Department of Special Botany (later renamed as Plant Systematics and

Phytogeography) (1936 – 1951) at the Faculty of Physics and Mathematics at the University of Sofia and academician Prof. Daki Jordanov (1893 – 1978), who was Head of the Department of Plant Systematics and Plant Geography, Head of the Faculty of Physics and Mathematics (1947 - 1950), and Rector of the University of Sofia (1956 – 1962). Later, Prof. Boris Kitanov (1912 - 1996) who was Head of the Department of Plant Systematics and Phytogeography at the Biological Faculty from 1965 - 1973 (Stanev 1993, Stanev 2008).

In 1955, by order of the Minister of Culture and Education, the University Botanic garden in the town of Balchik was founded, under the guidance of Prof. D. Jordanov. The design of the garden is done with the participation of the garden designer Nicola Minchev and the first technical park manager Nikola Momchilov (Pavlov 2002). Prof. D. Jordanov was director of the University Botanic garden in Balchik until 1978.

Until 1977 the Botanic Garden in Sofia was under the management of the garden designer Nicola Minchev. In 1977 the specialist biologist Spas Popov was appointed as manager of the garden, under the direction of Prof. Elissaveta Bozhilova from the Department of Botany. During these years, an exchange of seeds has already taken place both with the Botanic Garden in Balchik and with Botanical Gardens from all over the world. According to S. Popov, seeds from about 300 plant species were exchanged annually, thus the plant collections were enriched (personal conversation).

In 1977 the University Botanic Garden "Eco-Park" in the city of Varna was established. Therefore, the University Botanic Gardens become three in different cities – Sofia, Varna, and the town of Balchik.

In 1996 the botanist Dr. Krasimir Kosev, graduate of the Royal Botanic gardens - Kew and currently a member of the European Consortium of Botanic gardens, was appointed as a Director of the Botanic Gardens of the University of Sofia. Under his leadership in the last 26 years, with a lot of work and perseverance, the Botanic Gardens acquire its modern vision.

Today the University Botanic Garden in Balchik covers an area of approximately 195 decares, of which: 61 decares of architectural and park complex, a garden of 7 decares, farm buildings, and an 800$^{th}$ square meters greenhouse. The garden is divided into three different parts, historically and functionally:

A landscape park already shaped at the time of Queen Marie of Romania is now a monument of garden and park art.

A garden, spreading over an area of 0.7 ha, shows visitors the beauty of seasonal flower compositions (**Fig. 1**), alpine spots, water areas, cacti, and succulents in typical stone beds cut in Balchik rocks.

Protected area: In 2005 the territory of the Botanic Garden was declared Protected Area.

The garden is specialized in growing collections of tropical and subtropical exotic plants. It is famous for its collection of succulents and cacti - around 4000

**Fig. 1.** Seasonal flower compositions - University Botanic Garden in Balchik.

**Fig. 2.** Rosary - University Botanic Garden “Eco - Park”, Varna.

species. The total number of plant species in all collections is more than 4900. It is a national center for rare and endangered species under the Washington Convention and holder of the Order of Cyril and Methodius II degree for merits in science and education.

University Botanic Garden "Eco-Park" Varna is the first ecological park in the country, which combines artificial and natural ecological systems. The park is situated in an area of 360 decares. The plant collections include more than 300 species of exotic trees and shrubs, and the herbaceous plants species are more than 100 (**Fig. 2**).

The University Botanic Ggarden in Sofia on Moskovska str.№49 is the smallest and up till now it is located at its original site in the center of Sofia. It covers 5 decares divided into a park, a rosary and greenhouses (**Fig. 3**). One of the attractions of the park area is the old oak (*Quercus robur* L.) which rises in the center of the garden and is believed to be 130 years old. According to some authors, at the opening ceremony of the University Botanic garden (1982) a tree has been planted, and in its roots the Bulgarian king Ferdinand put a golden coin (Kitanov & Chakarov 2017). However, according to stories by the garden designer N. Minchev, the oak was in this place before the opening of the Botanic garden and is still older. Tsavkov & Zafirov (2016) and Ivanova (2020) provide data on an important event – the 1000th anniversary of the St. Methodius death, that took place in Sofia, in the yard of the Classical High school (today the yard of the Botanic Garden) in April 1885. Twelve young trees were then planted at a special ceremony.

To determine the age of the oak, a dendrochronological study was performed. In the winter of 2015/2016, two samples were taken with a Pressler's borer, which was processed by a special method. The number of annual growth rings was counted. According to this study, the age of the tree is set at about 140 years (Tzavkov & Zafirov 2016).

In the park rock gardens, a water pond with lilies, a wetland with moisture-loving plants, and a perennial garden are separated. The perennial garden covers an area of 350 m². The display of collections perennial herbaceous plants has been accomplished based on systematic principles. In the park area of the Botanic Garden, visitors can see remarkable tree species such as *Metasequoia glyptostroboides* Hu & W.C.Cheng, *Ginkgo biloba* L., *Eucommia ulmoides* Oliver and many others. The two trees of *M. glyptostroboides* have been grown from seeds brought by Acad. N. Stoyanov from his expedition to China in 1947 (Shopova 2019). *M. glyptostroboides* is the only species in the genus *Metasequoia*, which belongs to the Cupressaceae family. The species is a relict from the Mesozoic era.

The plants in the greenhouses are mainly cultivated for scientific and educational purposes and include well-documented collections of subtropical, tropical species and succulents. In the tall central greenhouse (**Fig. 4**) grow different Gymnosperms - species of the genus *Araucaria*, *Cycas*, *Zamia*, *Dioon*, as well as different species of palms, including *Livistona chinensis* (Jacq.) R.Br. ex Mart. and high variety of

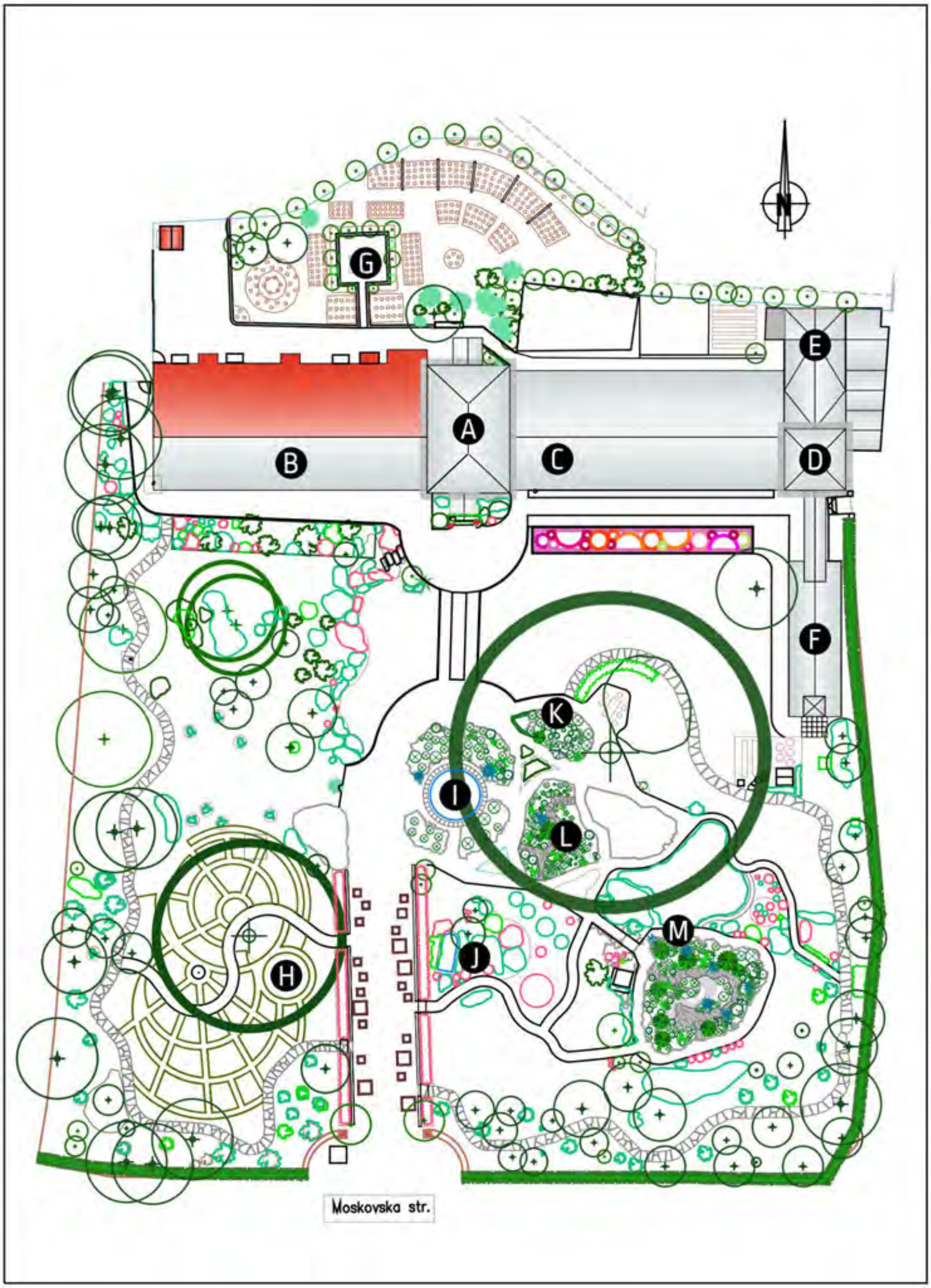


**Fig. 3.** Map of the University Botanic Garden in Sofia. **Legend:** A, B, C, D, E, F – Greenhouses; G – Rosary garden; H – Perennial garden; I - Pond with lilies; J - Wetland with moisture-loving plants; K, L, M - Rock gardens.

**Fig. 4.** The tall central greenhouse - University Botanic Garden in Sofia.

tropical plant species - *Strelitzia nikolai* Regel & K.Koch, *Monstera deliciosa* Liebm, *Musa mannii* H.Wendl. ex Baker etc. In the greenhouse "Humid tropic conditions" the visitors can see epiphytes such as orchids, bromeliads, and ferns. There is also a greenhouse with succulents and a tall greenhouse with large size species of Cactaceae and Euphorbiaceae family, as well as a greenhouse with Mediterranean plant species. The University Botanic Garden in Sofia keeps and preserves more than 1500 plant species.

In recent years, a park place called Pharmacy Garden has been developed, where visitors and students can get acquainted with various medicinal plants and learn more about their importance for medical use.

University Botanic Gardens are members of the World Botanical Gardens Council (BGCI), the European Botanic Gardens Consortium (EBGC), and the Botanical Gardens Environmental Education Network (EBGEN) and participate with their collections in the exchange of Index Seminum seeds with botanical gardens from the whole world.

University Botanic Gardens play an important role in promoting and protecting plant biodiversity. The collections of the gardens are an important base for research and education in plant sciences.

## CONFLICT OF INTERESTS

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

## References


Cheney J., Navarro J. N. & Jackson P. W. (Eds.) 2000. Action Plan for Botanic Gardens in the European Union. CIP Royal Library Albert I, Brussel, 68 pp.

Ivanova I. 2020. The 1000th anniversary of the death of St. Methodius is celebrated with three-day celebrations in 1885. https://glasove.com/izgubenata-bylgariya/1000-godishninata-ot-smyrtta-na-sv-metodij-se-otbelqzva-s-tridnevni-tyrjestva-prez-1885-g on 10 Sep 2020.

Kiehn M. 2008. The Botanical Garden of the University of Vienna. - University Museum and Collections Journal 1: 91-93.

Kitanov B. & Chakarov V. 2017. University botanic gardens – 125 years later. - Silva Balcanica 18 (2): 53-54.

Pavlov D. V. 2002. Guidebook of the University Botanic Garden Balchik. Sofia University, Sofia 16 pp.

Shopova Y. 2019. The Metasequoia – a live relict from the Mesozoic Era. – Leaf 7: 4-7.

Spencer R. & Cross R. 2017. The origins of botanic gardens and their relation to plant science, with special reference to horticultural botany and cultivated plant taxonomy. – Muelleria 35: 43-93.

Stanev S. 1993. Daki Jordanov (1. 9. 1893 – 5. 4. 1978). – In: Stars extinguished in the mountains. Publishing house of the Bulgarian Academy of Sciences, Sofia, 113-132 (in Bulgarian).

Stanev S. 2008. Founders of Bulgarian Botany. University Press “Paisii Hilendarski”, Plovdiv, 276 pp. (in Bulgarian).

Stanev S. 2010. The history of botany in Bulgaria (till 1944). University Press “Paisii Hilendarski”, Plovdiv, 701 pp. (in Bulgarian).

Stanev S. 2017. Prof. Dr. Stefan Georgiev the founder of the Bulgarian Academic botany and the University Botanical Garden. – Leaf 4: 4-7.

Stearn W.T. 1971. Sources of information about botanic gardens and herbaria. - Biological Journal of the Linnean Society 3: 225-233.

Tsavkov M. & Zafirov N. 2016. The English Oak at the University Botanic Garden “talks”. – Leaf 2: 5-9.



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# REVIEW ON BIOLOGICAL AND BIOTECHNOLOGICAL CHARACTERISTICS OF THE TERRESTRIAL ORCHID *LUDISIA DISCOLOR*

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**Abstract.** The present paper presents a review of the distribution, taxonomy, ecology, biochemical properties and usage of the orchid *Ludisia discolor*.

**Keywords:** DNA barcoding, jewel orchid, micropropagation, mycorrhiza, photosynthesis, phytochemical potential

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## INTRODUCTION

*Ludisia discolor* (Ker-Gawl.) A. Rich. is a species of terrestrial orchid from subfamily Orchidoideae originating in parts of the Asian continent but commonly grown as an ornamental plant in many countries outside of its natural range. Similarly to many of its closest relatives, it is often described as a jewel orchid, a nickname applied due to the characteristic coloration of its leaves which give the plant its attractive appearance. Taxonomic relationships among the members of the jewel orchid group have been established through molecular approaches, such as DNA barcoding serving as a valuable tool for quick species identification of morphologically similar individuals. The overall morphology of *L. discolor* is a product of the orchid's natural environment which involves shady and forested habitats with reduced levels of sunlight. Hence, the plant requires special adaptations for better efficiency of the photosynthetic process. As a member of the orchid family, it forms mycorrhizal associations with different species of fungi, many of which remain largely undiscovered so far. Another important feature of *L. discolor* is that it can produce a large variety of phytochemicals, especially plant glycosides such as kinsenoside, goodyeroside and gastrodin among others. Many of these compounds have been studied for their potential pharmacological activity both in vitro and in vivo thus giving a promising perspective for the development of new sets of drugs targeting various medical conditions. This finding sparks an interest in the plant's secondary metabolism since many of the biochemical processes responsible to produce the orchid's bioactive chemicals have not been completely elucidated yet. These potential applications as well as the demands of the floral industry stimulate the research in the mass propagation of *L. discolor*. Vegetative reproduction is achieved relatively easy and explant cultures involving sterile conditions and in vitro techniques have been successfully established.

## RESULTS

### Distribution – natural and by trade

*Ludisia discolor* (Ker-Gawl.) A. Rich. is distributed in southeastern Asia, as its native geographical range spans countries in the mainland such as southern China, northeastern India, Thailand, Vietnam, Laos and Myanmar but also extends into various islands off the shore which includes territories of Indonesia, Malaysia and the Philippines (Kew Science 2020) (**Fig. 1**). These regions are characterized by relatively stable warm climates with high levels of air moisture. *L. discolor* is often found growing in swampy habitats or in the understory of tropical and subtropical forests where the degree of illumination is relatively low (Averyanov et al. 2003).

Even though the orchid is widespread in its native geographical range and is common in cultivation, some of its populations have been decreasing. This is mostly due to habitat loss as well as to beliefs in folk medicine which attribute various

**Fig. 1.** Map of the natural distribution of *L. discolor*.

medicinal properties to the plant. As a result, uncontrolled gathering of specimens from their natural environment and trade of the plant have been often (Teoh 2016). *L. discolor* is commonly grown as an ornamental plant in many countries outside of its natural range (**Fig. 2**). *L. discolor* is the principal orchid species accounting for 45% of live plant trade mostly exported by Amazon countries and imported by the Netherlands (Sinovas et al. 2017).

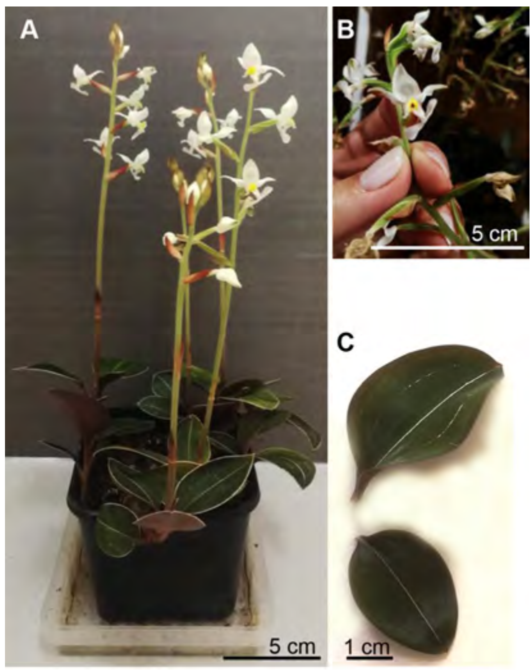


**Fig. 2.** *L. discolor* plant as a house plant: **a** – plant view; **b** – inflorescence; **c** – young leaves. Scale bars are shown.

