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DNA BARCODING OF XYLOBIONT SPECIES OF FUNGI AND LICHENS FROM THE SAMURSKY NATIONAL PARK (REPUBLIC OF DAGESTAN, RUSSIA): FIRST RESULTS

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DNA barcoding is one of the most effective and modern approaches to obtaining new information on biodiversity of fungi and lichens in poorly studied and/or unique regions. As a result of this research, a total of 16 DNA barcodes were obtained for new regional finds of xylobiont ascomycetous fungi and lichens inhabiting the territory of the Samursky National Park (Republic of Dagestan, Russia). Among them, not only nucleotide sequences of ITS nrDNA for specimens identified based on classical micromorphological methods are presented, but also new information on cryptic species differentiated using molecular genetic approach. The taxonomic spectrum of the studied objects includes representatives of the genera *Coniophora*, *Dendrographa*, *Diploicia*, *Dirina*, *Evernia*, *Hyphoderma*, *Lyomyces*, *Mycoacia*, *Opegrapha*, *Peniophorella*, *Phanerochaete*, and *Xylodon*. *Mycoacia aurea* is registered for the first time for the Republic of Dagestan and the Eastern Caucasus.

Keywords: corticioid fungi, DNA barcode, internal transcribed spacer, liana forest, lichenized fungi, *Phlebia aurea*

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DNA barcoding is a standardised system for the identification of biological samples based on the use of relatively short nucleotide sequences from highly variable genome sequences, which are unique for taxonomic identification of species. The approach was initially developed and tested on a range of animal groups (Hebert et al., 2003; Kerr et al., 2007; Hubert et al., 2008), and was subsequently applied to plants and fungi. A substantial body of genomic research has demonstrated that different groups of organisms have distinctive genome sequences that are optimally suited for DNA barcoding. In accordance with the original concept of universal DNA barcoding (Begerow et al., 2010), an internal transcribed spacer (ITS) fragment comprising two internal transcribed spacers and a 5.8S gene was proposed as the first universal molecular marker for DNA barcoding in fungi, offering satisfactory resolution across a diverse range of taxa (Schoch et al., 2012; Xu, 2016).

The considerable increase in the volume of newly generated data pertaining to the primary structure of fungal DNA ITS regions has underscored the necessity

for the development of specialized databases. One such comprehensive and publicly accessible repository is the National Center for Biotechnology Information (NCBI) RefSeq (Targeted Loci), which has become the main resource for the curation of fungal ITS reference sequences. The principal benefit of such curated databases is that the profile representatives of the scientific community conduct the curation, annotation and expansion of the database (Lücking et al., 2020). In addition to ITS, other secondary barcoding markers are used for fungi, including intergenic spacer (IGS), fragments of β -tubulin II (*TUB2*), DNA-dependent RNA polymerase II (*RPB1*, *RPB2*), translation elongation factor 1 α (*TEF1*), and others.

The advent of DNA barcoding and high-throughput sequencing techniques has given rise to a novel methodological approach for the study of fungal biodiversity. While these methods of classification and identification based on nucleotide sequences are powerful tools for rapid detection of hidden diversity, it is essential to exercise caution when interpreting the data obtained to

Table 1. Data of the specimens collected in the Samursky National Park, Republic of Dagestan, Russia

