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## The first records of two rare polyporoid fungi *Picipes submelanopus* and *Picipes ulleungensis* in Russia

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**Summary.** Molecular genetic studies of the morphological genus *Polyporus* s. l. recently made it possible to detect the presence of several phylogenetic lineages among its representatives, to clarify the boundaries and scope of the taxon. Species with a black cuticle covering the stipe of the basidiomata have been separated in the genus *Picipes*. Most representatives of this genus are known from China. In Russia, out of 29 species of the genus, there are three widespread species of *Picipes* – *P. badius*, *P. melanopus*, *P. tubaeformis*, and one rare species – *P. rhizophilus*. Phylogenetic analysis of the nucleotide sequences of selected nrDNA loci obtained from the herbarium materials collected by us, originally assigned to *Picipes melanopus*, revealed the presence of two rare, morphologically similar species, *P. submelanopus* and *P. ulleungensis*, which turned out to be new to Russia. *Picipes submelanopus* is characterized by a terrestrial fruiting bodies and differentiated from *P. melanopus*, that grows on dead wood. All specimens of fruiting bodies of *Picipes submelanopus* collected by us in Western Siberia were found on the roots of woody plants, as in the type locality in China. *Picipes ulleungensis* differs from *P. melanopus* by larger fruiting bodies, slightly larger pores. In order to differentiate *Picipes submelanopus* and *P. ulleungensis* from morphologically similar species, in addition to morphological characters and ecological preferences, it is desirable to use DNA barcoding methods.

## Первые находки двух редких полипороидных грибов *Picipes submelanopus* и *Picipes ulleungensis* в России

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**Ключевые слова:** ДНК-штрихкодирование, Западная Сибирь, новые находки, полипороидные грибы, редкие виды.

**Аннотация.** Молекулярно-генетические исследования морфологического рода *Polyporus* s. l. за последнее время позволили обнаружить среди его представителей наличие нескольких филогенетических линий, уточнить границы и объем таксона. Виды с черной кутикулой, покрывающей ножку плодового тела, были выделены в род *Picipes*. Большая часть представителей этого рода известна из Китая. В России из 29 ви-

дов рода встречается три широко распространенных вида *Picipes* – *P. badius*, *P. melanopus*, *P. tubaeformis* и один редкий вид – *P. rhizophilus*. Филогенетический анализ нуклеотидных последовательностей избранных локусов ярдНК, полученных из собранных нами гербарных материалов, первоначально отнесённых к *Picipes melanopus*, позволил выявить наличие двух редких, морфологически сходных видов – *P. submelanopus* и *P. ulleungensis*, оказавшихся новыми для России. *Picipes submelanopus* характеризуется наземными плодовыми телами и отличается от *P. melanopus*, растущего на мертвой древесине. Все образцы плодовых тел *Picipes submelanopus*, собранных нами в Западной Сибири, были обнаружены на корнях древесных растений, как и в типовом местонахождении в Китае. *Picipes ulleungensis* отличается от *P. melanopus* более крупными плодовыми телами, несколько более крупными порами. Для дифференциации *Picipes submelanopus* и *P. ulleungensis* от морфологически сходных видов, помимо морфологических признаков и экологических предпочтений, желательнее использовать методы ДНК-штрихкодирования.

## Introduction

The genus *Picipes* was described by Ivan Zmitrovich and Aleksandr Kovalenko (Zmitrovich, Kovalenko, 2016) with the type species *Picipes badius* (Pers.) Zmitr. et Kovalenko. Three species were assigned to the new taxon, including also *Picipes melanopus* (Pers.) Zmitr. et Kovalenko and *P. tubaeformis* (P. Karst.) Zmitr. et Kovalenko, segregated from the morphological genus *Polyporus* P. Micheli ex Adans., belonging to a separate phylogenetic lineage. Earlier, these species were combined into the “Melanopus” morphological group based on the presence of a black cuticle covering the entire or most of the stipe of the basidiomata (Núñez, Ryvarde, 1995). Later, polyporoid fungi of this group were actively studied by mycologists from China, where their high species diversity is observed. Molecular genetic studies made it possible to clarify the taxonomic status and transfer another 10 species to the genus *Picipes*, and another 18 species were described as new to science in the genus, two of which were transferred to the genus *Polyporus* s. str. (Dai et al., 2014; Zhou et al., 2016; Cui et al., 2019; Bhunjun et al., 2022; Ji et al., 2022). Thus, based on the data below, the genus *Picipes* currently includes 29 taxa.

