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The first record of *Pyrenopeziza protrusa* (Dermateaceae s. l.) in Russia on the Kunashir Island has made it possible to correct the data on the limits of its range

A. V. Bogacheva[1, 2*], A. Yu. Nikulin[1, 3]

*[1] Federal Scientific Center of the East Asia Terrestrial Biodiversity FEB RAS, 100-letiya Vladivostoka Pr.,
159, Vladivostok, 690022, Russian Federation*

[2] E-mail: bogacheva@biosoil.ru; ORCID iD: https://orcid.org/0000-0002-1661-1573

[3] E-mail: artyrozz@mail.ru; ORCID iD: https://orcid.org/0000-0001-6113-2136

** Corresponding author*

Keywords: biogeography, fungi, Honshu Island, integrative approach, ITS rDNA, Japan, Kurile Islands, magnolia leaves.

Summary. Using the integrative approach, we identified the specimen from the central veins of last year's fallen magnolia leaves collected on Kunashir Island as *Pyrenopeziza protrusa* (Berk. et M. A. Curtis) Sacc. The species known from a small area on the Honshu Island (Japan) and the only one in the state of New Jersey (USA) was newly reported from Kunashir Island (Russia). We suggest that the expansion of the range of the fungus northwards from the previously established site is a response to the changing climatic conditions of modern times. It is likely that highly specialized fungal species tend to expand their range northwards in response to global warming.

Первая в России находка *Pyrenopeziza protrusa* (Dermateaceae s. l.) на острове Кунашир позволила скорректировать данные о границах его ареала

А. В. Богачева, А. Ю. Никулин

*Федеральный научный центр биоразнообразия наземной биоты Восточной Азии Дальневосточного отделения
Российской академии наук, пр. 100-летия Владивостока, д. 159, г. Владивосток, 690022, Россия*

Ключевые слова: грибы, биогеография, интегративный подход, Курильские острова, листья магнолии, остров Хонсю, Япония, ITS rDNA.

Аннотация. С помощью интегративного подхода мы идентифицировали экземпляр из центральных жилок прошлогодних опавших листьев магнолии, собранных на о. Кунашир, как *Pyrenopeziza protrusa* (Berk. et M. A. Curtis) Sacc. Вид, который, как было известно, имеет небольшой ареал на острове Хонсю (Япония) и отмечен лишь единично в штате Нью-Джерси (США), был впервые обнаружен на острове Кунашир (Россия). Мы предполагаем расширение ареала гриба к северу от ранее установленного местонахождения как реакцию на изменение климатических условий современности. Вероятно, узкоспециализированные виды грибов стремятся расширить свой ареал на север в ответ на глобальное потепление климата планеты.

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The fungal diversity of the Russian Far East region, particularly its southern part, is exceptional compared to other regions of Russia. In each of our publications, we repeat this theory and prove it with new facts (Bogacheva, 1996, 2005, 2010, 2018; Rebriev et al., 2021, 2022, 2023). In this paper, we will discuss the finding of *Pyrenopeziza protrusa* (Berk. et M. A. Curtis) Sacc., which was noted on *Magnolia* sp. leaves in the Russian Far East (Fig. 1A). Both the fungus and the plant have a small species ranges. The latter (e. g. *Magnolia obovata* Thunb.) is protected and listed in many Red Data Books. In this context, any information on the magnolia consort mycobiota is particularly valuable. In the Russian Far East, 17 species of the genus *Pyrenopeziza* Fuckel have been recorded to date. The Far Eastern Regional Herbarium (VLA) maintains samples of these species collected by scientists during seven decades of mycological research.

