

## TWO RARE AND INTERESTING SPECIES OF PEZIZALES (FUNGI) FROM BOSNIA AND HERZEGOVINA – *Peziza montirivicola* and *Trichophaea flavobrunnea*

DVIJE RIJETKE I ZANIMLJIVE VRSTE REDA PEZIZALES (GLJIVE) U BOSNI I HERCEGOVINI - *Peziza montirivicola* i *Trichophaea flavobrunnea*

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### Abstract

Two interesting and rarely occurring species of fungi from the order Pezizales found in Bosnia and Herzegovina are presented here. Ecology, morphology, microscopic characters and conservation status of *Peziza montirivicola* and *Trichophaea flavobrunnea* are briefly discussed in the paper. Notes on the similar species of operculate discomycetes are given.

**Key words:** Ascomycota, conservation, IUCN, *Peziza*, *Trichophaea*

## 1. INTRODUCTION / UVOD

Pezizalean fungi are generally underexplored in Bosnia and Herzegovina. Some information about ecology and taxonomy of certain species can be found in Focht (1979, 1990), Usčuplić & Treštić (2003), Usčuplić (2004, 2012) and Matočec & Ozimec (2013). Only few species of Pezizales are mentioned as endangered in the Red List of Fungi in the Federation of Bosnia and Herzegovina (Đug et al., 2013). *Sarcosphaera coronaria* (Jacq.) J. Schröt. and *Sowerbyella imperialis* (Peck) Korf are listed as critically endangered (CR) and have the highest conservation status among Pezizales in the Federation of Bosnia and Herzegovina. *Caloscypha fulgens* (Pers.) Boud. has conservation status of vulnerable species (VU), and all other species of Pezizales are listed under data deficient category (DD). Currently, fungi are completely left out from the similar strategic document in the

Republic of Srpska (Crvena lista zaštićenih vrsta flore i faune Republike Srpske, 2012).

In the last few years more comprehensive field research and study of ascomycetous fungi in Bosnia and Herzegovina were undertaken. Some of the results are presented in Jukić & Omerović (2011), Omerović & Jukić (2015) and Jukić (2016). Apart from the inventory study, the research was conducted in order to make quality risk assessment and to recognize vulnerable species and most endangered type of habitats. Among registered findings, a couple of globally rare species from the order Pezizales (Ascomycota) have been recorded: *Peziza polarispinosa* J. Moravec and *Marcellina brevicostatispora* J. Moravec (Omerović & Jukić, 2015).

In order to properly conserve endangered species from the order Pezizales and fungi in gener-

al, as well as to properly revise existing strategic conservation documents, it is necessary to continue with publishing relevant studies and other mycological data from the territory of Bosnia and Herzegovina.

Therefore, a detailed description of two threatened species of Pezizales is presented here. Ecological importance of *Peziza montirivicola* Perić and *Trichophaea flavobrunnea* (Richon) Priou, Perić, Van Vooren & Hairaud is discussed and their conservation status is recommended.

## 2. MATERIALS AND METHODS / MATERIJAL I METODE

The material analysed in this study was collected by the author during the field research in 2016 on two different localities. All analyses were performed on freshly collected living material, as proposed by Baral (1992). Nikon SE type 102 compound light microscope was used for observing micromorphological characters. Macro-photographs were taken by Canon 40D camera combined with different macro lenses. Micro-photographs were taken using a Sony DSC-H2 camera.

All measurements of basic micromorphological characters were made using the Piximètre software (Piximètre, 2016).

Specimens were first analysed in tap water mount and afterwards were treated with different chemical reagents: IKI - Lugol's solution (Baral, 1987) for testing amyloidity and for determining the type of reaction in the apical portion of the asci; Congo red (0.04% aqueous solution) mainly for staining excipular tissues

and for measuring ascospores (in this type of media); LCB - Lactophenol Cotton Blue (Aneja, 2003) for staining the ascospore ornamentation and acetocarmine (Harmaja, 1974) for staining the nuclei.

Fresh material was cut by hand using a razor blade.

In all media, unless otherwise stated, 20 ascospores were measured (N) in order to determine their relevant length and width. Ascospores length/width ratio mean value is given (Qe).

All analysed material has been preserved and stored as exsiccata in a private fungarium (N.J. - Nedim Jukić) with relevant data entered into Amateur Mycological Association Electronic Database (FAMU).

IUCN categories and recommended conservation measures are given for the two species of Pezizales presented here. Risk assessment was done according to relevant criteria (IUCN, 2016).

