

Notes on the fungal diversity in the Botanical Garden of the National Museum of Bosnia and Herzegovina

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Abstract: Over the past three years, initial mycological studies have been carried out in the Botanical Garden of the National Museum of Bosnia and Herzegovina. As a result of these sporadic surveys, 22 different species of fungi were found, including *Octospora pseudoampezzana*, *Xylaria oxyacanthae*, *Orbilia carpoboloides* and *Calathella eruciformis*, which were recorded for the first time in Bosnia and Herzegovina. The aim of this study is to initiate scientific research on fungal occurrence and diversity in this urban floral oasis and thus also to improve national knowledge about the fungal species that inhabit such anthropogenic habitats. This work can also serve as an additional argument to emphasise the importance of botanical gardens in urban areas, and their contribution to the diversity of mycobiota in anthropogenic habitats in general.

Key words: mycobiota, urban areas, botany, Sarajevo

INTRODUCTION

Although man-made green spaces in the urban environment are mostly represented by parks and other landscaped green areas, whose primary functions are to provide quality space for recreation and relaxation, without any particular interest for maintaining a high level of biodiversity, there are also botanical gardens formed with the opposite intention, i.e. designed to create small biodiversity oases deep within urban centres.

Botanical gardens have multiple purposes in everyday life: They can represent important refuges for the survival of certain highly endangered endemic and/or rare plant species; they provide great opportunities for multidisciplinary scientific research that could provide a better understanding of phyto-diversity in general; they are a great place for education *in situ*; they provide a perfect setting for daily relaxation, which is beneficial for both physical and mental health. They can also be a useful tool in biosecurity research by serving as sentinel monitoring sites to detect threats from pathogens and pests (Wondafrash et al., 2021). Their high biological and conservational importance is indicated by the fact that 31% of the total number of accepted and currently known plant species grow in botanical gardens all over the world (Antonelli et al., 2020).

Alongside trees and plants, botanical gardens are home to other closely associated organisms that are usually much less investigated than the flora itself. Such other organisms occasionally become subject of inventory primarily on the initiative of employed scientists or in the context of regular institutional project activities (see, for example, Glushakova & Kachalkin, 2017 for yeasts; Matveev et al., 2018 for slime moulds; Wilkie, 2020 for lichens; Cathrine et al., 2022 for arachnids, etc.).

The real value of inventory studies within botanical gardens largely lies in the fact that they help us to determine the true magnitude of biodiversity in these urban oases, which brings us one step closer to *ex situ* conservation of endangered and rare organisms, while at the same time allowing us to closely monitor structural changes in biodiversity levels through temporal observation and analysis of the diversity of certain species.

Fungi, as an inevitable and crucial component of the biodiversity of botanical gardens, had largely remained under-studied in the past. With the exception of isolated earlier individual studies (for example Currey, 1874), only recently have significant efforts been undertaken in terms of the mycological research of botanical gardens (Eckstein & Eckstein, 2009; Folcz & Börcsök, 2015; Kartika et al., 2018; Watling et al., 2019; Kruse et al., 2020; Bradshaw et al., 2022). In Bosnia and Herzegovina, to date there has been no evidence of mycological studies being carried out in botanical gardens.

On the other hand, the presence of taxonomic studies of relative significance in similar anthropogenic habitats in urban areas are worthy of note (Moravec, 1971; Jukić et al., 2018).

The Botanical Garden of the National Museum of Bosnia and Herzegovina is located near the centre of Sarajevo, in the oldest cultural and scientific institution in the state. The garden itself is an object of special educational purpose, and represents an important monument of natural and botanical heritage.

Although it occupies a significantly smaller area in comparison to other European botanical gardens (14,270 m² according to Bečić et al. (2011)), the diversity of the plant fund has gradually increased over the years, having reached a maximum number of 3,000 different plant species (Šilić, 1988). Currently, however, official sources list much smaller number of the plants (1,700 according to the National Museum's own website).

The Botanical Garden was founded by the famous Austrian botanist Karl Malý at the beginning of the 20th century, and has undergone certain structural and physiognomic changes over time. However, its main role as an invaluable and an effective instrument of *in situ* teaching at different levels of education remained unchanged.

A detailed overview of and insight into the diversity and abundance of living plants in the Botanical Garden of the National Museum of Bosnia and Herzegovina has been provided by Šilić (1988) and Bečić & Ljujić-Mijatović (2010). However, no other systematic and comprehensive inventories of other taxonomic groups – including fungi – have been conducted to date.