## Material and methods

We collected specimens in the Western Siberia, the Novosibirsk Region and the Altai Territory. We took photographs of fresh fungal fruiting bodies, as well as dry basidiomata from the herbarium collection. We used a Zeiss Axio Imager A1 light microscope (Carl Zeiss Microscopy, Germany) for the detailed morphological examination.

### DNA extraction and sequencing

To extract DNA, we crushed fragments of dried fungal fruiting bodies (0.01 g) using aluminium oxide ( $Al_2O_3$ ). Then, we homogenized them in the

Phyto-Sorb kit lysis buffer (Synthol, Moscow). We amplified the ITS1–5.8S–ITS2 nrDNA regions with ITS1F and ITS4B primers (Gardes, Bruns, 1993). We used HS Taq DNA Polymerase (Evrogen, Moscow). We used C1000 Thermal Cycler (Bio-Rad, USA) and visualized amplicons in Gel Doc XR+ Imager (Bio-Rad, USA) to perform the PCR. We executed Sanger DNA sequencing at SB RAS Genomics Core Facilities (ICBFM SB RAS, Novosibirsk, Russia).

### Sequence alignments and phylogenetic analyses

We analyzed the *Picipes* species' complete ITS1–5.8S–ITS2 sequenograms in Chromas, version 2.6.6 (<http://technelysium.com.au/wp/chromas/>). We compared these sequences with the existing data in GenBank using the Nucleotide BLAST tool (Altschul et al., 1990). Based on the BLAST search, we incorporated additional ITS sequences of other *Polyporus* s. l. species into the analyses.

### Sequences and alignment

The total ITS dataset consists of 23 sequences including an outgroup. For this study we produced newly generated ITS sequences. Table provides an overview of the taxa we used, information on the herbarium specimens, GenBank accession numbers, and references. We aligned the sequences online in MAFFT (Kato et al., 2002), version 7 (<http://mafft.cbrc.jp/alignment/server/>) using the E-INS-I strategy (Kato, Toh, 2008). We chose *Trametes conchifer* (Schwein.) Pilát as an outgroup (Justo, Hibbett, 2011).

### Phylogenetic analysis

We used MEGA X software (Kumar et al., 2018) for the statistical analyses of our nucleotide datasets. We reconstructed phylogenetic relations using the Maximum Likelihood (ML) method (Felsenstein, 2004). We built phylogenetic trees in the online version (Trifinopoulos et al., 2016, <http://iqtree>).

cibiv.univie.ac.at/) of IQ-TREE software (Nguyen et al., 2015) with the standard parameters. We used nonparametric bootstrapping with 1000 replicates

as statistical support in this analysis. We visualized the phylogenetic trees in Fig Tree version 1.4.4 (Rambaut, 2018).

Table

Sequences we used for the alignments with their corresponding GenBank access numbers and references

Species	Herbarium voucher/ isolate (ITS)	Genbank accession numbers (ITS)	Reference
<i>Lentinus (Polyporus) brumalis</i>	Dai 14946	KX851609	Zhou et Cui (GenBank)
<i>Lentinus tigrinus</i>	–	KY565250	Akbarikiarood et Rahnama (GenBank)
<i>Lentinus substrictus (Polyporus ciliatus)</i>	H6012970	LN610426	Miettinen (GenBank)
<i>Neofavolus alveolaris</i>	CBS 207.48	MH856311	Vu et al., 2019
<i>Picipes badius</i>	UBC:F19745	HQ604799	Berbee et al., (GenBank)
<i>Picipes rhizophilus</i>	Dai 16082	KX851634	Ji et al., 2022
<i>Picipes submelanopus</i>	HMAS 290127	MK966675	Wei (GenBank)
<i>Picipes submelanopus</i>	NSK 1014847	OP458518	in this study
<i>Picipes ulleungensis</i>	Cui12410	KX900022	Ji et al., 2022
<i>Picipes ulleungensis</i>	SFC 20120814-41	KY038467	Tibpromma et al., 2017
<i>Picipes ulleungensis</i>	NSK 1014990	OP964916	in this study
<i>Picipes ulleungensis</i>	KUC20130809-19	KJ668475	Jang et Kim (GenBank)
<i>Podofomes mollis</i>	CLZhao 4429	MK343523	He et Zhao, 2022
<i>Polyporus chozeniae</i>	LE301310	KJ595567	Zmitrovich et al., 2014
<i>Polyporus fraxineus</i>	Dai 2494	KC572023	Zhou et al., 2016
<i>Polyporus melanopus</i>	H 6003449	JQ964422	Xue et Zhou, 2012
<i>Polyporus pseudobetulinus</i>	TFM:F-27567	AB587644	Zhou et al., 2011
<i>Polyporus squamosus</i>	SP-16	MK386841	Farooq et al., (GenBank)
<i>Polyporus tuberaster</i>	LE-BIN 3865	MG735335	Senik et al., (GenBank)
<i>Polyporus tubaeformis</i>	CLZhao 4429	MK894074	He et Zhao, 2022
<i>Polyporus umbellatus</i>	–	AY322495	Xing et Guo, 2005 (GenBank)
<i>Polyporus varius</i>	MHHNU 30391	MK182303	Chen et al., (GenBank)
<i>Trametes conchifer</i>	FP106793sp	JN164924	Justo et Hibbett, 2011