Species	Host tree species	Herbarium specimen number	Genbank Accession number (NCBI)
<i>Dendrographa decolorans</i> (Turner et Borrer) Ertz et Tehler	<i>Populus nigra</i>	DAG 1511	OR936146
<i>Diploicia canescens</i> (Dicks.) A. Massal.	<i>Populus nigra</i>	DAG 1510	OR936147
<i>Dirina ceratoniae</i> (Ach.) Fr.	<i>Populus nigra</i>	DAG 1512	OR936145
<i>Evernia prunastri</i> (L.) Ach.	<i>Pinus brutia</i> var. <i>eldarica</i>	DAG 1514	OR936143
<i>Opegrapha vulgata</i> (Ach.) Ach.	<i>Pinus brutia</i> var. <i>eldarica</i>	DAG 1513	OR936144
<i>Coniophora arida</i> (Fr.) P. Karst.	<i>Pinus brutia</i> var. <i>eldarica</i>	LE F-342607	OR936149
<i>Hyphoderma setigerum</i> (Fr.) Donk	<i>Carpinus betulus</i>	LE F-342603	OR936153
	<i>Carpinus betulus</i>	LE F-342601	OR936155
<i>Lyomyces crustosus</i> (Pers.) P. Karst.	<i>Pinus brutia</i> var. <i>eldarica</i>	LE F-342605	OR936150
<i>Mycoacia aurea</i> (Fr.) J. Erikss. et Ryvar den	<i>Quercus robur</i>	LE F-342598	OR936158
<i>Peniophorella praetermissa</i> (P. Karst.) K.H. Larss.	<i>Pinus brutia</i> var. <i>eldarica</i>	LE F-342606	OR936151
<i>Phanerochaete livescens</i> (P. Karst.) Volobuev et Spirin	<i>Populus alba</i>	LE F-342604	OR936152
	<i>Quercus robur</i>	LE F-342602	OR936154
	<i>Carpinus betulus</i>	LE F-342600	OR936156
	<i>Quercus robur</i>	LE F-342608	OR936148
<i>Xylodon quercinus</i> (Pers.) Gray	<i>Carpinus betulus</i>	LE F-342599	OR936157

ensure accurate conclusions. It is important to note that individual nucleotide sequences may not be associated with a particular species or genus of fungi. In some cases, these fungi represent described and well-known taxa for which there are no nucleotide sequences in specialised databases (Nilsson et al., 2016; Truong et al., 2017).

It is frequently the case that DNA barcoding cannot be achieved without the involvement of herbarium specimens. However, the success of DNA isolation from them depends on a number of factors, including the age of the specimen (which is notably reduced in specimens older than 50 years) and the method used to dry them. Additionally, the chemicals used to treat the premises from pests, as well as the systematic affiliation, can also have an impact on the outcome (Shneyer, Rodionov, 2019). In this regard, the use of fresh or recently collected specimens is clearly preferable to the use of herbarium materials from long-term storage collections for the purpose of obtaining high-quality molecular genetic data.

In the course of ongoing studies of biodiversity and the peculiarities of the ecology of xylobiont aphyllophoroid fungi and lichens in the territory of the Samursky National Park (Ismailov et al., 2017, 2023a, 2023b; Ivanushenko, Volobuev, 2020; Volobuev, 2020, 2021, 2023; Ismailov, Volobuev, 2022; Volobuev, Shakhova, 2022), a number of collected specimens underwent DNA barcoding.

The identifications that had previously been made on the basis of classical micromorphological characters were confirmed by molecular genetic methods for taxa listed below (Table 1). For DNA extraction, PCR amplification and sequencing we used the standard protocols described in Volobuev et al. (2021). For the first time, the ITS nucleotide sequences from authentic specimens of studied species from the Caucasus were obtained and deposited in the GenBank database. *Mycoacia aurea* (Fr.) J. Erikss. et Ryvar den is a new record of the species to the Republic of Dagestan and the Eastern Caucasus.

Dendrographa decolorans (Turner et Borrer) Ertz et Tehler is an epiphytic crustose lichen that grows on the bark of deciduous trees and shrubs, particularly on old oaks. The specimen was found on the bark of *Populus nigra*. It is a sterile sorediate crust which identification was done according to thin layer chromatography (detected an unidentified fatty acid). DNA was isolated from the sorediate part of thallus. The ITS sequence of 759 bp in 99.74% identity at 100% coverage corresponded to the reference sequence of *D. decolorans* from the Czech Republic (GenBank accession number OQ717831).

Diploicia canescens (Dicks.) A. Massal. is a species that can be found on a wide variety of substrates, including those that are base-rich or -enriched, such as bark, calciferous sandstone, and limestone. The specimen of *D. canescens* was discovered growing on the bark of *Populus nigra*. The specimen was identified based on

standard morphological and anatomical characteristics of the thallus and data on secondary metabolites obtained through thin layer chromatography. The revealed secondary metabolites are atranorin, chloroatranorin, and diploicin. DNA was isolated from a portion of the thallus. The ITS sequence of 560 bp in 98.73% identity at 98% coverage corresponded to the reference sequence of *D. canescens* from Italy (GenBank accession number AJ421992).

