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First record of the Mediterranean species *Ciboria brunneorufa* in the Balkan Peninsula

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To date, there are no published studies or official papers on mycobiota in coastal Bosnia and Herzegovina. In light of this fact, the author conducted a brief mycological study in the Mediterranean part of the country in February 2016. This paper offers a morphological description of the rare Mediterranean species *Ciboria brunneorufa*, an analysis of microscopic characters and a comparison with data of other authors. It also discusses the global distribution pattern and abundance of *C. brunneorufa*.

An increased level of negative anthropogenic influence is expected on the Klek Peninsula in the future. In order to conserve this habitat unique in Bosnia and Herzegovina and to protect *C. brunneorufa* and other fungal species, it is necessary to make an environmental risk assessment and to carry out further systematic mycological field studies.

Key words: *Helotiales*, *Sclerotiniaceae*, Bosnia and Herzegovina, coastal habitat, anthropogenic influence.

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Pobřeží Bosny a Hercegoviny je územím, o jehož mykobiotě dosud nebyly publikovány žádné studie. Ve světle této skutečnosti provedl autor krátký mykologický průzkum středomořské části země v únoru 2016. Článek předkládá morfologický popis vzácného mediterránního druhu *Ciboria brunneorufa*, analýzu mikroskopických znaků a srovnání s údaji jiných autorů. Diskutováno je též celkové rozšíření a hojnost výskytu *C. brunneorufa*.

V budoucnu je očekáván nárůst negativního vlivu lidské činnosti na poloostrově Klek. V zájmu ochrany zdejšího biotopu – unikátního v rámci Bosny a Hercegoviny – a vzácných druhů hub, jako je právě *Ciboria brunneorufa*, je nezbytné posouzení environmentálních rizik se zahrnutím dalších systematických terénních průzkumů zdejší mykobioty.

INTRODUCTION

Although Bosnia and Herzegovina has a very narrow access to the Adriatic Sea (20 km of coastline), its maritime region is part of a distinct geographical area and plays a significant role in terms of Mediterranean biodiversity and its

conservation. This small mainland area should be particularly considered an important part of the Mediterranean biodiversity corridor.

The vegetation of the Neum-Klek bay is quite similar to that of the surrounding areas and ecosystems in other Mediterranean countries. Consequently, this area may represent a very interesting habitat in Bosnia and Herzegovina, with a fungal diversity worth investigated. The main feature of the coastal region of Bosnia and Herzegovina is its rather degraded vegetation of the *Orno-Quercetum ilicis* association, typical of this climate zone (Barudanović et al. 2015), with a predominance of *Quercus ilex*, *Carpinus orientalis*, *Fraxinus ornus*, *Laurus nobilis*, *Juniperus oxycedrus*, *Erica arborea*, *Pistacia lentiscus*, *Pistacia terebinthus* and other Mediterranean species.

In the past Mediterranean species of fungi were only poorly inventoried in Bosnia, with no published data or official list. Only a couple of recent papers and works address ascomycetes and their distribution in Bosnia and Herzegovina in general. The main content of these notes and publications are preliminary checklists and taxonomic classifications and descriptions of ecological preferences of certain species (Jukić & Omerović 2011, Usćuplić 2012, Omerović & Jukić 2015).

On the other hand, research, inventories and monitoring of certain species of ascomycetes in neighbouring Mediterranean countries have been undertaken more systematically (Matočec 2000, Matočec & Kušan 2008, Van Vooren 2010, Doveri 2011, Kušan et al. 2014, Kušan et al. 2015, Van Vooren et al. 2015a, Van Vooren et al. 2015b, Loizides et al. 2016).

In February 2016, the author conducted a brief mycological study in the Neum-Klek bay and a find of the Mediterranean fungus *Ciboria brunneorufa* is presented in this paper. The presence of other ascomycetes was not recorded at this time of the year.

The only previously published notes dealing with the *Sclerotiniaceae* family in Bosnia and Herzegovina can be found in Palmer et al. (1994), while some records of different species of the *Ciboria* genus are mentioned in Jukić & Omerović (2011).

Ciboria brunneorufa was first described by Bresadola (1903). Saccardo (1906) later cited the same description. More recently, Ormad & García (2007), Ormad et al. (2010) and Pancorbo & Ribes (2010) provided additional information on its morphology and ecology including a description of relevant microscopic characteristics.

In the past, more detailed and comprehensive studies of the *Sclerotiniaceae* family and *Ciboria* genus were conducted by Whetzel (1945), Buchwald (1949), Dumont & Korf (1971), Galán et al. (1996), and Holst-Jensen et al. (1997).