Materials and methods

Fungus samples were collected on Kunashir Island in the spring, 23rd May 2020, in the territory of the Kuril State Natural Reserve by A. N. Tikhonov. A considerable number of ascomas were observed in the central veins of last year's fallen leaves. The laboratory processing of the collected material was carried out in accordance with the methods generally accepted in mycology (Hawksworth, 1974; Baral, 1987a, b, 1992) at the Laboratory of Botany, the Federal Scientific Center for Biodiversity, Far East Branch of the Russian Academy of Sciences. To observe ascospores, asci, paraphyses and other microstructures of the dried specimens, a mounting fluid (water) was used to rehydrate them on a slide glass and gently squashed with a cover glass. The microstructures were observed in detail by a microscope Nikon Eclipse E200 (Nikon, Tokyo, Japan). The dimensions of 20 ascospores and 10 asci from each specimen were determined using an ocular micrometer, with measurements taken in both length and width.

Magnolia is observed in its natural habitat in the Sakhalin region of Russia, where it is found on Kunashir Island, as well as on the territory of China and on the islands of Japan (Dandy, 1973; Ueda, 1986; Kharkevich, 1987). The exact species of magnolia from which the fungus was sampled is intentionally omitted. The taxonomic position of the species *Magnolia hypoleuca* Siebold et Zucc., as indicated in the flora of the Kuril Reserve, is not generally accepted. In the flora of Japan, a synonym for the taxon is provided, namely *M. obovata* (Itagaki et al., 2019). The aim of this study is not to analyze the taxonomic permutations within the genus of the host plant. Consequently, only the plant genus name is indicated.

Genomic DNA was extracted from the dried apothecia by the method described previously by Echt et al. (1992) with some modifications of Abdullin et al. (2021). The sequence of the internal transcribed spacer region of ribosomal DNA (rDNA), including the 5.8S gene (ITS), was obtained for the purpose of species identification. The ITS rDNA was amplified using a primer combination (ITS1 and ITS4) and temperature profile, following Han et al. (2014), in a T100 Thermal Cycler (Bio-Rad Laboratories, Inc., Hercules, CA, U.S.A.). The PCR products were sequenced in both directions with the same primers at the Instrumental Centre of Biotechnology and Gene Engineering of Federal Scientific Centre of the East Asia Terrestrial Biodiversity FEB RAS using an ABI 3500 Genetic Analyzer (Applied Biosystems, Waltham, MA, USA) with a BigDye Terminator v. 3.1 sequencing kit (Applied Biosystems, Waltham, MA, USA). The sequences were assembled using the Staden Package v.1.4 (Bonfield et al., 1995) and subsequently deposited into GenBank under the accession number OM423713.

The ITS rDNA sequence of the fungus was compared with those from authentic and reference strains available at the National Center for Biotechnology Information (NCBI, Bethesda, USA) by means of a BLAST search (https://blast.ncbi.nlm.nih.gov/Blast.cgi; accessed on 25 May 2023) in order to estimate its taxonomic position. The selection of representative NCBI accessions for phylogenetic analysis was performed according Han et al. (2014) and Itagaki et al. (2019) datasets. For the dataset, three taxa representing phylogenetically distant lineages were included as an outgroup. The sequences (taxa, accession number and strain/voucher name are given as listed in the NCBI) were aligned in the SeaView program (Galtier et al., 1996) with manual corrections.

The best evolutionary model, TIM1ef+G, was determined using jModelTest 2.1.1 (Darriba et al., 2012). Phylogenetic trees were constructed using the maximum likelihood (ML) method in RAxML v.7.2.6 (http://embnet.vital-it.ch/raxml-bb/, accessed on 28 May 2023) (Kozlov et al., 2019) and Bayesian inference (BI) in MrBayes v.3.1.2 (Huelsenbeck, Ronquist, 2001). In BI, four runs of four Markov chains were executed for 1 million generations, sampling every 100 generations for a total of 10000 samples. The convergence of the chains was assessed, and stationarity was determined according to the "sump" plot, with the first 2500 samples (25 %) discarded as burn-in; posterior probabilities were calculated from trees sampled during the stationary phase. The robustness of the ML trees was estimated by examining the bootstrap percentages (BPs; Stamatakis et al., 2008) and posterior probabilities (PPs) in BI. Those with BPs < 50 % and PPs < 0.95 were not considered.

