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Dirina ceratoniae (Arthoniales, Ascomycota): first record from Russia

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Keywords: Caucasus, Daghestan, *Dirina*, lichenized fungi, molecular phylogeny, new record.

Summary. The Mediterranean species *Dirina ceratoniae* (Roccellaceae, Arthoniales) is reported for the first time for Russia, specifically the Greater Caucasus in Daghestan, based on morphological and molecular evidences. The specimen collected on *Populus nigra* in a coastal lowland region is sorediate and sterile. The sorediate morph is not common in this species and currently known only from a few specimens from the Canary Islands. In the phylogenetic analysis, the ITS1-5.8S-ITS2 sequence obtained for the material grouped in a well-supported clade with all *D. ceratoniae* specimens available in GenBank, within a lineage including three other species: *D. canariensis*, *D. fallax*, and *D. massiliensis*. The discovery of *D. ceratoniae* in Daghestan substantially expands the known distributional range of this taxon to the east of Eurasia.

Dirina ceratoniae (Arthoniales, Ascomycota): первая находка для России

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Ключевые слова: Дагестан, Кавказ, лихенизированные грибы, молекулярная филогения, новые находки, *Dirina*.

Аннотация. Впервые для России и Большого Кавказа приводится средиземноморский вид *Dirina ceratoniae* (Roccellaceae, Arthoniales) на основе морфологических и молекулярных данных. Образец, собранный на *Populus nigra* в прибрежном широколиственном лесу дельты р. Самур (Республика Дагестан), отличается сорediозным и стерильным талломом. Сорediозная форма не является обычной для этого вида и в настоящее время известна только по нескольким экземплярам с Канарских островов. В результате филогенетического анализа нуклеотидная последовательность ITS1-5.8S-ITS2 области ярДНК, полученной из собранного материала, сформировала общую со всеми имеющимися в базе данных GenBank образцами *D. ceratoniae* хорошо поддерживаемую кладу внутри более крупной группы, включающей три других вида: *D. canariensis*, *D. fallax* и *D. massiliensis*. Находка *D. ceratoniae* в Дагестане существенно расширяет известный ареал этого таксона на восток Евразии.

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Introduction

The genus *Dirina* Fr. (Roccellaceae, Arthoniales) combines crustose lichens with usually pruinose apothecia. There are also producing soralia in some taxa. It is predominantly subtropical, with species mostly restricted to coastal habitats, growing on rocks or bark. The genus is mainly distributed in the Northern Hemisphere and has a preference for areas with Mediterranean climate. *Dirina* contains 24 species, with the greatest diversity found in the Mediterranean region and in Macaronesia (Tehler, 1983; Tehler et al., 2013).

Within Russia, the genus *Dirina* is known exclusively from the Caucasus. The work "A checklist of the lichen flora of Russia" includes one species of this genus: *Dirina massiliensis* Durieu et Mont. (Urbanavichus, 2010, cited as *Dirina stenhammarii* (Fr. ex Stenh.) Poelt et Follmann). *Dirina massiliensis* is one of the most widespread species of *Dirina*, typically growing on coastal and inland calcareous rocks (Tehler et al., 2013). It was reported for the first time from the Western Caucasus (Pisút, 2002) and subsequently also found in the Republic of Adygea, in the vicinity of Mt Malyy Thach (Otte, 2007, as *D. stenhammarii*), the Republic of North Ossetia – Alania, in the North Ossetia Nature Reserve (Urbanavichene, Urbanavichus, 2019, as *D. stenhammarii*), in Stavropol Territory, namely the "Lermontova Skala" protected area (Urbanavichene, Urbanavichus, 2018, as *D. stenhammarii*), the Republic of Daghestan, on the Gunib Plateau (Urbanavichus, Ismailov, 2013, as *D. stenhammarii*), and in Armenia's Dilijan National Park (Gasparyan et al., 2016), always on rock. In 2017, *Dirina fallax* De Not. was reported from sandstone in sub-Mediterranean forests of the Abrau Peninsula (Utrish Reserve) on the eastern Black Sea coast, being the second species of the genus *Dirina* for Russia and the Caucasus (Urbanavichus, Urbanavichene, 2017). This species has its main distribution in the western part of the Mediterranean region and along the Atlantic coasts of Europe and Africa, north to Scotland and south to Morocco (Tehler et al., 2013). Currently, *D. fallax* is known in Russia only from Utrish.