### Taxonomic affiliation

*Ludisia* is generally considered a monotypic genus with *L. discolor* being the only known member (Chen et al. 2019). *Ludisia* belongs to the Orchidoideae subfamily of orchids which spans most of the terrestrial members of Orchidaceae. It is placed in Cranichideae tribe, which is overall considered monophyletic and further classified in the subtribe of Goodyerinae (Chase et al. 2015; Chen et al. 2019). Goodyerinae contains about 35 genera with many of the species colloquially referred to as "jewel orchids" for reasons like those for *Ludisia*, namely their variegated foliage, which provides them with a high ornamental value (Glicenstein 2009; Hayden 2016). Jewel orchids are universally terrestrial plants. Most of them are photosynthetic but some Goodyerinae display myco-heterotrophic character. They usually produce white or yellowish flowers that emerge centrally from the plant's stem, which is attached to a well-developed underground rhizome. Leaves are often, albeit not always, variegated in coloration. Many of the species are common in cultivation, including members of *Anoectochilus*, *Macodes* and *Dossinia* among others.

**Taxonomic affiliation via DNA barcoding**

*L. discolor* is very close morphologically to other Orchidaceae species, such as genera *Anoectochilus* and *Goodyera*. Therefore, molecular approach such as chloroplast genome comparison and DNA barcoding is applied for rapid and reliable taxonomic determination (Chen et al. 2019; Yu et al. 2019). The chloroplast genome of *L. discolor* is fully sequenced and it is characterized by the presence of a large signal-copy section of 82 922 base pairs and a small single-copy section of 26 572 base pairs (Yu et al. 2019). Such regions are very common in the genome of flowering plants and can be involved in a wide variety of functions (Han et al. 2014). They are often used to infer various phylogenetic relationships and to monitor key evolutionary events in the history of a taxonomic group or species in plants. In addition, the chloroplast genome of *L. discolor* includes another 132 genes, of which a total of 86 are protein-encoding. Thirty-eight genes are responsible for tRNA synthesis and eight genes for rRNA synthesis. Analysis and comparison of chloroplast genomes between individual plant populations show that the number and type of genes are highly conserved, but the length may vary. Accordingly, the genealogy of a given set of populations could be established depending on the length (Yu et al. 2019).

Plastid genes can be used as markers both for species identification and establishment of phylogenetic relationships through the marker methodological method DNA barcoding. The method relies on the amplification of short DNA fragments of genes whose genetic variation reflects the evolutionary history of plants. The set of such genes include *matK* (maturase K, tRNA-Lys), *psaB* (reaction centre protein of PSII), *rbcL* (ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit), *trnL*, *trnL-F* (*trnL-F* region composed of trnL intron and *trnL-F* intergenic spacer), and nuclear are: *ITS* (internal transcribed spacer 1 and 2 in rRNA) and *Xdh* (xanthine dehydrogenase gene; Chen & Shiau 2015; Chen et al. 2019). The goal of DNA barcoding is to create a shared source of DNA sequences that can be used to identify and determine taxonomic affiliation (Hollingsworth et al. 2011). Barcode of Life Data System (BOLD) is an information platform and repository that helps to acquire, store, and analyze DNA barcodes with open-public access (Ratnasingham & Hebert 2007).

To demonstrate the representation of available *in silico* data into individual phylogenetic trees for *L. discolor* we used the BOLD data base (**Fig. 3**). The DNA barcode sequences were pulled out from BOLD by search for the name of the plant species. The resulting list of sequenced *L. discolor* marker genes included information about two chloroplast and one nuclear DNA barcoding markers – *matK*, *rbcL* and *ITS*, respectively. The phylogenetic tree based on the *matK* marker (**Fig. 3a**) distinguished *Ludisia* as a sister genus of *Anoectochillus*, which is consistent with the ecological and morphological similarities between the two genera. The genus *Goodyera*, in turn, forms its own branch, and genus *Rhomboda* appears as a sister branch to *Ludisia*. In comparison with *rbcL* (**Fig. 3b**) and *ITS* (**Fig. 3c**), *matK* showed highest resolution. Combinations of the markers did not provide precise phylogenetic information (data not shown).

**Photosynthetic adaptations in *L. discolor* and *Anoectochillus* sp.**

*L. discolor* and other jewel orchids are commonly characterized by an insufficient access to sunlight in their natural habitat. The uppermost layer of a forest usually receives between 1000-2500 μmol $m^{-2}$ $s^{-1}$ photosynthetically active radiation, while only about 5 μmol $m^{-2}$ $s^{-1}$ reach the understory (Bolhàr-Nordenkampf et al. 2013). Due to that, species ecologically described as shade plants like *L. discolor*, *Anoectochillus* as well as many other *Goodyerinae* sp., have developed a set of photosynthetic adaptation traits such as distinct leaf structure, pigmentation and cytological features that help them to overcome these challenges. Sectioning coupled with microscopic analyses indicates that leaves of *L. discolor* and *Anoectochillus* sp. possess structural organization which is unique and unusual for monocotyledonous plant species (Poobathy et al. 2018). It is characterized by the presence of a dorsiventral arrangement of photosynthetic parenchyma differentiated into two layers, one of which is composed of spongy, while the other of palisade mesophyll (**Fig. 4**). Experiments with different types of light resulted in different fluorescent profiles of the two layers hinting at differences in pigmentation. Subsequent cytological observations as well as chromatographic analyses provide more information about the distribution and chemical nature of these pigments. The spongy mesophyll fluoresces in bright red when exposed to blue, green and ultraviolet wavelengths, which is considered as an indication for the presence of anthocyanins that are well known for their photoprotective role in plants. The vacuoles of both *L. discolor* and *Anoectochillus* sp. were discovered to possess large amounts of anthocyanins and fill the entire cellular space of the cells in the abaxial leaf layer. Chloroplasts are present irrespective of the anthocyanin content of the cells and demonstrate strong fluorescence under blue and ultraviolet light. The anthocyanins seem responsible only for the fluorescence under green light, since UV and blue light exposure results in a very dim glow. The cells that contain these pigments are spherical in shape and usually located right below the palisade layer. Furthermore, cyanidin was discovered as a major pigment in methanol extracts of both jewel

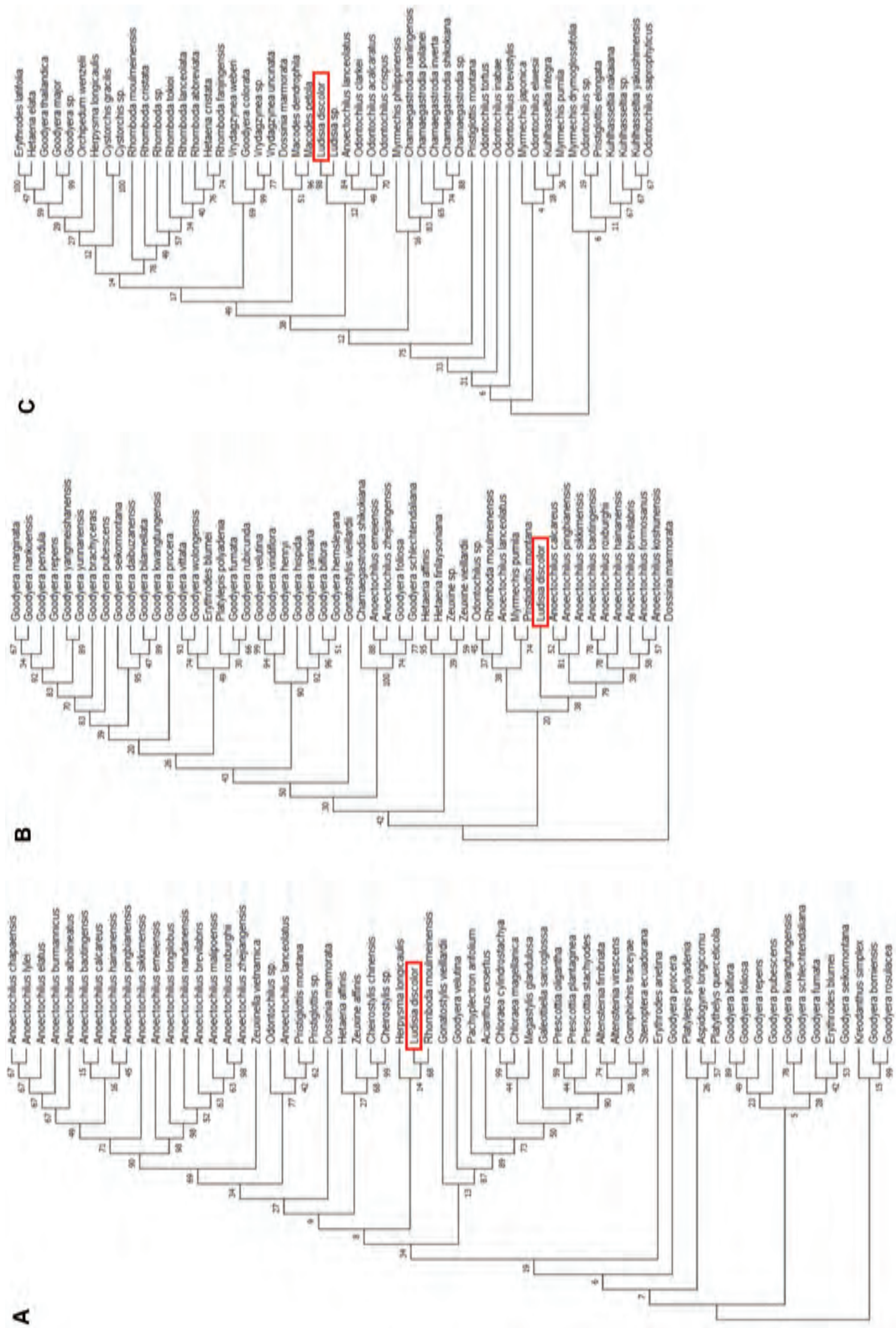
A
B
C

**Fig. 3.** Phylogenetic trees based on DNA barcoding markers. **A** – *matK* plastid gene. **B** – *rbcL* plastid gene. **C** – *ITS2* nuclear intergenic spacer. The sequences were pooled out from the BOLD database. The BOLD identification code and the one linked to the GenBank of NCBI are as follows: *matK* gene GBVR1719-13/AJ543911.1; *rbcL* gene GBVB3936-11/AJ542395.1; *ITS2* gene ITSAB3571-14/AJ539483.1. Each gene sequence is compared in the BLAST database to determine available proximity sequences. The obtained alignment of the *L. discolor* gene sequence with the 100 closest sequences available in the database (deposited by different experimental groups) was downloaded in FASTA format for subsequent phylogenetic analysis. To determine the evolutionary interspecific relationships, evolutionary history is traced by applying the Neighbor-Joining method (Saitou & Nei 1987). A "bootstrap" phylogenetic tree was constructed (Felsenstein 1985), in which the branches correspond to clusters of similar species, and the percentage of similarity (based on a bootstrap test with 500 copies and similarity over 50%) is shown by number in the branches. Evolution distances were calculated using the Maximum Composite Likelihood method (Tamura et al. 2004) and are in units of the number of major field substitutions. The evolutionary analyzes were performed with the MEGA X program (Kumar et al. 2018).

orchids but chromatographic analysis indicated that the mixture is complex and contains other related compounds such as procyanidin and pro-anthocyanidin, which serve as precursors in the biochemical processes leading to cyanidin.

Studies have also been conducted to establish the link between the photosyn-thetic processes in *Anoectochillus* and its metabolism, in particular its ability to produce various flavonoids that have potential antioxidant activity (Ma et al. 2010). Light as a factor on the ability of orchids to inhibit various oxidative processes has been studied in *Phalaenopsis* (Ali et al. 2005). The data show that higher intensity increases superoxide dismutase levels in in vitro propagated individuals. *Anoectohillus*, on the other hand, it is a typical shade-loving plant, suitable for growing at photosynthetic photon flux (PPF) values in the range of 30 to 50 µmol $m^{-2}$ $s^{-1}$. The saturation point is at about 60 µmol $m^{-2}$ $s^{-1}$. Values of 90 µmol $m^{-2}$ $s^{-1}$ caused stress, inhibited the natural course of photosynthesis through loss of activity in Photosystem II (PSII) and led to a decrease in the concentration of flavonoids. There was also a significant

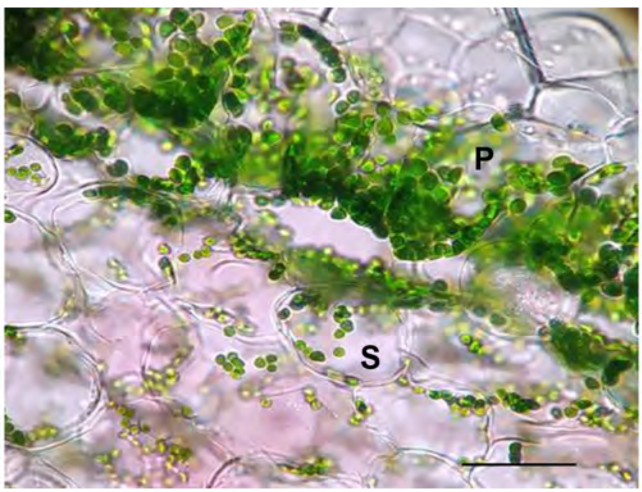


**Fig. 4.** Leaf lamina cross-section of *L. discolor*. Photosynthetic parenchyma differentiated into two layers: palisade (P) and spongy (S) mesophyll. Scale bar 50 µm. (section is made by V.A.H.)

decrease in the concentration of chlorophyll, while its highest levels are at PPF values of 10 μmol $m^{-2}$ $s^{-1}$. In the range of increase from 10 to 60 μmol $m^{-2}$ $s^{-1}$ there was an increase in the ratio between chlorophyll *a* and chlorophyll *b* and a decrease in the size of the light-gathering complex of PSII. This was accompanied by an increase in the concentration of flavonoids which can be explained by the fact that a greater light intensity increases oxidative stress, and the plant changes its biochemical parameters to prevent the negative impact. However, very high levels of illumination led to a sudden drop in flavonoid levels. Research sug-gests that light could be successfully used as a factor by which the biosynthesis of various biologically active substances can be purposefully regulated.

**Mycorrhizal interactions**

Mycorrhizal associations represent a widespread form of symbiotic interaction in plants and recent research reveals that they can be formed by nearly 90% of all land species (Soudzilovskaia et al. 2020). For orchids, these associations are not only universal but quintessential in the early stages since the lack of endosperm in the miniature seed requires the presence of a fungal partner to achieve successful protocorm development (Rasmussen 2002). Some orchids, ecologically belonging to the group of myco-heterotrophic plants, spend their entire life in an obligatory symbiosis with fungi. Such species are described within the jewel orchid group, with the genus *Chamaegastrodia* as a major example. Analysis of *L. discolor* specimens collected from various locations in Thailand resulted in the isolation of *Ceratorhiza goodyerea-repentis* (Athipunyakom et al. 2004). As the name implies, this species is associated with a temperate relative of *L. discolor*, namely *Goodyera repens*. *Ceratorhiza goodyerea-repentis* was also isolated from another species of *Goodyera*, namely *G. procera*, which also demonstrates the presence of *C. cerealis*. However, genetic analyses of fungi from the related *G. pubescens*, which grows in North America, identifies species from the genus *Tulasnella* as the only symbiont that is present in this orchid (McCormick et al. 2004). Studies on the genetic identification of fungal isolates from *L. discolor* and different *Anoectochillus* species collected from China have provided intriguing data regarding their effect on *A. roxburghii* (Ye et al. 2020). A total of 277 different strains have been successfully isolated that belonged to a diverse set of genera such as *Gliomastix*, *Bjerkandera*, *Auricularia*, *Helminthosporium*, *Colletotrichum*, *Acremonium*, *Bionectria*, *Fusarium*, *Hypoxylon*, Xylariaceae, *Diaporthe*, *Phomopsis* and *Chaetomium*. A growth promoting and biomass increasing effect was observed for those isolates when co-cultivated with *Arabidopsis thaliana*. Two fungal strains, which were subsequently identified as *Chaetomium globosum* and *Colletotrichum gloeosporioides*, were also revealed to have a prominent beneficial effect on *A. roxburghii*. They appear to promote the accumulation of various chemical components such as flavonoids, kinsenosides and polysaccharides, many of which represent an interesting object for research given their potential medical application. Histochemical analysis shows that the two species of fungi are endophytic in nature and colonize the intercellular gap of xylem parenchyma cells in roots without inhibiting the development of the host. Isolation of fungi such as *Ceratorhiza* and *Tulasnella* is not surprising since they belong to the group of the so called *Rhizoctonia* complex, a taxonomically loose set of fungi which are commonly associated with orchids and considered to be their major symbionts (Yang & Li 2012). The species isolated from the Chinese-collected plants, however, unfold an important object of interest. Many of these fungi such as *Acremonium*, *Hypoxylon*, *Diaporthe* and many others are ascomycetes, which is considered very unusual since the only orchid known to form symbiosis with members of this phylum is the unrelated *Epipactis*, a genus associated with species in the genus *Tuber* (Selosse et al. 2004). More research on this topic would be necessary to uncover the enigmatic biological nature of these potentially novel interactions in the orchid family and give more details about its effect on the development of *Ludisia* and *Anoectochillus* species in their native environment.