## Results and discussion

***Picipes submelanopus*** (H. J. Xue et L. W. Zhou) J. L. Zhou et B. K. Cui ( $\equiv$  *Polyporus submelanopus* H. J. Xue et L. W. Zhou) (Fig. 1A, B, E).

**Specimens examined.** RUSSIAN FEDERATION: “Altai Territory, Troitsky district, Borovlyanka village, 52.6170°N, 84.4379°E, 229 m a. s. l., birch-pine forest, on the roots of *Betula pendula* in soil. 11 VIII 2009. V. A. Vlasenko” (NSK 1014992, GenBank OP964915 (ITS)); “Novosibirsk Region, Novosibirsk City, Akademgorodok, Botanical Garden, 54.8148°N, 83.1019°E, 166 m a. s. l., plantations of willows, on the roots of *Salix* sp. in soil. 21 VII 2009. V. A. Vlasenko” (NSK 1014993); *ibid*, “Akademgorodok, st. Tereshkovoy 9, 54.8431°N, 83.1058°E, 175 m a. s. l., planting birches along the road, on the roots of *Betula pendula* in soil. 03 VII

2021. V. A. Vlasenko” (NSK 1014847, GenBank OP458518 (ITS), OP965388 (LSU)).

**Distribution.** North America – Canada, Europe – Estonia, Latvia; Asia – Russia (in this study), China, Pakistan (Xue, Zhou, 2012; Runnel et al., 2021; *Picipes submelanopus*, 2023).

**Comments.** The ML analysis of the ITS1–5.8S–ITS2 nrDNA region confirmed that the studied specimen belongs to the *Picipes submelanopus* (Fig. 2). The species as an independent taxon was introduced only in 2012, based on molecular genetic studies. On a global scale, the species is known from four localities, with a type locality in China. *Picipes submelanopus* is morphologically similar to *P. melanopus*.

*Picipes submelanopus* is characterized by a terrestrial habit, centrally or laterally black-stipitate basidiomata, straw-yellow to honey-yellow pore surface,



circular to angular pores, two types of generative hyphae bearing either simple septa or clamp connections, and cylindrical basidiospores. However, *Picipes melanopus* has smaller pores and clamped generative hyphae only (Xue, Zhou, 2012). All specimens of the species collected by us in Western Siberia were found on the roots of woody plants – on a birch and willow. In the type locality, in China, *Picipes submelanopus* has also been found on the soil on tree roots (Xue, Zhou, 2012).

According to this ecological feature, the species is differentiated from *Picipes melanopus*, which develops on dead wood. Records from China, Pakistan, and Russia have been confirmed by molecular genetic studies. In Latvia, the species is known from metagenomic soil studies data (*Picipes submelanopus*, 2023).