To (partially) address this issue, the authors conducted such research, with the preliminary results presented here representing the first real insight into the diversity of fungi in botanical gardens in Bosnia and Herzegovina.

MATERIAL AND METHODS

Six field studies were carried out between 2021 and 2023 in the Botanical Garden of the National Museum of Bosnia and Herzegovina, Sarajevo. The main focus of the research was ascomycetous fungi, their ecology and diversity. At the same time, seven species from the phylum Basidiomycota were also recorded, mainly those that are easy to identify *in situ* by recognizing relevant macro-characters.

The ascomata and basidiomata of the registered fungi species were photographed both *in situ* and *ex situ* using photographic equipment and a stereomicroscope. Macro-photographs *in situ* were taken with a Canon 700D camera, a 100mm f/2.8L Macro IS USM lens, a tripod and a Canon Macro Ring Lite MR-14EX II, while *ex situ* macrophotographs were taken with a Nikon SMZ445 stereo zoom microscope. A

Nikon SE type 102 compound light microscope was used for laboratory analyses and observation of micromorphological characters. Photomicrographs were taken with a Sony DSC-H2 camera.

Samples for microscopy were prepared by cutting fresh fungal apothecia by hand using a razor blade, and, in the case of very small apothecia, samples were taken with a teasing needle.

The descriptions of macro- and micromorphological characters are mainly based on the analysis of living structures of fresh collections (Baral, 1992) from the Botanical Garden of the National Museum of Bosnia and Herzegovina, and are combined with relevant literature data where indicated.

All measurements were carried out in a tap water mount. For each species analysed, 20 ascospores were measured to determine their average length and width. The extreme values of measured ascospores and other structures are given in parentheses. The ornamentation of the ascospores was stained with lactophenol cotton blue (LCB); the presence or absence of an iodine reaction in the apical part of the asci was checked using Lugol's solution (IKI); cresyl blue (CRB) was used to highlight cytoplasmic inclusions such as spore bodies in *Orbiliomycetes* and for staining the nucleus and nucleolus in *Sclerotiniaceae*.

In order to define the simple spore shape in accordance with the Q value (length:width ratio of ascospores) the geometric delimitation criteria from Kušan et al. (2014) were used.

The abbreviation "FAMU" and the corresponding database codes are given for each material deposited in the Fungarium of the Mycological Society MycoBH.

RESULTS

1. ***Arrhenia spathulata* (Fr.) Redhead**
Fig. 1 – j)
Among shoots of *Tortula ruralis* covering rocks, 20 Feb 2021.
2. ***Octospora gyalectoides* Svrček & Kubička**
Fig. 2 – d), f)
Rocky and base-poor site, probably on shoots of *Crossidium squamiferum*, 14 Feb 2021, 07 Jan 2023, N.J./070123-Y2; FAMU – 0959.
3. ***Octospora pseudoampezzana* (Svrček) Caillet & Moyne**
Figs. 1 – b), d); 3 – i), o), p), q)
Description: Ascomata apothecial, 1–4 (5) mm in diam., yellow-orange, orange to brown-orange; margin conspicuous and quite pronounced, first whitish and silky, later getting darker, finally in mature specimens be-

coming blackish; hymenium finely granulose. Asci cylindrical, *250–340 × 22–28.7 µm, 8-spored, uniseriate. Ascospores *(19.7) 20.2–23.8 × (14.3) 14.5–15.9 (16.9) µm, ellipsoid to broadly ellipsoid, containing one large oil droplet, usually eccentric; ornamentation consists of roundish isolated warts, evenly scattered over the spore surface and of more or less the same diam. (0.5–1.0–1.2 µm). Paraphyses cylindrical, barely exceeding asci, slightly enlarged in the upper part, *7–10 µm wide in the apical part, containing light orange pigmentation.

Material examined: On shoots of *Schistidium crassipilum*/ *S. helveticum* complex covering limestone rocks, 14 Feb 2021, 20 Feb 2021 & 07 Jan 2023, N.J./070123-Y1; FAMU – 1389.

Notes: As stated in Eckstein & Eckstein (2009) and Németh et al. (2022), host species is almost always *Schistidium crassipilum*. Rare cases with *Racomitrium heterostichum* as a host moss were reported in Eckstein et al. (2020). First record for Bosnia and Herzegovina.