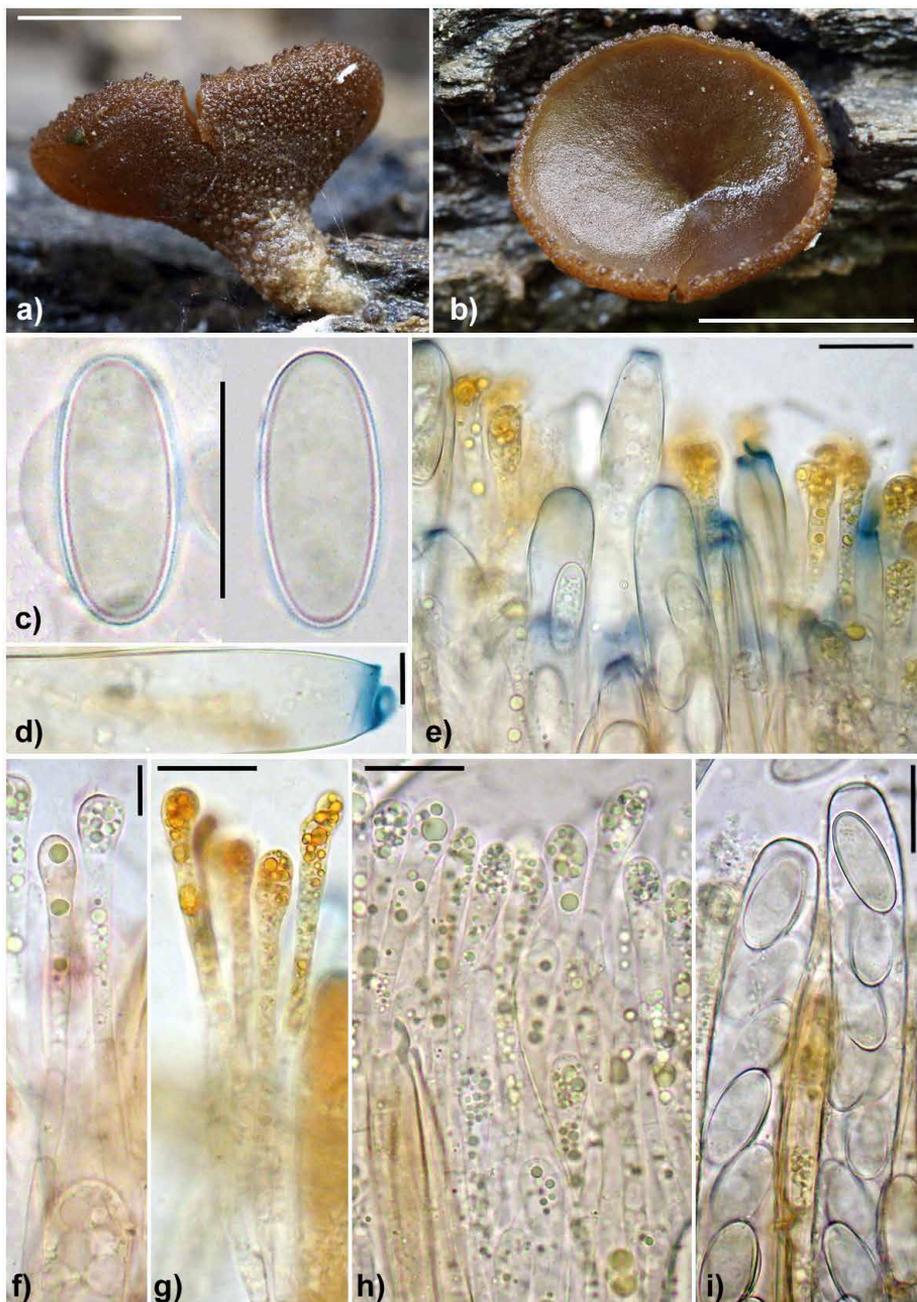
## 3. RESULTS AND DISCUSSION / REZULTATI I DISKUSIJA

### *Peziza montirivicola* Perić 2015      Figures 1–2

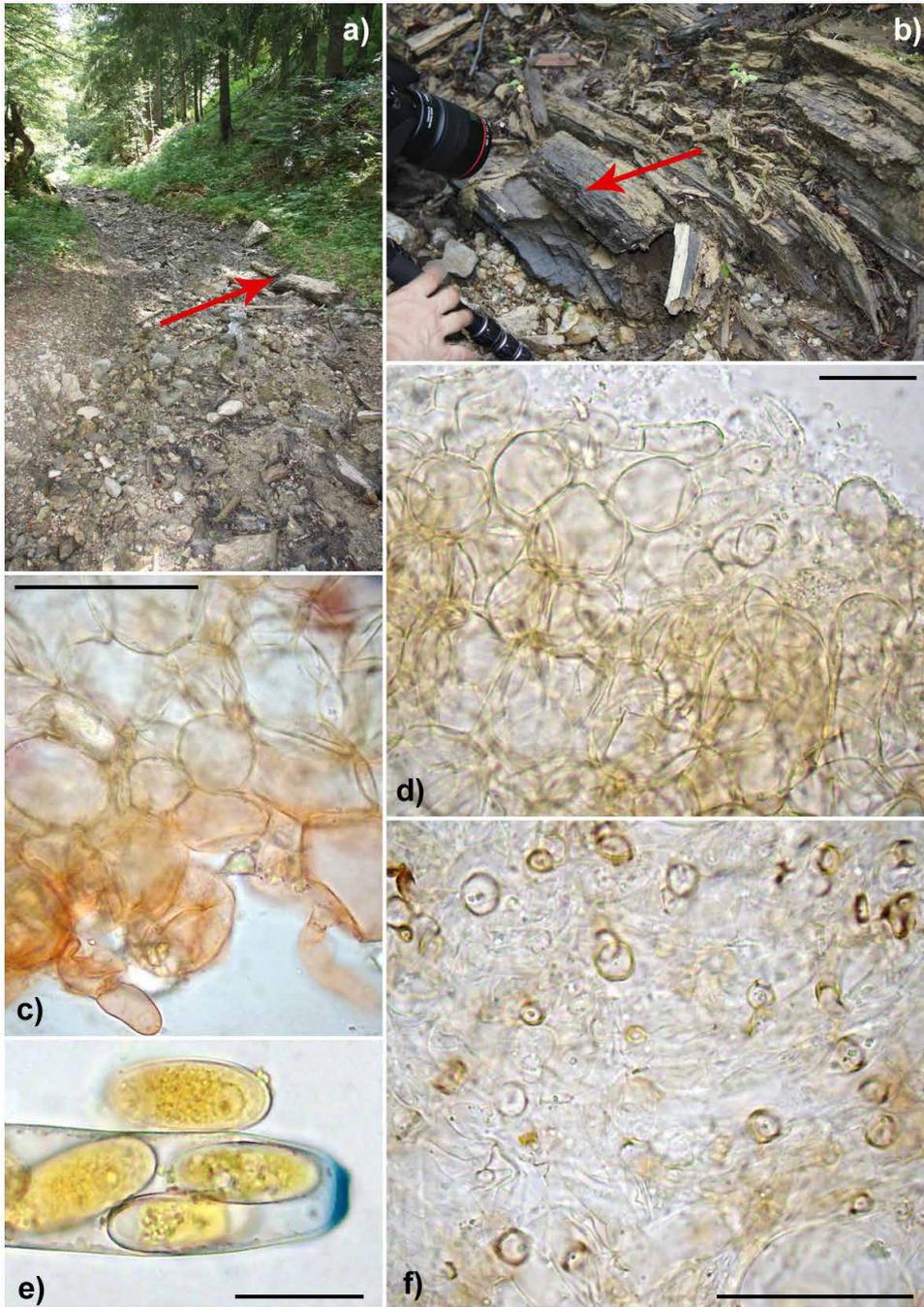
**Description:** Ascoma apothecial, stipitate, slightly above 2 cm in diam. and 1.7(1.8) cm high, hymenium finely granulated, brown olivaceous, brownish red to chocolate brown. Margin conspicuous and crenate, sporadically with darker granules. Outside of the apothecia darker, chocolate brown, distinctly granulated, with dark brown granules towards the margin, and brighter granules on the surface of the stipe. Flesh rather fragile. Stipe furfuraceous and granulated, whitish yellow, 0.7–1 × 0.5 cm, at base covered with white mycelium (Fig. 1-a), as described in Perić & Grebenc (2015).