The collection of *Ciboria brunneorufa* from the Neum-Klek bay represents the first record of this species in the territory of Bosnia and Herzegovina and the first record from the Balkan Peninsula as well.

MATERIAL AND METHODS

Fruitbodies of *Ciboria brunneorufa* were found and collected at a single locality on 11 February 2016. The collection included the substratum in order to obtain vital characteristics of the collected material.

The material was analysed according to vital taxonomy methods (Baral 1992). The analysis was performed using a Nikon SE type 102 compound light microscope with an oil-immersion lens and 1000× magnification.

Micro-photographs were taken using a Sony DSC-H2 camera. Ascospores and other microscopic elements were measured using the Piximètre software (Henriot & Cheype on-line).

Measurements and observations were made on multiple preparations. Living cells were first observed in a tap water mount, later IKI – Lugol's solution (Baral 1987) and Congo red (0.04% aqueous) were used. Afterwards, additional analyses were conducted on herbarium specimens using Melzer's reagent (Huhtinen 1990).

In all media, 20 spores were measured to statistically analyse their characteristics (length, width, Qe). Qe stands for ascospore length/width ratio and is given here as the mean value.

The exsiccate has been preserved and adequately stored in a private herbarium (N.J. – Nedim Jukić). Relevant data have been entered into the Amateur Mycological Association's electronic database (FAMU).

RESULTS AND DISCUSSION

Ciboria brunneorufa Bres. 1903 Figs. 1–4

Description. Ascomata apothecial, stipitate, 2–4 mm high and 1–3 mm in diam. Hymenium smooth, beige to brown with a pinkish or rust tint (but paler colours are quite dominant during dry weather conditions). Margin conspicuous in mature apothecia, usually slightly darker than hymenium. Outer side of apothecia brighter than hymenium, with fine furfuraceous, sometimes almost hairy surface all over the stipe.

Asci cylindrical, eight-spored, 105–135(140) × 8–12.5 µm, emerging from croziers, in IKI euamyloid, type bb (Baral 1987).

Ascospores biseriate, in tap water mount (13.4)14–16.8(17.4) × (4.5)5–5.6(5.8) µm, Qe = 2.9; treated with Lugol's solution (11.2)12.9–15.3(15.5) × (3.3)4.6–5.4(5.5) µm; in Congo red (12.7)13–14.9(15.6) × (4.4)4.7–5.6(6.3) µm; in herbarium material treated with Melzer's reagent (9.9)11.4–13.1(13.9) × (3.4)3.7–4.2(4.3) µm, Qe = 3.1; smooth and hyaline, aseptate, ellipsoid to fusiform, often slightly inequilateral, with 1–4 larger lipid bodies of 0.7–1.5 µm diam. and several smaller ones in

each polar half. The number of lipid bodies seemed to be reduced when treated with Congo red. This is probably a consequence of either coalescence of minute lipid bodies with larger ones or minute lipid bodies becoming masked in this medium.

Ascospores containing a single central nucleus, 3 µm diam. Presence of a nucleolus, about 1 µm diam., was also recorded.

Paraphyses cylindrical, septate, up to 130 µm in length, usually as long as asci. Two types observed: one type rather wide, 3.5–5.5 µm at apex, septate, containing refractive vacuoles with pink-reddish pigment, the other one narrower, 2.2–3.7 µm, septate, non-refractive and hyaline, rarely bifurcate.

Medullary excipulum composed of cylindrical elongated, subhyaline to brownish, slightly curved cells with rounded ends forming a *textura porrecta*, 5.4–9.2 µm wide and 50–110 µm in length.

Ectal excipulum consisting of irregularly globose cells, 7–35 µm diam., forming a *textura globulosa-subangularis*. Towards the external part cells become gradually elongated, resembling short hyphoid hairs, 4.5–6 µm wide at the top, containing refractive vacuoles and a pigment similar to those in the paraphyses (Fig. 4c).

Comparison of microscopic characters. Some small inconsistencies and differences are revealed by comparison of data sets from other papers and the present data (Tab. 1).

The measurements of ascospores, asci and paraphyses in Ormad et al. (2010) correspond best to the measurements from the Neum-Klek bay collection. Ascal length and description of aseptate paraphyses in Ormad & García (2007) were a result of errors in the description, as stated and corrected afterwards in Ormad et al. (2010).

The presence of an interior nucleolus, 1 µm diam., is pointed out in Ormad et al. (2010) as well.