4. ***Ciboria amentacea* (Balb.) Fuckel**
Figs. 1 – e), g); 3 – b), c), g), h), l) r), s)
On fallen and buried male catkins of *Corylus avellana*, apparently emerging from thali of *Marchantia polymorpha*, 20 Feb 2021, 28 Feb 2021 & 07 Jan 2023.
5. ***Auricularia mesenterica* (Dicks.) Pers.**
On the dead *Ulmus* sp. trunk, 20 Mar 2021 & 07 Jan 2023.
6. ***Xylaria oxyacanthae* Tul. & C. Tul.**
Fig. 1 – k)
Description: Stromata usually slender, simple or rarely branched, 2–5 cm in height and up to 2–4 mm wide, cylindrical, slightly enlarged in the upper part and often laterally compressed. Apices finely pronounced, sometimes mammiform, pinkish-orange to orange-brown. Middle part more whitish or greyish, while lower part of stromata remains black or greyish-black
Material examined: On partially buried and mummified *Crataegus monogyna* fruits probably mixed with excrements of *Columba palumbus*, 20 Mar 2021.
Notes: First record for Bosnia and Herzegovina. The material was identified on the basis of the description of relevant macro and micro characteristics from Kujawa & Karasiński (2007) and Himani & Krishnappa (2020).
7. ***Piceomphale bulgarioides* (Rabenh.) Svrček**
Figs. 1 – c); 3 – m), n)
On fallen *Picea omorika* cones, 20 Mar 2021. First record of *P. bulgarioides* on *Picea omorika* in Bosnia and Herzegovina.



Figure 1. Some fungal species registered in the Botanical Garden of the National Museum of Bosnia and Herzegovina: **a)** *Geopora sumneriana* under *Cedrus libani*; **b), d)** *Octospora pseudoampezzana*, bryophilous species on shoots of *Schistidium crassipilum*/ *S. helveticum* complex; **c)** *Piceomphale bulgarioides* on cones of *Picea omorika*; **e), g)** *Ciboria amentacea* ascomata seemingly emerging from the liverwort *Marchantia polymorpha* thalli; **f), i)** *Ciboria coryli* sharing the same habitat type with *C. amentacea*; **h)** *Ciboria rufofusca* on fallen *Abies alba* cones; **j)** *Arrhenia spathulata*, bryophilous species; **k)** *Xylaria oxyacanthae* (anamorph) on buried seeds of *Crataegus* sp.

8. ***Ciboria coryli*** (Schellenb.) N.F. Buchw.
Fig. 1 - f), i)
On fallen and partially buried male catkins of *Corylus avellana*, 28 Feb 2021.
9. ***Ciboria rufofusca*** (O. Weberb.) Sacc.
Fig. 1 - h)
On lying cone scales of *Abies alba*, 08 May 2022.
10. ***Geopora sumneriana*** (Cooke) M. Torre
Figs. 1 - a); 3 - j), k)
Under *Cedrus libani*, among the litter of needles, 08 May 2022.
11. ***Orbilbia aurantiorubra*** Boud.
Figs. 2 - a); 3 - a), e)
On dead dry semi-decorticated twigs of *Fraxinus* sp.?, 08 May 2022.
12. ***Orbilbia carpoboloides*** (P. Crouan & H. Crouan) Baral
Figs. 2 - b), c); 3 - d), f)
Description: Ascomata apothecial, 0.5–1.5 (1.8) µm wide., light rose-carneous, light ochraceous, yellow-ochraceous or orange-ochraceous, sometimes slightly reddish, irregularly rounded or broadly elliptical, concave, with conspicuous margin usually made of whitish “teeth” or fimbriate hair tufts (Baral et al., 2020). Asci cylindrical, *(62.4) 65.4–78.8 (79.7) × (4.9) 5.6–6.4 (6.7) µm, 8-spored, bi- or triseriate. Ascospores *(12.4) 12.5–15.6 (15.9) × (2.3) 2.3–2.8 (2.9) µm, fusiform to fusoid, rarely straight, more often slightly curved at the base; spore body vermiform, barely enlarged at the base, 4.2–7 µm in length. Paraphyses septate, quite straight, or slightly flexuous, mammiform to spatulate (Baral et al., 2020), with more or less pronounced apical narrowed part.
Material examined: On corticated part of dead twig of undetermined deciduous tree, 08 May 2022.
Notes: First record for Bosnia and Herzegovina.
13. ***Propolis farinosa*** (Pers.) Fr.
Fig. 2 - i)
On dead deciduous twigs, 08 May 2022.
14. ***Pyrenopeziza rubi*** (Fr.) Rehm
Fig. 2 - h), j)
On dead *Rubus* sp. twig, 08 May 2022.
15. ***Xylaria carpophila*** (Pers.) Fr.
On fallen dead beechmasts, 08 May 2022.
16. ***Dacrymyces capitatus*** Schwein.
Fig. 2 - e)
On dead twigs of unknown deciduous tree, 08 May 2022.
17. ***Calathella eruciformis*** (P. Micheli ex Batsch) D.A. Reid
Description: Basidiomata cyphelloid, tubular in shape, sometimes distinctly narrowed at the base or campanulate, 0.5–1.5 mm wide and up to 2 mm high; outer side and marginal region densely covered in long whitish hairs, later becoming more ochre, grey to ochre-brownish, surface more brownish; hymenial inner part smooth, pale cream to ochre cream.
Material examined: On dead *Populus* sp. twigs, 07 Jan 2023, N.J./070123-Y3; FAMU – 1391.
Notes: First record for Bosnia and Herzegovina. The material was identified based on the description of relevant macro and micro characteristics from Halama et al. (2019).
18. ***Xylaria longipes*** Nitschke
On remnants of unknown deciduous tree, 07 Jan 2023.
19. ***Schizophyllum commune*** Fr.
On dead *Populus alba* remnants, 07 Jan 2023.
20. ***Auricularia auricula-judae*** (Bull.) Quéf.
On living *Berberis* sp. branches, 07 Jan 2023.
21. ***Candolleomyces typhae*** (Kalchbr.) D. Wächt. & A. Melzer
Fig. 2 - g)
On decaying dead stem and/or leaves of *Typha angustifolia*, 25 Jun 2023, N.J./250623-Y1; FAMU – 1390.
22. ***Psilachnum inquilinum*** (P. Karst.) Dennis
On dead partially inundated stem of *Equisetum hiemale*, 25 Jun 2023, N.J./250623-Y2; FAMU – 1392.



