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Species of *Odontia* and *Tomentella* (Thelephorales, Basidiomycota) new to Dagestan, Russia

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Abstract
Aim. To obtain new data on the species diversity, phylogenetic structure, and ecological characteristics of thelephoroid fungi (Thelephorales, Basidiomycota) in the Republic of Dagestan.
Material and Methods. Both micromorphological and molecular analyses were used for studying of the fungal specimens collected by the authors in 2018-2019 in the Gunibsky and Magaramkentsky Districts of Dagestan. Additional specimens from the Mycological Herbarium of the Komarov Botanical Institute of the Russian Academy of Sciences (LE) were studied. The ITS region of nrDNA was amplified with two pairs of primers, ITS1F/ITS4 and ITS5/ITS4.
Results. Sixteen ITS sequences belonging to eight species were obtained from the studied material. Of them, 14 sequences clustered in the *Tomentella* clade and two sequences nested within the *Odontia* clade. Four species – *Odontia duemmeri*, *Tomentella lapida*, *T. radiosa*, *T. terrestris* – were registered for the first time for Dagestan. Detailed information on the specimens studied is presented. Species identification of *Odontia fibrosa*, *Tomentella badia*, *T. ferruginea*, and *T. stuposa* was confirmed by ITS nrDNA analysis.
Conclusion. Data on the species richness of the genera *Odontia* and *Tomentella* in Dagestan is updated, and the species *T. lilacinogrisea* is excluded from the regional funga. To date the genera *Odontia* and *Tomentella* in the Republic of Dagestan are represented by three and fifteen species, respectively.
Key Words
Biodiversity, basidiomycetes, distribution of fungi, ITS phylogeny, DNA barcodes, *Tomentella*, Dagestan, Caucasus.

Новые для Дагестана виды родов *Odontia* и *Tomentella* (Thelephorales, Basidiomycota)

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Резюме

Цель. Получить новые данные о видовом разнообразии, филогенетической структуре и экологических характеристиках телефоровых грибов (Thelephorales, Basidiomycota) Республики Дагестан.

Материал и методы. В работе были использованы микроморфологический и молекулярный анализы для изучения образцов грибов, собранных авторами в 2018-2019 гг. в Гунибском и Магарамкентском районах Дагестана. Были изучены дополнительные образцы из микологического гербария Ботанического института им. В.Л. Комарова Российской академии наук (LE). Участок внутреннего траскрибируемого спейсера (ITS) ярДНК был амплифицирован для исследованных образцов с использованием двух пар праймеров, ITS1F / ITS4 и ITS5 / ITS4.

Результаты. Впервые получены 16 ITS последовательностей, которые отнесены к восьми видам грибов. 14 нуклеотидных последовательностей оказались в пределах клады, сформированной видами рода *Tomentella*, а две других последовательности вошли в кладу, образованную видами рода *Odontia*. Четыре вида – *Odontia duemmeri*, *Tomentella lapida*, *T. radiosa*, *T. terrestris* – впервые отмечены в Дагестане. Находки видов *Odontia fibrosa*, *Tomentella badia*, *T. ferruginea*, *T. stuposa*, ранее известных для Дагестана, подтверждены на основе анализа ITS области ярДНК.

Заключение. Обновлены данные о видовом богатстве родов *Odontia* и *Tomentella* в Дагестане, при этом вид *T. lilacinogrisea* исключен из региональной микобиоты. К настоящему времени роды *Odontia* и *Tomentella* в Республике Дагестан представлены 3 и 15 видами соответственно.

Ключевые слова
Биоразнообразие, базидиомицеты, распространение грибов, ITS-филогения, ДНК-штрихкодирование, *Tomentella*, Дагестан, Кавказ.

INTRODUCTION

Despite rather detailed scientific research on the flora and fauna of Dagestan, mycological studies have not been given due attention; they have been episodic and unsystematic in nature. In general, the data on the diversity of aphyllophoroid fungi are available for the protected natural territories [1-4]. This publication continues the series of works devoted to the inventory of species diversity and ecological characteristics of aphyllophoroid fungi in Dagestan [2-8], in particular of the genera *Odontia* and *Tomentella* [9].