Dirina ceratoniae (Ach.) Fr. is a crustose lichen with a predominantly Mediterranean distribution, with an outpost locality in the Canary Islands. It has also been identified in Russia, specifically the Greater Caucasus in Dagestan (Ismailov, Volobuev, 2022). The specimen was collected from a *Populus nigra* tree. The ITS sequence of 625 bp in 99.83% identity at 95% coverage corresponded to the reference sequence of *D. ceratoniae* from Spain (GenBank accession number KC107886).

Evernia prunastri (L.) Ach. is a widespread fruticose epiphytic lichen, most commonly observed on deciduous and coniferous trees. The specimen was observed growing on the twigs of *Pinus brutia* var. *eldarica* within a man-made pine plantation, exhibiting a distinctive morphology that deviated from the typical characteristics of the species. The taxonomic identification of the specimen was confirmed through a detailed microscopic investigation of its morphology. Furthermore, thin-layer chromatography demonstrated the presence of characteristic secondary metabolites for this species, namely evernic and usnic acids, along with atranorin. DNA was isolated from a portion of the thallus. The ITS sequence of 564 bp in 100% identity at 99% coverage corresponded to the reference sequence of *E. prunastri* from Poland (GenBank accession number MN387119).

Opegrapha vulgata (Ach.) Ach. is a species that is widely distributed, though not particularly abundant, in the temperate zone. It is a crustose lichen that exhibits optimal growth in humid forests. The specimen was observed on a decorticated and weathered pine branch. The specimen was identified based on macro- and micromorphological features. The species can be distinguished from close relatives by the presence of longer, curved, or occasionally straight conidia. DNA was extracted from apothecia. The ITS sequence of 615 bp in 99.83% identity at 96% coverage corresponded to the reference sequence of *O. vulgata* from the United Kingdom (GenBank accession number OQ717995).

Coniophora arida (Fr.) P. Karst. is a corticioid fungus that causes a brown rot of coniferous wood. It is widely distributed across the temperate zone. The specimen of *C. arida* was collected on dead fallen trunk of *Pinus brutia* var. *eldarica* in herb-rich pine-dominated forest with oak. The specimen was identified based on hypochnoïd

resupinate basidiomata and dextrinoid basidiospores. DNA was extracted from a piece of basidioma. The ITS sequence of 558 bp in 99.57% identity at 84% coverage corresponded to the reference sequence of *C. arida* from the United Kingdom (GenBank accession number AJ345007).

Hyphoderma setigerum (Fr.) Donk is one of the most commonly occurring corticioid species, which has been observed to grow on both deciduous and coniferous dead wood. In the Republic of Dagestan, the fungus has previously been registered on the wood of the following tree species: *Betula* sp., *Carpinus betulus*, *Populus tremula*, *Quercus macranthera*, and *Pinus kochiana*. The specimens of *H. setigerum* were observed in a herb-rich hornbeam forest with oak, on both living and fallen trunks of *Carpinus betulus*. The specimens were identified microscopically on the basis of the presence of characteristic septate cystidia with clamped septa. DNA was extracted from pieces of basidiomata. The first ITS sequence of 745 bp in 100% identity at 99% coverage corresponded to the reference sequence of *H. setigerum* from Russia (Altai Krai) (GenBank accession number ON869344). The second ITS sequence of the same length (745 bp) showed 99% identity at 99.73% coverage with the same reference sequence (ON869344).

Lyomyces crustosus (Pers.) P. Karst. is a widespread corticioid fungus causing a white rot of wood. It occurs on both deciduous and coniferous wood. The specimen was recorded from fallen branches of *Pinus brutia* var. *eldarica* in a man-made pine plantation. Identification was based on microscopic features, including a monomitic hyphal system, subulate hyphal ends, and subcylindrical basidiospores. DNA was extracted from a piece of basidioma. The ITS sequence of 787 bp in 98.98% identity at 99% coverage corresponded to the reference sequence of *L. crustosus* from Finland (GenBank accession number DQ873614).

Mycoacia aurea (Fr.) J. Erikss. et Ryvarden is a lignicolous fungus characterised by resupinate basidiomata of cream to yellowish appearance, with a hydroid hymenophore comprising short cylindrical aculei. The specimen was collected from fallen twigs of *Quercus robur* in an herb-rich hornbeam forest with oak. This finding is the first discovery of this species in the Republic of Dagestan. The specimen was identified based on the presence of hyphae with clamps and subballantoid basidiospores. DNA was extracted from a piece of basidioma. The ITS sequence of 756 bp in 100% identity at 80% coverage corresponded to the reference sequence of *M. aurea* from Turkey (GenBank accession number HQ153409).