Asci are operculate, cylindrical, octosporous, amyloid, 16.5–20 × 310–400 μm. Ascospores uniseriate, smooth, hyaline, oblong ellipsoid, with conspicuous mucilaginous sheath. In tap water mount (23.2)24–27(27.4) × (9.2)9.8–11.1(11.5) μm, Qe = 2.4, N = 20; in IKI (23.1)23.8–25.4(25.6) × (9.5)9.9–11.1(11.2) μm, Qe = 2.3, N = 10. Nucleus (3.8)4.1–4.7 μm wide, usually positioned centrally.

Paraphyses cylindrical-clavate, septate, at the apex up to 9 μm wide, very distinctive due to the large number of greenish to greenish yellow refractive vacuoles 0.3(0.4)–4.5 μm in diam.



**Figure 1.** *Peziza montirivicola* (N.J./260616-Y1): **a), b)** - single apothecia registered on the Igman Mt.; **c)** - mature ascospores with mucilaginous sheath and visible nucleus; **d), e), g)** - asci and paraphyses in IKI; **f)** - paraphyses in Congo red; **h)** - paraphyses with distinctive refractive vacuoles (tap water); **i)** - asci and ascospores (tap water). Bars: **a)** - 1cm; **b)** - 1,5 cm; **c)** - 20  $\mu\text{m}$ ; **d)** - 10  $\mu\text{m}$ ; **e)** - 20  $\mu\text{m}$ ; **f)** - 10  $\mu\text{m}$ ; **g), h), i)** - 20  $\mu\text{m}$  / Slika 1. *Peziza montirivicola* (N.J./260616-Y1): **a), b)** - usamljeni apotecij registrovan na planini Igman; **c)** - zrele askospore sa uočljivim mucilaginoznim plaštom i nukleusom; **d), e), g)** - askusi i parafize tretirani Lugolovom otopinom; **f)** - parafize u kongo crvenom; **h)** - parafize sa uočljivim refraktivnim vakuolama (vodovodna voda); **i)** - askusi i askospore (vodovodna voda). Mjerne skale: **a)** - 1cm; **b)** - 1,5 cm; **c)** - 20  $\mu\text{m}$ ; **d)** - 10  $\mu\text{m}$ ; **e)** - 20  $\mu\text{m}$ ; **f)** - 10  $\mu\text{m}$ ; **g), h), i)** - 20  $\mu\text{m}$ . (© N. Jukić)



**Figure 2.** *Peziza montirivicola* (N.J./260616-Y1): **a), b)** - natural habitat of *P. montirivicola* (Javornik, Igman); **c), d)** - marginal hyphoid and ectal excipulum cells, Congo Red - **c)**, tap water mount - **d)**; **e)** - apical portion of asci in IKI; **f)** - septal rings in the subhymenium and medullary excipulum. Bars: **c)** - 50  $\mu\text{m}$ ; **d), e)** - 20  $\mu\text{m}$ ; **f)** - 50  $\mu\text{m}$  / **Slika 2.** *Peziza montirivicola* (N.J./260616-Y1): **a), b)** - prirodno stanište *P. montirivicola* (Javornik, Igman); **c), d)** - marginalni hifoidni završeci i vanjski dio ekscipuluma, kongo crveno - **c)**, vodovodna voda - **d)**; **e)** - vršni dio askusa u Lugolovoj otopini; **f)** - septalni ringovi u subhimeniju i medularnom ekscipulumu. Mjerne skale: **c)** - 50  $\mu\text{m}$ ; **d), e)** - 20  $\mu\text{m}$ ; **f)** - 50  $\mu\text{m}$  (© J. Jukić - b), N. Jukić - a), c), d), e), f)

In Perić & Grebenc (2015), these vacuoles are described as bright yellow to brownish yellow, surrounded by numerous hyaline and smaller droplets.