### Traditional medicine

Orchids (including *L. discolor*) are commercially used in Chinese and South Asian traditional medicine systems (Teoch 2016; Leon & Lin 2017). Numerous patents were generated using *L. discolor* as few of them are shown in **Table 1**. In Thailand's primary health care practices, *L. discolor* is listed among the herbs used in the treatment of venomous snakebites (Wongkongkathep et al. 2017).

**Table 1. *L. discolor* patents.**

| Patent | Description |
|---|---|
| CN105963562A, 2016 | The traditional Chinese medicine composition with *L. discolor*, all herbs are combined to treat muscular atrophy side effects caused by creotoxin and also play an auxiliary treatment role on cerebral trauma spasm. |
| CN103705742B, 2016 | The emophysematous Chinese medicine composition for the treatment of deficiency type of QI of the lung and kidney. In this drug composition is made by *L. discolor*, herba; *Juglans regia*, smen; *Davallia solida*, rhizome; *Oroxylum indicum*, semen; *Trichosanthes kirilowii*, pericarpium. |
| CN105053354A, 2015 | Patent about the kyllinga monocephala rottb lung-dispersing and cough-relieving tea is prepared by nine herbs, one of them is *L. discolor,* and has the effects of ventilating lung, relieving cough, clearing heat, moistening lung and eliminating phlegm when being taken for a long time. |
| CN104940625A, 2015 | Traditional Chinese Medicine composition for treating chronic obstructive lung disease. Herbal composition from 16 ingredients, include *L. discolor,* and used as decoction with water. |

According to the Thai ancient textbook, *L. discolor* (rhizome) is an ingredient of a recipe used for relieving the symptoms of hyperacidity syndrome (Sireeratawong et al. 2013). The whole plant is utilized in Traditional Chinese Medicine and it may be collected throughout the year and used fresh or dry (sun-dried). The herb is known as sweet and astringent, efficient against lung complaints, regulator of body fluids, blood purifier and anti-inflammatory and blood clotting agent. It is also used to treat haemoptysis caused by pulmonary tuberculosis, neurasthenia, anorexia and as a cough relive and decoction (dry or fresh herb). The herb can be chewed fresh or used to produce beverages (Li 1998; Teoch 2016). An ethnobotanical study on medicinal plants in Hainan Island, China showed that local people use whole plants of *L. discolor* for external treatments of injuries (Zheng & Xing 2009). *Anoectochilus roxburghii* is officially designated as the only source of local medicinal herb Jinxianlian (Wu et al. 2020). Some clinical applications of *Goodyera* and *Ludisia* species are similar to those of *A. roxburghii*, such as their utilization in the treatments of tuberculosis hemoptysis, rheumatism, rheumatoid arthritis and after snake bite (Ai 2013).

### Phytochemistry and biological activities

Three genera of jewel orchids, namely *Ludisia*, *Anoectochillus* and *Goodyera*, have been studied for their phytochemical content and its potential biological activity (Du et al. 2008; Wu et al. 2020). Key compounds extracted from these plants are kinsenoside, goodyeroside A and goodyeroside B. Their structures have been confirmed through mass spectroscopic analyses (**Table 2**; Rehman et al. 2016). These molecules that represent isomers of each other belong to the class of lactone glycosides – they have been extensively isolated from jewel orchids, with kinsenoside and goodyeroside A also extracted from the unrelated *Crocus sativus* (Righi et al. 2015).

**Table 2. Phytochemical content.**

| Metabolite | Chemical formula | PubChem Identifier https://pubchem.ncbi.nlm.nih.gov/ | References |
|---|---|---|---|
| Gastrodin | $C_{13}H_{18}O_7$ | 115067 | Pang et al. 2018 |
| Goodyerin | $C_{36}H_{40}O_{19}$ | 102460727 | Du et al. 2002 |
| Goodyeroside | $C_{10}H_{18}O_9$ | 10445498 | Zhang et al. 2005 |
| Kinsenoside | $C_{10}H_{16}O_8$ | 10422896 | Zhang et al. 2005 |

The lactone glycosides derivatives are characterized by the presence of a glucopyranosyl moiety bonded via a glycosidic bond to a butyrolactone ring. There are a total of 6 chiral centers, one of them located in a ring giving the potential for a variety of other isomers (Zhang et al. 2005). There is evidence that these phytochemicals demonstrate hepatoprotective, antihyperglycemic, antioxidant, anti-inflammatory and antihyperliposis activities (**Table 3**). Early experiments

**Table 3. Biological activities.**

| Biological activity | Extraction notes | Reference |
|---|---|---|
| Anti-diabetic | Pre-extracted kinsenoside | ZHANG ET AL. 2007 |
| Anti-hyperliposis | Reverse-phase silica fel column chromatography without methanol | DU ET AL. 2001 |
| Antioxidant | Ethanol treatment, chromatography | LIU ET AL. 2014 |
| Hepatoprotective | Water extraction, filtration, concentrating at reduced pressure | LIN ET AL. 1993 |
| Sedative and anti-convulsant | Methanol extraction at room temperature | DU ET AL. 2002 |
| Vascular protective | Water extraction at 90°C | LIU ET AL. 2017 |

investigated the effect of *A. formosanus* extracts on tissues from rats with acute hepatitis that was induced through carbon tetrachloride and acetaminophen treatment (LIN ET AL. 1993). Analysis of the samples shows that the induced cell damage has been inhibited in primary cultured hepatocytes isolated from the animals. Later research discovered that the active constituents of these extracts are kinsenoside and its related compounds (WU ET AL. 2007). Its potential antidiabetic activity has been evaluated through in vivo studies, which indicated that orally administering different doses of kinsenoside to rats results in treated groups having a higher number of intact β cells in the islets of Langerhans (ZHANG ET AL. 2007). The anti-hyperliposis activity of the jewel orchid compounds was demonstrated on rat models where the animals were fed with a high fat diet. Animals that were given kinsenoside showed a reduction in weight, however goodyeroside A failed to exert such effect (DU ET AL. 2001). The latter compound is considered to have a generally lower biological activity according to the available literature stressing on the absence of anti-diabetic and anti-autoimmune effect. On the other hand, goodyeroside B appears to be poorly studied and its potential activity needs to be further investigated. By contrast to its related compounds, there exist an increased number of preclinical studies regarding the metabolic stability of kinsenoside. The studies employ liver microsomes, subcellular structures representing vesicles containing a set of common liver enzymes that often damage various xenobiotics and impede the potential exploitation of novel drugs with various biological activities. Recent research involving HPLC allowed to determine the quantitative content of lactone glycosides in the three major members of the terrestrial orchid group and showed variation depending on both taxonomy and growth conditions. *Anoectochillus* species differ drastically in their kinsenoside content with values ranging from 3.38 to 229.17 mg/g (WU ET AL. 2020). Interestingly, tissue-cultured samples of *A. roxburghii* and *A. formosanus* display higher yields of kinsenoside compared to specimens collected from the wild. It implies that artificially grown plants are better candidates for mass extraction compared to orchids in their native habitat. The presence of kinsenoside has also been confirmed in both *Ludisia* and *Goodyera*, with the highest concentration registered for *G. schlechtendaliana*. Both genera are rich in goodyeroside A with a concentration from 79.6 up to 150.61 mg/g, while the values for kinsenoside are lower compared to what has been determined for *Anoectochillus*. Besides being rich in lactone glycosides, *Anoectochillus*, *Ludisia* and *Goodyera* contain an abundant variety of other biological compounds. Another type of glycoside, goodyerin, which belongs to the flavonol class has been demonstrated to have sedative properties (Du et al. 2002). Tests with phenol and sulfuric acid show high polysaccharide content in most sampled species of jewel orchids, apart from *G. biflora* and *A. burmannicus*, where the concentration appears relatively low. Such polysaccharides have been studied for their biological activity as well and there is evidence for hepatoprotective, antidiabetic and vascular protective properties similarly to data for kinsenoside. Other phytochemicals are various flavonoids, gastrodin, isorhamnetin, quercetin, narcissin and kempherol that were isolated from these plants, too (Wu et al. 2020). Many of them are reported to have antioxidant, anti-cancer or anti-autoimmune activities, however, further in vivo tests are necessary for a better evaluation of any potential pharmacological effect.

**Micropropagation**

In contrast to many other members of its family, *L. discolor* can be readily propagated via rooting of stem cuttings or separation of individuals from a larger tuft (Jackson 2005). The plant does not require any special conditions and usually demonstrates vigorous development. The combination of these factors make *L. discolor* an excellent model organism for the orchid family, which sparks interest in its application for plant research in the lab. Skills in its micropropagation are vital for this purpose since they allow the maintenance of many individuals in a sterile environment and provide an important tool for analyses of its pharmacological properties. Sterile conditions keeping the explants free from contamination has a key role for the success of this process. Sterilization procedures involving a combination of ethanol rinses plus various concentrations of sodium hypochlorite or mercury chloride have been tested (Poobathy et al. 2019). The analyses show that mercury chloride at concentration 0.4 w/v is the most suitable choice for this purpose. Various growth media have been tested as well, including half strength Murashige and Skoog (MS), modified Knudsen C and MS basal medium combined with tryptone and gelrite (**Table 4**). Half strength MS with added naphthyl acetic acid (NAA), thidiazuron and active charcoal gives optimal development of the explants, since *L. discolor* appears to be sensitive to high concentration of nutrients. Acclimatization of individuals can be achieved with coconut coir, coconut husk and peatmoss (Poobathy et al. 2019). The formation of adventitious bud formation in *Ludisia* has been studied as well and the effects of 6-benzylaminopurine (6-BA), NAA, $Cu^{2+}$ and $Ag^{2+}$ were tested.

**Table 4. Media variants used for the micropropagaion of *L. discolor*.**

| Variant | Composition | Reference |
|---|---|---|
| T1 | Half-strength Murashige and Skoog (Murashige and Skoog, 1962) basal medium, 0.2% (w/v) activated charcoal, 8% (w/v) Mas banana cultivar homogenate, 3% (w/v) sucrose, 3.5 g $L^{-1}$ Gelrite, 1.0 mg $L^{-1}$ 1-naphthaleneacetic acid (NAA), and 0.1 mg $L^{-1}$ thidiazuron (TDZ) | Poobathy et al. 2019 |
| T2 | Knudson C | |
| T3 | Modified Knudson C | |
| T4 | MS basal medium, 3 g $L^{-1}$ tryptone, 30 g $L^{-1}$ sucrose, 2.75 g $L^{-1}$ Gelrite (pH 5.2) | |
| Adventitious bud induction | MS basal, 6-BA, NAA, $CuSO_4$, AgCl, sucrose, inositol | Liu et al. 2021 |
| Seed germination A | Half strength Murashige and Skoog | Shiau et al. 2005 |
| Seed germination B | Half strength Hyponex № 1, 2.00 mg/L 6-BA, 0.6 mg/L NAA, coconut milk | Hongyang et al. 2016 |

The experiment indicates that best results are produced with a combination of 1.0 mg/L 6-BA, 0.75 mg/L NAA plus 0.25 mg/L $CuSO_4$ or 6.4 mg/L AgCl. The phytoeffectors 6-BA, $Ag^{2+}$ and $Cu^{2+}$ show an inhibitory effect as the concentration increases, however, high concentration of NAA is well tolerated and leads to a better regeneration rate (Liu et al. 2021).

Successful asymbiotic germination of seeds from *L. discolor* is also reported in at least two studies (Shiau et al. 2005; Hongyang et al. 2016). Both use immature capsules produced after hand pollination. This avoids the sterilization of the seeds since the interior of the capsule is considered free of contaminants and only its surface is subjected to treatments with both bleach and mercury chloride resulting in effective sterilization. Similarly to other orchid species, immature seeds demonstrate a higher germination rate. However, more tests would be helpful to determine the optimal sowing medium since both studies contradict each other with respect to what is considered the best choice for *L. discolor*. In one of the studies, half strength MS leads to the most desired results (Shiau et al. 2005), while in the other study (Hongyang et al. 2016) Hyponex is reported to produce the highest germination rate with half-strength MS appearing to be inappropriate. Nevertheless, the addition of regulatory substances such as NAA, TDZ or 6-BA has an unambiguously simulative effect on the seedling's development. About four to five months are usually required for seedlings to reach an early stage of maturity after which they can be transferred to greenhouse conditions.

## CONCLUSION

*Ludisia discolor* could serve as a model organism for studying fundamental features in the biology of orchids – plants that are usually more demanding in terms of cultivation and reproduction conditions. For this reason, the exploration of its biochemical, physiological, reproductive, and other characteristics is essential to reveal the great potential that *L. discolor* can offer. DNA marker techniques like DNA barcoding open new horizons for taxonomic characterization of orchids both in terms of better understanding the genetic variability and species dynamics at ecology level but also to elucidate the complex network of mycorrhizal associations within this plant family.

*Ludisia discolor* is also rich in biologically active substances, including some key compounds such as kinsenoside, gudieroside and gudierin, which are of pharmacological interest. The study of orchids and the development of successful techniques for its reproduction can open new opportunities for mass production of these compounds and their potential application in medicine. In summary, *L. discolor* could be used for decorative purposes, as well to produce economically important products, which increases the interest of more detailed studies.

## CONFLICT OF INTERESTS

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


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## AUTHORS CONTRIBUTION

All authors contributed equally to the design, discussion and writing of the manuscript.

## References


Ai T.-M. 2013. Medicinal Flora of China. Volume 12. Peking University Medical Press, Beijing, China, 797 pp.

Ali M. B., Hahn E. J. & Paek K. Y. 2005. Effects of light intensities on antioxidant enzymes and malondialdehyde content during short-term acclimatization on micropropagated *Phalaenopsis* plantlet. - Environ. Exp. Bot. 54 (2): 109–120.

Athipunyakom P., Manoch L. & Piluek C. 2004. Isolation and identification of mycorrhizal fungi from eleven terrestrial orchids. - Agric. Nat. Resour. 38 (2): 216–228.

Averyanov L. V., Loc P. K., Hiep N. T. & Harder D. K. 2003. Phytogeographic review of Vietnam and adjacent areas of Eastern Indochina. - Komarovia 3: 1–83.

Barcode of Life Data System. https://www.boldsystems.org/ (Last accessed on 31.05.2022).

Chase M. W., Cameron K. M., Freudenstein J. V., Pridgeon A. M., Salazar G., Van den Berg C. & Schuiteman A. 2015. An updated classification of Orchidaceae. - Bot. J. Linn. Soc. 177 (2): 151–174.

Chen S. P., Tian H. Z., Guan Q. X., Zhai J. W., Zhang G. Q., Chen L. J., Liu Z. J., Lan S. R. & Li M. H. 2019. Molecular systematics of Goodyerinae (Cranichideae, Orchidoideae, Orchidaceae) based on multiple nuclear and plastid regions. - Mol. Phylogenet. Evol. 139: 106542.

Chen J.-R. & Shiau Y.-J. 2015. Application of internal transcribed spacers and maturase K markers for identifying *Anoectochilus*, *Ludisia*, and *Ludochilus*. - Biol. Plant. 59 (3): 485–490.

Du X.M., Irino N., Furusho N., Hayashi J. & Shoyama Y. 2008. Pharmacologically active compounds in the *Anoectochilus* and *Goodyera* species. - J. Nat. Med. 62 (2): 132–148.

Du X. M., Sun N. Y., Takizawa N., Guo Y. T. & Shoyama Y. 2002. Sedative and anticonvulsant activities of goodyerin, a flavonol glycoside from *Goodyera schlechtendaliana*. - Phytother Res. 16 (3): 261–263.

Du X., Sun N., Tamura T., Mohri A., Sugiura M., Yoshizawa T., Irino N., Hayashi J. & Shoyama Y. 2001. Higher yielding isolation of kinsenoside in *Anoectochilus* and its anti-hyperliposis effect. - Biol. Pharm. Bull. 24 (1): 65–69.

Glicenstein L. 2009. Jewel and painted-leaf orchids: I. *Ludisia* and *Anoectochilus*. - Orchids 78 (11): 664–669.

Bolhàr-Nordenkampf H. R. & Draxler G. 2013. Functional leaf anatomy. In: Hall D. O., Scurlock J. M. O., Bolhar-Nordenkampf H. R., Leegood R. C. & Long S. P. (Eds.), Photosynthesis and production in a changing environment: a field and laboratory manual. Springer Science & Business Media, Dordrecht, 326 pp.