Peniophorella praetermissa (P. Karst.) K.H. Larss. is a wood-inhabiting, resupinate basidiomycete that is frequently collected from all forest ecosystems. The

specimen was collected from fallen branches of *Pinus brutia* var. *eldarica* in a man-made pine plantation. The key diagnostic features of the species are broadly ellipsoid basidiospores, lepto- and gloecystidia, and strophanocysts, which are globose cells engaged in nematode trapping. DNA was extracted from a piece of basidioma. The ITS sequence of 793 bp in 99.83% identity at 75% coverage corresponded to the reference sequence of *P. praetermissa* from the United Kingdom (GenBank accession number DQ647460).

Phanerochaete livescens (P. Karst.) Volobuev et Spirin was highlighted as a result of taxonomical revision of *Ph. sordida* species complex (Volobuev et al., 2015). *Ph. livescens* is widely distributed in nemoral and hemiboreal zones of Eurasia, inhabiting angiosperms. The specimens were collected from dead wood of *Populus alba*, *Quercus robur*, and *Carpinus betulus* in deciduous and mixed forests. The specimens were identified based on sharp-tipped, strongly encrusted cystidia with equally thickened walls, and basidiospores micromorphology. DNA was extracted from pieces of basidiomata. All ITS sequences (718–760 bp) in 100% identity at 100% coverage corresponded to the reference sequences of *Ph. livescens* from Finland (GenBank accession number KP994376).

Xylodon quercinus (Pers.) Gray is a quite common corticioid fungus with odontoid hymenophore. It is distributed throughout the temperate areas of the Northern Hemisphere. The specimen was collected from fallen twigs of *Carpinus betulus* in an herb-rich hornbeam forest. The specimen was identified based on cylindrical to subballantoid basidiospores, sterile hyphal ends, and capitate hyphoid cystidiols. DNA was extracted from a piece of basidioma. The ITS sequence of 766 bp in 99.74% identity at 99% coverage corresponded to the reference sequence *X. quercinus* from Russia (Nizhny Novgorod Oblast) (GenBank accession number OK273841).

Consequently, as a result of these studies, a series of novel DNA nucleotide sequences were obtained from specimens of xylobiont aphyllorphoroid fungi and lichens inhabiting the territory of the Samursky National Park. Among them, both DNA barcodes for new regional finds identified on the basis of classical micromorphological methods and new information on cryptic species differentiated using a molecular genetic approach were obtained. It is evident that the systematic collection, documentation and sequencing of fresh specimens from understudied regions represents the most effective and relevant approach to obtaining new biodiversity information, as well as to improving the quality of the DNA sequence information available in publicly accessible repositories.

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ДНК-баркодинг ксилобионтных видов грибов и лишайников национального парка “Самурский” (Республика Дагестан, Россия): первые результаты

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ДНК-штрихкодирование является одним из наиболее эффективных и современных подходов к получению новых сведений о биологическом разнообразии грибов и лишайников в малоизученных и/или уникальных регионах. В результате проведенных исследований получены 16 ДНК-штрихкодов для новых региональных находок ксилобионтных афиллофороидных грибов и лишайников, обитающих на территории Самурского национального парка (Республика Дагестан, Россия). Среди них представлены не только нуклеотидные последовательности ITS ярдНК для образцов, идентифицированных на основании классических микроморфологических методов, но и новые сведения о криптических видах, дифференцируемых с использованием молекулярно-генетического подхода. Таксономический спектр изученных объектов включает представителей родов *Coniophora*, *Dendrographa*, *Diploicia*, *Dirina*, *Evernia*, *Hypodermia*, *Lyomyces*, *Mycoacia*, *Opegrapha*, *Peniophorella*, *Phanerochaete* и *Xylodon*. Вид *Mycoacia aurea* приводится впервые для Республики Дагестан и Восточного Кавказа.

Ключевые слова: внутренний транскрибируемый спейсер, ДНК-штрихкод, кортициоидные грибы, лиановый лес, лишенизированные грибы, *Phlebia aurea*