Results and discussion

The collected specimens were identified as *Pyrenopeziza protrusa* (Berk. et M. A. Curtis) Sacc. The systematic position of the species is still under discussion. In accordance with the electronic resource Index Fungorum (https://www.indexfungorum.org/), the species is currently being proposed for consideration within the scope of the genus *Mollisia* (Fr.) P. Karst. 1871 (*Mollisia protrusa* (Berk. et M. A. Curtis) Baral) (http://www.indexfungorum.org/names/Names.asp). In terms of morphology, these two taxa are highly similar, with only minor outward differences (Korf, 1973; Jaklitsch et al., 2016). Previously, they were classified as Dermateaceae (Kirk et al., 2008). However, based on molecular data, both taxa are now placed in separate families, namely Ploettnerulaceae and Mollisiaceae, respectively (https://www.speciesfungorum.org/Names/Fundic.asp). In the present paper, we maintain the classification of Dermateaceae sensu lato as the family of *Pyrenopeziza* Fuckel in the traditional sense.

Pyrenopeziza protrusa (Berk. et M. A. Curtis) Sacc.: "Russian Federation, Sakhalin Region, Kunashir Island, Kuril State Natural Reserve, 49º06'31"N, 142º10'63"E. 23 V 2020. A. N. Tikhonov" (VLA D – 4653). – On the central veins of the underside of *Magnolia* sp. leaf, litter, apothecia numerous, scattered, sessile, discoid with slightly raised margin, 1–1.5 mm in diameter, 0.1–0.3 mm in height, rough on the outside due to the protruding cells of the excipulum, light brown; when dried, the color does not change; white hyphae at the base. The excipulum consists of spherical cells, the outer cells are elongated, club-shaped, 20 μm long, with 2–3 granules at the apex (Fig. 1B). Asci are cylindrical, amyloid (J+), 35–42.5 × 3–4 μm, eight-spore; ascospores are arranged in two rows (Fig. 1C). Spores ellipsoid to slightly club-shaped, 6–7.5 × 2–1.5 μm, unicellular, colorless. Paraphyses few, simple, cylindrical, slightly widening at the umbo, not exceeding the length of the asci.