Han F., Peng Y., Xu L. & Xiao P. 2014. Identification, characterization, and utilization of single copy genes in 29 angiosperm genomes. - BMC Genom. 15 (1): 1–9.

Hayden W. J. 2016. Jewels of the Orchidaceae. - Sempervirens Quarterly (Fall 2016), 6–7.

Hollingsworth P. M., Graham S. W. & Little D. P. 2011. Choosing and using a plant DNA barcode. - PloS ONE, 6 (5): e19254. doi:10.1371/journal.pone.0019254

Hongyang L. I., Yang L. I. U., Guanming C. H. E. N. & Zhijuan Y. A. N. G. 2016. Aseptic Seedling and Rapid Propagation of *Ludisia discolor*. - Agric. Sci. Technol. 17 (11): 2473–2476, 2482.

Jackson A. M. 2005. Determination of optimal rooting conditions for stem cuttings of *Ludisia discolor*. Master Thesis, Mississippi State University, 51 pp.

Kew Science, 2020. Plants of the world online. http://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:641773-1 (Last accessed on 31.05.2022).

Kumar S., Stecher G., Li M., Knyaz C. & Tamura K. 2018. MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. - Mol. Biol. Evol. 35 (6): 1547–1549.

Leon C. & Lin Y.-L. 2017. Chinese medicinal plants, herbal drugs and substitutes: an identification guide. Kew Publishing, Royal Botanic Gardens, Kew, 850 pp.

Li N.H. (Ed.) 1998. Chinese medicinal herbs of Hong Kong, vol. 2. Hong Kong Chinese Medicinla Research Institute, Hong Kong, 198 pp.

Lin J.M., Lin C.C., Chiu H.F., Yang J.J. & Lee, S.G. 1993. Evaluation of the anti-inflammatory and liver-protective effects of *Anoectochilus formosanus*, *Ganoderma lucidum* and *Gynostemma pentaphyllum* in rats. - Am. J. Chinese Med. 21 (01): 59–69.

Liu Y., Li X., Chen J., Xue Y. & Zhu Y. 2021. Effects of different factors on adventitious bud induction from stem explants of *Ludisia discolor*. - In: Duan L. & Abdullah A.Z. (Eds.), E3S Web of Conferences 245, 5th International Conference on Advances in Energy, Environment and Chemical Science, 26-28 February 2021, Shanghai, China, p. 03024.

Ma Z., Li S., Zhang M., Jiang S. & Xiao Y. 2010. Light intensity affects growth, photosynthetic capability, and total flavonoid accumulation of *Anoectochilus plants*. - HortScience 45 (6): 863–867.

McCormick M. K., Whigham, D. F. & O'neill J. 2004. Mycorrhizal diversity in photosynthetic terrestrial orchids. - New Phytol. 163 (2): 425–438.

Poobathy R., Zakaria R., Murugaiyah V. & Subramaniam S. 2018. Autofluorescence study and selected cyanidin quantification in the Jewel orchids *Anoectochilus* sp. and *Ludisia discolor*. - PLoS ONE 13 (4): e0195642.

Poobathy R., Zakaria R., Murugaiyah V. & Subramaniam S. 2019. Surface sterilization and micropropagation of *Ludisia discolor.* - Biocatal. Agric. Biotechnol. 22: 101380.

Ratnasingham S. & Hebert P. D. 2007. BOLD: The Barcode of Life Data System (http://www. barcodinglife. org). - Mol. Ecol. Notes 7 (3): 355–364.

Rasmussen H. N. 2002. Recent developments in the study of orchid mycorrhiza. -

Plant soil 244 (1): 149–163.
Rehman S. U., Kim I. S., Choi M. S., Luo Z., Yao G., Xue Y., Zhang Y. & Yoo H. H. 2016. Development of a hydrophilic interaction liquid chromatography–tandem mass spectrometric method for the determination of kinsenoside, an antihyperlipidemic candidate, in rat plasma and its application to pharmacokinetic studies. - J. Pharm. Biomed. Anal. 120: 19–24.
Righi V., Parenti F., Tugnoli V., Schenetti L. & Mucci A. 2015. *Crocus sativus* petals: waste or valuable resource? The answer of high-resolution and High-resolution magic angle spinning nuclear magnetic resonance. - J. Agric. Food Chem. 63 (38): 8439–8444.
Saitou N. & Nei M. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. - Mol. Biol. Evol. 4 (4): 406–425.
Selosse M. A., Faccio A., Scappaticci G. & Bonfante P. 2004. Chlorophyllous and achlorophyllous specimens of *Epipactis microphylla* (Neottieae, Orchidaceae) are associated with ectomycorrhizal septomycetes, including truffles. - Microb. Ecol. 47 (4): 416–426.
Shiau Y. J., Nalawade S. M., Hsai C. N. & Tsay H. S. 2005. Propagation of *Haemaria discolor* via in vitro seed germination. - Biol. Plant. 49 (3): 341–346.
Sinovas P., Price B., King E., Hinsley A. & Pavitt A. 2017. Wildlife trade in the Amazon countries: an analysis of trade in CITES listed species. Technical report prepared for the Amazon Regional Program (BMZ/DGIS/GIZ). UN Environment - World Conservation Monitoring Centre, Cambridge, UK, 113 pp.
Sireeratawong S., Nanna U., Vannasiri S., Chiruntanat N., Srithiwong S., Subcharoen P., Termwiset P., Tungsukruthai P., Chuthaputti A. & Jaijoy K. 2013. Acute and subchronic toxicity study of Tud-Rak-Ka-Sai-Puu recipe in rats. - Afr. J. Tradit. Complement. Altern. Med. 10 (1): 142–148.
Soudzilovskaia N. A., Vaessen S., Barcelo M., He J., Rahimlou S., Abarenkov K., Brundrett M. C., Gomes S. I., Merckx V. & Tedersoo L. 2020. FungalRoot: global online database of plant mycorrhizal associations. - New Phytol. 227 (3): 955–966.
Tamura K., Nei M. & Kumar S. 2004. Prospects for inferring very large phylogenies by using the neighbor-joining method. - Proc. Natl. Acad. Sci. U.S.A. 101 (30): 11030–11035.
Teoh E. S. 2016. Medicinal orchids of Asia. Springer Nature Switzerland AG, 770 pp.
Wongkongkathep S., Prasertsiripong N., Stienrut P., Chantraket R. & Sonksakda M. (Eds.). 2017. Thai traditional and alternative health profile: Thai traditional medicine, Indigenous medicine and alternative medicine 2014-2016. Technical Services Bureau, Department for Development of Thai Traditional and Alternative Medicine, Ministry of Public Health, 357 pp.
Wu J. B., Lin W. L., Hsieh C. C., Ho H. Y., Tsay H. S. & Lin W. C. 2007. The hepatoprotective activity of kinsenoside from *Anoectochilus formosanus*. -

Phytother Res. 21 (1): 58–61.
Wu Y. B., Peng M. C., Zhang C., Wu J. G., Ye B. Z., Yi J., Wu J. Z. & Zheng C. J. 2020. Quantitative determination of multi-class bioactive constituents for quality assessment of ten *Anoectochilus*, four *Goodyera* and one *Ludisia* species in China. - Chin. Herb. Med. 12 (4): 430–439.
Yang, G. & Li, C. 2012. General Description of *Rhizoctonia* species complex. In: Cumagun, C. J. R. (Ed.). Plant Pathology [Internet]. IntechOpen, London, 41–52.
Ye B., Wu Y., Zhai X., Zhang R., Wu J., Zhang C., Rahman K., Qin L., Han T. & Zheng C. 2020. Beneficial effects of endophytic fungi from the *Anoectochilus* and *Ludisia* species on the growth and secondary metabolism of *Anoectochilus roxburghii*. - ACS Omega 5 (7): 3487–3497.
Yu X., Xie T. X., Liu B., Zhang D. Y., Liu X. D., Ai Y. & Liu Z. J. 2019. The complete chloroplast genome sequence of *Ludisia discolor* from Hainan of China. - Mitochondrial DNA Part B 4 (2): 3663–3664.
Zhang X., Huang H. H. & Chen Q. H. 2005. A novel total synthesis of kinsenoside and goodyeroside A relying on the efficient reaction of the chiral 2 (5H)-furanones. - J. Asian Nat. Prod. Res. 7 (5): 711–721.
Zhang Y., Cai J., Ruan H., Pi H. & Wu J. 2007. Antihyperglycemic activity of kinsenoside, a high yielding constituent from *Anoectochilus roxburghii* in streptozotocin diabetic rats. - J. Ethnopharmacol. 114 (2): 141–145.
Zheng X. & Xing F. 2009. Ethnobotanical study on medicinal plants around Mt.Yinggeling, Hainan Island, China. - J. Ethnopharmacol. 124 (2): 197–210.

**Patents information:**

Patent CN104940625A (2015) Traditional Chinese medicine composition for treating chronic obstructive lung disease, CN Patent.
Patent CN105053354A (2015) *Kyllinga monocephala* rottb lung-dispersing and cough-relieving tea and preparation method thereof, CN Patent, Harbin Lingdan Hongyun Tea Co., Ltd.
Patent CN103705742B (2016) Traditional Chinese medicine for curing emphysema, CN Patent, Haining Qianjiang Xingye Investment Development Co., Ltd
Patent CN105963562A (2016) Traditional Chinese medicine preparation for relieving side effects after treatment of patients with cerebral trauma spasm by creotoxin, CN Patent.

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# APPLICATION OF REMOTE SENSING IN ENVIRONMENTAL STUDIES: ADVANTAGES AND CHALLENGES

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**Abstract.** Growing concern about environmental challenges has led to the development of new observation tools to perform monitoring and assessment in a broad range of environments, application to conservation management and for mapping of natural resources. Although, the emerging methods and technologies of remote sensing are a powerful tool, they meet some difficulties and limitations in their real applications. This paper overview several projects and initiatives in Lithuania and Bulgaria related with application of both unmanned aerial vehicles and satellite imagery in various types of environment assessments. The benefits and limitations that emerged during the investigations have been discussed in the international workshop organised by the EU project of LIFE programme ALGAESERVICE for LIFE.

**Key words:** remote sensing methods, environmental sampling, field work, algal blooms

## INTRODUCTION

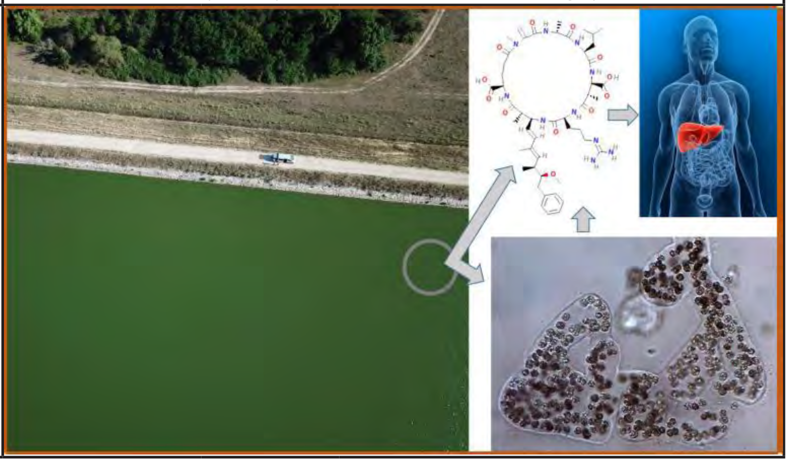
European Union outlines the main needs and potential applications of remote technologies, especially in the areas related to agriculture, forestry, biodiversity, plant health, soil, inland waters and coastal areas, fisheries and aquaculture, etc. (https://knowledge4policy.ec.europa.eu/earth-observation/eu%C2%A0policy-areas_en). Therefore, nowadays remote sensing technologies (unmanned aerial vehicles (UAV), satellites, radars, etc.) have become great tool for observation and analysis of various processes in environment *e.g.*, changes of land cover, spread of invasive plant species, waterbody eutrophication. It was an inevitable process as technologies evolved and commonly used point or ground-based observations which are limited, costly, time-consuming and labor intensive became insufficient. Especially it is important in cases when territories of interest are remote, difficult to reach or cover large areas. Thus, to observe and analyze the processes that are crucial for proper interpretation of results and decision making requires modern remote sensing techniques. To maintain accuracy and avoid errors these new methods as well as other traditional methods require verification and proper processing of obtained data which is gathered using various remote sensing techniques (Noor et al. 2018, Li et al. 2020, Mertikas et al. 2021).

In order to discuss and share knowledge about the use of remote sensing methods in environmental research the scientific-practical workshop "Remote sensing in environmental studies: advantages and challenges" was organized in the Nature Research Center (Vilnius, Lithuania) on 12th of October, 2021 within the EU project of LIFE programme "Algae – economy based ecological service of aquatic ecosystems" (LIFE17 ENV/LT/000407 ALGAESERVICE for LIFE). Eight presentations from representatives of different institutions from Bulgaria and Lithuania were presented during the workshop also the technical possibilities of unmanned aircrafts were presented during the breaks of the workshop. More than 100 participants took place in the workshop from different institutions from Bulgaria, Poland and Lithuania. Main results of the researches of the seminar speakers are presented in the article.

RESULTS

## 1. Application of unmanned aerial vehicles in environmental studies

### 1.1. Drone applications in Bulgarian algological studies

| | | | |
|---|---|---|---|
| **Project** | *Cyanoprokaryotes – a new potential risk factor for malignant diseases in Bulgaria?* | | |
| **Funded** | Scientific Research Fund of the Bulgarian Ministry of Education, grant number DN 13-9/15.12.2017 | | |
| **Duration** | 2017 - 2023 | **Website** | https://cyanoprokaryota.weebly.com/ |
| **Implementers** | Sofia University "St. Kliment Ohridski"<br>Leaders: Prof. Maya Stoyneva and Assoc. Prof. Blagoy Uzunov | | |
| **Graphical abstract** |  | | |

The project is aimed at the study of specific blue-green algae (cyanoprokaryotes, cyanobacteria) capable of releasing cyanotoxins related to cancer and other diseases of social importance in the country. An interdisciplinary approach has been applied to successfully solve the added tasks. Sampling was done according to an innovative methodology with selection of sites as a result of aerial drone observations. This was the first in Europe and the fifth in the world realized and published application of a drone in working with hazardous algal blooms in freshwaters and the first in studies of thermophilic habitats. Furthermore, for the first time in Bulgaria a method for determining the abundance of phytoplankton by high performance liquid chromatography (HPLC) has been applied.

As a result, new data were collected on the abundance and biodiversity of cyanoprokaryotes in the country with the finding of 127 species (46 new for Bulgaria) and proving their significant role in the phytoplankton of the shallow waterbodies. New data have been obtained on cyanotoxins in the country, with first records for Bulgaria of cylindrospermopsin from Lake Vaya and Reservoir Mandra, and first finding of microcystins in Reservoir Sinyata Reka. Microcystins were found also in the Lake Durankulak, where for the first time saxitoxins have been also detected. Polyphasic approach, based on microscopic, chemical and molecular genetic studies, was applied in investigation of toxigenic species and evidence for the genetic ability for microcystins production in *Microcystis wesenbergii*, long considered to be non-toxic, was provided. Data on malignant diseases were collected from the University Hospital "St. Marina" - Varna and their primary treatment was performed.

### 1.2. Application of unmanned aerial systems to investigate macroalgae agglomerations

<table>
<tr><td>Project</td><td colspan="3">Algae – economy based ecological service of aquatic ecosystems (LIFE17 ENV/LT/000407)</td></tr>
<tr><td>Funded</td><td colspan="3">EU LIFE Programme, the Ministry of Environment of the Republic of Lithuania, the National Fund for Environmental Protection and the Water Management in Poland, and by the project partners</td></tr>
<tr><td>Duration</td><td>2018 - 2023</td><td>Website</td><td>www.algaeservice.gamtostyrimai.lt/</td></tr>
<tr><td>Implementers</td><td colspan="3">Nature Research Centre (coordinator), Nature Heritage Fund, Baltic Environment, Spila, A. Mickiewicz University in Poznan, Institute of Nature Conservation (Polish Academy of Sciences)</td></tr>
<tr><td>Graphical abstract</td><td colspan="3">
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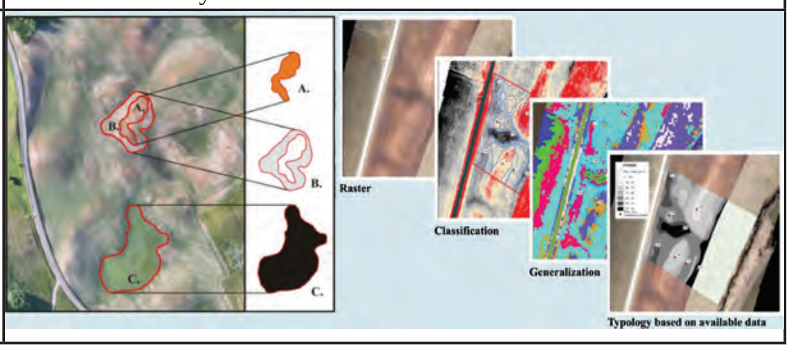
Remote studies of rivers in Lithuania were carried out using an unmanned aerial system that consisted of a fixed-wing UAV and built-in visual or infrared spectral cameras. The main purpose was to map the vegetation cover in the riverbeds in order to identify the areas overgrown with filamentous macroalgae, to estimate their quantities and to account for potential resources. Prior to implementation, the best hydrometeorological conditions suitable for the maximum accuracy of the results of remote sensing of algae and the minimum impact of possible disturbances were determined.