To date fifteen species of the genus *Tomentella s. lato* are known for mycobiota of Dagestan: *Tomentella atramentaria*, *T. badia*, *T. bryophila*, *T. cinerascens*, *T. crinalis* (=*Odontia ferruginea*), *T. ellisii*, *T. ferruginea*, *T. fibrosa* (=*Odontia fibrosa*), *T. lateritia*, *T. lilacinogrisea*, *T. pilosa*, *T. punicea*, *T. stuposa*, *T. subtestacea*, and *T. umbrinospora* [7-10].

Taking into account the widespread use of molecular techniques in mycology both to describe new taxa and to study the modern species composition of regional mycobiotas, we carried out a comparative study of the internal transcribed spacer (ITS) region of the nuclear ribosomal DNA (nrDNA) from the specimens of the genera *Odontia* and *Tomentella*, which allowed us to identify species new to Dagestan.

The *aim* of the study was to obtain new data on the species diversity, phylogenetic structure, and ecological characteristics of thelephoroid fungi (Thelephorales, Basidiomycota) in Dagestan.

MATERIAL AND METHODS

Specimens of basidiomycetes were collected during a routine survey of forest ecosystems in the Gunibsky and Magaramkentsky Districts of the Republic of Dagestan within the protected areas: the Upper Gunib Nature Park and the Samursky National Park in May and September-October 2018-2019. Additionally, specimens stored in the Mycological Herbarium of the Komarov Botanical Institute of the Russian Academy of Sciences (LE) were studied. Microscopy-based identification of fungi as well as re-examination of herbarium specimens was done at magnifications up to ×1000 using LOMO Mikmed-6 optical microscope, Carl Zeiss AxioImager A1 microscope and a standard set of reagents (5% potassium hydroxide solution, Melzer's reagent).

DNA was extracted from small pieces of dried basidiocarps using the FitoSORB DNA extraction kit (Syntol, Russia) according to the manufacturer's instructions. PCR reactions were performed in 25 µL of reaction mixtures containing 5 µL of Fidelity Buffer (5X), 0.5 µL of KAPA HiFi HotStart DNA Polymerase, 0.75 µL of dNTPs, 0.5 µL of each PCR primer, 12.75 µL of deionized H_2O, and 5 µL of template DNA. The ribosomal ITS1–5.8S–ITS2 region was amplified with two pairs of the primers: ITS1F and ITS4 or ITS5 and ITS4 [11; 12]. PCR products were visualized using agarose gel electrophoresis and GelRed staining, and subsequently purified with the Fermentas Genomic DNA Purification Kit (Thermo Fisher Scientific, Lithuania). Purified PCR products were sequenced on an ABI model 3130 Genetic Analyzer (Applied Biosystems, CA, USA). Raw data were edited and assembled in MEGA 6 [13]. Newly generated sequences were deposited in the GenBank. Additionally, 46 ITS sequences were retrieved from GenBank [14] and UNITE [15] (Table 1). Sequences were aligned with the MAFFT version 7 web tool [16; 17] using the E-INS-1 option. Maximum Likelihood (ML) analysis was performed in the IQ-TREE Web Server [18] with 1000 ultrafast bootstrap replicates.

Table 1. Specimens and sequences used in this study
Таблица 1. Образцы и нуклеотидные последовательности, использованные в данном исследовании