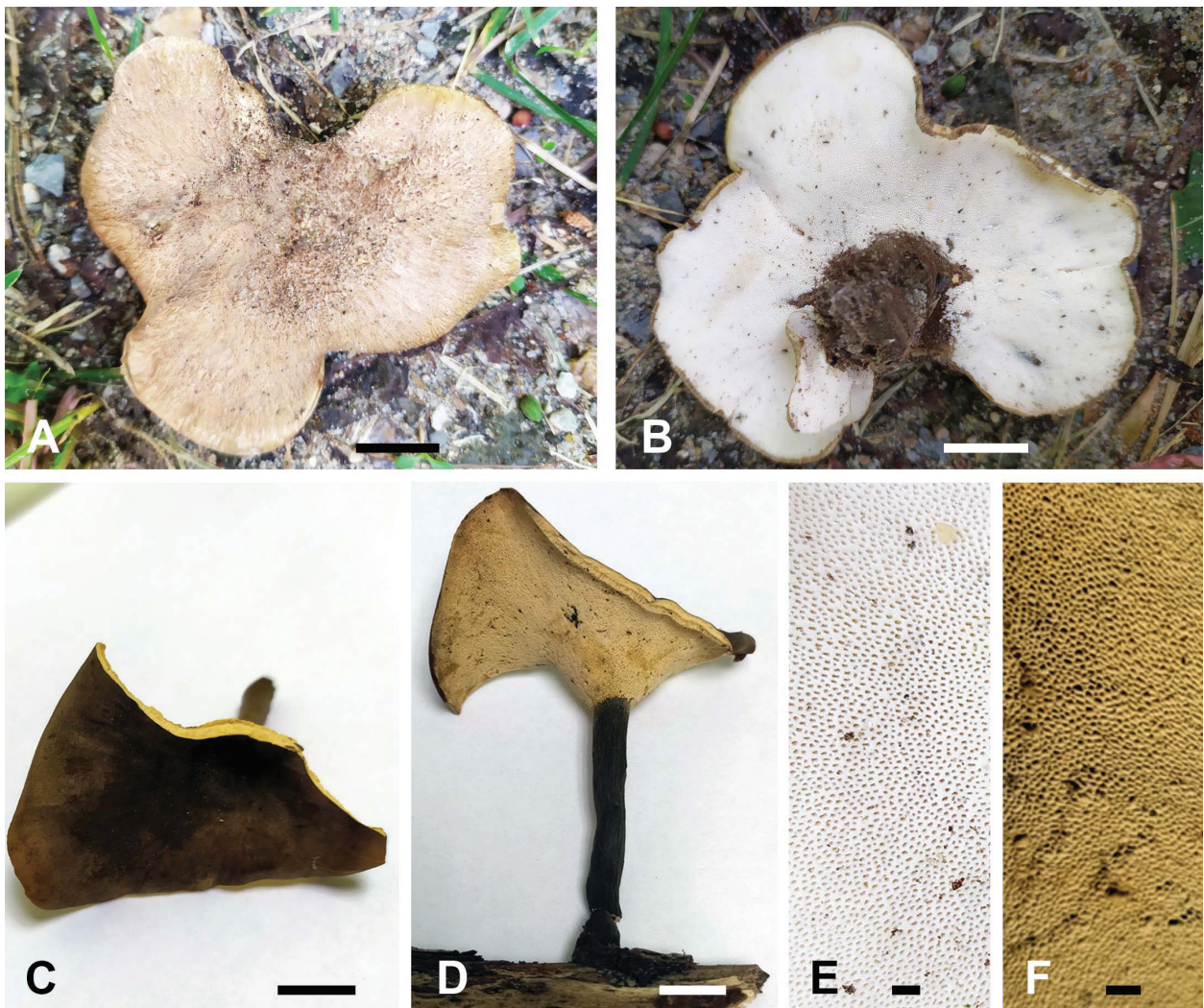


Fig. 1. *Picipes submelanopus*: A, B – fresh basidiomata; E – hymenial surface; *Picipes ulleungensis*: C, D – dry basidiomata; F – hymenial surface. Bars: A–D – 1 cm; E–F – 1 mm.

***Picipes ulleungensis*** (H. Lee, N. K. Kim et Y. W. Lim) B. K. Cui, Xing Ji et J. L. Zhou ( $\equiv$  *Polyporus ulleungensis* H. Lee, N. K. Kim et Y. W. Lim) (Fig. 1C, D, F).

**Specimens examined.** RUSSIAN FEDERATION: “Altai Territory, Talmensky district, near Vypolzovo village, 53.8296°N, 83.7521°E, 162 m a. s. l., birch forest, on a fallen trunk of *Betula pendula*. 09

VII 2008. V. A. Vlasenko” (NSK 1014990, GenBank OP964916 (ITS)).

**Distribution.** Europe – Estonia; Asia – Russia (in this study), China, South Korea (Tibpromma et al., 2017; Runnel et al., 2021; Ji et al., 2022).

**Comments.** The ML analysis of the ITS1–5.8S–ITS2 nrDNA region confirmed that the studied specimen nests within the *Picipes ulleungensis* (Fig. 2). The species as an independent taxon was de-

scribed in 2017, based on molecular genetic studies. On a global scale, the species is known from four localities, with a type locality in South Korea. *Picipes ulleungensis* is morphologically similar to *P. melanopus*. Specimens of the species, collected by us in Western Siberia, were found on dead wood – on a birch. In the type locality in Korea, *Picipes ulleungensis* has also been found on a woody plant

belonging to the genus *Betula* – *B. platyphylla* var. *japonica* (Tibpromma et al., 2017). *Picipes ulleungensis*, according to the original description, differs from *P. melanopus* larger basidiomata and slightly larger pores (5–6 on mm vs. 6–8 on mm). Records from South Korea, China, and Russia have been confirmed by molecular genetic studies.