The preparation and analysis of the collected material included three steps: 1) Building an orthophotomosaic by combining a set of aerial photographs; 2) Analysis of raster images based on different colour characteristics of the aerial photographs. Heterogeneous areas were identified and grouped in accordance with the turbidity data. On the basis of the classification, as well as expert opinion and direct studies, polygons were automatically assigned to one of the classification types using software; 3) An inventory of macroalgal agglomerations using ArcGIS software according to a number of parameters (*e.g.* the area and volume occupied by algae, hotspots of agglomerations). Raster segmentation and classification of the orthophoto maps of the studied channels allow the effective identification of river sections with different concentrations of algae and the calculation of their amount. Riverbed scanning using an infrared wave (thermal imaging) sensor confirmed the results obtained from the visual spectrum orthophotography analysis and captured the polygons with the highest concentrations of macroalgae.

Transparency, insolation and the degree of shading of the water surface were the main limiting factors for the accuracy of the study. So, if the factors limiting quality of the assessment are taken into account, the application of remote sensing technologies provides qualitatively new high-detail information, significantly reduces time and money, and enables more broad assessment.

### 1.3. Opportunities and strengths of ecosystem research using remote sensing methods

<table>
<tr><td>Project</td><td colspan="3">Development of Innovative Service "Remote Sensing Studies of Soil and Consultantions"</td></tr>
<tr><td>Funded</td><td colspan="3">Rural development 2014-2020 for Operational Groups (in the sense of Art 56 of Reg.1305/2013)</td></tr>
<tr><td>Duration</td><td>2018 - 2021</td><td>Website</td><td>https://ec.europa.eu/eip/agriculture/en/find-connect/projects/inovatyvios-pa-slaugos-dirvo%C5%BEemio-aerodistanciniai</td></tr>
<tr><td>Implementers</td><td colspan="3">Lithuanian Agricultural Advisory Service and Institute of Geosciences, Vilnius University</td></tr>
<tr><td>Graphical abstract</td><td colspan="3">
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</table>

The idea of remote sensing of the soil is based on the aim to perform detailed mapping of the soil cover of the study area using unmanned aerial systems (UAS), at the same time distinguishing soil facies - homogeneous habitats. These areas have identical or very close physical and chemical characteristics (granulometric structure, humus content, moisture content, degree of erosion) within their boundaries, and for which the same agrotechnical measures could be applied individually or to the whole complex of related habitats and achieve the same impact result. The results of this work can become a basis for the concept of precision agriculture.

The most appropriate (technical and natural) test conditions were identified and clearly defined, limiting the parameters that significantly reduce the quality of the final results. It should be noted that due to the negative shading effect, which has a significant negative effect on the test results, it is necessary to perform the tests at a cloud cover of less than 3 points, or at full cloud cover, when only scattered sunlight reaches the ground. For both arable land the height of the sun above the horizon, which should be at least 25 degrees, remains an important factor in ensuring the accuracy of the primary data. Adherence to these conditions eliminates most of the external noise affecting the image quality of the surface under study.

With regard to the properties of the test object, it is necessary to emphasize that the uniformity of the test surface is incomparably more important than the nature of the surface coating, which was considered a significant and even limiting factor at the beginning of the tests. Studies have shown that if the surface of the land is even (nature of cultivation, type of grazing), regardless of whether it is ploughed or covered with grassy vegetation, it is easy to see and record the differences in soil properties caused by natural processes. In the case of cultivated soil, these signs are directly visible, and in the case of grassy cover, they are reflected through differences in the nature of the vegetation. The latter observation is particularly important and significant, as it allows to obtain reliable research results not only in the presence of open surfaces, but also in the study of surfaces with grass cover, crops in the initial germination phase, or even the surfaces affected by no-till technology.

### 1.4. Can drones make botanical field work easier?

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|---|---|
| **Project** | *Development of Innovative Service "Remote Sensing Studies of Soil and Consultantions"* |
| **Funded** | Project (Contract No. 05.5.1-APVA-V-018-01-0012) co-financed by the European Union Structural Funds according to the 5th Priority of the European Union Funds Investment<br>Operational Program for 2014–2020 "Environment, Sustainable Use of Natural Resources and Adaptation to Climate Change" under the measure “Biodiversity protection” (05.5.1-APVA-V-018) |

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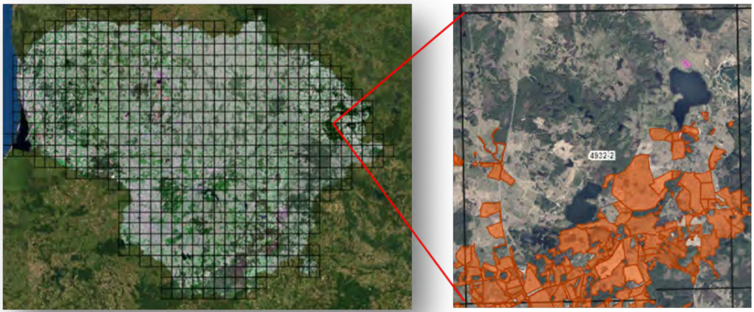
| **Duration** | 2019 - 2022 | **Website** | www.gamtostyrimai.lt/ |
| **Implementers** | Nature Research Centre | | |
| **Graphical abstract** |  | | |

During the planning of the investigation of the status of invasive and alien plant species in Lithuania, one of the main goals was to map 64 plant species. Most of the species are being mapped using a grid, approximately 5 x 5 kilometers. However, Invasive Alien Species of Union concern must be mapped as precisely as possible. Of these species, three of them occur in Lithuania: *Asclepias syriaca*, *Heracleum sosnowskyi* and *Impatiens glandulifera*. To achieve precise mapping, three unmanned aircraft vehicles are being used: i) two quadcopters (dji mavic pro and dji mavic 2 zoom); ii) one fixed wing drone (UAV Birdie) with a parrot sequoia camera.

*Impatiens glandulifera* mostly occurs in wet natural habitats such as alluvial forests, hygrophyte belts close to rivers or lakes, *etc*.; therefore, it is almost unrecognizable via drone. *Asclepias syriaca* does not cover vast areas in Lithuania and can be easily found during investigations of other species.

*Heracleum sosnowskyi*, as previous knowledge shows, in total, covers more than 10K ha across the country. In some regions, it grows only in small patches or individually. The mapping of *H. sosnowskyi* was carried out using drones at sites where intensive invasion is historically known and at sites which were reported by field workers as invaded. Currently, an area of more than 15K ha is mapped as invaded by *H. sosnowskyi*. The drones flew more than 280 km over approximately 16 hours of flight time. A huge number of images were captured and after used to map this invasive species.

During the field work complications arose, these could be classified into two types. The first is related with species biology and ecology. There is a relatively short period (approximately two weeks of blooming) when *H. sosnowskyi* can be effortlessly recognized. In some places, this species is controlled and as a result it is difficult to observe vegetative individuals. Sometimes it invades forest edges and is not visible via drone. The second group of complications is more technical. Fixed wing drones are generally only effective in large invaded areas. Also, one must have a suitable place for drone landing, which is not always possible at certain sites. Furthermore, the success of the investigation and quality of images depend on weather conditions. Additionally, drones have several issues, for example, relatively short flight time and sometimes drones (or pilots) fail – drones fall down or fly away.

Despite the challenges, drones have made the investigation of invasive plant species more precise, simpler, and have saved time and human resources.

### 1.5. Assessing recreational fishing effort using remote methods

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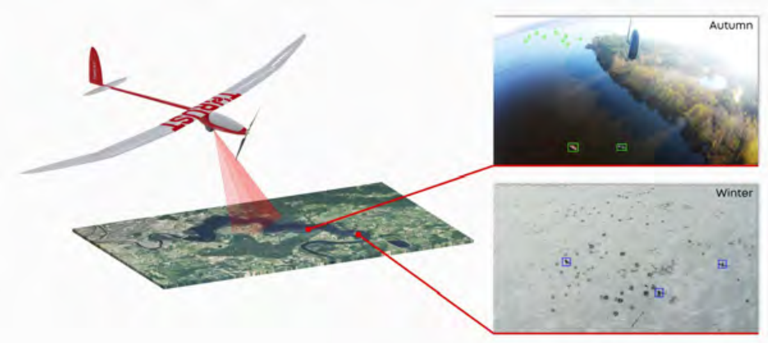
|---|---|---|---|
| **Project** | *Sustainable inland fisheries* | | |
| **Funded** | European Regional Development Fund (project No 01.2.2-LMT-K-718-02-0006) under grant agreement with the Research Council of Lithuania (LMTLT) | | |
| **Duration** | 2019 - 2022 | **Website** | www.sif.lt |
| **Implementers** | Nature Research Centre, UAB Aerodiagnostika | | |
| **Graphical abstract** |  | | |



Recreational fishing has become a major force on inland and coastal aquatic ecosystems, with nearly 15% of Europeans at least occasionally engaging in recreational fishing activities. Assessing spatial and temporal distribution of anglers, and total fishing effort has been a major challenge for inland and coastal ecosystem management. This assessment is also important from a socio-economic perspective, because recreational fishing contributes to local economies and is important for human wellbeing. In most countries, recreational fishing effort estimates are highly uncertain, or simply non-existent. In this study, we applied two novel approaches to assess recreational fishing effort in Lithuania – aerial inspection using autonomous fixed wing drones and anonymous data from a popular GPS-enabled angler fish finder device. We show that drone-based angler counts are highly accurate and efficient, and the method can be used to survey large areas in short time with minimum $CO_2$ emissions, disturbance to anglers and wildlife. By employing both standard and infrared cameras, as well as machine learning algorithms, video post-processing time can be greatly shortened and angler count accuracy improved further, especially under visually challenging conditions. Using nearly 40 missions distributed over one year in a popular fishing destination Kaunas Reservoir we developed a statistical model to predict angler numbers across seasons and weekdays. Further, we calibrate observed angler numbers with daily anonymous fish finder device usage, to produce daily angler number estimate in Kaunas Reservoir, and, potentially, anywhere in Lithuania. We are currently developing improved machine learning algorithms for drone video analytics and expanding angler number calibration to other locations in Lithuania.

## 2. Application of satelite images in environmental studies

### 2.1. Remote sensing solutions in water quality monitoring and management

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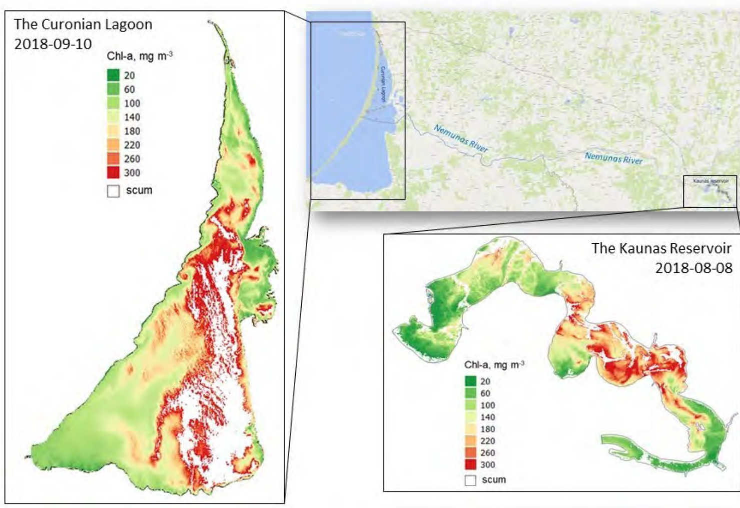
|---|---|---|---|
| **Project** | • *"Phosphorus as driver of cyanobacterial hyperblooms in the Curonian Lagoon (Patchy)" [S-MIP-17-11]*<br>• *Horizon 2020 EOMORES project [730066]*<br>• *contract TODAY [4000122960/18/NL/SC]* | | |
| **Funded** | • Research Council of Lithuania (LMT) grant<br>• European Union<br>• European Space Agency PECS | | |
| **Duration** | 2017 - 2020 | **Website** | https://eomores-h2020.eu/, http://apc.ku.lt/ |
| **Implementers** | Klaipeda University (all projects), EOMORES consortium: Water Insight, the Netherlands (coordinator); Deltares, the Netherlands; CNR-IREA, Italy; SYKE, Finland; Tartu Observatory, Estonia; Klaipeda University, Lithuania; The University of Stirling, United Kingdom; PML, United Kingdom; Evenflow, Belgium | | |
| **Graphical abstract** |  | | |

The projects are related to remote sensing solutions in water quality monitoring and management with a focus on cyanobacteria hyperblooms mapping in two Lithuanian water bodies: the Curonian Lagoon and the Kaunas Reservoir. The long-term analysis (1985–2018) in the Curonian Lagoon was based on a significant amount (over 500) of synoptic satellite images: Landsat series, MERIS on-board Envisat, MSI on-board Sentinel-2A/B, and OLCI on-board Sentinel-3A. Despite the pronounced seasonal variations in the growth of cyanobacteria during the time series, the duration of the period, when cyanobacteria hyperblooms are present, has been consistently getting longer since 2008 (Vaičiūtė et al. 2021). Dense cyanobacteria blooms, so-called hotspots, were more frequently observed in the southern and central parts of the lagoon, i.e., the mostly stagnant south-southwestern part of the lagoon (Ferrarin et al. 2008). The spatial distribution patterns were related to the presence of muddy sediments that are rich in phosphorus, water renewal time and internal hydrodynamics features. Similarly, the ongoing eutrophication significantly alters cyanobacteria hyperblooms during summer period in the Kaunas Reservoir (a dammed part of the River Nemunas). Chlorophyll-*a* concentration estimated from MSI Sentinel-2 A/B images can range from 4.6 to more than 5000 mg $m^{-3}$ exceeding the threshold of high-risk for public health proposed by the World Health Organization (WHO 2021). We hypothesize that characteristic hydrological patterns and meteorological conditions alter the main, longstanding, well-established spatial patterns of cyanobacteria hyperblooms in the Kaunas Reservoir. This hypothesis will be tested by adapting the methodology used in the case of the Curonian Lagoon. The investigations confirmed that remote sensing methods are providing a significant data and information about the status quo of ongoing eutrophication, the spatial variability of cyanobacteria hyperblooms, a risk for public health, and can support the planning of eutrophication mitigation actions in both waterbodies and the River Nemunas basin as a whole.

### 2.2. Sentinel satellite images for observation of hotspots of cyanobacteria blooms

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|---|---|---|---|
| **Project** | *Algae – economy based ecological service of aquatic ecosystems (LIFE17 ENV/LT/000407)* | | |
| **Funded** | EU LIFE Programme, the Ministry of Environment of the Republic of Lithuania, the National Fund for ENVIRONMENTAL PROTECTION and the Water Management in Poland, and by the project partners | | |
| **Duration** | 2018 - 2023 | **Website** | www.algaeservice.gamtostyrimai.lt/ |
| **Implementers** | Nature Research Centre (coordinator), Nature Heritage Fund, Baltic Environment, Spila, A. Mickiewicz University in Poznan, Institute of Nature Conservation (Polish Academy of Sciences) | | |

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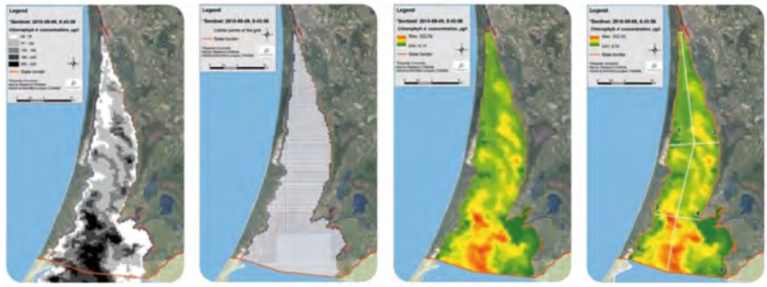
| **Graphical abstract** |  |

The Horizon 2020 project EOMORES (in cooperation with Klaipėda University) has a series of services for monitoring the quality of inland and coastal water bodies based on a combination of the most up-to-date satellite data, innovative *in situ* instruments. This tool to measure chlorophyll-*a* concentrations was applied to identify hot-spots of cyanobacteria blooms in the Curonian Lagoon. GIS analysis tools were used to model data for the period of 2018–2019.