In 2021, during the field work in remnants of a subtropical type lowland forests at the western shore of the Caspian Sea, we found a sorediate, otherwise sterile specimen with a trentepohlioid photobiont that could not be immediately identified. We therefore employed molecular techniques using the ITS barcoding marker to assess the taxonomic status of this material.

Materials and Methods

Habitat

The specimen was collected in Samurskiy forest in October 2021. This forest is located in the south-east part of Daghestan, at the border with Azerbaijan, in the delta of the Samur River. The elevation of the Samurski forest ranges between –25 to 35 m a. s. l. Its climate is warm-temperate; average annual temperature is 12.6 °C (1.4 °C in January, 24.5 °C in August, the latter being the warmest month). Average annual rainfall is low (400 mm), but the humidity is high due to the vicinity of the Caspian Sea, a dense river system and high ground water level; average relative air humidity is 78 % (Gadzhieva, Solovyev, 1996).

Core forests with dense tree cover are dominated by *Acer campestre* L., *Carpinus betulus* L., *Quercus robur* L., and *Ulmus campestris* L. Woodlands with sparse tree cover are formed by *Alnus barbata* C. A. Mey., *Juglans regia* L., *Populus nigra* L., *Pyrus caucasica* Fed., and *Salix alba* L. with some lianas (*Clematis orientalis* L., *C. vitalba* L., *Hedera pastuchowii* Woronow, *Humulus lupulus* L., *Lonicera caprifolium* L., *Periploca graeca* L., *Smilax excelsa* L., and *Vitis silvestris* C. C. Gmel.) (Ismailov et al., 2017).

Methods

Morphological and microscopic investigations were performed using light microscopy (LOMO Mikmed-6 and MSP-2) and with the use of chemical spot tests (potassium hydroxide, potassium hypochlorite, and paraphenylenediamine) and UV light. Thin layer chromatography of acetone extracts was performed on 10 × 10 cm Sorbfil aluminium plates F254 layer in solvent system C according to the methods summarized by Orange et al. (2001). *Parmelia sulcata* Taylor were used as a species for the identification of control substances. Photos of specimen were prepared using stereomicroscope Zeiss Axio Zoom V16.

DNA was amplified without prior purification directly from small portion of the dried lichen thallus using Phire Plant Direct PCR Master Mix (Thermo Scientific, Lithuania) according to the manufacturer's protocol. Amplification of the ITS region was done with the ITS1F/ITS4 primer pair (White et al., 1990; Gardes, Bruns, 1993). The PCR product was purified with the GeneJET PCR Purification Kit (Thermo Scientific, Lithuania). Sequencing was performed using the same primers on an ABI 3500 Genetic Analyzer (Applied Biosystems). The raw sequence reads were edited and assembled using MEGA

6.0 (Tamura et al., 2013). The newly generated sequence was deposited in GenBank (accession no. OM200107).

The phylogenetic tree was constructed using the maximum-likelihood method on the IQ-TREE web server (Trifinopoulos et al., 2016), with 1000 ultrafast bootstrap replicates. For this, 22 ITS nrDNA sequences of selected *Dirina* species were retrieved from GenBank following the dataset of Tehler et al. (2013) after the previous BLAST search. *Dirina candida* (Müll. Arg.) Tehler et Ertz (accession no. KC107870 and KC107873) were chosen as outgroup. All sequences were aligned with a MAFFT version 7 web tool (Katoh et al., 2019) using the E-INS-1 option. Ambiguous regions were not deleted from the final dataset before starting the analysis.

Results

Dirina ceratoniae (Ach.) Fr., 1831, Lich. Eur. Reform.: 194.

≡ *Lecanora ceratoniae* Ach., 1810, Lich. Univ.: 361.

Lectotype (designated by Tehler, 1983): [Spain] (H-ACH1068A, H9501809; iso – UPS BOT L-000636).

Description. Thallus small (1.4 cm diam.), well developed, crustose, corticolous, epiperidermal, continuous, 0.3–0.5 mm thick. Surface matt, rugose-verruculose, tuberculate, byssoid around the edges, epruinose, white. Cortex 30–45 μm thick, without crystals. Medulla white, with loose hyphae near the substratum; Prothallus white, loose. Ascomata absent. Soralia present, numerous, white greenish, maculate to confluent mainly in central part of thallus (Fig. 1). Photobiont trentepohlioid. Spot tests: thallus C+ red, KC+ red, K–, P–, UV–; medulla C–.

Secondary metabolites: erythrin, lecanoric acid.

