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 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 fungi. 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 анализ 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 транскрибируемого спейсера (ITS) ярДНК был амплифицирован для исследованных образцов с использованием двух пар приампермоных, 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 микробиоты. К настоящему времени виды 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.

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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. bryphila, T. cinerascens, T. crinalis (=Odontia ferruginea), T. ellisi, T. ferruginea, T. fibrosa (=Odontia fibrosa), T. lateritica, T. lilacinogrisea, T. pilosa, T. puricea, T. suposa, T. subtestacea, and T. umbrospora [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 mycobios, we carried out a comparative study of the internal transcribed spacer (ITS) region of the nuclear ribosomal DNA (rDNA) 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, phylegetic 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 Axioslager A1 microscope and a standard set of reagents (5% potassium hydroxide solution, Melzer’s reagent).

DNA was extracted from small pieces of dried basidicarps 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 H2O, 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-L 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

| 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       | TAA201283        | 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 |
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 microscopic-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.) Köljalg
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](image)

*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 re­determined 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°
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.) Zmirt. [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 basidio-carpers 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. ilicinigrisea* is excluded from the regional funa. Currently, the genera *Odontia* and *Tomentella* in the Republic of Dagestan are represented by three and fifteen species, respectively.

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