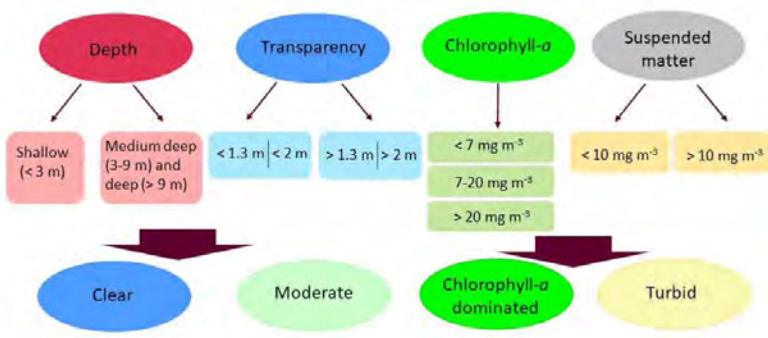
The first step included raw raster data conversion to actual points of chlorophyll-*a* concentrations. Further vector data was modelled using IDW tool via ArcMap 10.8.1 software to better distinguish hot spots of potential blooming zones. Such approach allowed not only getting maps of spatial distribution of chlorophyll-*a* concentrations throughout different months and days but also precise calculations. In this way it is possible to distinguish hot spots of cyanobacteria blooms where harvesting would be the most effective.

Analysis of the concentrations of chlorophyll-*a* shows that in year 2018 the highest mean and maximum values of concentrations were distributed in zones 5 and 6 of Lithuanian part of Curonian Lagoon. The highest mean and maximum concentrations of chlorophyll-*a* were observed at September (max of mean = 169.5 mg $m^{-3}$ and max = 322.0 mg $m^{-3}$). The trend of concentrations between zones remained quite stable till the end of September, while during October these values distributed unevenly. Probably due to the change of meteorological conditions which leads to form different distribution of currents in the Curonian Lagoon. Nevertheless, the analysis of minimum concentrations of chlorophyll-*a* proved the highest possibility of distribution of potential cyanobacteria blooms in 5 and 6 zones of the Curonian Lagoon as lowest concentrations of chlorophyll-*a* distributed in the rest of the zones with minimal exceptions.

The Sentinel data obtained and analyzed for 2019 complemented and proved the same period as well as zones with the potentially highest concentrations of chlorophyll-*a*. Data of 2019 also showed that the 5 and 6 zones of the Curonian Lagoon experienced the highest risk of cyanobacteria blooms (concentrations of chlorophyll-*a*: max of mean = 59.3 mg $m^{-3}$ and max = 200.5 mg $m^{-3}$).

According to the analysis of Sentinel data the most relevant period for cyanobacteria harvesting in the Curonian Lagoon could be from the end of July till the first part of November as the Sentinel data showed that even at the end of October of 2018 the concentrations of chlorophyll-*a* can reach maximum of 263.7 mg m$^{-3}$ (90.2 mg m$^{-3}$ in average).

### 2.3. Water quality parameters assessment in Lithuanian lakes using remote sensing and machine learning

| | | | |
|---|---|---|---|
| **Project** | *PhD studies* | | |
| **Funded** | Vilnius University PhD study programme | | |
| **Duration** | 2018 - 2022 | **Website** | www.hkk.gf.vu.lt/en/ |
| **Implementers** | Dalia Grendaitė, supervisor Edvinas Stonevičius | | |
| **Graphical abstract** |  | | |



Remote sensing data help to observe water bodies in large areas frequently. However, remote sensing data come with uncertainties. The largest ones are from atmospheric influence and accuracy of biophysical or geophysical parameter retrieval algorithms. To be able to use remote sensing data reliably and effectively we need well performing atmospheric correction (AC) and accurate parameter retrieval algorithms. The analysis of various AC algorithms (Acolite, Acolite Rayleigh, iCOR, Sen2Cor, C2RCC, C2X, and POLYMER) for chlorophyll-*a* concentration retrieval showed high uncertainty of reflectance values related to AC product selection (Grendaitė & Stonevičius 2021). Nevertheless, some chlorophyll-*a* algorithms (band difference algorithms) were less dependent on the AC product selection and provided closest to *in situ* chlorophyll-*a* concentrations regardless of the AC product selected. In addition, since most algorithms and AC products show relatively higher bias for low chlorophyll-*a* concentrations (<10 mg m$^{-3}$), deriving algorithms for groups of lakes may improve the chlorophyll-*a* concentration retrieval from Sentinel-2 MSI data. We classify lakes to water types based on often routinely measured in monitoring programmes water quality parameters, such as, chlorophyll-*a* concentration, water transparency, and suspended matter. We aim to create a data-driven model that use lake spectra from satellite and machine learning methods and assign a lake a class that is defined by water quality parameters. This method could be used in areas where *in situ* spectral data is scarce. We test machine learning algorithms (logistic regression, support vector machine, random forest, adaboost, xgboost) to separate lakes into four classes: clear, moderate, turbid based on chlorophyll-*a* concentration, and turbid due to other reasons. Machine learning model inputs are derived from lake spectra (reflectance amplitude, band ratios, the derived water colour parameter). The separated classes are used for construction of water parameter retrieval algorithms from satellite data. The developed class of lakes and parameter algorithms can be tested and adapted to other regions.

### 3. Demonstrations of unmanned aircrafts

| **Antanas Gedvilas**, the President of the Unmanned Aircraft Association | |
|---|---|
| **Graphical abstract** |  |

During the workshop fixed wing original aircrafts were introduced, their characteristics, advantages over drones and the examples of their application in the various types of assessment were discussed.

## CONCLUSIONS

The discussion of results obtained after application of remote sensing vehicles during the Workshop revealed their important role in time-saving and better orientation for observation and selection of sampling sites despite the different technical challenges that yet have to be faced in each specific study.

## CONFLICT OF INTERESTS

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

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## References

Ferrarin C., Razinkovas A., Gulbinskas S., Umgiesser G. & Bliudžiute L. 2008. Hydraulic regime-based zonation scheme of the Curonian Lagoon. - Hydrobiologia 611: 133–146. https://doi.org/10.1007/s10750-008-9454-5

Grendaitė D. & Stonevičius E. 2021. Uncertainty of atmospheric correction algorithms for chlorophyll α concentration retrieval in lakes from Sentinel-2 data. - Geocarto Int. https://doi.org/10.1080/10106049.2021.1958014

Li J., Pei Y., Zhao S., Xiao R., Sang Z. & Zhang C. 2020. A review of remote sensing for environmental monitoring in China. - Remote Sens. 12 (7): 1130. https://doi.org/10.3390/rs12071130

Mertikas S. P., Partsinevelos P., Mavrocordatos C. & Maximenko N. A. 2021. Chapter 3 - Environmental applications of remote sensing. - In: Abdel-Mohsen O. M., Paleologos E. K. & Howari F. M. (Eds), Pollution assessment for sustainable practices in applied sciences and engineering, Butterworth-Heinemann, 107–163. https://doi.org/10.1016/C2015-0-06451-6

Norzailawat M. N., Abdullah A. & Hashim M. 2018. Remote sensing UAV/drones and its applications for urban areas: a review. - IOP Conf. Ser.: Earth Environ. Sci. 169: 012003. doi:10.1088/1755-1315/169/1/012003

Vaičiūtė D., Bučas M., Bresciani M., Dabulevičienė T., Gintauskas J., Mėžinė J., Tiškus E., Umgiesser G., Morkūnas J., De Santi F. & Bartoli M. 2021. Hot moments and hotspots of cyanobacteria hyperblooms in the Curonian Lagoon (SE Baltic Sea) revealed via remote sensing-based retrospective analysis. - Sci. Total Environ. 769: 145053. https://doi.org/10.1016/j.scitotenv.2021.145053

WHO 2021. Guidelines on recreational water quality, Volume 1 Coastal and Fresh Waters. World Health Organization, Geneva. Licence: CC BY-NC-SA 3.0 IGO, 138 pp.

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ГОДИШНИК НА СОФИЙСКИЯ УНИВЕРСИТЕТ „СВ. КЛИМЕНТ ОХРИДСКИ“

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# REPORT ON THE MEETING OF COST ACTION “APLICATIONS FOR ZOOSPORIC PARASITES IN AQUATIC SYSTEMS” (CA20125 PARAQUA), LARNACA, CYPRUS

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**Abstract.** This short paper reports on the meeting of the COST Action “Applications for zoosporic parasites in aquatic systems” (CA20125 ParAqua, 02.11.2021-30.10.2024) held from 4th to 7th of July 2022 in Larnaca, Cyprus. The meeting was organised by I.A.CO Environmental & Water Consultants Ltd and was an opportunity to discuss the progress of the Action, to program the activities for the next year and to undertake collaborative work for the implementation of the scientific objectives of the Action.

**Key words:** cross-sectoral collaboration, European network, microalgal biotechnology

COST stands for European Cooperation in Science and Technology, an EU-funded program to support cross-sectoral and interdisciplinary research networks in Europe and beyond. In this context, the COST Action ParAqua, “Applications for zoosporic parasites in aquatic systems”, aims to organise and coordinate an innovative and dynamic European network, connecting academia, industry and water management authorities to advance and apply knowledge and expertise on aquatic fungi and fungi-like parasites and the relation with their hosts in natural and

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industrial systems. The Action was recently introduced on the COST News (https://www.cost.eu/aquatic-parasites-paraqua/) and more information can be found on the Action webpage (paraqua-cost.eu).

Currently, zoosporic parasites represent a major threat for microalgal biotechnology and industrial production (Carney & Lane 2014). Microalgal biotech plays an important role in the Bioeconomy Strategy and Action plan for a sustainable economy outlined by the European Commission and represent a very high potential for an economy based upon renewable resources. Like any other living organism, however, algae are vulnerable to parasitic infections. Parasitism is the most common consumer strategy (Lafferty et al. 2008), and a large number of biotic interactions are driven by parasites such as viruses, pathogenic bacteria and parasitic eukaryotes (Kagami et al. 2014, Tijdens et al. 2008). ParAqua will compile and share knowledge on the occurrence of zoosporic parasites and their relationships with hosts, elucidate drivers and evaluate impacts of parasitism in aquatic environments and algal biotech production. The aim is to implement new tools that can be used to promote better and safer production, such as methods for monitoring and early detection, or strategies of mitigating parasitic infections. But not only. ParAqua will also explore ways for valorisation of aquatic parasites for the production of essential biomolecules and advocate the importance of diversity and full ecosystem approaches to promote food web stability and resilience in aquatic systems and introduce zoosporic parasites as bioindicators for ecosystem health.

Of the 38 COST Members countries, 24 are already represented in the ParAqua Action Management Committee (Albania, Austria, Bosnia and Herzegovina, Bulgaria, Croatia, Cyprus, Estonia, Finland, Germany, Hungary, Iceland, Italy, Latvia, Montenegro, The Netherlands, The Republic of North Macedonia, Poland, Portugal, Serbia, Slovenia, Spain, Switzerland, Turkey, United Kingdom), together with Israel which is a Cooperating Member and has full rights to participate in the COST action. Representatives of Romania, Czech Republic, Egypt, Azerbajan and Japan are also Action participants as Working Group Members.

Four interconnected working groups were established to organise the scientific activities: WG1, Occurrence and early detection of zoosporic diseases in natural and artificial aquatic systems (Leader Albert Reñé and Co-Leaders Laura Garzoli and Andrea Tarallo); WG2, Drivers underlying the dynamics of zoosporic diseases in algal biotech and natural systems (Leader Ivana Trbojevic and Co-Leader Hans-Peter Grossart); WG3, Control strategies and valorisation of research for application (Leader Gabriel Acien Fernandez and Co-Leader Maja Berden Zrimec) and WG4, Integration and Dissemination (Co-leader Miloš Stupar). Together with the Working Group, the Action Committees assure the good functioning of the Action in several aspects, including providing guidance and advice for inclusive communication within the Network (Moderators Committee Leader Bastiaan Ibelings, Co-Leader Dedmer van de Waal), coordinating the scientific communication (Science Communication Coordinator Ana Gavrilović and Co-Leader Füsun Akgül) and oversee the good functioning of the Action Grants awarding to support the mobility of Researchers and Innovators (Grant Awarding Coordinator Kristel Panksep and Co-Leader Veljio Kisand). The Action Chair is Serena Rasconi and the Vice-Chair is Alena Gsell.

The Action is constantly growing, one of the main capacity building goals is to establish an active network of representatives from academic research, biotech and water management, where each sector brings their own experience and expertise and achieves a strong representation throughout Europe and beyond. These meetings are an important opportunity for Action Members to discuss progress and plan common activities, but also a good chance to expand the network by inviting local and external participants potentially interested to join the Action.

The four-day hybrid conference was held in Cyprus from July 4 to 7 2022 at the Multifunctional Center for Social Activities and Welfare of the Larnaka Municipality. The Local Organiser was I.A.CO Environmental & Water Consultants Ltd, an industrial network member specialised in environmental surveys and consultancy.

The first meeting day was dedicated to the Action Management Committee, during which the Action Chair, the Working Group Leaders and the Committees Coordinators reported to the Management Committee and to Action Members the Action progress and started discussing about the goals and activities for the next year. The following days were dedicated to the WG1 and 2 workshop and meeting, during which the participants worked on the topics "Zoosporic parasites interactive database" and "Needs and solutions for zoosporic parasites detection".

Participants from a number of countries attended on site (**Fig. 1a, b, c**) (Bulgaria, Italy, Croatia, Poland, North Macedonia, Spain, Serbia, Iceland, France, Switzerland and Cyprus) and 33 participants attended online (**Fig. 1d, e**) (from Albania, Switzerland, Cyprus, Germany, Egypt, Spain, Estonia, Finland, France, Hungary, Israel, Montenegro, Netherlands, Poland, Portugal, Serbia, Slovenia and UK).

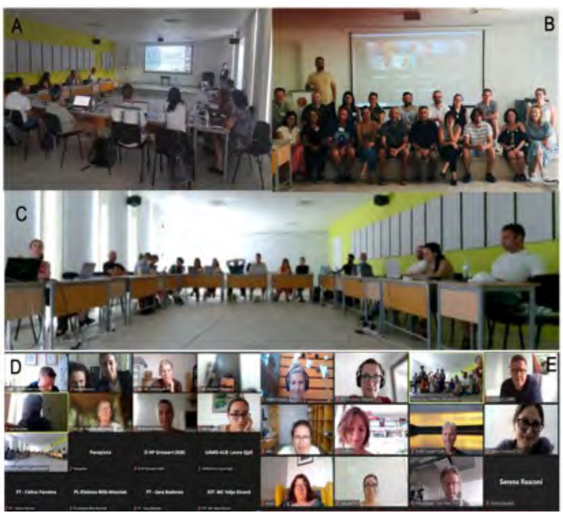


**Fig. 1.** Management Committee meeting and WG1 and 2 workshop of the COST Action ParAqua (CA 20125): **a**, **b**, **c** - participants at the Multifunctional Center for Social Activities and Welfare of the Larnaka Municipality, Larnaca, Cyprus; **d**, **e** - participants on the virtual session.

During the conference, a site visit was organised to the Wastewater Treatment Plant of the Sewerage Board of Larnaka (**Fig. 2**). The Wastewater Treatment Plant covers the Larnaca district and uses the sewage for producing two end products for reuse. Purified water is returned free of charge to farmers for irrigation purposes, and the remaining solids are compressed into pellets and used as fertiliser. First, the Plant was presented by Mr Haris Papanikolaou (Laboratory Technician) and Ms Ioanna Ioannidou (Chemical Engineer) (**Fig. 2a, b**), illustrating the purpose of the infrastructure and its importance to the community. After that, the participants visited the model of the infrastructure (**Fig. 2c**) and toured the premises and the tanks (**Fig. 2d**) where different stages of the treatment take place, with interesting information about the process.


**Fig. 2.** Site visit at the Wastewater Treatment Plant of the Sewerage Board of Larnaka: **a**, **b** - presentation by Mr Haris Papanikolaou (Laboratory Technician) and Ms Ioanna Ioannidou (Chemical Engineer); **c, d** - the participants visited a model of the infrastructure and toured the premises and the tanks.

The following days the conference was dedicated more specifically to collaborative work on the ParAqua research coordination objectives. The first workshop day was dedicated to "Assemble and integrate all available information on occurrence and drivers of zoosporic parasites into an interactive database". The workshop was chaired by Ilaria Rosati and Andrea Tarallo (CNR Italy), which are in charge of several management aspects within LifeWatch Italy (the Italian node of LifeWatch ERIC) and linked the Action with the prestigious European Research Infrastructure Consortium. They will coordinate the activities to collect and organise the data provided by the Action participants for the implementation of the database to gather and synthesise existing information on zoosporic parasites and make them available to the end users through a user-friendly interface. After an informative introduction about LifeWatch and the e-Science Infrastructure for Biodiversity and Ecosystem Research (**Fig. 3a**), the collaborative work started with a discussion on the data resources available from the members. The afternoon session was dedicated to create database templates based on the resources to be included and how these will be organised in the database interface (**Fig. 3b**).

