

УДК 582.284.52:581.95(579.154-751.2)

Boletoid fungi (Boletaceae, Basidiomycota) of the Bidoup – Nui Ba National Park (Vietnam)

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Keywords: biodiversity, Boletaceae, boletoid basidiomycetes, Central Highlands of Vietnam, protected areas, Tây Nguyên, tropical forests.

Summary. Data on the diversity of boletoid fungi of the Bidoup – Nui Ba National Park are presented here and analyzed. An annotated list of 13 species are published for the first time for the National Park and 8 for the first time for Vietnam (*Porphyrellus nigropurpureus*, *Phylloporus pachycystidiatus*, *Ph. rubiginosus*, *Pulveroboletus brunneopunctatus*, *Strobilomyces brunneolepidotus*, *S. calidus*, *Veloporphyrellus pseudovelatus*, *Xerocomus subparvus*). Among plant communities of the National Park the mountain evergreen coniferous-broad-leaved forests dominated by Fagaceae, Lauraceae and Magnoliaceae and with the participation of representatives of Podocarpaceae and Pinaceae are characterized by the highest diversity of boletoid fungi. The species are illustrated with color photographs. The nucleotide sequences obtained during the study were deposited in NCBI GenBank.

Болетовые грибы (Boletaceae, Basidiomycota) национального парка Бидуп – Нуйба (Вьетнам)

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Ключевые слова: биоразнообразие, болетоидные базидиомицеты, особо охраняемые природные территории, тропические леса, Тэйнгунэн, Центральное нагорье Вьетнама, Boletaceae.

Аннотация. В настоящей работе представлены сведения о разнообразии болетовых грибов национального парка Бидуп-Нуйба и дан их краткий анализ. Приведен аннотированный список из 13 видов, впервые публикуемых для национального парка, из которых 8 – впервые для Вьетнама (*Porphyrellus nigropurpureus*, *Phylloporus pachycystidiatus*, *Ph. rubiginosus*, *Pulveroboletus brunneopunctatus*, *Strobilomyces brunneolepidotus*, *S. calidus*, *Veloporphyrellus pseudovelatus*, *Xerocomus subparvus*). Среди растительных сообществ национального парка наибольшим разнообразием болетовых грибов характеризуются горные вечнозеленые хвойно-широколиственные леса с преобладанием Fagaceae, Lauraceae, Magnoliaceae и участием представителей Podocarpaceae и Pinaceae. Виды проиллюстрированы цветными фотографиями. Нуклеотидные последовательности, полученные в ходе исследования, депонированы в NCBI Genbank.

Introduction

Boletoid fungi due to well-developed mycelium and more or less large basidiomata play a significant role in tropical ecosystems, forming mycorrhizal associations with forest-forming tree species of more than 10 families, including Dipterocarpaceae, Fabaceae, Fagaceae, and Pinaceae, improving their nutrition and growth, increasing resistance to adverse environmental influences, as well as participating in the decomposition of organic matter (Thoen, Bâ, 1989; Sato et al., 2007; Becerra, Zak 2011). Many of them are important as a source of nutrition for humans and as potential producers of biologically active substances for medical purposes, what is very actual in recent years. The Southeast Asia region is the center of diversity for this group.

The aim of the present work was to study the diversity of boletoid fungi in the Bidoup – Nui Ba National Park, located in the central part of the Dalat Plateau. Large tracts of mountain and foothill forests have been preserved here. The study of the fungal diversity of typical tropical forests complements the information on the biota of macromycetes in Vietnam and may be useful in the development of measures for the conservation of these valuable nature areas and species inhabiting them.

Bidoup – Nui Ba National Park

Bidoup – Nui Ba National Park (VQG Bidoup Núi Bà) is located in the northwest of Lam Dong province, Lac Doung District, in the Central Highlands of Vietnam (Tây Nguyên), in the central part of the Dalat plateau between 12°00'–12°19'N and 108°21'–108°44'E, occupying an area of 648 km². The park relief is dominated by mountains with heights of more than 1400 m a. s. l., the highest peaks exceed 2000 m – Bidoup (2287 m), Lang Bian (2167 m) and Hon Giao (2062 m). The hydrographic network of the National Park belongs to Krong-No (Krong Nô) River basin (flowing to Mekong (Mê Kông)) and Da Nhim River (flowing to Dong Nai (Đông Nai)). Climate is tropical monsoon. The average annual temperature is 18 °C, in winter in the mountains there can be short-term frosts down to –0.1 °C. Average annual rainfall is about 1800 mm (Eames, 1995; Sourcebook..., 2004; Brodribb, Field, 2008).