Species	GenBank / UNITE accessions	Specimen voucher	Origin
Odontia duemmeri	UDB011121	KHL10605	Jamaica
Odontia duemmeri	UDB018552	TU115185	Mexico: Municipality of Lazaro Cardenas
Odontia duemmeri	UDB033701	TU115587	Germany: Bavaria
Odontia duemmeri	**MT981503**	LE 314777	Russia: Dagestan
Odontia ferruginea	UDB032228	TU111186	Estonia
Odontia fibrosa	MK602775	TU115028	China
Odontia fibrosa	**MT981502**	LE F-332368	Russia: Dagestan
Thelephora terrestris	AF272921	JS17996 (O)	Unspecified
Thelephora terrestris	AF272923 / UDB000215	TAA162083	Estonia
Tomentella alpina	EF655702	IB20060231 (holotype)	Austria
Tomentella atramentaria	AF272904	TAA149211	Unspecified
Tomentella atramentaria	EF644115	IB2004189	Austria
Tomentella atramentaria	KT353045	GO-2009-248	Mexico: Mexico State
Tomentella badia	AF272917 / UDB000239	TAA164600	Estonia
Tomentella badia	AF272937 / UDB000238	TAA159022	Russia
Tomentella badia	KJ140664	CFMR:DLL2011-166	United States: central Wisconsin
Tomentella badia	UDB000961	NF.S103 (O)	Norway
Tomentella badia	**MT981507**	LE 299095	Russia: Kaluga Region
Tomentella badia	**MT981508**	LE 299096	Russia: Kaluga Region

Tomentella badia	**MT981506**	LE 313862	Russia: Lipetsk Region
Tomentella badia	**MT981495**	LE 314772	Russia: Dagestan
Tomentella badia	**MT981499**	LE 314775	Russia: Dagestan
Tomentella cinerascens	UDB003309	TU100735	Finland
Tomentella cinerascens	UDB016193	TU108037	Estonia
Tomentella cinerascens	UDB016498	TU111378	Italy
Tomentella crinalis	UDB032224	TU105627	Estonia
Tomentella ellisii	UDB011603	TU115347	Finland
Tomentella ellisii	UDB000226	TU123494	Germany
Tomentella ferruginea	AF272909	TAAM166877	Estonia
Tomentella ferruginea	MH310801	SS367B	Unspecified
Tomentella ferruginea	**MT981504**	LE 314778	Russia: Dagestan
Tomentella ferruginea	**MT981501**	LE F-332319	Russia: Dagestan
Tomentella fuscocinerea	DQ974776	src813	United States
Tomentella fuscocinerea	GU214810	TU108229	Iran
Tomentella globosa	MG136838	Yuan11618	Finland
Tomentella globosa	MG136839	Yuan11603	Finland
Tomentella lammiensis	MG136840	Yuan11617	Finland
Tomentella lammiensis	MG136841	Yuan11597	Finland
Tomentella lapida	UDB003322	TU100884	France: Vaucluse
Tomentella lapida	UDB016346	TU115491	Estonia
Tomentella lapida	UDB016370	TU115604	Estonia
Tomentella lapida	**MT981496**	LE F-332369	Russia: Dagestan
Tomentella lilacinogrisea	AF272910	TAA159499	Unspecified
Tomentella lilacinogrisea	UDB016500	TU111381	Italy
Tomentella lilacinogrisea	UDB018468	TU108189	Estonia
Tomentella longisterigmata	MG136836	Yuan11610, holotype	Finland
Tomentella longisterigmata	MG136837	Yuan11602	Finland
Tomentella radiosa	**MT981500**	LE 314776	Russia: Dagestan
Tomentella ramosissima	U83480	LT19, Bruns Herbarium, UCB	United States
Tomentella sp.	UDB018564	TAAM150657	Estonia
Tomentella sp.	UDB025528	TU116751	Morocco: Douar Tala Atia
Tomentella stuposa	AF272944	JS20510 (O)	Unspecified
Tomentella stuposa	UDB000247	TAAM159822	Sweden
Tomentella stuposa	**MT981509**	LE 286822	Russia: Oryol Region
Tomentella stuposa	**MT981505**	LE 292220	Russia: Oryol Region
Tomentella stuposa	**MT981498**	LE 314774	Russia: Dagestan
Tomentella stuposa	**MT981510**	LE 314779	Russia: Dagestan
Tomentella terrestris	AF272911 / UDB000221	TAA159557	Estonia
Tomentella terrestris	UDB003315	TU100886	France: Vaucluse
Tomentella terrestris	**MT981497**	LE 314773	Russia: Dagestan

RESULTS AND DISCUSSION

The ITS dataset includes 16 newly generated sequences and 46 sequences of 19 species downloaded from public databases (GenBank, UNITE). The genus *Odontia* was used as an outgroup. The final ITS alignment contained 858 positions (including gaps). The ML tree is shown in Fig. 1. Our 16 sequenced specimens appeared in eight separate well-supported clades, which correspond to different species. Among them, 14 sequences clustered in the clade comprised of *Tomentella* species and two sequences nested within the *Odontia* clade.