Subhymenium is composed of *textura intricata*. Medullary excipulum predominantly of *textura globulosa*. Ectal excipulum of *textura globulosa-angularis*, cells 14.4–45.7 µm wide, forming external elongated hyphoid cells at the margin.

Highly refractive and thickened septal rings are present in the subhymenium and medullary excipulum. (Figure 2-f).

**Ecology and phenology:** It is usually found in the highlands or mountains, near the water stream, on fallen, partially submerged and soaked trunks of *Fagus sylvatica* and *Picea abies* or on sandy soil with moss and different kind of wood debris from June to the end of September.

**Distribution:** *Peziza montirivicola* is semi-aquatic riparian species. It has been registered so far in three countries: Montenegro, Switzerland and Bosnia and Herzegovina. Previously, this species was reported in Bosnia and Herzegovina from the territory of the National Park Sutjeska (Perić & Grebenc, 2015). Material treated here was found on the Igman Mt. which is the fifth known locality for this species on a worldwide scale.

**Material examined:** Igman Mt. (25 km SW from Sarajevo, Bosnia and Herzegovina), nearby locality Javornik, 1496 m asl, 43.74752° N, 18.23294° E, single apothecia on wet, decaying, laying trunk of *Picea abies*, in semi-dry alpine streambed used as a forest path, 26 June 2016, leg. N. Jukić (N.J./260616-Y1; assign database code: FAMU-0909).

Unidentified immature ascomata of *Pachyella* sp. are registered sharing the same substrate.

**Comments:** There are several recently described semi-aquatic or aquatic species from the genus *Peziza* Dill. ex Fr. (Frank, 2013; Perić & Grebenc, 2015; Pfister et al., 2016). *Peziza oliviae* J.L. Frank is North American, stipitate aquatic species, growing submerged in small streams. It has been reported so far only from the state

of Oregon (Frank, 2013). Besides aquatic species *Aquapeziza globispora* D.M. Hu, L. Cai & K.D. Hyde (Hu et al., 2012), *Peziza oliviae* is the only member of the *Pezizaceae* which produce fruitbodies underwater. Substrate of the species from the genera *Adelphella* Pfister, Matočec & I. Kušan and *Pachyella* Boud. is usually only partially inundated. *Peziza nordica* Kristiansen, LoBuglio & Pfister is a very similar species to *P. montirivicola* discovered in Norway. It inhabits dead logs and debris of *Betula* and *Salix* and has slightly larger brown to brownish orange apothecia, wider paraphyses and smaller asci (Pfister et al., 2016).

*Peziza lohjaensis* Harmaja is usually a more robust species described in Finland (Harmaja, 1986). It differs by having distinctly warted and smaller ascospores and unlikely other species of *Peziza* from this group, it does not have conspicuous and well-defined stipe. All of the so far known species of *Peziza* that live in or near the running water have quite similar ascospore size, grows on the submerged or water-soaked wood trunks, logs and other debris and have stipitate (*P. oliviae*, *P. nordica* and *P. montirivicola*) to sub-stipitate apothecia (*P. lohjaensis*). According to molecular analysis presented in Pfister et al. (2016) and relevant phylogenetic data in Perić & Grebenc (2015), *Peziza nordica* and *Peziza lohjaensis* are the most closely related species to *P. montirivicola*.

*Peziza montirivicola* can be microscopically confused with a couple of other similar species from the genus *Peziza*, like *Peziza paludicola* (Boud.) Sacc. & Traverso and *Peziza ampliata* Pers. The first one differs by having smaller and less ellipsoid ascospores without mucilaginous sheath, more clavate paraphyses with not so distinctive greenish yellow refractive vacuoles, slightly different excipulum cells and more or less different type of habitat (Cacialli et al., 2011). *Peziza ampliata* differs by having smaller yellowish brown to brown apothecia. It can be found on large trunk of hardwood trees or on smaller branches and wood debris (Hansen et al., 2002).

Since *Peziza montirivicola* is a very rare species, generally found on quite limited and endan-

gered type of habitat, it would be advisable to include it in conservation activities and measures in Bosnia and Herzegovina. This fungus needs clean water courses and enough fallen trunks and logs on riverbanks left to decay naturally. Small population size, habitat loss and fragmentation are major threats for this species. It is recommended to be placed under category critically endangered (CR), based on the IUCN criteria D.

***Trichophaea flavobrunnea* (Richon) Priou, Perić, Van Vooren & Hairaud 2015**      **Figures 3–5**

**Description:** Ascomata disc shaped, first concave, later expanding, becoming more or less flattened, up to 10(12) mm in diam. Hymenium porcelain or coconut white, dirty white, greyish to whitish yellow, smooth and often slightly shiny. Outside of the apothecia darker, ochraceous, covered with short hairs. Margin conspicuously hairy.