The vegetation cover is dominated by mountain and alpine evergreen broadleaf and mixed forests. Secondary pyrogenic pine forests with *Pinus kesiya* are widespread, accounting for about a third of the park's area.

The species of Fagaceae (*Castanopsis pseudoserrata*, *C. indica*, *Lithocarpus stenopus*, *L. truncatus*, *Quercus braianensis*) and Lauraceae (*Cinnamomum*, *Litsea*) dominate in the stand of evergreen broadleaf forests. In mixed forests, conifers complement the upper tier: *Pinus dalatensis*, *P. krempfii*, *Fokienia hodginsii*, *Dacrycarpus imbricatus*, *Keteleeria evelyniana*. Juglandaceae (*Engelhardtia roxburghiana*), Theaceae (*Schima wallichii*, *Anneslea fragrans*), Magnoliaceae, Myrtaceae, Hamamelidaceae (*Rhodoleia championii*), Aceraceae (*Acer campbellii*), Simplicaceae (*Symplocos lucida*) are also common in the stand. Above 1700 m a. s. l., crooked cloud forests with dominance of Ericaceae (*Vaccinium sprengelii*, *V. viscifolium*), Fagaceae (*Lithocarpus silvicularum*, *L. dalatensis*, *Castanopsis wilsonii*, *Quercus poilanei*) Sapotaceae (*Palaquium ridleyi*) are widespread (Eames, 1995; Tordoff et al., 2004; Kuznetsov et al., 2006; Tran, 2011; Phạm, Le, 2012; Novozhilov et al., 2020).

Materials and methods

Collections were made in different types of tropical forests in Bidoup – Nui Ba National Park during 2010–2014 in course of the investigation of mycobiota of the Central Highlands (Tay Nguyen). Boletoid fungi in the territory of the National Park were collected in the following main biotopes:

Mountain evergreen coniferous-broadleaved forests dominated by Fagaceae, Lauraceae and Magnoliaceae and with the participation of representatives of the Podocarpaceae and Pinaceae (1500–1700 m a. s. l.).

Middle mountain open pyrogenic coniferous forest dominated by *Pinus kesiya* (1500–1700 m a. s. l.).

High mountain cloud forests dominated by Ericaceae and Fagaceae (1700–2000 m a. s. l.).

The collected specimens were examined using light and electron microscopy, as well as by analyzing DNA sequences (ITS and *tefla* sites). Macromorphological features have been studied in fresh collections and later through detailed photographs taken in the field. Microscopical characters were studied with a light Zeiss Axioscope A1 microscope with AxioCam 1Cc 3 camera and program tools AxioVisionRel.4.6. (Carl Zeiss, Germany). SEM photos were also used for the identification of material. They have been made by L. A. Kartseva on a scanning analytical electron microscope JEOL JSM-6390LA of Core Facility Centre 'Cell and Molecular Technologies in Plant

Science' of Komarov Botanical Institute and by A. V. Alexandrova (Lomonosov State University) on scanning analytical electron microscopes JSM-6380LA and Camscan-S2 (Cambridge Instruments, 1990). Basidiospores, basidia, and hymenial cystidia were observed in squash preparations of small parts of the tubes in 5 % KOH. The pileipellis was examined on a radial section of the pileus, the stipitipellis – on longitudinal slice of the stipe in 5 % KOH. Basidiospore dimensions are based on 20 measurements, whereas cystidia and basidia dimensions are based on observing at least 10 structures per collection.

The molecular study is based on a phylogenetic species recognition (Taylor et al., 2000). To assess the taxonomic status of the identified phylogenetic lineages and put forward species hypotheses, we relied on the accepted boundaries of the possible variability of the ITS1-5.8S-ITS2 and *tefla* nDNA regions within the species (Petersen et al., 2008; Hughes et al., 2009).

DNA was extracted from herbarium material using NucleoSpin® Plant II kit (Macherey-Nagel, Düren, Germany). The ribosomal ITS1–5.8S–ITS2 region was amplified with primers ITS1F and ITS4B (Gardes, Bruns, 1993), and elongation translation factor (*tefla*) with Boletaceae-specific primers EF1-B-F1 and EF1-B-R (Wu et al., 2014). PCR products were purified with the Fermentas Genomic DNA Purification Kit (Thermo Fisher Scientific, Waltham, MA) and sequenced on an ABI model 3130 Genetic Analyzer (Applied Biosystems, Waltham, MA). Raw data were edited and assembled in MEGA X (Kumar et al., 2018). Newly generated sequences were deposited in NCBI GenBank (Table 2).