The ITS nrDNA analysis confirmed the microscopy-based taxonomic assignment of the specimens from the Republic of Dagestan belonging to three species from the genus *Tomentella* (*T. badia*, *T. ferruginea*, *T. stuposa*) and one species from the genus *Odontia* (*O. fibrosa*) [7-10].

The first finding of *Tomentella badia* from Dagestan was recorded on a fallen trunk of *Juniperus oblonga* from the Gunib Plateau (Gunibsky District) as a result of the special study of juniper-associated aphyllophoroid fungi [7]. This fungus was also collected from fallen trunks of *Betula* sp. in the same area by Sergey Volobuev and Aziz Ismailov in October 2018 and these specimens (LE 314772, LE 314775) were sequenced now (Table 1).

The species *Tomentella ferruginea* was registered for Dagestan based on four specimens mentioned by U. Kõljalg [10], one specimen (LE F-332319) from the Samursky National Park (Magaramkentsky District) [8], and the specimen from the Gunib Plateau (Gunibsky District) collected from a fallen trunk of *Betula* sp. by Yuliya Ivanushenko in May 2019 (LE 314778). The last two specimens mentioned were sequenced in this study.

T. stuposa, one of the most common species of the genus *Tomentella,* was reported for Dagestan [10], but the specimens from the Gunib Plateau (Gunibsky District) collected from fallen trunks of *Betula* sp. by Sergey Volobuev and Aziz Ismailov in October 2018 (LE 314774, LE 314779) were sequenced and included in the phylogenetic analysis for the first time (Table 1, Fig. 1).