Figure 2. Some fungal species registered in the Botanical Garden of the National Museum of Bosnia and Herzegovina: **a)** *Orbilbia aurantiorubra* on dead *Fraxinus* sp.? twig; **b), c)** *Orbilbia carpoboloides* on dead twig of undetermined deciduous tree; **d), f)** *Octospora gyalectoides* on shoots of *Crossidium squamiferum*; **e)** *Dacrymyces capitatus* on dead twigs of undetermined deciduous tree; **g)** *Candolleomyces typhae* on decaying stem of *Typha angustifolia*; **h), j)** *Pyrenopeziza rubi* on dead *Rubus* sp. stem; **i)** *Propolis farinosa* on undetermined deciduous twigs.

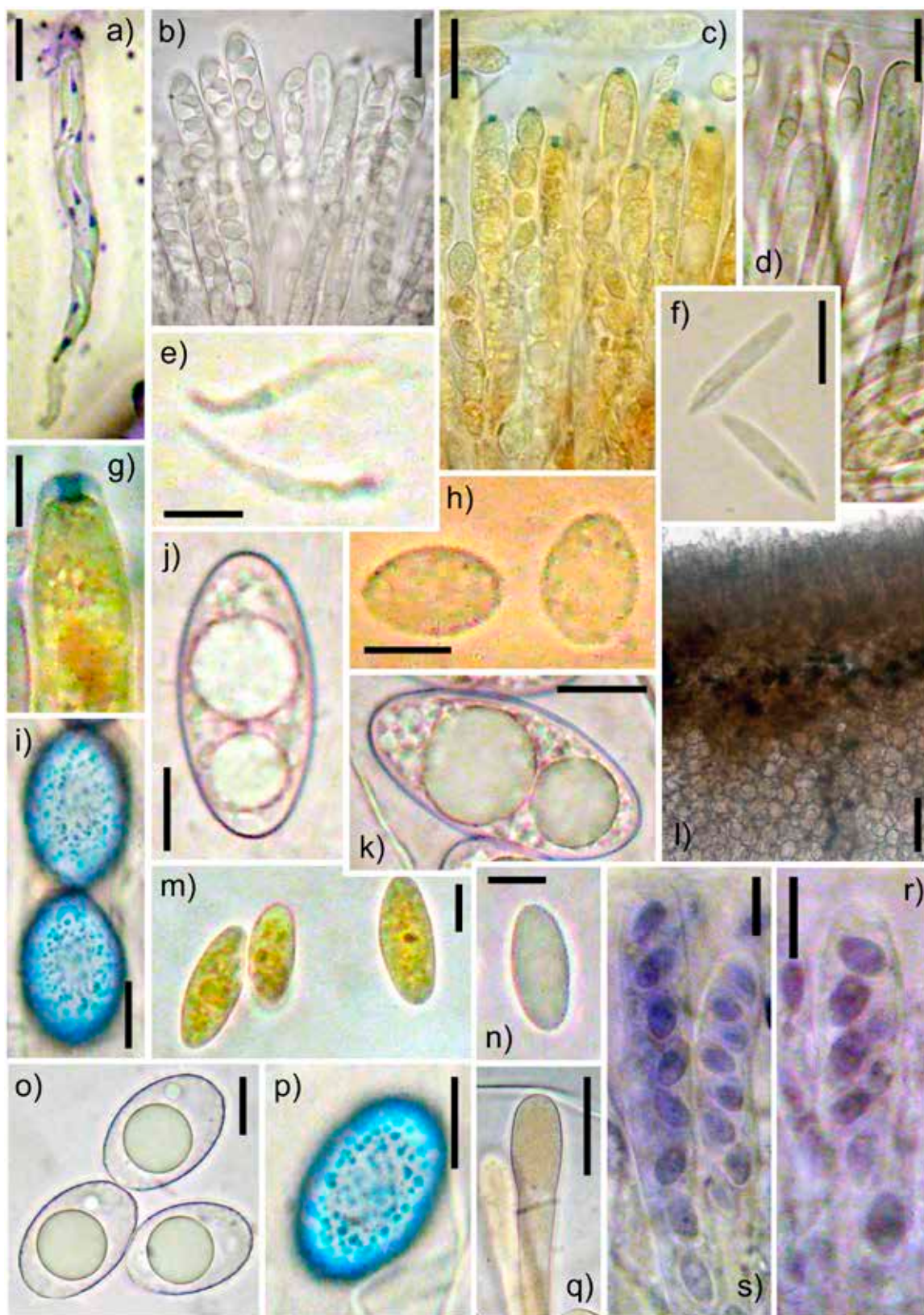


Figure 3. Micro characters of some fungal species registered in the Botanical Garden of the National Museum of Bosnia and Herzegovina: **a), e)** *Orbilia aurantiorubra* – a) asci and ascospores in CRB mount, e) *mature ascospores; **b), c), g), h), l) r), s)** *Ciboria amentacea* – b) *immature ascospores and asci, c) *asci and ascospores in IKI mount, g) *apical apparatus of the asci in IKI mount, h) – *mature ascospores, l) *vertical section of the apothecia, r), s) asci and ascospores in CRB mount; **d), f)** *Orbilia carpoboloides* – d) *apical part of the paraphyses and asci, f) *mature ascospores; **j), k)** *Geopora sumneriana* – *mature ascospores; **m), n)** *Piceomphale bulgarioides* – m) *mature ascospores in IKI mount, n) *mature ascospores; **i), o), p), q)** *Octospora pseudoampezzana* – o) *immature ascospores, i), p) *mature ascospores in LCB, q) *apical part of the paraphyses. Bars: l) – 50 µm; a), d), f), i), j), k), o), p), r), s) – 10 µm; e), g), h), m), n) – 5 µm.

DISCUSSION

Botanical gardens in urban areas should be considered unique sources of biodiversity enrichment. These small areas represent a profound substitutional link between human and natural environments, especially in the modern and accelerated lifestyles of today.

Diverse floristic components and structures, the heterogeneity of the flora, the vegetation of different bioclimates, the combination of normally incompatible components, and the special care and treatment of the plants are the cause of the great and highly specific biodiversity in these small "oases" and are also the main reasons for botanical gardens' need to be one of the highest priorities in urban planning.

This short and narrowly focused mycological study clearly shows that botanical gardens are highly interesting and intriguing man-made habitat types, and should be recognized as fungal diversity hotspots within contemporary urban areas. This has also been argued by other mycologists in the past (Eckstein & Eckstein, 2009; Folcz & Börcsök, 2015; Kartika et al., 2018; Kruse et al., 2020; Bradshaw et al., 2022). A number of long-term studies and corresponding results, such as those of Folcz & Börcsök (2015) are quite impressive and show the true value of botanical gardens and their importance for the biodiversity of fungi, and the conservation of fungi in general.