The dimensions of the *textura porrecta* cells in Ormad et al. (2010) are 8–10 × 100–130 µm and are slightly longer and wider than those in the collection from the Neum-Klek bay.

Tab. 1. Table comparing characters of all published collections of *Ciboria brunneorufa*.

Publication / Herbarium	Date	Asci	Ascospores	Paraphyses width
Bresadola (1903)	–	120–135 × 6–8 µm	10–12 × 3–4 µm	2–3 µm
Ormad & Garcia (2007)	13 Dec 2006, 25 Oct 2007, 28 Dec 2007	232 × 10 µm	10.5–16 × 3–5 µm	< 4 µm
Pancorbo & Ribes (2010)	06 Dec 2009	88.8–135 × 6.5–10.3 µm	11.2–14.8 × 4.3–5.2 µm	< 3 µm
Ormad et al. (2010)	22 Dec 2009, 02 Jan 2010	110–135 × 8–10 µm	11–15 × 4–5 µm	< 4(4.5) µm
Jukić (this study) / N.J./110216-Y1	11 Feb 2016	105–140 × 8–12.5 µm	13.4–17.4 × 4.5–5.8 µm	two types (2.2–3.7 and 3.5–5.5 µm)

Fig. 1. *Ciboria brunneorufa* (N.J./110216-Y1): **a, b –** apothecia growing on fallen leaves of *Pistacia lentiscus*. Photo N. Jukić.

Fig. 2. *Ciboria brunneorufa* (N.J./110216-Y1): **a –** apothecia photographed in situ, NE part of Klek Peninsula, Bosnia and Herzegovina; **b –** evergreen shrub of *Pistacia lentiscus*; **c –** illustrative photo of typical vegetation on Klek Peninsula with predominance of *Quercus ilex*. Photo N. Jukić.