Specimen examined: "Russia, North Caucasus, Republic of Daghestan, Magaramkentskiy district, Samurskiy National Park, on bark of *Populus nigra* among sparse vegetation with old poplar trees, –23 m. 41°51'59.40"N, 48°33'19.80"E. 19 X 2021. A. B. Ismailov" (DAG 1392).

The specimen identification by alone morphological, anatomical and chemical characters was difficult and supported with molecular analysis results. Initial BLAST of the ITS sequence from our specimen suggested its identity with *D. ceratoniae*, with similarity values of 99.83 % and 99.66 % with specimens of the latter (accession nos. FJ639084 and KC107881). In our phylogenetic tree, the specimen clustered within a well-supported clade with all available in GenBank *D. ceratoniae* specimens (Fig. 2). *Dirina ceratoniae* therefore formed part of a clade including three other species: *D. canariensis* Tehler et Ertz, *D. fallax*, and *D. massiliensis* (Fig. 2), all forming well-supported clades.

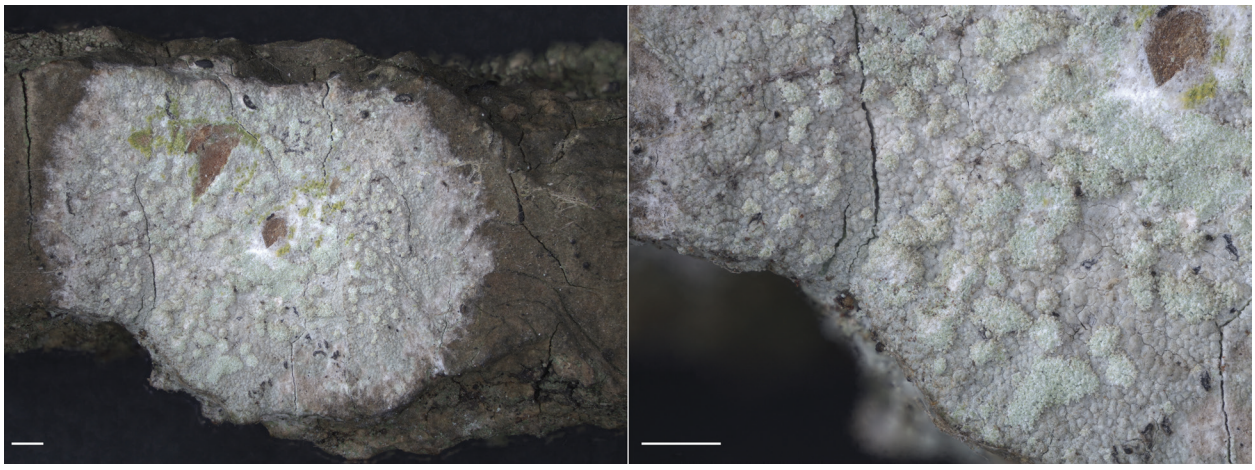

Fig. 1. Habitus (left) and enlargement part of thallus (right) of *Dirina ceratoniae*. Scale – 1 mm.

Discussion

Dirina ceratoniae is mainly distributed in the Mediterranean region, with an outpost locality in the Canary Islands; it is more common in the western Mediterranean and only occasionally found in the eastern parts. The discovery of *D. ceratoniae* in Daghestan substantially expands the known distributional range of this taxon eastwards. The known locations nearest to Daghestan are in Cyprus and Israel (Tehler, 1983; Tehler et al., 2013). It is included in the Italian red list of epiphytic lichens under the

"Least Concern" category (Nascimbene et al., 2013). *Dirina ceratoniae* prefers open, dry habitats, with a long continuity near the sea, and is usually corticolous (Tehler, 1983; Tehler et al., 2013).

From the strictly saxicolous *D. massiliensis*, *D. ceratoniae* can always be separated by its corticolous habit and less pruinose thallus surface (Tehler, 1983). But *D. ceratoniae* may also occur on calciferous rocks. Saxicolous specimens of *D. ceratoniae* are very similar to *D. massiliensis*. In some cases these two species are indistinguishable without DNA analysis. Sorediate forms of phylogenetically close saxicolous species of *D. canariensis* and *D. fallax* differs from *D. ceratoniae* by dark, usually greyish or greenish brown thallus, punctiform to maculate soralia (*D. ceratoniae* has maculate to confluent soralia) and confined to siliceous and acidic rocks.