Collections studied are kept in the Herbarium of the Komarov Botanical Institute (LE).

History of the studies on of boletoid fungi in the Bidoup – Nui Ba National Park

Information on the history of the investigation of macromycetes in the Central Highlands of Vietnam, including the Bidoup – Nui Ba National Park, is presented in the works of Morozova et al. (2012) and Pham et al. (2018).

The table below (Table 1) summarizes the published data on Boletaceae in Bidoup – Nui Ba National Park, including species described as new to science from this area at different times.

A series of works on the diversity of macromycetes of the Central Highlands, containing information on 24 species of boletoid fungi, belongs to Lê Bá Dũng (Le, 2001, 2003). However, these works do

not indicate the exact locations of the fungi, so we cannot consider these findings as collected in the territory of the Bidoup – Nui Ba National Park.

It should be noted that previously species were identified on the basis of morphological and ecological characters only, and often for identification of paleotropical species European and North American keys were used. Species described from Madagascar also present in the list. In light of recent data on the geographical distribution of fungi, such determinations are questionable and require confirmation by the molecular methods. In this regard, we take into account only the species, which collections were confirmed by molecular data (including those determined only to the genus) and / or described directly from the territory of the National Park. Attempts to isolate DNA from old type specimens, unfortunately failed.

Results and discussion

Thirteen species not previously recorded for the Bidoup – Nui Ba National Park are listed below, including eight species published for the first time for Vietnam (marked with “!”).

Annotated list of species reported for the first time for the Bidoup – Nui Ba National Park

Subfamily Austroboletoidae G. Wu et Zhu L. Yang

Austroboletus* cf. *mucosus (Corner) Wolfe: “Vicinity of Giang Ly Ranger Station, 12°11'5.6"N, 108°41'12.3"E, 1540 m a. s. l., on soil in a medium-mountain broad-leaved forest with the participation of conifers. 29 V 2014” (LE 315558, fig. 1a). Our collections differ from the protologue (Corner, 1972) in a very light color. Young basidiomata are almost white, turn yellowish with age, while in the original description they are completely yellow.

! ***Veloporphyrillus pseudovelatus*** Yan C. Li et Zhu L. Yang: “Vicinity of Klong Lan Village, the left bank of the Da Nhim River, path to Bidoup Mt, 12°07'44"N, 108°39'22"E, 1570 m a. s. l., at the base of the *Pinus kesiya* in the secondary pine forest (*Pinus kesiya*). 25 VI 2010” (LE 315608, fig. 1b).

Subfamily Boletoidae

! ***Porphyrellus nigropurpureus*** (Hongo) Y. C. Li et Zhu L. Yang: “Vicinity of Giang Ly Ranger Station, the left bank of the Da Nhim River, on soil in a medium-mountain broad-leaved forest with the participation of Lauraceae, Myrtaceae, Fagaceae, Magnoliaceae. 24 VI 2010” (LE 254399, fig. 1c); *ibid.* 29 VI 2010 (LE 254400).