Asci cylindric, octosporous, hyaline, inamyloid, sometimes eccentric in the apical part, 290–350 × 12.5–17 μm. Operculum quite sublaterally positioned (Figure 5-e). Ascospores ellipsoid fusiform, hyaline, slightly or often inconspicuously inequilateral, in tap water mount (22.7)22.9–25.2(25.9) × (10)10.6–11.6(12.2) μm, Qe = 2.4, N = 20; in IKI (21.1)21.6–24.7(24.8) × (9.5)9.6–11.1(11.5) μm Q = 2.2, N = 20; in acetocarmine (23.2)23.7–26.1(27.2) × (9.1)9.7–10.9(11.7) μm, Qe = 2.4, N = 20; in LCB (20.9)22.4–24.1(25.7) × (10.4)10.8–11.4(11.8) μm, Qe = 2.1, N = 10. Ascospores contain 2–4(5) larger hyaline lipid bodies (guttules), 3–6.5 μm in diam., and numerous smaller ones. Ascospores's innermost wall layer thickened in the polar area.

Paraphyses cylindrical, septated, hyaline, quite numerous and dense, only slightly enlarged at the apex, 2.7–4 μm wide.

Medullary excipulum composed of *textura intricata* mixed with irregularly cylindrical or rarely pyriform cells, 18–50 × 2.5–9 μm. Ectal excipulum composed of *textura globulosa-angularis*, cells 10.8–29 × 7–21.5 μm. Marginal hairs brownish, septate, base directly connected with excipulum cells, 100–900 × 4.5–9 μm, pseudohairs present.

**Ecology and phenology:** This species has been recorded only on needles litter and other remnants of *Cupressaceae*. Fallen needles and twigs of *Juniperus communis*, *J. oxycedrus* and *Cupressus* sp. are reported as a substrate (Perić et al., 2015).

Although it is quite rare, it seems that it has a wide range of ecological tolerance. It has been usually reported from the coastal territories, in a period from November to the end of February. The only previously recorded finding from the continental part of Europe is actually a holotype (Aulnay-l'Aître commune) (Richon, 1879).

**Distribution:** Material treated here was found in the vicinity of Šipovo (Bosnia and Herzegovina) which is the fifth known locality for this species on a worldwide scale. It has been reported so far from France and Montenegro (Perić et al., 2015).

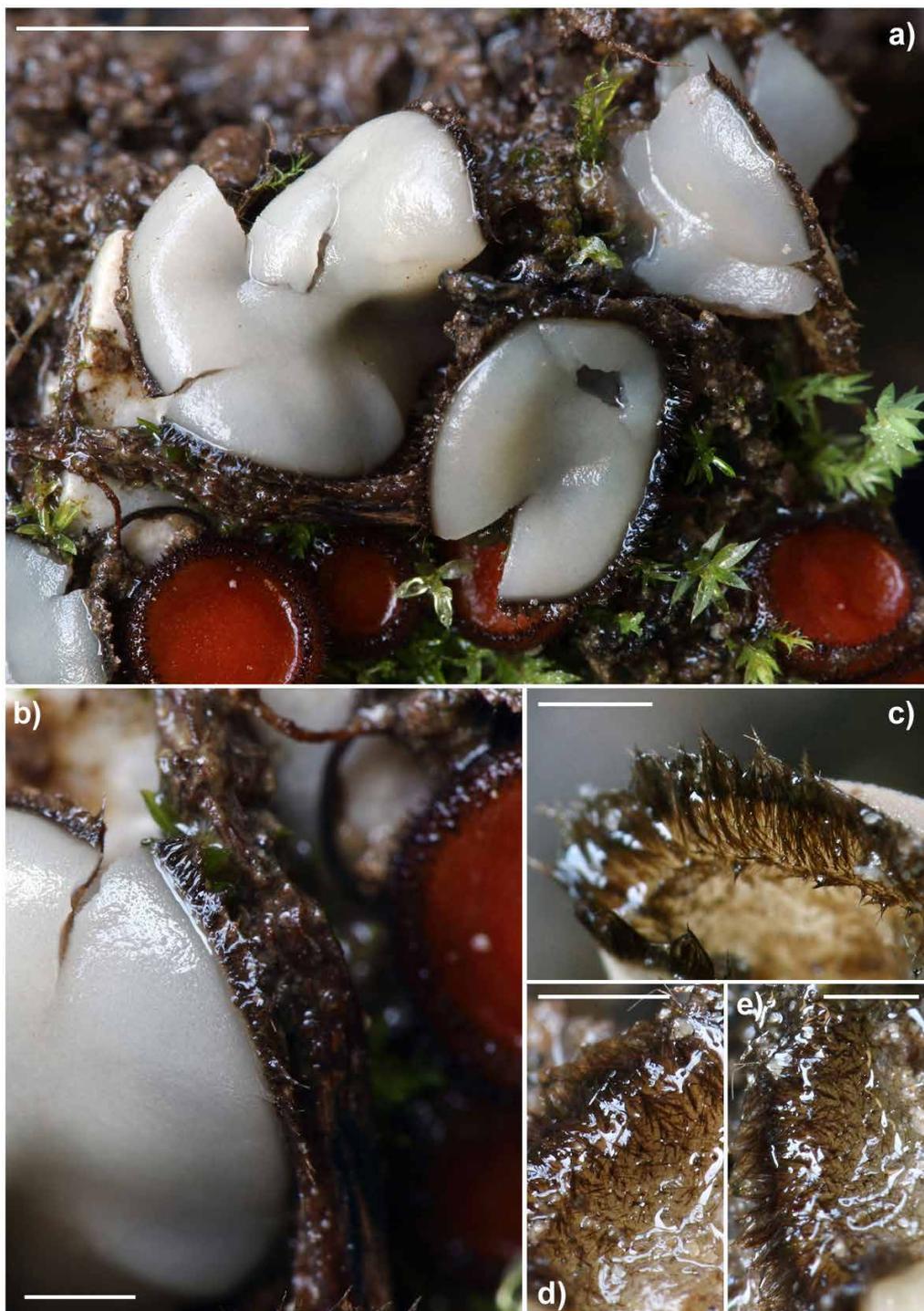
**Material examined:** Vicinity of Janj pristine forest (20 km SE from Šipovo, Bosnia and Herzegovina), 1094 m asl, 44.16198° N, 17.27167° E, gregarious, alongside forest path, at the edge of submontane dominant coniferous forest of *Abies alba* and *Picea abies* with *Fagus sylvatica*, on soil mixed with *Juniperus communis* needles and other debris, 18 September 2016, leg. N. Jukić (N.J./180916-Y14; assign database code: FAMU-0923). Accompanied and sharing the same substrate with unidentified *Scutellinia* species.