The sorediate morph of *D. ceratoniae* is known only for few specimens from the Canary Islands. Tehler et al. (2013) suggested that the sorediate morph of *D. ceratoniae* is a result of not favourable habitat conditions and developed in unhealthy specimens. This might also be the case in the Russian

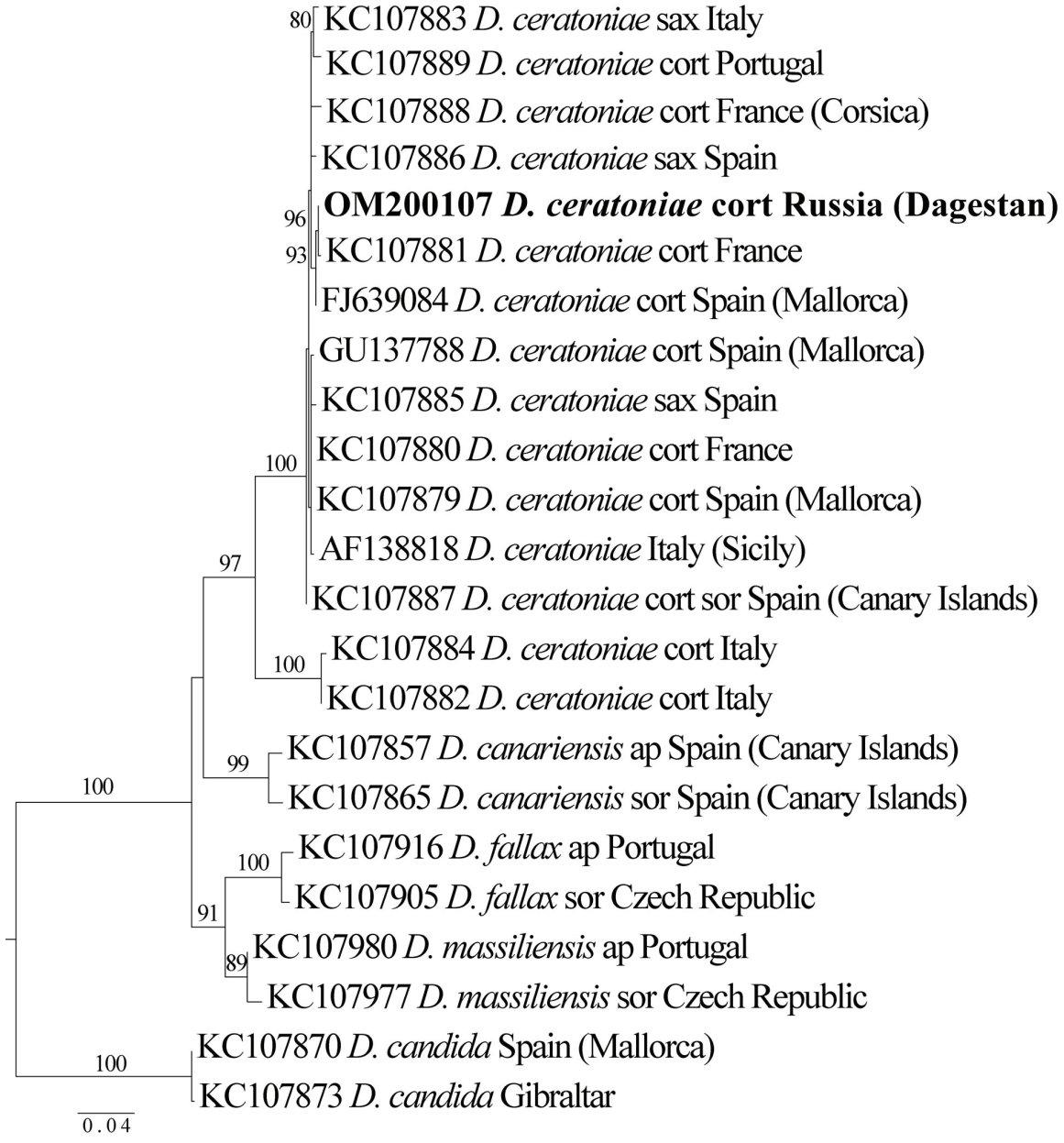

Fig. 2. The Maximum Likelihood tree illustrating the ITS nrDNA-based phylogeny of *Dirina* species. Ultrafast bootstrap values (%) not less than 80 are shown above the branches. Sequence accession numbers (GenBank) are indicated before species names. The name of the newly sequenced specimen in this study is written in bold.

locality, because our specimen grew in remnants of lowland forest among anthropogenic transformed and degraded landscapes. Sorediate specimens from the Canary Islands also were found within not natural habitat, in parkland conditions within a city.

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