Table 1

Species noted in publications, indicating the geographical origin of their types

Виды*	Patouillard, 1909	Perreau, Joly, 1964	Dörfelt et al., 2004	Ton, 2009	Nguyen et al., 2015	Pham et al., 2015	Crous et al., 2018	Crous et al., 2019	Geographical origin of the type
<i>Austroboletus subflavidus</i> (Murrill) Wolfe					+				North America
<i>Boletellus ananas</i> (M. A. Curtis) Murrill		+							North America
<i>Boletellus annamiticus</i> (Pat.) E.-J. Gilbert [= <i>Strobilomyces annamiticus</i> Pat.; = <i>Boletellus emodensis</i> (Berk.) E.-J. Gilbert ss. auct.]	+								Vietnam: Bidoup – Nui Ba
<i>Boletus auripes</i> Peck					+				North America
<i>Boletus bouriquetii</i> R. Heim		+							Madagascar
<i>Boletus edulis</i> Bull.				+					Europe
<i>Boletus queletii</i> var. <i>aurantiacus</i> Dörfelt, Kiet et A. Berg [? = <i>Crocinoletus pinetorum</i> N. R. Zeng, L.-L. Wu, Zhi Q. Liang, S. Jiang]			+	+					Vietnam: Bidoup – Nui Ba
<i>Boletus speciosus</i> Frost						+			North America
<i>Fistulinella olivaceoalba</i> T. H. G. Pham, Yan C. Li et O. V. Morozova							+		Vietnam: Bidoup – Nui Ba
<i>Neoboletus erythropus</i> (Pers.) C. Hahn [= <i>Boletus erythropus</i> Pers.; = <i>Tubiporus erythropus</i> (Pers.) Maire]		+							Europe
<i>Phylloporus rhodoxanthus</i> (Schwein.) Bres.					+				Europe
<i>Phylloporus sulcatus</i> (Pat.) E.-J. Gilbert [= <i>Paxillus sulcatus</i> Pat.]	+	+							Vietnam: Bidoup – Nui Ba
<i>Pulveroboletus ravenelii</i> (Berk. et M. A. Curtis) Murrill			+						North America
<i>Rugiboletus extremiorientalis</i> (Lj. N. Vassiljeva) G. Wu et Zhu L. Yang [= <i>Leccinum extremiorientale</i> (Lar. N. Vassiljeva) Singer]						+			Russian Far East
<i>Strobilomyces strobilaceus</i> (Scop.) Berk. [= <i>Strobilomyces floccopus</i> (Vahl) P. Karst.]		+							Europe
<i>Veloporphyrellus vulpinus</i> T. H. G. Pham, O. V. Morozova, A. V. Alexandrova et E. S. Popov								+	Vietnam: Bidoup - Nui Ba
<i>Xerocomus langbianensis</i> Dörfelt, Kiet et A. Berg bis			+	+					Vietnam: Bidoup - Nui Ba
Total: 17	2	5	3	2	3	2	1	1	

* The names of taxa are given in accordance with the data of the Index Fungorum resource (as of July 2020) with indication of the synonyms under which they were indicated in the corresponding publications.

! *Strobilomyces brunneolepidotus* Har. Takah et Taneyama: “Vicinity of Hon Giao Ranger Station, path to Hon Giao Mt, 12°11'18.1"N, 108°42'50.6"E, 1700 m a. s. l., on soil in upper montane mossy evergreen broadleaf forest. 28 VI 2010” (LE 315609, fig. 1d).

! *S. calidus* Li H. Han, J. Xu & Zhu L. Yang: “Vicinity of Giang Ly Ranger Station, 12°10'42.1"N, 108°41'00.7"E, 1510 m a. s. l., on

soil in a medium-mountain broad-leaved forest with the participation of conifers. 27 V 2014 (LE 315562, fig 1e.).

Subfamily Leccinoideae G. Wu et Zhu L. Yang
Pseudoaustroboletus valens (Corner) Y. C. Li et Zhu L. Yang, in Li, Li, Zeng, Cui et Yang: “Vicinity of Giang Ly Ranger Station, 12°11'05.6"N, 108°41'12.3"E, 1540 m a. s. l., on soil in a medium-

mountain broad-leaved forest with the participation of conifers. 28 VI 2010” (LE 315593). “Ibid., leg. A. E. Kovalenko, Ngoc Nguyen Bao. 04 XI 2012” (LE 312475). “Ibid. 29 V 2014” (LE 315553, fig. 1f).

Table 2

Distribution of Boletaceae species of the Bidoup – Nui Ba National Park
by biotope types with Genbank numbers