Although botanical gardens are usually very limited in space and may seem somewhat irrelevant factors in terms of fungal diversity (or overall biodiversity) at the national, regional and global level, it is quite the contrary. These islands of biodiversity play a significant role in enriching fungal heterogeneity, on national and regional scales in particular. Also, botanical gardens are the only locations where certain species of fungi (or other organisms) with natural habitats that are very distant from one another can be found living together. As a result, they provide a great opportunity for scientists around world to closely study and monitor different types of organisms, but such studies should also be tak-

en with caution, due to the negative effects of outbreeding depression on population persistence (Chen & Sun, 2018). They can, however, provide ideal conservation environments for *ex situ* conservation of fungi (Folcz & Börcsök, 2015), with the results presented here going some way to support this hypothesis. In addition, botanical gardens provide a wide range of mycological interpretation and educational activities, including those aimed at preserving fungal diversity (Hu & Zhang, 2008).

Collections of *Octospora pseudoampezzana*, *Xylaria oxyacanthae*, *Orbilia carpoboloides* and *Calathella eruciformis* from the Botanical Garden of the National Museum of Bosnia and Herzegovina represent the first registered findings of these species in Bosnia and Herzegovina, and provide solid arguments for emphasizing the important role that botanical gardens can potentially play in modern science. This preliminary checklist of fungi from the Botanical Garden of the National Museum of Bosnia and Herzegovina is arguably only partial in nature, and minute projection of the overall level of fungal diversity within this man-made habitat. It is for this reason that the authors strongly outline the (largely self-implied) need for continuation of this mycological research.

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SAŽETAK

Botanički vrtovi općenito predstavljaju vrlo značajne oaze bioraznolikosti koje je stvorio čovjek i obično su prostorno smještene unutar same jezgre urbanih sredina. Njihova uloga danas je višestruka i nezamjenjiva. Oni prije svega služe za edukaciju različitih interesnih grupa, pomažu pri *ex situ* konzervaciji ugroženih vrsta biljaka i drugih organizama te u značajnoj mjeri obogaćuju biodiverzitet u urbanim zonama, gdje on inače, ne dolazi do izražaja. Botanički vrtovi su interesantna staništa i s aspekta mikologije, prvenstveno zbog prisustva obilja vaskularne, ali i nevaskularne flore, odnosno brojnih biljnih vrsta koje učestvuju u različitim tipovima interakcija s gljivama. O tome svjedoče pojedini naučni radovi u okviru kojih su obrađene različite taksonomske skupine gljiva: Eckstein & Eckstein (2009) – brioofilne vrste, Folcz & Börsök (2015) – više različitih skupina, Kartika et al. (2018) – više različitih skupina, Watling (2019) – nelihenizirane vrste, Kruse et al. (2020) – više različitih skupina, Bradshaw et al. (2022) – gljive porodice *Erysiphaceae* i dr. Ovim istraživanjem utvrđeno je prisustvo pojedinih vrlo značajnih i rijetkih vrsta gljiva te je valorizovana pretpostavljena važna uloga botaničkih vrtova u raznolikosti gljiva u modernim urbanim zonama. S druge strane, premda postoji više od 100 godina i sadrži izuzetno vrijedne zbirke briofita, viših biljaka i dendroflora, Botanički vrt Zemaljskog muzeja Bosne i Hercegovine do danas nije bio predmet mikoloških istraživanja. U ovom radu su prvi put prezentovani rezultati istraživanja diverziteta gljiva Botaničkog vrta Zemaljskog muzeja Bosne i Hercegovine koja su realizovana u posljednje dvije godine. Kratka istraživanja su provedena u zimskim, proljetnim i ljetnim dijelovima godine, a ukupno je u periodu februar 2021. – juni 2023. realizovano 7 istraživanja. Predmet ovih istraživanja bile su gljive odjeljka Ascomycota, ali su uporedo, u manjem obimu, registrovane i neke karakteristične i značajne vrste odjeljka Basidiomycota. Sav prikupljeni materijal je naknadno laboratorijski obrađen u skladu s principima vitalne taksonomije, a radi ispravne determinacije vrsta i registrovanja najbitnijih specijskih obilježja. U okviru provedenog seta istraživanja determinisane su 22 vrste gljiva, od čega 15 vrsta iz odjeljka Ascomycota i 7 iz odjeljka Basidiomycota. Vrste *Octospora pseudoampezzana*, *Xylaria oxyacanthae*, *Orbilbia carpoboloides* i *Calathella eruciformis* zabilježene su prvi put u Bosni i Hercegovini. Za navedene vrste je u okviru rada dat kraći deskriptivni opis koji sadrži podatke o najbitnijim morfološkim i mikrokarakteristikama.