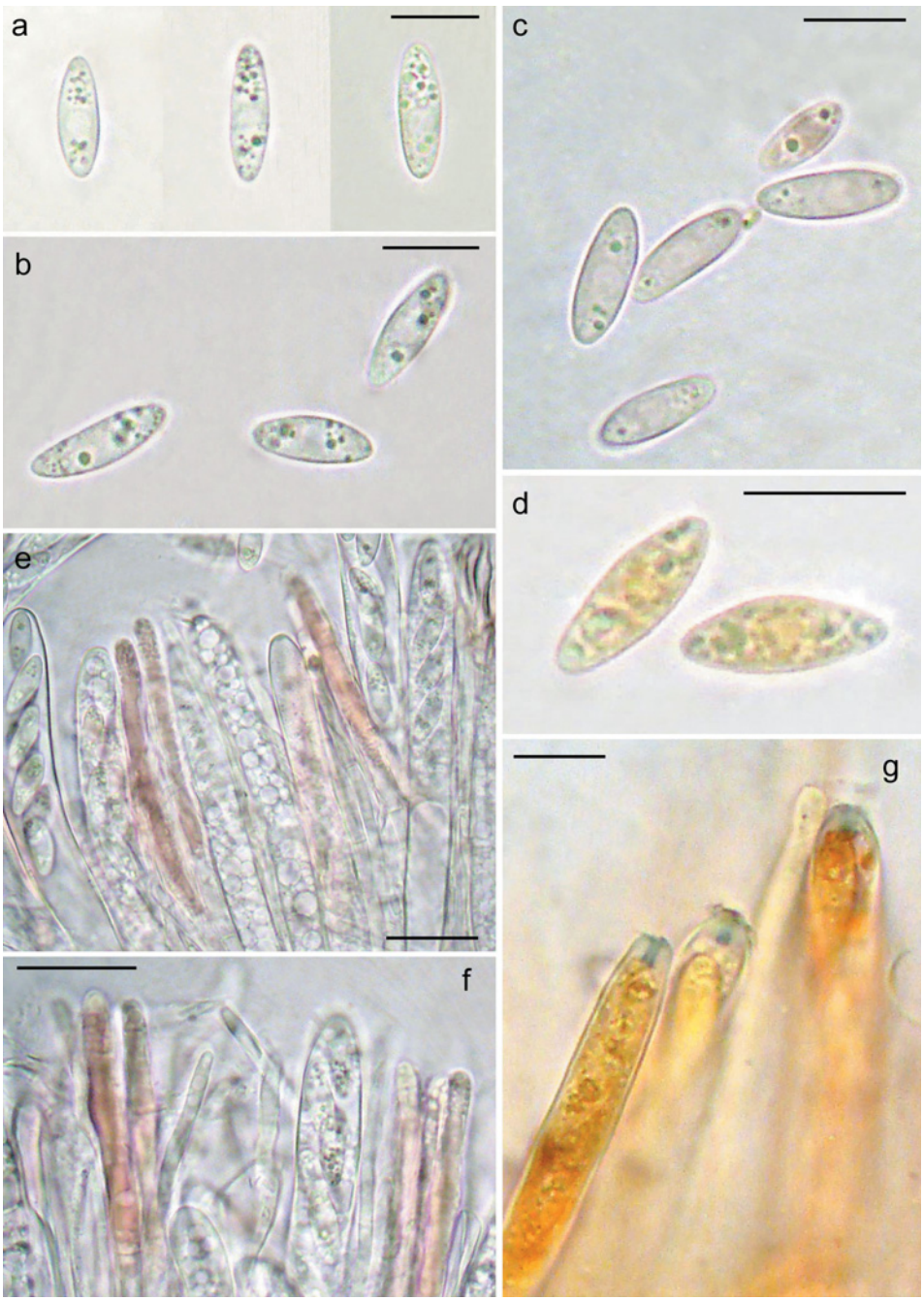

Fig. 3. *Ciboria brunneorufa* (N.J./110216-Y1): **a, b –** ascospores in water mount; **c –** ascospores in Congo red; **d –** ascospores treated with Lugol's solution; **e**, **f –** pigmented paraphyses with refractive vacuoles, hyaline paraphyses and asci; **g –** euamyloid apical rings of the asci. Bars: **a**, **b**, **c**, **d**, **g** = 10 µm; **e**, **f** = 20 µm. Photo N. Jukić.