The second day of the workshop was dedicated to the detection methods of the zoosporic parasites and to discuss solutions for most effective, feasible and cost-effective techniques for the early detection of zoosporic parasites in natural and artificial ecosystems. In the morning, the program included presentations from the Action members which provided an overview of the current methods used for investigations on zoosporic parasites. The Action organised in May a Training School on “Detection and identification of zoosporic parasites” hosted by Hans-Peter Grossart at IGB in Germany. The lectures from the Training School are available on the ParAqua Channel (https://www.youtube.com/channel/UC0tL1TyHqKCN8XY6IAFqtVw/playlists). The workshop was the continuation of the Training School, where participants were invited to present their experience regarding the learned methods and share feedback on their application for parasites investigations. The session started with the microscope methods, Pini Marco presented his experience in assessing parasites in algal cultures (Marco, 2022, **Fig. 4a**). Géza Selmeczy presented his experience with staining and video analysis (Selmeczy, 2022) and Slawek Cerbin shared his experience on apply image analysis in zoosporic parasite research and metabarcoding (Cerbin, 2022. **Fig. 4b**). The session continued then with a focus on molecular methods, Oliver Barić presented a nice protocol to use Next-Generation Sequencing (NGS) for identification of parasites in cultured microalgae (Barić et al. 2022, **Fig. 4c**) and Athina Papatheodoulou the application of DNA metabarcoding methodology in the European project Watdimon (Papatheodoulou, 2022). The sessions concluded with presentations on perspectives and applications of methods. Blagoy Uzunov presented opportunities for implementation of the knowledge acquired during the TS in Neuglobsow in Bulgarian projects (Uzunov, 2022, **Fig. 4d**), Miloš Stupar explored ways to apply the lessons from Stechlinsee in limnological research in


**Fig. 3.** WG1 and 2 workshop on the database construction: **a** - Andrea Tarallo presents the LifeWatch infrastructure and the Data Management; **b** - the participants during the working session on the database implementation.

Serbia (Stupar & Trbojević, 2022, **Fig. 4e**) and Jovica Leshoski shared some ideas on possibilities and prospects in a start-up lab, notably for molecular methods (Leshoski, 2022, **Fig. 4f**).

Two local Cyprus Government officials were invited and participated in the workshop on the techniques for the detection of zoosporic parasites, M. Lavrendios Vasiliades from the Department of Fisheries and Marine Research and M. Iakovos


**Fig. 4.** WG1 and 2 workshop on zoosporic parasites detection methods: **a**-**f** - presentations from the participants; **g**, **h** - collaborative writing session on the methods booklet.


**Fig. 5.** Impressions from the guided visit of the city: **a** - group picture at the Saint Lazarus church; **b** - details of a typical street and buildings in Larnaca; **c** - the medieval castle; **d**, **e** - the pottery studio and the prehistoric (3000 B.C.) cross-shaped idol symbol of the island art.

Tziortzis from the Water Development Department. The invited participants appreciated the well organised conference and were interested to obtain more information on the topic of algae parasites to explore opportunities to apply the presented methods to their needs.

The afternoon session was dedicated to collaborative writing (**Figs. 4g-h**) to create a booklet of methods and techniques for end-users to conduct research on zoosporic parasites. The booklet will be published open access and will contain protocols, recommendations and practical hints on techniques for identification and quantification of parasites infections and will serve as guideline for early detection and monitoring methods.

During the meeting, the participants had the opportunity to visit the city of Larnaca with a walking tour of the downtown guided by M. Demetriou Demetris, from the Municipality tourist office. Cyprus has a long history, it is one of the oldest civilisations in the Mediterranean and its position at the crossroad of three continents made the island a unique mosaic of different cultures. The visit started at the Saint Lazarus church (**Fig. 5a**), founded in the 9th century, then the visit moved along the Foinikoudes promenade (**Fig. 5b**) to the medieval castle (**Fig. 5c**), built in the 12th century to protect the harbour of the town. The visit ended at the ceramics and pottery studio (**Fig. 5d**), where the group was able to observe some homemade exhibitions of museum replicas of the prehistoric and ancient (3000 B.C.) world of Cyprus (**Fig. 5e**).

After the successful meetings, the ParAqua participants continued and expanded networking experiencing traditional Cypriot culinary delicacies. Eating in Cyprus is serious business, and usually you can see Cypriots gathered around a table with a couple of plates called meze to snack on. With a rich history, the island has seen a variety of influences, all of which add to the local gastronomy. Halloumi is probably Cyprus' most famous product that is produced by combining a mixture of goat's and sheep milk, before being set with rennet. This is an unusual practice due to the absence of acid-producing bacteria in any part of the process, a standard for most dairy products. Halloumi's high melting point means it can be easily fried or grilled, and is less often served cold. Beside Halloumi, traditional Cypriot foods include souvlakia (grilled meat kebabs), sheftalia (grilled pork), afelia (pork marinated in coriander), cous-cous with Greek yoghurt, pitta bread, kolokasi (root vegetables), different fish dishes, lamb, artichokes, chickpeas, olives and many more...


**Fig. 6.** Part of various meze dishes - **a**, **b**.


**Fig. 7.** Self-exploration of Cypriot food on the first night upon our arrival (**a**). With help of hosts and meeting organisers, I.A.CO, we are getting familiar with rich Cypriot cuisine (**b**, **c**).

In order to taste all these delicacies in a few days, the best way is to order Cypriot traditional meat or fish meze, what could consist of as many as 30 tasting plates (usually 20+) designed for sharing (**Figs. 6a, b**). These are savoury dips and vegetables and a wide variety of fish and meat dishes prepared in several different ways. And of course, these great meals go well with ouzo, local wine or local bear.

This is how we started the exploration of Cypriot food on the first night upon our arrival (**Fig. 7a**). Our hosts and meeting organisers, colleagues and friends from I.A.CO, organised the conference Gala Dinner and helped us in the next days to become even more familiar with this rich cuisine (**Figs. 7b, c**).

## CONFLICT OF INTERESTS

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

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## References


Barić O., Radočaj T., Vardić I. & Gavrilović A. 2022. The use of Next-Generation Sequencing (NGS) for identification of parasites in cultured microalgae. Workshop of the COST Action ParAqua - Applications for zoosporic parasites in Aquatic Ecosystem (CA20125), Larnaca - Cyprus, July 6, 2022.

Carney, L.T. & Lane, T. W. 2014. Parasites in algae mass culture. – Front. Microbiol. 5: 278. https://doi.org/10.3389/fmicb.2014.00278

Cerbin S. 2022. Beginner’s impressions on applying metabarcoding and image

analysis in zoosporic parasites research. Workshop of the COST Action ParAqua - Applications for zoosporic parasites in Aquatic Ecosystem (CA20125), Larnaca - Cyprus, July 6, 2022.
Lafferty, K. D., Allesina, S., Arim, M., Briggs, C. J., De Leo, G., Dobson, A. P., & Thieltges, D. W. 2008. Parasites in food webs: the ultimate missing links. - Ecol. Lett. 11: 533-546.
Leshoski J. 2022. Identification of zoosporic parasites, possibilities, and prospects in a start-up lab. Workshop of the COST Action ParAqua - Applications for zoosporic parasites in Aquatic Ecosystem (CA20125), Larnaca - Cyprus, July 6, 2022.
Kagami, M., Miki, T. & Takimoto, G. 2014. Mycoloop: chytrids in aquatic food webs. - Front. Microbiol. 5: 166.
Marco P. 2022. Lesson from the Training School Microscopy session to assess parasites in algal cultures. Workshop of the COST Action ParAqua - Applications for zoosporic parasites in Aquatic Ecosystem (CA20125), Larnaca - Cyprus, July 6, 2022.
Papatheodoulou A. 2022. Project Watdimon- DNA metabarcoding methodology for European benthic diatoms. Workshop of the COST Action ParAqua - Applications for zoosporic parasites in Aquatic Ecosystem (CA20125), Larnaca - Cyprus, July 6, 2022.
Selmeczy G. 2022. Experience at the TS: staining and video analysis. Workshop of the COST Action ParAqua - Applications for zoosporic parasites in Aquatic Ecosystem (CA20125), Larnaca - Cyprus, July 6, 2022.
Stupar M. & Trbojević I. 2022. Could the lessons from Stechlinsee be applied in further limnological research in Serbia? Workshop of the COST Action ParAqua - Applications for zoosporic parasites in Aquatic Ecosystem (CA20125), Larnaca - Cyprus, July 6, 2022.
Tijdens, M., Van De Waal, D. B., Slovackova, H., Hoogveld, H. L. & Gons, H. J. 2008. Estimates of bacterial and phytoplankton mortality caused by viral lysis and microzooplankton grazing in a shallow eutrophic lake. - Freshwater Biol. 53: 1126-1141.
Uzunov B. 2022. Implementation of the knowledge acquired during TS in Neuglobsow in Bulgarian projects. Workshop of the COST Action ParAqua - Applications for zoosporic parasites in Aquatic Ecosystem (CA20125), Larnaca - Cyprus, July 6, 2022.

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# REPORT ON THE SIXTH INTERNATIONAL “FASCINATION OF PLANTS DAY” (FoPD) AT THE FACULTY OF BIOLOGY OF SOFIA UNIVERSITY "ST. KLIMENT OHRIDSKI"

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**Abstract.** The sixth international “Fascination of Plants Day” (FoPD) was organized on 18th of May 2022 in the Faculty of Biology of Sofia University together with the Agrobioinstitute of the Agricultural Academy. Guests were welcomed by exhibition of plants grown in the Faculty’s greenhouse and educational stand of the student club SKOREC consisting of a rich palette of volunteer activities related to expanding students' knowledge of biodiversity and its conservation. The event aimed at popularization of plant science and its significance for life on Earth. The fascination of medicinal, crop and ornamental plants, as well as microalgae, was discussed in respect to their importance and functional characteristics. Curious facts about plants were demonstrated by practical demonstrations. Students from different degrees, PhDs, MSc, BSc, were involved in visual presentations on research projects funded by Sofia University and Ministry of Education and Science. The overall participation of students was major achievement since this promoted the formation of additional professional competencies in real environment. Moreover, pupils from higher schools participated in a drawing/photo contest organized to draw their attention to the plant world from an entertaining point of view.

**Key words:** Bulgaria, Faculty of Biology, plant science, professional competencies, students

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## INTRODUCTION

### How is organized FoPD worldwide, in Europe and in Bulgaria?

All information about the FoPD plant scientists' initiative can be accessed via www.plantday18may.org. Fascination of Plants Day is supported world-wide by a network of National Coordinators who voluntarily promote and disseminate the activity within their countries and turn it into a success story. To organize an event in your country, one should contact the National Coordinator on the FoPD country page (https://plantday18may.org/countries/). Scientific institutions, universities, botanical gardens, and museums, together with farmers and companies, open their doors.

For 2022, nearly 56 countries joined the 6$^{th}$ edition of FoPD worldwide to offer over 820 plant-based outreach activities for all interested people from toddlers to grandparents (https://plantday18may.org/statistics/). In Bulgaria, FoPD has been organized every two years since 2015 when plant-related institutions organize events to explain facts about plants (Zhiponova et al. 2021).

### What is the significance of plant science today?

The goal of plant scientists is to reveal how important are plants for our life – the air we breathe, the food we eat, for health, for building our homes, for decoration. The significance of plant organisms for the life is sometimes underestimated and we aim to make people enthusiastic and fascinated by plants, as well as to explain the need to make research on them. This year we demonstrated scientific aspects of medicinal, crop and ornamental plants, as well as microalgae, suggesting how these organisms work and how this can be used in practice for food, health, and sustainable agriculture. The research is fundamental, and we try to engage students who are interested to work in this field and help us. Together, seniors and students, we explained strategies that are conducted together with the Bulgarian Academy of Sciences and the Agricultural Academy. We participate and present projects supported by the National Research Fund of Ministry of Science and Education and by the Research Fund of Sofia University.

## RESULTS

### How is organized FoPD in the Faculty of Biology of Sofia University?

The Faculty of Biology of Sofia University participates each time together with the Agrobioinstitute of Agricultural Academy. This year different events were organised (**Table 1**; **Figs. 1-10**). In FoPD participate colleagues interested in animal biology and other directions because plants take place there, too. There is interaction between different disciplines, and we use FoPD to show how fascinating plants are.

Major target groups are students, pupils, children, who we want to ignite attention and educate them to respect nature. We involve young people to participate and present the fundamental data and results in a popular way. In this way we

**Table 1. FoPD events in the Faculty of Biology of Sofia University.** Presentations and practical demonstrations were performed in the Faculty's Lobby. In total, the participants included: 40 BSc, 4 MSc, and 5 PhD students; one pupil from the American College of Sofia; 4 senior researchers from Agrobioinstitute; 15 members of the Faculty of Biology. Nearly 40 children participated in the drawing/photo contest.

| Participating Organization | Information – Topics, Participants and Funding |
|---|---|
| Agrobioinstitute, Agricultural Academy (**Fig. 2**) (**Funding:** EU & National Science Fund of Ministry of Education and Science) | **"LEGUMES AND THEIR IMPORTANCE TO ANIMALS AND HUMANS AND THE ENVIRONMENT"**<br>Prof. Anelia Iantcheva, Assoc. Prof. Mariana Radkova<br>**Project:** "Translating knowledge for legume-based farming for feed and food systems" with the acronym "Legumes Translated" ID 817634, 11.2018-04.2022, funded by the EU;<br>**Project:** "Functional and bioinformatics analyses of GRAS transcription factors related to the response of abiotic and biotic stress in annual (Medicago truncatula) and perennial (Medicago sativa) alfalfa" DN 16/9 from 12.2017-May 2022 funded by the National Science Fund of Ministry of Education and Science<br>**"PHYTOPATHOGENIC FUNGI"** Assist. Prof. Dr. Aneta Lyubenova<br>**"*IN VITRO* PLANTS"** Assist. Prof. Dr. Lilia Georgieva |
| **Green house of Faculty of Biology** (**Fig. 1** and **3**) (**Funding:** EU) | **"PLANTS ARE FASCINATING"**<br>Aneliya Raycheva, MSc (Department of Plant Physiology)<br>**Project:** Center of Competence: BG05M2OP001-1.002-0012-C01, 2018-2023: "Sustainable utilization of bio-resources and waste from medicinal and aromatic plants for innovative bioactive products" funded by Operational Program "Science and Education for Smart Growth" – 2018, co-financed by European Union via European Regional Development Fund |
| **SKOREC (Fig. 4)** | **NATURE CONSERVATION AND EDUCATIONAL CLUB** in the Faculty of Biology, University of Sofia (https://www.facebook.com/groups/170251143796/about) Yana Skordeva, Plamen Petrov, Martina Rangelova, Andrey Kolev, Vyara Ivanova, Yuzelim Komarevska, Miglena Sashkova<br>Supervisor: Assist. Prof. Atanas Grozdanov |
| **Projects of PhD students** (**Fig. 5**) (**Funding:** Fund for scientific investigations of Sofia University "St. Kliment Ohridski") | **"POSSIBILITIES FOR APPLICATION OF PROMISING STRAINS OF MICROALGAE IN THE PROCESSES OF PHYCOREMEDIATION"**<br>Zornitsa Karcheva (Department of Plant Physiology)<br>Supervisor: Assoc. Prof. Ganka Chaneva; Project № 80-10-13/10.05.2022<br>**"AEROTERRESTRIAL ALGAE FROM MEGALITHIC COMPLEXES IN HASKOVO DISTRICT"**<br>Miroslav Ivov Androv (Department of Botany)<br>Supervisor: Prof. Maya Stoyneva-Gärtner; Project № 80-10-47/10.05.2022<br>**"AQUAPONICS AS A PHYTOEFECTOR FOR THE TERRESTRIAL ORCHID *LUDISIA DISCOLOR*"**<br>Alexander Tomov (Department of Plant Physiology)<br>Supervisor: Assoc. Prof. Miroslava Zhiponova; Project № 80-10-69/11.05.2022 |