Species	Types of biotops			Altitude, m a. s. l.	Voucher numbers	Genbank numbers	
	1	2	3			ITS	<i>tefla</i>
<i>Aureoboletus longicollis</i>	+			1500	LE 315562 LE 312514	MT893598	MW033312
<i>Austroboletus</i> cf. <i>mucosus</i>	+			1540	LE 315558	MT893602	–
<i>Austroboletus</i> sp.	+			1550	LE 312524	MT893601	–
<i>Boletus queletii</i> var. <i>aurantiacus</i> [? = <i>Crocinoletus pinetorum</i>]		+		1600	V 136, HAL 1768, holotype	–	–
<i>Boletellus annamiticus</i>	+			1500	FH 3720 (LBA 161), lectotype	–	–
<i>Boletellus</i> sp.	+			1600	LE 315630 LE 254394	–	–
<i>Crocinoletus rufoaureus</i>	+		+	1700	LE 315594 LE 315561 LE 312520	–	–
<i>Fistulinella olivaceoalba</i>			+	1850	LE 312004, holotype	MH718344	MH733592
<i>Leccinellum</i> sp.	+			1550	LE 312515	–	MW033313
<i>Phylloporus pachycystidiatus</i>	+			1550	LE 312522	MT893596	–
<i>Phylloporus rubiginosus</i>	+			1480	LE 312523	MT893597	–
<i>Phylloporus sulcatus</i> (Pat.) E.-J. Gilbert [= <i>Paxillus sulcatus</i> Pat.]	+			1500	FH 3725 (LBA 210), holotype	–	–
<i>Phylloporus</i> sp.	+			1550	LE 312519	–	MW033321
<i>Porphyrellus nigropurpureus</i>	+			1500	LE 254399 LE 254400	MT893603 MT893604	–
<i>Pseudoaustroboletus valens</i>	+			1540	LE 312475 LE 315593 LE 315553	MT893599	MW033322
<i>Pulveroboletus brunneopunctatus</i>	+			1520	LE 315598	–	–
<i>Strobilomyces brunneolepidotus</i>			+	1700	LE 315609	–	MW033320
<i>Strobilomyces calidus</i>	+			1510	LE 312518	–	MW033317
<i>Strobilomyces</i> sp. 1	+			1500	LE 312516	–	MW033316
<i>Strobilomyces</i> sp. 2	+			1400	LE 312517	–	MW033319
<i>Veloporphyrellus pseudovelatus</i>		+		1570	LE 315608	–	MW033318
<i>Veloporphyrellus vulpinus</i>	+			1500	LE 315544, holotype	MN511177	MN597966
<i>Xerocomus langbianensis</i>		+		1600	DI 9903, HAL 769, isotype	–	–
<i>Xerocomus subparvus</i>	+			1490	LE 315595	MT893600	MW033315
<i>Zangia roseola</i>	+			1500	LE 315610	–	MW033314
Total: 25	20	3	3				

Subfamily Xerocomoideae Singer

Aureoboletus longicollis (Ces.) N. K. Zeng et Ming Zhang, in Zeng, Zhang et Liang: “Vicinity of Giang Ly Ranger Station, 12°10'46.8"N, 108°41'08.5"E, 1500 m a. s. l., on soil in a medium-mountain broad-leaved forest with the participation of conifers. 25 V 2014” (LE 312514, fig. 2a). “Ibid. 12°10'43.3"N, 108°41'05.3"E. 27 V 2014” (LE 315562).

! *Phylloporus pachycystidiatus* N. K. Zeng, Zhu L. Yang & L. P. Tang: “Vicinity of Klong Lanh Village, the left bank of the Da Nhim River, path to Bidoup Mt, 1550 m a. s. l., on soil in a medium-mountain broad-leaved forest with the participation of Lauraceae, Myrtaceae, Fagaceae, Magnoliaceae. 25 VI 2010” (LE 312522, fig. 2b).

! *Ph. rubiginosus* M. A. Neves & Halling: “Vicinity of Giang Ly Ranger Station, 12°10'54.1"N, 108°41'00.5"E, 1480 m a. s. l., on soil in a medium-mountain broad-leaved forest with the participation of conifers. 25 V 2014” (LE 312523, fig. 2c).

! *Xerocomus subparvus* Xue T. Zhu et Zhu L. Yang, in Wu, Li, Zhu, Zhao, Han, Cui, Li, Xu et Yang: “Vicinity of Giang Ly Ranger Station, 12°10'46.8"N, 108°41'00"E, 1490 m a. s. l., on soil in a medium-mountain broad-leaved forest with the participation of conifers. 25 V 2014” (LE 315595, fig. 2d).

Subfamily Zangioideae G. Wu, Y. C. Li et Zhu L. Yang

Zangia roseola (W. F. Chiu) Y. C. Li et Zhu L. Yang, in Li, Feng et Yang: “Vicinity of Klong Lanh Village, path to Bidoup Mt, 12°07'57.3"N, 108°39'02.8"E, 1500 m a. s. l., on soil in a medium-mountain broad-leaved forest with the participation of conifers. 24 V 2014” (LE 315610, fig. 2e).

***Pulveroboletus*-group**

Crocinoletus rufoaureus (Masse) N. K. Zeng, Zhu L. Yang et G. Wu, in Zeng, Wu, Li, Liang et Yang: “Vicinity of Hon Giao Ranger Station, path to Hon Giao Mt, 12°11'18.1"N, 108°42'50.6"E, 1700 m a. s. l., on soil in upper montane mossy evergreen broadleaf forest. 28 VI 2010” (LE 315594). “Ibid. 26 V 2014” (LE 315561, fig. 2f). “Vicinity of Klong Lanh Village, path to Bidoup Mt, 12°07'57.3"N, 108°39'02.8"E, 1500 m a. s. l., on soil in a medium-mountain broad-leaved forest with the participation of conifers. 24 V 2014” (LE 312520).