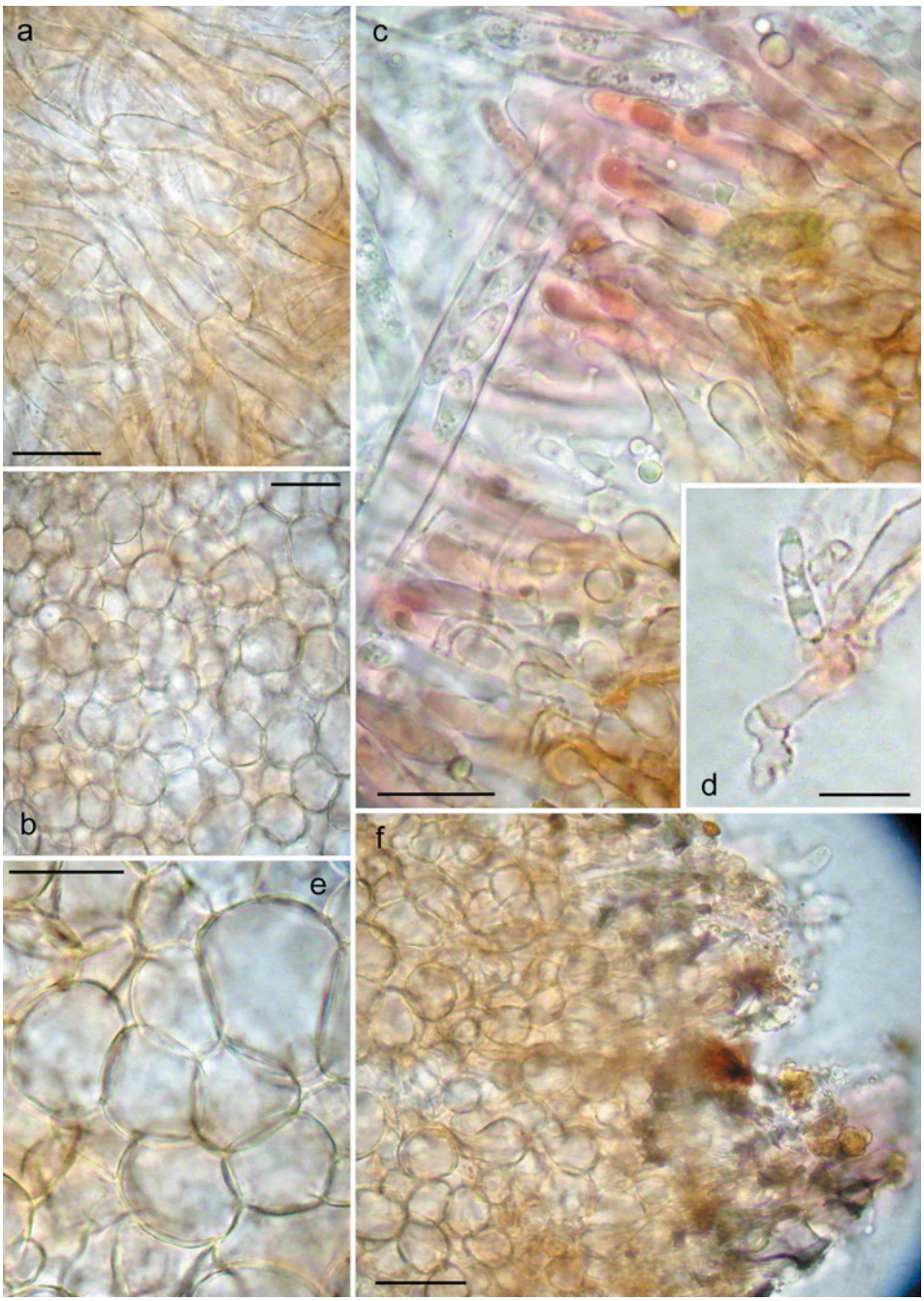

Fig. 4. *Ciboria brunneorufa* (N.J./110216-Y1): **a –** medullary excipulum; **b**, **e –** ectal excipulum; **c**, **f –** margin of apothecium; **d –** ascogenous system. Bars: **a**, **b**, **c**, **e**, **f** = 20 µm; **d** = 10 µm. Photo N. Jukić.

It is worth mentioning that most of the above cited authors did not mention the existence of two different types of paraphyses. The presence and absence of refractive vacuoles and differences between paraphyses are only briefly discussed in Ormad et al. (2010). It is not clear whether these phenomena are the result of a standard maturing process or not. Also, the presence of refractive vacuoles with pink or pale red pigmentation in the walls of outer excipular cells, similar to those in the paraphyses, is described only in Ormad et al. (2010).

Distribution. According to published data and relevant literature, *Ciboria brunneorufa* is only known from Spain (Ormad & García 2007, Ormad et al. 2010, Pancorbo & Ribes 2010) and Portugal (Bresadola 1903; holotype).

The collection from the Neum-Klek bay in Bosnia and Herzegovina represents the northernmost locality of *C. brunneorufa* currently known.

Ciboria brunneorufa is probably much more widespread than currently known, but is easily overlooked due to its small size and type of habitat. It possibly also inhabits other Mediterranean countries, in which its host *Pistacia lentiscus* is quite abundant.

Pistacia lentiscus is common and widespread in the coastal and insular regions of Croatia and Montenegro (Šilić 2005) and in all other countries with a Mediterranean vegetation. It reaches Turkey, Lebanon, Syria and Jordan in the east, the Canary Islands and Iberian Peninsula in the west, and North Africa in the south (Al-Saghir & Porter 2012). This area represents the potential ecological and distributional boundaries of *Ciboria brunneorufa*.

Since *Pistacia lentiscus* inhabits a relatively small area in Bosnia and Herzegovina, it would be advisable to conduct a continuous field study and to establish a long-term monitoring process for *C. brunneorufa* in order to properly conserve the species.

Material examined

Bosnia and Herzegovina. Klek Peninsula, near the town of Neum, 42.91359° N, 17.61787° E, alt. 34 m, gregarious, on wet fallen leaves of *Pistacia lentiscus*, 11 February 2016, leg. N. Jukić (private herbarium N.J./110216-Y1; assign code: FAMU-0912).

CONCLUSIONS

Since the Neum-Klek bay is the only area in Bosnia and Herzegovina where coastal or maritime tourism can develop, this area is heavily influenced by various types of anthropogenic activity.

Unlike Neum, the Klek Peninsula has not been much urbanised so far, but has already been suggested as a location for building new tourist facilities. Urbanisation will most probably lead to the destruction of the already severely degraded

coastal habitat and might also lead to the extinction of some rare and important species in this geographically very limited area.

Therefore, the record of *Ciboria brunneorufa* from February 2016 indicates that the mycobiota of Neum-Klek bay needs to be explored soon. At the same time, it provides a valuable argument to discuss the conservation of Klek Peninsula and the Neum-Klek bay area in general.

In order to make an environmental risk assessment of the mycobiota in the Mediterranean part of Bosnia and Herzegovina, it is advisable:

1) to establish a long-term monitoring process and to track the appearance and fructification of this and other important fungal species;
2) to implement more comprehensive field research in order to provide relevant data on the distribution, abundance and risk assessment of certain species.

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