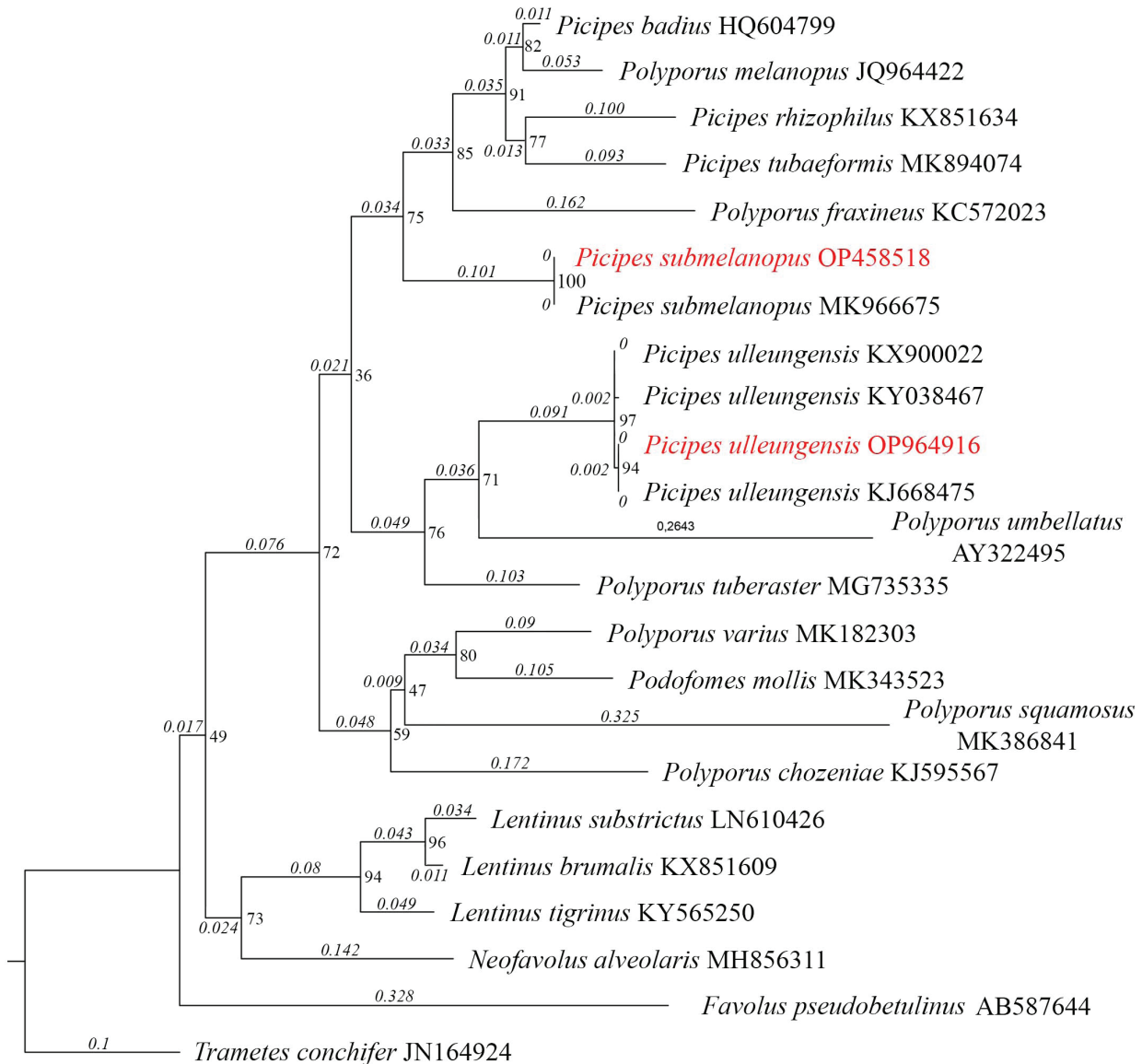


Fig. 2. The maximum likelihood tree based on ITS1–5.8S–ITS2 nrDNA sequences shows the phylogenetic relationships between *Picipes submelanopus*, *P. ulleungensis*, and other closely related *Polyporus* s. l. species. Numbers on the tree demonstrate genetic distances (branches) and bootstrap values (nodes). Each sequence name contains its GenBank access number.

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