**Comments:** The genus *Trichophaea* Boud. has been created by Boudier (1885) with *Trichophaea woolhopeia* (Cooke & W. Phillips) Boud. as a type species. Today, *T. woolhopeia* is considered as species complex (Rubini et al., 2011) and needs detailed taxonomic revision.

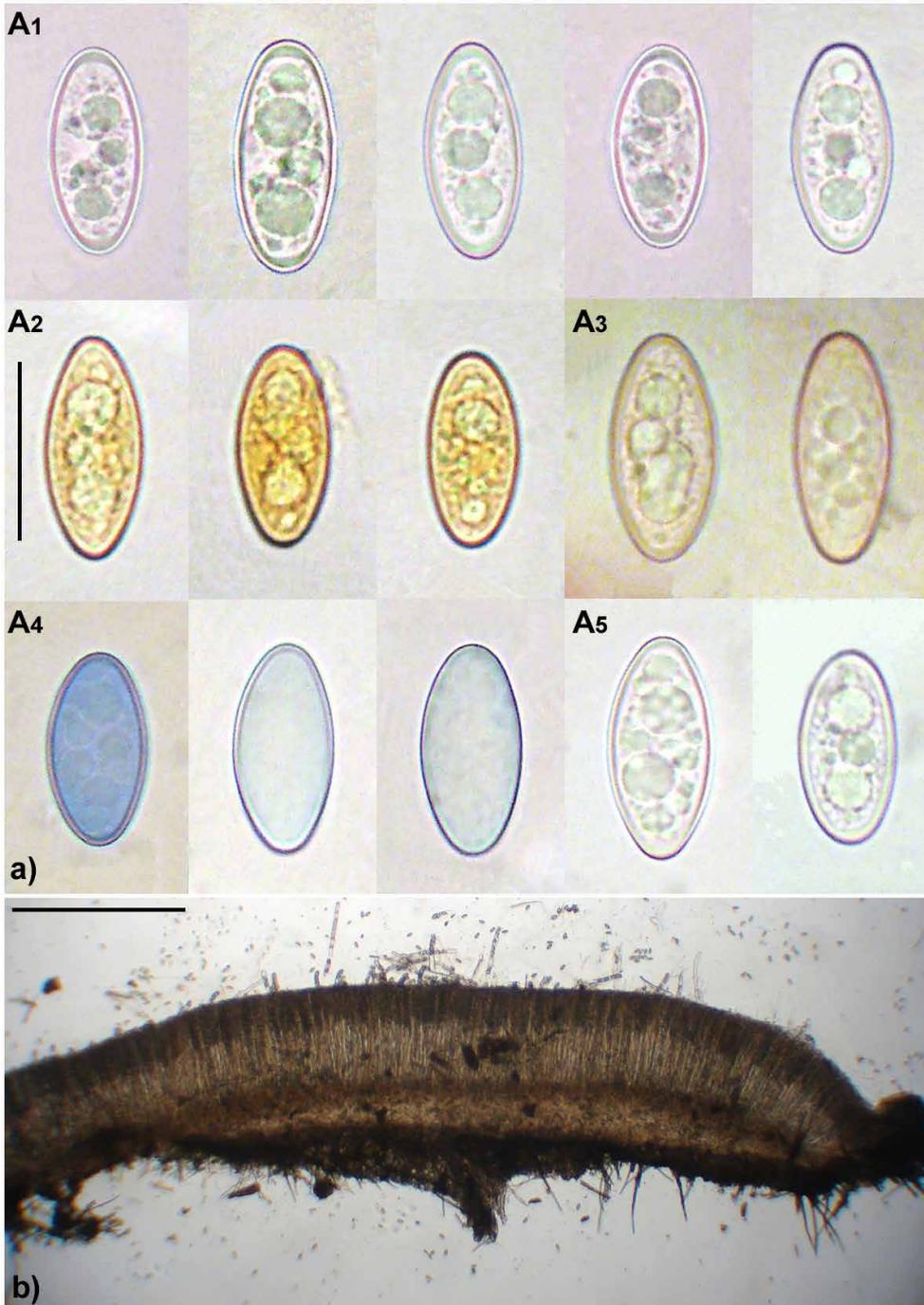
*Trichophaea flavobrunnea* is described by Richon (1879) as *Peziza flavo-brunnea* Richon.

Soon Saccardo (1889) transferred it to the genus *Lachnea* (Fr.) Gillet (*nom. illeg.*). Boudier (1910) provided description and illustration of the most relevant microscopical and macroscopical characters based on Richon's collection.