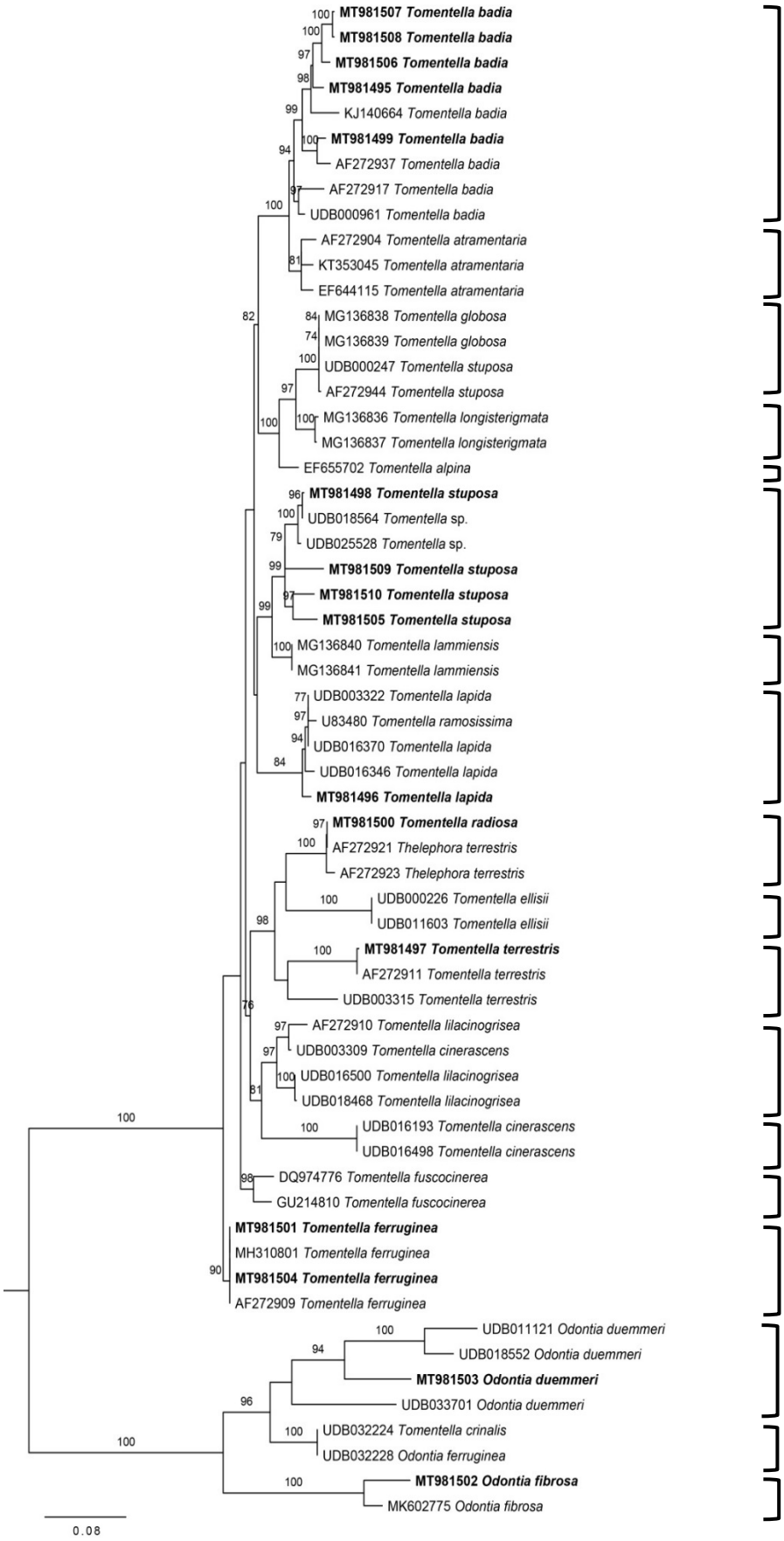

Figure 1. The Maximum Likelihood tree illustrating the phylogeny of *Odontia* and *Tomentella* species, based on ITS sequence dataset. Ultrafast bootstrap values (%) not less than 70 are shown above the branches. Sequence accession numbers (GenBank or UNITE) of selected species are indicated before species names. The bold font shows the names of the sequences obtained in this study

Рисунок 1. Дерево, построенное методом максимального правдоподобия и иллюстрирующее филогению видов *Odontia* и *Tomentella*, на основе ITS-последовательностей. Значения ultrafast bootstrap (не менее 70%) показаны над ветвями. Номера последовательностей (в базах данных GenBank или UNITE) отобранных таксонов указаны перед видовыми названиями. Жирным шрифтом выделены названия нуклеотидных последовательностей, полученных в данном исследовании

The newly generated ITS sequence of *Odontia fibrosa* was obtained (Table 1) for the specimen from the Samursky National Park (Magaramkentsky District) [8], which is the second finding of the species besides the record in the Upper Gunib Nature Park (Gunibsky District) [9].

At the same time, four other species are new to Dagestan – *Odontia duemmeri*, *Tomentella lapida*, *T. radiosa*, and *T. terrestris*. Detailed annotations for specimens of these species and some taxonomic and distributional remarks are presented below.

Odontia duemmeri (Wakef.) Kõljalg

Specimen examined: Russia, Republic of Dagestan, Gunibsky District, Gunib Plateau, 42.400873° N, 46.910158° E, 1905 m a.s.l., herb-rich birch forest, on fallen trunk of *Betula* sp. (LE 314777), 1 October 2019, coll. and det. Sergey V. Volobuev and Yuliya Yu. Ivanushenko.

The second species of the *Odontia* genus, followed by *O. fibrosa* [9], which is reported for Dagestan. As stable isotope analyses showed, all representatives of *Odontia* possess a non-ectomycorrhizal lifestyle, but their nutrition differs from typical xylotrophic basidiomycetous fungi [19]. Micromorphology of *O. duemmeri* is carefully described and illustrated by E. Martini [20]. This species occurs both on deciduous (*Quercus robur*) and coniferous (*Juniperus communis*) trees, but it was not previously collected from the wood of birch. Our finding (Fig. 2) is the first one of the species on the Caucasus.