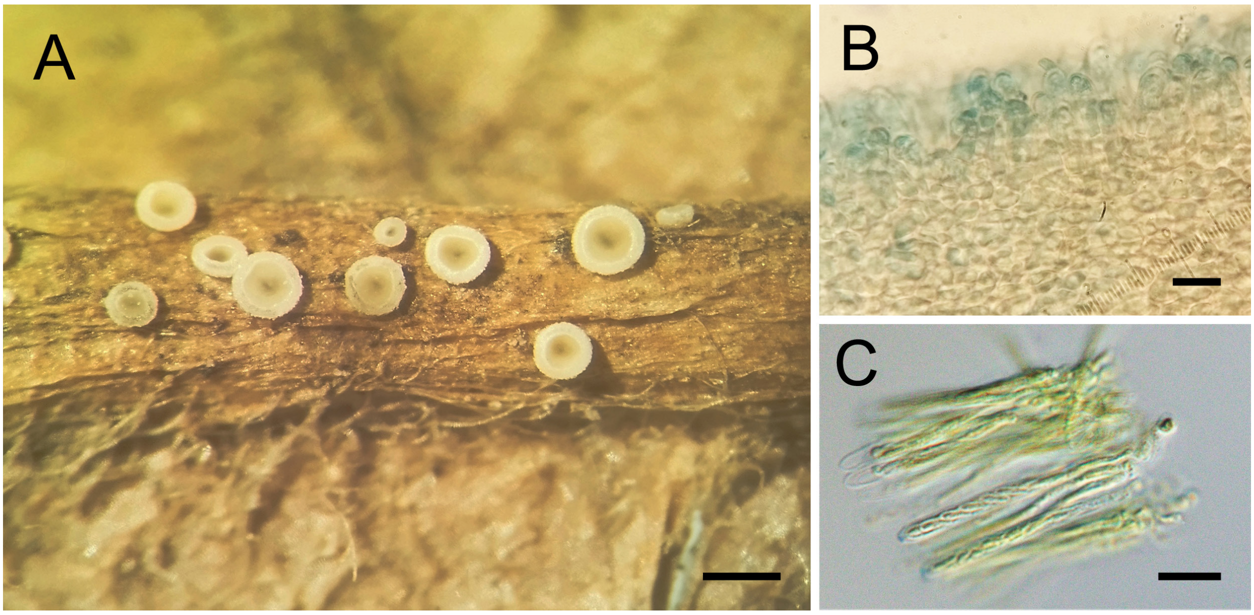

Fig. 1. Morphology of *Pyrenopeziza protrusa* on *Magnolia* sp. fallen leaves: A – fresh apothecia; B – external cells of excipulum (in CottonBlue); C – asci with ascospores (in Melzer's reactive). Scale bars: A – 1 mm; B, C – 10 μm.

Previously, the species had only been recorded once in North America, in the state of New Jersey (V 1876, USA) and annually on the island of Honshu (Japan) (https://www.gbif.org/occurrence/1998433930).

To aid the morphology-based identification process we assembled a dataset comprising 24 ITS rDNA sequences representing the major relative genera and three outgroup taxa. The close relationship between *Pyrenopeziza* and the genera *Vibrissea* Fr., *Mollisia*

(Fr.) P. Karst., and *Loramyces* W. Weston has been shown previously demonstrated by Han et al. (2014). The topologies of the ML and BI trees based on the ITS rDNA dataset were similar, with the exception of some differences in clade support (Fig. 2). The results of the analysis positioned our specimen in a basic position within the examined group, exhibiting a phylogenetic proximity to other available *Pyrenopeziza* accessions (Fig. 2). Based on the tree topology, it can be confirmed that the sample belongs to the species *P. protrusa*. All specimens of this species exhibited a high degree of genetic similarity, followed by *Pyrenopeziza* sp. (without support) and *P. nervicola* (73/0.99, for BP and PP, respectively). The *Vibrissea*, *Mollisia*, and *Loramyces* accessions formed a distinct terminal clade on the phylogenetic tree (95/1.00).

In the Asia-Pacific region, the species was previously recorded on Honshu Island (Itagaki et al., 2019) with a single host plant species of *Magnolia* (Itagaki, Hosoya, 2021). The present study documents the range expansion of *P. protrusa*. Previous mycological studies of the vegetation of Kunashir Island have not identified this species among the island's mycobiota (Bogacheva, 1996). Over the past two decades, climate change has occurred, resulting in alterations to the phenotype of plants and, consequently, to fungal species. In 2015, employees of the Botanical Garden-Institute of the Far Eastern Branch of the Russian Academy of Sciences (BGI FEB RAS, Vladivostok) discovered a self-seeding *Magnolia* (*M. sieboldii* s. l.) not only on the territory of the botanical garden but also in natural ecosystems. Since then, the invasive potential of *Magnolia* in natural ecosystems, along with the observed tendency towards expansion in a north-easterly direction, has been monitored and recorded (Bogachev et al., 2022).