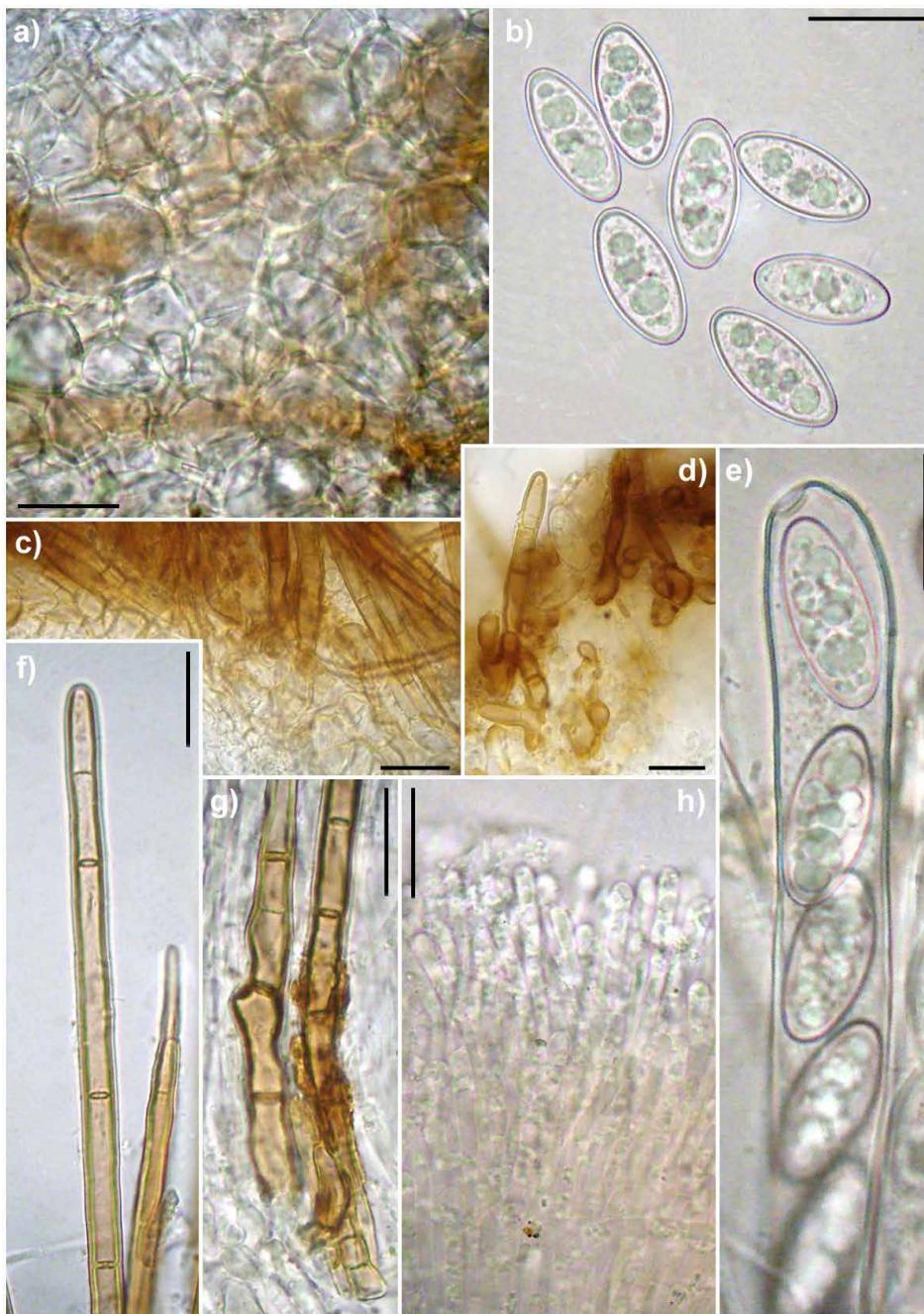
Perić et al. (2015), after more than 100 years, published a detailed description based on recently collected material and established a novel



**Figure 3.** *Trichophaea flavobrunnea* (N.J./180916-Y14): **a), b)** - ascomata photographed *ex situ*; **c), d), e)** - marginal hairs soaked in transparent gel. Bars: **a)** - 1 cm; **b)** - 0.2 cm; **c), d), e)** - 0.1 cm / *Slika 3.* *Trichophaea flavobrunnea* (N.J./180916-Y14): **a), b)** - askomate fotografisane *ex situ*; **c), d), e)** - marginalne dlačice natopljene u providnom gelu. Mjerne skale: **a)** - 1 cm; **b)** - 0,2 cm; **c), d), e)** - 0,1 cm (© N. Jukić)



**Figure 4.** *Trichophaea flavobrunnea* (N.J./180916-Y14): **a)** - mature ascospores in different types of media (A1 - tap water; A2 - IKI; A3 - Acetocarmine; A4 - LCB; A5 - Congo Red); **b)** - vertical median section of apothecia. Bars: **a)** - 20  $\mu$ m; **b)** - 500  $\mu$ m / **Slika 4.** *Trichophaea flavobrunnea* (N.J./180916-Y14): **a)** - zrele askospore u različitim medijima (A1 - vodovodna voda; A2 - Lugolova otopina; A3 - acetokarmin; A4 - pamučno plavo; A5 - kongo crveno); **b)** - vertikalni/poprečni presjek apotecija. Mjerne skale: **a)** - 20  $\mu$ m; **b)** - 500  $\mu$ m (© N. Jukić)