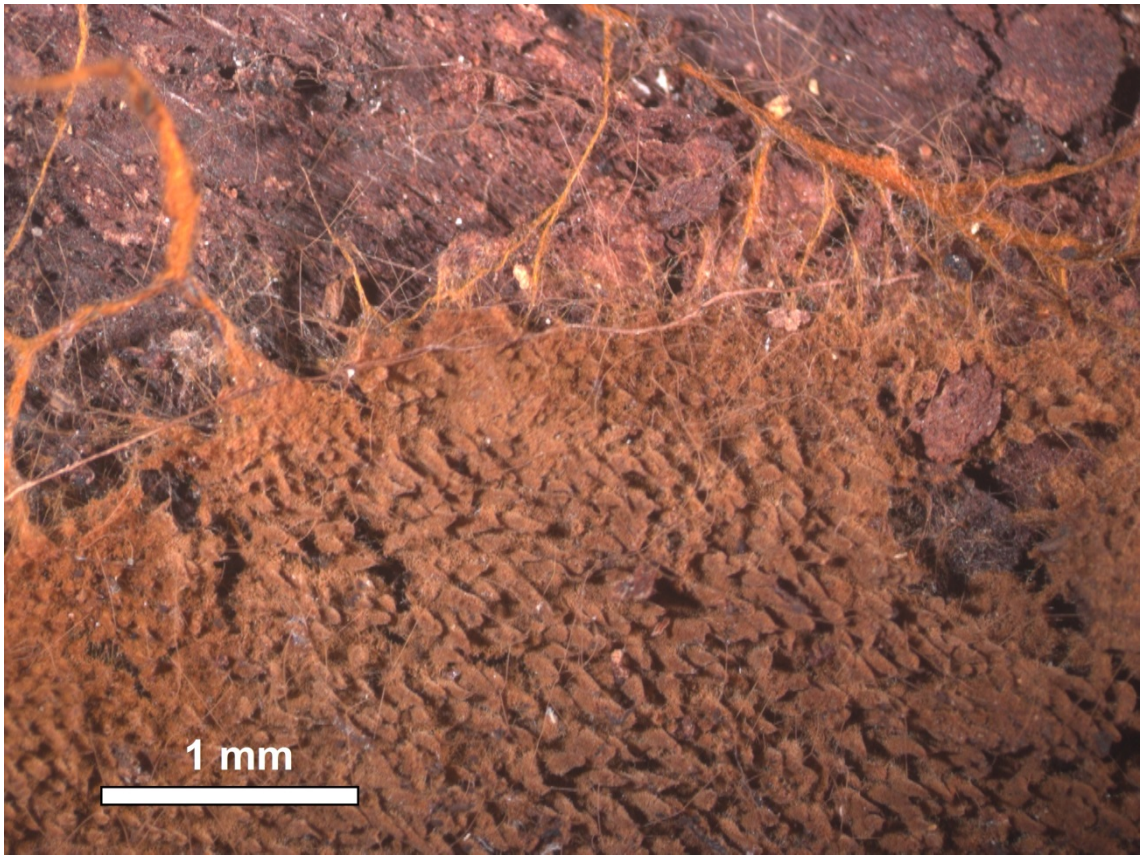

Figure 2. Basidiocarp of *Odontia duemmeri* (LE 314777): details of hymenophore with rhizomorphs
Рисунок 2. Плодовое тело *Odontia duemmeri* (LE 314777): внешний вид гименофора с ризоморфами

Tomentella lapida (Pers.) Stalpers

Specimen examined: Russia, Republic of Dagestan, Magaramkentsky district, Samursky National Park, 41.845944° N, 48.560056° E, –9 m a.s.l., herb-rich mixed forest dominated by *Carpinus betulus* and *Quercus robur* subsp. *pedunculiflora*, on fallen trunk of *Crataegus* sp. (LE F-332369), 5 October 2019, coll. and det. Sergey V. Volobuev.