! *Pulveroboletus brunneopunctatus* G. Wu et Zhu L. Yang, in Wu, Li, Zhu, Zhao, Han, Cui, Li,

Xu et Yang: “Vicinity of Giang Ly Ranger Station, 12°11'06.7"N, 108°41'13.9"E, 1520 m a. s. l., on soil in a medium-mountain broad-leaved forest with the participation of conifers. 29 V 2014” (LE 315598, fig. 2g).

In addition, thanks to molecular genetic methods, 6 genotypes were revealed, which could not be attributed to any known species either by molecular or morphological data. There are: *Austroboletus* sp., *Boletellus* sp., *Leccinellum* sp., *Phylloporus* sp., and two species of *Strobilomyces*.

Thus, we can say about the presence of 25 species of Boletaceae family in the studied area, which were confirmed by the molecular data or were taken into account as species with a type locality in a National Park (Table 2). The species are distributed between 14 genera, belonging to five subfamilies and the *Pulveroboletus* group.

Among plant communities of the National Park the mountain evergreen coniferous-broad-leaved forests dominated by Fagaceae, Lauraceae and Magnoliaceae and with the participation of representatives of Podocarpaceae and Pinaceae are characterized by the highest diversity of boletoid fungi. The most favorable conditions for their growth have developed in these types of forest: the largest number of possible symbionts grows; the temperature and humidity indicators are optimal. In addition, the largest number of observations was carried out in these biotopes. In secondary pine and high mountain foggy and crooked forests the diversity of the boletoid fungi was found to be distinctly lower.

The available information on the nutritional properties of tropical boletes is very limited. We can only assume them, based on knowledge of the phylogenetic relationships in this group of fungi. *Boletellus annamiticus* and *Crocinoletus rufoaureus* are most likely edible, as the very closely related species *Boletellus emodensis* and *Crocinoletus laetissimus* are eaten (Læssøe et al., 2018; Wu et al., 2019). *Zangia roseola* considered edible (Wu et al., 2019). *Aureoboletus viscosus* and some species of the genus *Strobilomyces* regarded as potentially edible (Læssøe et al., 2018). Therefore, the related *Aureoboletus longicollis* and other species of the genus *Strobilomyces* can also be considered as potentially edible. It is known that *Pulveroboletus brunneopunctatus*, as well as other species of this genus, are poisonous (Chen et al., 2014, Læssøe et al., 2018). However, this question requires further study.



Fig. 1. Basidiocarps *in situ*: a – *Austroboletus* cf. *mucosus* (LE 315558); b – *Veloporphyrillus pseudovelatus* (LE 315608); c – *Porphyrillus nigropurpureus* (LE 254399); d – *Strobilomyces brunneolepidotus* (LE 315609); e – *Strobilomyces calidus* (LE 315562); f – *Pseudoaustroboletus valens* (LE 315553).



Fig. 2. Basidiocarps in situ: a – *Aureoboletus longicollis* (LE 315562); b – *Phylloporus pachycystidiatus* (LE 312522); c – *Phylloporus rubiginosus* (LE 312523); d – *Xerocomus subparvus* (LE 315595); e – *Zangia roseola* (LE 315610); f – *Crocinoboletus rufoaureus* (LE 315561); g – *Pulveroboletus brunneopunctatus* (LE 315598).

Acknowledgments

The authors are grateful to staff of the Joint Russian-Vietnamese Tropical Research and Technological Center for organizing the expeditions and to management of the Bidoup – Nui Ba National Park for their help and for permission to collect in the forests. We thank Drs. E. S. Popov (Komarov Botanical Institute) for the help with collecting, A. V. Alexandrova (Moscow State University) and L. A. Kartseva (Komarov Botanical Institute) – for their

assistance with the SEM photos. We are grateful to Curators of Herbariums HAL and FH for the opportunity to study type collections. The work was conducted in the framework of a research project of the Komarov Botanical Institute of the Russian Academy of Sciences (no. AAAA-A19-119020890079-6) using equipment of its Core Facility Centre ‘Cell and Molecular Technologies in Plant Science’ with the financial support of Russian Foundation for Basic Research (project no. 20-04-00349).

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