**Figure 5.** *Trichophaea flavobrunnea* (N.J./180916-Y14): **a)** - medullary excipulum cells; **b)** - mature ascospores in tap water mount; **c), d)** - marginal hairs and pseudohairs base; **e)** - living ascospores and asci with laterally positioned operculum; **f), g)** - upper part and base of marginal hairs; **h)** - upper part of living paraphyses in tap water mount. Bars: **a), b), c), e), f), g), h)** - 20  $\mu\text{m}$ ; **d)** - 10  $\mu\text{m}$  / **Slika 5.** *Trichophaea flavobrunnea* (N.J./180916-Y14): **a)** - ćelije središnjeg ekscipuluma; **b)** - zrele askospore u vodovodnoj vodi; **c), d)** - baza marginalnih dlačica i pseudodlačica; **e)** - vitalne askospore i askus sa postrance pozicioniranim operkulomom; **f), g)** - vršni dio i baza marginalnih dlačica; **h)** - vršni dio vitalnih parafiza u vodovodnoj vodi. Mjerne skale: **a), b), c), e), f), g), h)** - 20  $\mu\text{m}$ ; **d)** - 10  $\mu\text{m}$ . (© N. Jukić)

taxonomic status within the genus *Trichophaea*, confirmed by molecular analysis.

Velenovsky (1934) and Svrček (1948) probably described some other, different species under the name *Lachnea flavo-brunnea* (Richon) Sacc. Ascospores drawing in Svrček (1948) present broadly ellipsoid ascospores containing numerous smaller guttules with clearly visible ornamentation units (warts and ribs). Marginal hairs are slightly longer and significantly wider than those described in Perić et al. (2015) and comparing to hairs dimensions in this collection as well.

Macroscopically, *T. flavobrunnea* can be easily overlooked due to the quite possible confusion with *Humaria hemisphaerica* (Hoffm.) Fuckel, but it differs by having less cupuliform and usually smaller apothecia, slightly darker marginal or excipular hairs and by inhabiting different kind of substrate and habitat. Microscopically, *T.*

*flavobrunnea* has longer and more fusiform ascospores, more similar to those of *Trichophaea gregaria* (Rehm) Boud. or *Trichophaea pseudo-gregaria* (Rick) Boud. *Trichophaea flavobrunnea* has the largest apothecia among other members of the genus *Trichophaea*.

It is a very rare and possibly quite sensitive species. Only currently known locality of this species in Bosnia and Herzegovina (area around virgin forest Janj near Šipovo) needs to be properly managed by local authorities. Small population and shrubs of *Juniperus communis* should be undisturbed. This way natural decomposition of dead plant debris will be secured. Habitat loss and fragmentation are major threats for this fungus. It has a very limited distribution in general so it is recommended to be considered as a critically endangered species (CR), based on the IUCN criteria B2ab (ii,iii) and D.

#### 4. CONCLUSIONS / ZAKLJUČCI

Pezizalean fungi in Bosnia and Herzegovina were poorly inventoried in the past. Only few species are considered and mentioned as relevant in a single available strategic conservation document in Federation of Bosnia and Herzegovina (Đug et al., 2013). In the other Bosnian entity, Republic of Srpska, none of the species from the order Pezizales, or fungi in general, are taken into consideration while preparing the official Red List of Flora and Fauna (Crvena lista zaštićenih vrsta flore i faune Republike Srpske, 2012).

Strategic documents in Bosnia and Herzegovina need to be revised and more relevant species of fungi from the order Pezizales and generally Ascomycota should be considered in future conservation plans and measures.

Since there are many severe active habitat destruction and fragmentation processes across the whole Bosnia and Herzegovina, it would be advisable to include all endangered species of Pezizales in all relevant conservation documents and strategies. Prior to this, it is important to implement comprehensive risk assessment studies and to publish field research results on regular basis. Consistency of appropriate systematic research should be continued.

Two species of Pezizales presented in this paper should be included in conservation programs and the Red List of Fungi of Bosnia and Herzegovina with the conservation status as recommended.

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## Sažetak

Gljive reda Pezizales, ali i gljive razreda Ascomycota općenito, u prošlosti nisu bile predmet ozbiljnih sistematskih istraživanja na prostoru Bosne i Hercegovine. U posljednjih nekoliko godina realizovana su detaljnija i sveobuhvatnija mikološka istraživanja. Dio rezultata višegodišnjih istraživanja objavljen je u nekoliko radova (Omerović & Jukić, 2015; Jukić, 2016).

U okviru istraživanja, uz proces inventarizacije značajna pažnja je usmjerena na analizu i procjenu ugroženosti određenih vrsta, te identifikaciju posebnih staništa i područja od značaja za gljive.

Kao rezultat ovih istraživanja zabilježen je određeni broj rijetkih i ugroženih gljiva reda Pezizales. U radu su predstavljene morfološke, mikromorfološke i ekološke karakteristike dvije takve vrste (*Peziza montirivicola* i *Trichophaea flavobrunnea*) te je ukratko prodiskutovano o sličnim vrstama operkulatnih diskomiceta sa kojima se obrađene vrste eventualno mogu zamjeniti.

S obzirom da gljive reda Pezizales nisu u dovoljnoj mjeri obuhvaćene postojećim konzervacijskim dokumentima u Bosni i Hercegovini (Đug et al., 2013; Crvena lista zaštićenih vrsta flore i faune Republike Srpske, 2012) neophodno je izvršiti dopunu ili/i reviziju istih s ciljem adekvatne zaštite najosjetljivijih vrsta i pripadajućih staništa.

**Ključne riječi:** Ascomycota, konzervacija, IUCN, *Peziza*, *Trichophaea*