This is a common species with a worldwide distribution. *T. lapida* is close to *T. stuposa* (Fig. 1), but it differs distinctly from the latter in its encrusted subicular hyphae and smaller basidiospores. At the same time, it was noted previously [10] that *T. lapida* is also close to *T. lilacinogrisea*. Apparently, the similarities in the incrustation of thick-walled and brown subicular hyphae as well as shape of basidiospores, which can be slightly globose in frontal and lateral face in both species, were taken into account. The main differences between *T. lapida* and *T. lilacinogrisea* are in the size of spores (6–7 µm in *T. lilacinogrisea* and 7.5–9.5 µm in *T. lapida*) and the diameter of subicular hyphae. Our specimen (LE F-332369) has a smaller size of spores (6.5–7(7.5) µm). In a previous microscopic study [8], the specimen was incorrectly identified as *T. lilacinogrisea*. Based on the molecular analysis and additional examination of the micromorphology, the specimen was re-determined as *T. lapida*. The species was known previously in the Caucasus from Russia (Krasnodar Territory) and Armenia [10].

Tomentella radiosa (P. Karst.) Rick

Specimen examined: Russia, Republic of Dagestan, Gunibsky District, Gunib Plateau, 42.392758° N, 46.935123°

E, 1573 m a.s.l., herb-rich pine-dominated forest with birch and hornbeam, on fallen trunk of *Betula* sp. (LE 314776), 30 September 2019, coll. and det. Sergey V. Volobuev.

A widespread species in the Caucasus, in particular, in its north-western part (Karachay-Cherkessia Republic, Krasnodar Territory) and Transcaucasia (Armenia, Azerbaijan, Georgia) [10]. This species grouped together with sequences of *Thelephora terrestris* Ehrh. in our phylogenetic tree (Fig. 1) that supports the nomenclature combination of *Thelephora terrestris* f. *radiosa* (P. Karst.) Zmitr. [21] for this taxon.

Tomentella terrestris (Berk. et Broome) M.J. Larsen

Specimens examined: Russia, Republic of Dagestan, Gunibsky District, Gunib Plateau, 42.409078° N, 46.901189° E, 1959 m a.s.l., herb-mosses pine forest, on fallen trunks of *Betula* sp. (LE 314790) and *Pinus kochiana* (LE 314791), 4 October 2018, coll. and det. Sergey V. Volobuev; 42.407591° N, 46.903117° E, 1920 m a.s.l., herb-mosses pine forest, on fallen trunk of *Pinus kochiana* (LE 314773), 6 October 2018, coll. Sergey V. Volobuev and Aziz B. Ismailov, det. Sergey V. Volobuev and Yuliya Yu. Ivanushenko; 42.396977° N, 46.922749° E, 1663 m a.s.l., herb-mosses pine-dominated forest with birch, on fallen trunk of *Pinus kochiana* (LE 314792) and on soil at the base of *Pinus kochiana* trunk (LE 314793), 28 September 2019, coll. and det. Sergey V. Volobuev and Yuliya Yu. Ivanushenko.

This is a remarkable species in the genus *Tomentella* due to the size of its basidia, which are up to 15–20 µm in diameter. This species is widely distributed in the Caucasus and is known from Russia (Karachay-Cherkessia Republic, Krasnodar Territory) and Azerbaijan [10]. The basidiocarps of *T. terrestris* were found during this study not only on well-decayed wood but also on soil.

CONCLUSION

Based on morphological and molecular evidence, four species of thelephoroid basidiomycetes – *Odontia duemmeri*, *Tomentella lapida*, *T. radiosa*, *T. terrestris* – were recorded for the first time to Dagestan. The data on the species richness of the genera *Odontia* and *Tomentella* in this region are updated and the species *T. lilacinogrisea* is excluded from the regional funga. Currently, the genera *Odontia* and *Tomentella* in the Republic of Dagestan are represented by three and fifteen species, respectively.

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Оба автора осуществляли сбор, морфологическое и молекулярное изучение материала, написание текста рукописи. Авторы в равной степени несут ответственность при обнаружении плагиата и других неэтических проблем.

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