| **Participating Organization** | **Information – Topics, Participants and Funding** |
|---|---|
| | **“TAXONOMIC CHARACTERISTICS OF STREPTOPHYTES IN THE LIVING ALGAE COLLECTION OF UNIVERSITY OF SOFIA "ST. KLIMENT OHRIDSKI (ACUS)”**<br>Kristian Rosenov Ivanov (Department of Botany)<br>Supervisor: Assoc. Prof. Blagoy Uzunov; Project № 80-10-95/13.05.2022<br>**“INFLUENCE OF LIGHT QUALITY ON THE BIOACTIVE POTENTIAL OF GREEN MICROALGA *COELASTRELLA* SP. BGV”**<br>Zhaneta Georgieva (Department of Plant Physiology)<br>Supervisor: Assist. Prof. Detelina Petrova; Project № 80-10-184/27.05.2022 |
| **Department Biophysics and Radiobiology** (**Fig. 6**) | **“HOW TO TALK WITH PLANTS?”**<br>Prof. Vasiliy Goltsev, Assoc. Prof. Margarita Kouzmanova, Assist. Prof. Momchil Paunov, Assist. Prof. Kolyo Dankov, Assist. Prof. Boyana Angelova |
| **Department of Biology Education** (**Fig. 7**) | **“PHOTOTROPISM”**<br>Tsvetomir Favliyanov (BSc 4y, Geography & Biology)<br>**“PROOF OF THE TRANSPORT OF SUBSTANCES IN THE PLANT ORGANISM”** Alex Atanasov (BSc 4y, Biology and Chemistry); Simona Georgieva (BSc 4y, Geography & Biology)<br>**“MICROSCOPIC OBSERVATION OF PLANT CELLS”**<br>Desislava Trenovska (BSc 4y, Geography & Biology); Katerina Damyanova (BSc 4y, Biology & English Language); Hristina Vylcheva (BSc 4y, Geography & Biology)<br>**“CHLOROPHYLL PRINTING”**<br>Radost Durchova (BSc 4y, Biology and Chemistry)<br>**“HOW TO GROW AVOCADO?”**<br>Viktoria Roseva (BSc 4y, Biology and Chemistry)<br>**“THE BEAUTIFUL VIOLETS”**<br>Pavel Veselinov (BSc 4y, Biology and Chemistry)<br>**“HOME EXPERIMENT WITH PLANTS”** Dimitrina Terzieva (BSc 4y, Biology and Chemistry)<br>**“LET’S RECOGNIZE CONIFERS QUICKLY AND EASILY”** Ivon Ivanova and Rositsa Spasova (BSc 3y, Teacher of natural sciences in the basic level of education)<br>Supervisor: Assoc. Prof. Kameliya Yotovska |
| **Department of Plant Physiology** (**Funding:** Bulgarian National Science Fund of Ministry of Education and Science) (**Fig. 8** and **9**) | **“COULD CATMINT PROTECT US FROM THE MICROBES AROUND US?”** Anna Zaharieva (BSc 3y, Biotechnology), Ana-Maria Nedelcheva (BSc 4y, Molecular Biology), Kalina Simeonova (BSc 4y, Molecular Biology), Muhammed Mohammed (BSc 4y, Molecular Biology), Mihaela Stoyanova<br>Supervisors: Assist. Prof. Detelina Petrova, Assoc. Prof. Miroslava Zhiponova<br>**“CATNIP – A NATURAL PROTECTOR OF THE HUMAN BODY”** Desislava Prinareva, Daniel Petkov, Yordan Ilinski, Kiril Stamboliyski, Vasil Stamenov, Vanesa Ivanova (all BSc 4y, Molecular Biology), Antoana Tsekova (scholar in American College of Sofia)<br>Supervisors: Assoc. Prof. Miroslava Zhiponova, Assist. Prof. Detelina Petrova |

| Participating Organization | Information – Topics, Participants and Funding |
|---|---|
| | **“INVESTIGATION OF THE EFFECT OF EXOGENOUS CYTOKININS ON THE ACTIVITY OF ENZYMATIC ANTIOXIDANTS IN IN VITRO CULTURED CATMINT (*NEPETA NUDA* L.)”** Alexandra Stoyanova, Desislava Stanoeva, Iva Varbacheva, Laura Yankova, Mario Marinov, Simona Svetlinova, Slaveya Kostadinova, Tsvetan Tsvetanov (all BSc 4y, Molecular Biology)<br>Supervisors: Assoc. Prof. Miroslava Zhiponova, Assist. Prof. Detelina Petrova<br>**“WHY DO CATS GO CRAZY OVER CATNIP (*NEPETA NUDA* L.)?”** Gabriela Kalafirova & Margarita Popova (BSc 3y, Molecular Biology)<br>Supervisors: Assist. Prof. Marieta Hristozkova, Assist. Desislava Mantovska<br>**“NEPETA NUDA FOR NATURAL COSMETICS”** Stefani Petrova, Tereza Georgieva, Alisa Marinkov, Vesela Balabanova-Bozushka (MSc 1y, Plant Biotechnology)<br>Supervisor: Assoc. Prof. Zhenya Yordanova<br>**Project:** № KP-06-N56/9/12.11.2021 “Interdisciplinary biotechnological approach for analysis and modulation of the biological potential of the medicinal plant Nepeta nuda” funded by Bulgarian National Science Fund and by the Ministry of Education and Science of Bulgaria<br>**Contributors:** Products including *N. nuda* were designed by the support of My Lavanda Skincare Essentials (https://www.facebook.com/MyLavanda/; https://www.instagram.com/mylavanda_bg/), Chocolaterie (https://chocolaterie.org/), Advertising agency Staks (www.staks.net), BaBka.handknitting (https://www.instagram.com/babka.handknitting/). |
| **Kids’ Corner (Fig. 10)** | Art place for children to create and socialize. |
| **CONTEST** | **Participants:**<br>(uploaded photos and paintings at https://plantday2015bg.wordpress.com)<br>• 19 High School "Elin Pelin", Sofia<br>• 18 High School "William Gladstone", Sofia<br>• 22 High School "G.S.Rakovski", Teacher: Maya Kenardzhieva<br>• High School "Britanica", Teacher: Tsvetelina Ivanova<br>• Second English Language High School "Thomas Jefferson", Teacher: Sevdalina Stoyanova<br>• Sofia Vocational High School "John Atanasov", Teachers: Nevenka Kostova, Elena Panayotova, Hristo Vasilev |
| **FoPD Links** | e-mail in Faculty of Biology: rastenia_bg@abv.bg; official site: https://plantday18may.org/;<br>Faculty of Biology site: https://www.uni-sofia.bg/index.php/bul/universitet_t/fakulteti/biologicheski_fakultet2/novini/den_na_ocharovanieto_na_rasteniyata_18_maj_2022_g<br>National Student Televison Alma Mater, Sofia, Report: https://www.youtube.com/watch?v=pMH_ZPa2tVs&ab_channel=AlmaMater |

engage the young people themselves who are part of our projects, which helps them to assimilate plant science and to get motivated and inspired for work with plants. We intend to describe fundamental data in a popular way with the aim to include BSc, MSc and PhD students who present mainly. The academical mentors are just to support and orientate the presentations and demonstrations.


**Fig. 1.** FoPD in the Faculty of Biology: **a** – Faculty's lobby; **b-d** – demonstration of ex vitro adaptation of petunia plant by the Department of Plant Physiology.

### By FoPD, a contest was organized for children

In this contest, major role have the colleagues from Department of Biology Education thanks to who we managed connection with teachers (**Table 1**). In this way we manage to engage early kids who are interested in nature and in plants. When we invite them for participation in FoPD, they also attend the organized activities with plants and animals and get excited. The contest itself did not aim to restrain kids' creativity but to give the freedom to express themselves. This year, the topic of the contest was "Plants – funny and comic", where nearly 40 children from different age groups took part. Students also participated. So, we feel satisfied with the attendance, which was very active and interesting for us, too.

### Educational focus – the competence approach in higher education

One of the main challenges for higher education is related to the quality of education and training of competent and competitive professionals. The expected

**Fig. 2.** Agrobioinstitute presents projects funded by EC and National Science Fund of Ministry of Education and Science: **a**, **b** – *in vitro* plants; **c** - phytopathogenic fungus; **d** – legumes; **e**, **f** – students as researchers. The research topics were illustrated with presentations, demonstration materials, posters, and flyers. A questionnaire on the importance of legumes was prepared for the participants in the event. For all those who filled in the questionnaire correctly, there was also a prize-prepared booklet with recipes for legumes.

results of higher education are described as a set of knowledge, skills and/or competencies acquired by the individual, which he is able to demonstrate after completing his studies. The competence approach is oriented towards achieving these goals (as expected results). The competence approach is related to the new educational paradigm and its application leads to improving the quality of education by making it practically oriented. The application of the competence approach in training changed the learning process from the statistical concept of acquiring knowledge to curriculum to the dynamic perception of competence as a complex of knowledge, skills and attitudes that develop and enrich throughout life.


**Fig. 3** (above). Greenhouse of the Faculty of Biology supported by EU fund of Center of Competence: **a-d** – ornamental and medicinal plants; **b, c** – *in vitro* plants.

**Fig. 4** (on the right). SKOREC – nature conservation and educational club: **a-e** - BSc students from SKOREC with art collection. SKORETC presented its traditional educational stand consisting of a rich palette of volunteer activities related to expanding students' knowledge of biodiversity and its conservation. The stand showed a rich exhibition of different types of plants painted by students and turned into posters and stickers. The student volunteers promote the activities of the club and demonstrate the achievements to the new students and the guests of the event.


A
B
C
D
E
КЛУБ СКОРЕЦ НА БИОЛОГИЧЕСКИЯ ФАКУЛТЕТ – ДЕСЕТ ГОДИНИ НАУКА И ОБРАЗОВАНИЕ В ЗАЩИТА НА ПРИРОДАТА


**Fig. 5.** PhD students presenting projects supported by Fund for scientific investigations of Sofia University "St. Kliment Ohridski": **a-e** – PhD students from the Departments of Botany and Plant Physiology exploring the microalgae's world; **c** – aquaponics used in PhD student projects; **f** – BSc students interested in microalgae.


**Fig. 6.** "HOW TO TALK WITH PLANTS?": **a-e** – members of the Department of Biophysics and Radiobiology reveal scientific approach to understand plants' state.


**Fig. 7.** BSc students from binary specialities: **a-e** – BSc students and the Department of Biology Education reveal plants' fascination by attractive demonstrations.

**Fig. 8.** Fascination of the catmint *Nepeta nuda* fundamental studies: **a-f** – BSc students from the specialities Molecular Biology and Agrobiotechnology and the Department of Plant Physiology reveal the properties of the medicinal plant *Nepeta nuda* such as antibacterial, antioxidant, attractant for cats, application in cosmetics; **g-j** – *Nepeta nuda in vitro* and *ex vitro* plants, and extracts. The project is funded by the National Science Fund of Ministry of Education and Science.


**Fig.9.** Catmint *Nepeta nuda* products for beneficial life: **a** – cosmetic cream (supported by My Lavanda Skincare Essentials https://www.facebook.com/MyLavanda/; https://www.instagram.com/mylavanda_bg/); **b** – chocolates (supported by Chocolaterie https://chocolaterie.org/); **c** – disinfectants (lab-made); **d-f** – cat toys (BaBka.handknitting (https://www.instagram.com/babka.handknitting/).

## Educational focus – the competence approach in higher education

One of the main challenges for higher education is related to the quality of education and training of competent and competitive professionals. The expected results of higher education are described as a set of knowledge, skills and/or competencies acquired by the individual, which he is able to demonstrate after completing his studies. The competence approach is oriented towards achieving these goals (as expected results). The competence approach is related to the new educational paradigm and its application leads to improving the quality of education by making it practically oriented. The application of the competence approach in training changed the learning process from the statistical concept of acquiring knowledge to curriculum to the dynamic perception of competence as a complex of knowledge, skills and attitudes that develop and enrich throughout life.


**Fig. 10.** Kids' corner: **a-e** – kids are introduced to plant art techniques.

The competence approach in higher education requires the formation of professionals in the following skills: knowledge of basic principles of functioning of the enterprises, develop creative thinking, skills of work in team, also such human qualities as self-awareness and self-esteem, etc. (Le Deist & Winterton 2005; Romanovtseva 2016). The application of the competence approach in higher education is determined by important socio-economic, social, and pedagogical prerequisites. The global preconditions include: the development of technological innovations in products and processes, as well as demographic changes that increase the importance of adaptive learning and work-based learning; the need to replace traditional educational models with results-related utility models; the lifelong learning strategy; initiatives for defining and validating competencies acquired through non-formal and informal learning; the need to improve the skills and qualifications of the workforce and to promote labor mobility by building common reference levels of professional competence; new technologies and complex problems in today's society, which require the creation of multidisciplinary teams of experts with world-class competencies (Song & Zhou 2021; Khutorskoy 2018; Lans et al. 2014).

The substantiated theoretical prerequisites for the use of the competence approach in the preparation of students are an adequate reaction to the change in the values of young people and the attempts to overcome the philosophical and psychological contradictions in modern education. Values are a necessary complement to knowledge because they structure and hierarchize goals and knowledge. The acquisition, transformation and use of knowledge is an active process, therefore, a prerequisite for the formation of emotional-volitional and motivational components of competence is the active position of the student (Gaybullaevna & Jonpulatovna 2021; Paulsen 2013).

## CONCLUSION

Competence is the achievement of compliance between the available cognitive resources of the individual and the requirements of the real environment. In the context of the formation of professional competencies is the participation of students and PhD students in this year FoPD. The young biologists were involved to get enthusiastic about science and to make other students and guests interested, too.

## CONFLICT OF INTERESTS

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


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We thank to our precious contributors: My Lavanda Skincare Essentials (https://www.facebook.com/MyLavanda/, https://www.instagram.com/mylavanda_bg/), Chocolaterie https://chocolaterie.org/), Advertising agency Staks www.staks.net, BaBka.handknitting (https://www.instagram.com/babka.handknitting/), National Student Televison Alma Mater.


## AUTHOR CONTRIBUTIONS

M.K.Z. and K.S.Y designed and wrote the manuscript, and A.V.I. contributed in adding information and improving the manuscript.

## References

Gaybullaevna D. N. & Jonpulatovna S. M. 2021. Innovative approach to education is a factor for developing new knowledge, competence and personal qualities. - Asian Journal of Multidimensional Research 10: 148-153.

Khutorskoy A. V. 2018. Methodological foundations for applying the competence approach to designing education. - Vysshee obrazovanie v Rossii = Higher education in Russia 12: 85-91.

Lans T., Blok V. & Wesselink R. 2014. Learning apart and together: towards an integrated competence framework for sustainable entrepreneurship in higher education. - Journal of Cleaner Production 62: 37-47.

Le Deist F. D. & Winterton J. 2005. What is competence? - Human resource development international 8: 27-46.

Paulsen M. (Ed.) 2013. Higher Education: Handbook of Theory and Research. Vol. 28. Springer, New York, 734 pp.

Romanovtseva O. V. 2016. The competence approach in education. - Contemporary Problems of Social Work 2: 83-91.

Song H., & Zhou M. 2021. STEM teachers' preparation, teaching beliefs, and perceived teaching competence: A multigroup structural equation approach. - Journal of Science Education and Technology 30: 394-407.

Zhiponova M. K., Yotovska K. S. & Iantcheva A. V. 2020. Fascination of plants day (FoPD) – reality and tradition in Bulgaria. - Annual of Sofia University "St. Kliment Ohridski" Faculty of Biology Book 2 – Botany 104: 85-102.

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# CONGRATULATION TO PROF. ELISSAVETA BOZILOVA AND ASSOC. PROF. STEFAN DRAGANOV ON THE OCCASION OF THEIR 90$^{TH}$ BIRTHDAYS

On the occasion of their 90$^{th}$ birthdays the Editorial Board congratulates Prof. Elissaveta Bozilova, DrSc and Assoc. Prof. Stefan Draganov, PhD respectful scholars and teachers in the Department of Botany of Sofia University and, respectively, former Editor-in-Chief and Secretary of the Editorial Board of the Book 2-Botany of the Annual of Sofia University!

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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.

**Original papers covering the entire field of scientific botany and mycology with a worldwide geographical scope** are published with special encouragement to the papers of students and young scientists. Five categories of contributions are published: 1) Research articles; 2) Review articles (invited or published with the editors‘ consent); 3) Short communications; 4) Book reviews; 5) Information about scientific events, past or forthcoming or, preferably, overview of the topics and contributions of the scientific meetings, as well as obituaries.

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Example:

* *corresponding author*: M. P. Stoyneva – Sofia University "St. Kliment Ohridski", Faculty of Biology, Department of Botany, 8 Blvd. Dr. Tsankov, BG-1164, Sofia, Bulgaria; mstoyneva@uni-sofia.bg

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 transliterated 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*.

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The **Acknowledgments** may be inserted at the end of the text, before the literature references. Their correctness and ethics are total responsibility of the authors.

**References** to the works cited are given **in the text** by the name of the author and the year of publication, *e.g.* Ivanov (1971), or (Ivanov 1971) and Ivanov & Petrov (1942) or (Ivanov & Petrov 1942), respectively. When more authors have to be cited in brackets semicolons between them should be used and works should be listed in chronological (not in alphabetical!) order, *e.g.* (Ivanov & Petrov 1942; Ivanov 1971; Babov 1987). In case of unchanged citation certain pages of a paper referenced should be indicated as follows: Ivanov 2013: 149–150, or Ivanov 2013: 169. The abbreviated citation et al. should be used in the text only in cases where three and more authors are involved, *e.g.* Ivanov et al. (1971), or (Ivanov et al. 1971). In case of editor/editors, they have to be indicated only in the reference list, but not in the text of the paper (*e.g.* Petrov (2013) in the text and Petrov I. A. (Ed.) 2013....in the references). In the same way, in the references, but not in the text, the Editor in chief is abbreviated as Ed-in-Chief and Compiler/Compilers are abbreviated as Comp. and Comps respectively (*e.g.* Petrov I. P. (Ed-in-Chief) 2013; Petrov A. V. (Comp.) 2015; Petrov A. V. & Draganov I. P. (Comps) 2017).

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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.

When the cited paper/chapter occupies only one page, it should be written as follows:

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.

When the cited paper/abstract/summary occupies only one page, it should be written as follows:

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*, Primorsko, Bulgaria, 8-12 May 2017, p. 36.

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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**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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