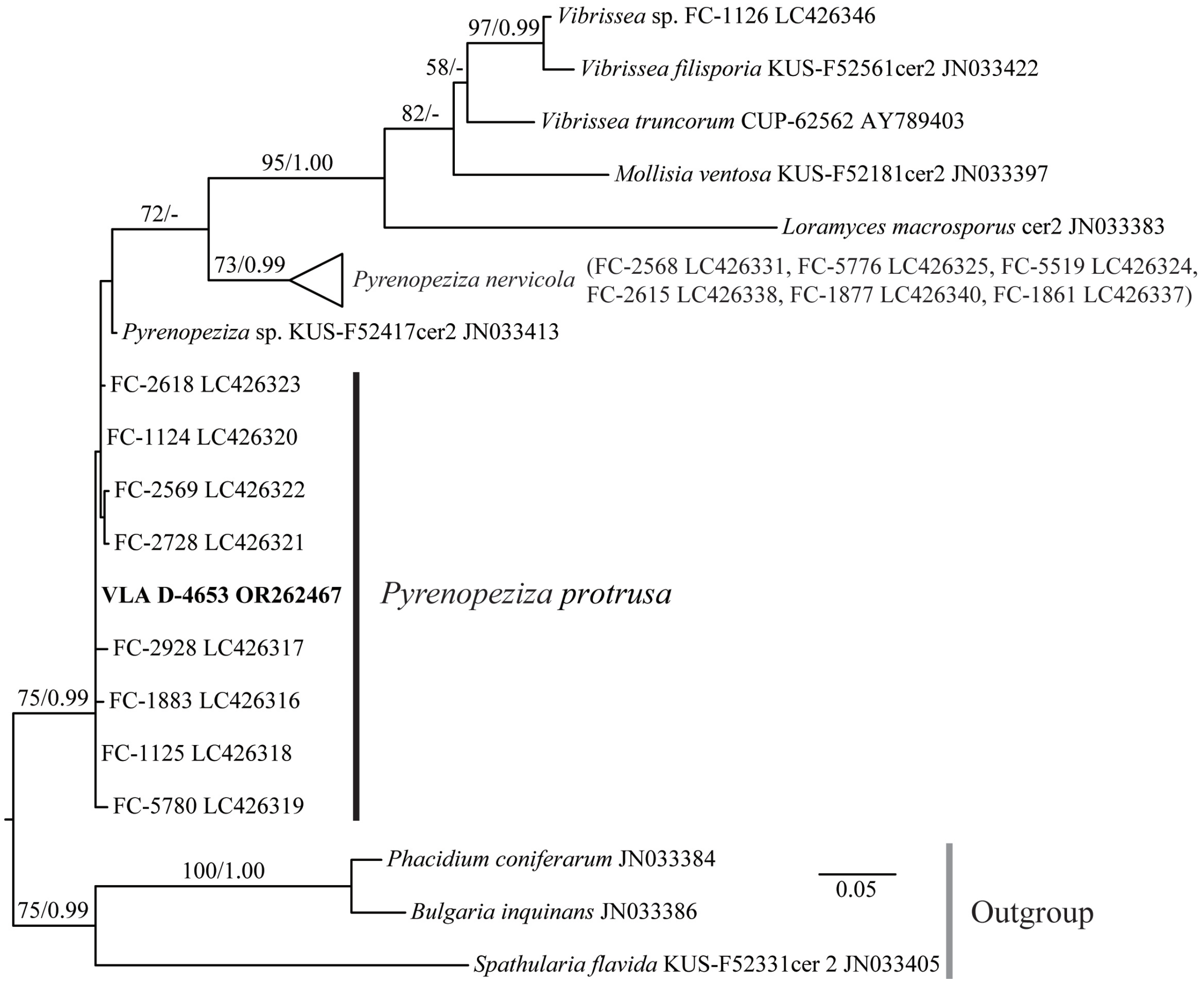

Fig. 2. ML phylogenetic tree representing the phylogenetic position of our specimen VLA D-4653 (boldface) based on ITS rDNA sequence data (24 accessions; 591 aligned positions; TIM1ef+G). Supports [(BP) ≥ 50% and (PP) ≥ 0.95: ML/BI] are given above the branches. The sequences have specimen/strain names (if provided) and NCBI GenBank accession numbers. Scale bar – substitutions per nucleotide position.

It is also possible that this finding can be explained by another hypothesis. In its life cycle, the fungus develops for a period of time in the magnolia leaf blade without forming a fruit body (Itagaki, Hosoya, 2021). It is plausible that the climatic conditions previously prevailing on Kunashir Island were not conducive to the completion of the full life cycle. Consequently, the *Pyrenopeziza protrusa* species has not been recorded within the island's biota.

In light of the findings of our study, we hypothesize that the fungus is gradually expanding its range northward from the previously established locality on Honshu Island. This expansion is believed to be a response to the changing climatic conditions of the modern era.

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