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Morphological characteristics and molecular phylogeny of *Disciseda hyalothrix* (Gasteromycetes) from Altai Mountains, a new record to Northern Asia

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Abstract

Morphological characteristics and molecular phylogeny of the gasteroid fungus, *Disciseda hyalothrix*, and data on its localities, habitat and distribution are provided. This rare species of *D. hyalothrix* was found in the protection zone of the Tigirek Reserve, Altai Territory, Russia (Altai Mountains, Western Siberia, Northern Asia). A fruiting body of *D. hyalothrix* was found in "Dragunskaya" cave. Detailed descriptions, illustrations of basidiocarp and basidiospores are given. The main diagnostic features of *Disciseda* species are the size of the spore and the nature of the ornamentation. Spores of *D. hyalothrix* are globose, brown, grossly verrucose, (6) 6.5–7.5 μm in diameter, without ornamentations, without pedicels or rarely with colorless pedicels 4–6 (up to 10) μm. In the SEM, ornamentation in the form of powerful pyramidal tufts consisting of thin spines pressed together to 1–1.3 μm in height. We present a scanning electron micrograph study and morphological characteristic comparison of *D. hyalothrix* and other *Disciseda* species found in Eurasia, which currently includes 5 taxa. We first generated new sequences for rDNA (ITS1-5.8S-ITS2 region and partly for LSU) of *D. hyalothrix* (NSK 1014099). The new record of *D. hyalothrix* broadens information on the ecology of the rare gasteromycete species, which was growing in the conditions of stony steppe communities. New sequence data for studied loci of rDNA will help clarify the phylogenetic relationships of species from the genera *Disciseda* and *Bovista*.

Key words – gasteroid fungi – molecular data – morphology – puffballs – new sequences

Introduction

The genus *Disciseda* was described by B. Chernyaev in 1845. It was later described by other authors as *Catastoma* Morgan and *Bovistina* Long et Stouffer, in 1982 and 1941 respectively (references are needed). In nature, this fungus is found in arid habitats such as deserts, sandy and stony steppes, and also in dry low-grass meadows, less often in drying light coniferous forests (Rebriev 2009). When totally mature, the exoperidium incrusted by the substratum breaks up in the lower part. The basidiocarp detaches from mycelium and turns over. The upper part of the exoperidium that is firmly attached at the apex, remains a cup-shaped structure. A small basal

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rupture appears, through which spores can escape. The cup-like residue of the exoperidium encrusted with the substrate, in which the fruiting bodies is located, is also observed in some representatives of *Bovista* genus, but the original development of fruiting bodies with inversion is observed only in the species of *Disciseda*.

Index Fungotum gives 46 names of *Disciseda* records (Index Fungorum 2019), a lot of them seem to be synonyms. Four species are found in Russia. Species of the genus *Disciseda* in Russia are most often found in arid areas, in steppes and deserts. The most widespread and common species among the genus are *D. bovista* (Klotzsch) Henn. and *D. candida* (Schwein.) Lloyd. The remaining species are of rare occurrence. For *Disciseda cervina* (Berk.) G. Cunn. about 20 records are known. Rare species of *D. hyalothrix* (Cooke et Massee) Hollós, in Russia, was previously recorded only for European part of the country (Rostov Region).

New findings and herbarium materials are important, as at present, due to the lack of modern monographic treatments and abundance of synonyms, it is difficult to determine the number of species in the genus *Disciseda*. The literature on the genus morphology is controversial. For example, presence of a parenchymal layer of exoperidia is indicated not only in *D. candida* (Moravec 1954), but also in *D. bovista* (Kers 1975, Jeppson 1997, Rebriev 2009). Some morphological differences in *D. candida* and *D. cervina* were revealed with SEM (Kers 1975, Rebriev 2009). SEM is currently the main method used to reliably differentiate species of the genus *Disciseda*, as well as to reveal the variability range for diagnostically significant morphological characteristics.

Phylogenetic studies of species of the genus *Disciseda* are poorly represented (Larsson & Jeppson 2008, Bates et al. 2009), data on the nucleotide sequences for ITS region was available for only two species before starts our study, and they were *D. candida* and *D. bovista*. New sequence data for studied loci of rDNA generated by us, can be used as barcodes to identify *D. hyalothrix*.

Materials & Methods

Field studies

A rare species of *D. hyalothrix* was found in the Altai Territory, in the protection zone of the Tigirek Reserve, in the vicinity of the Tigirek village (Fig. 1). This is the first finding of this species in the Northern Asia.

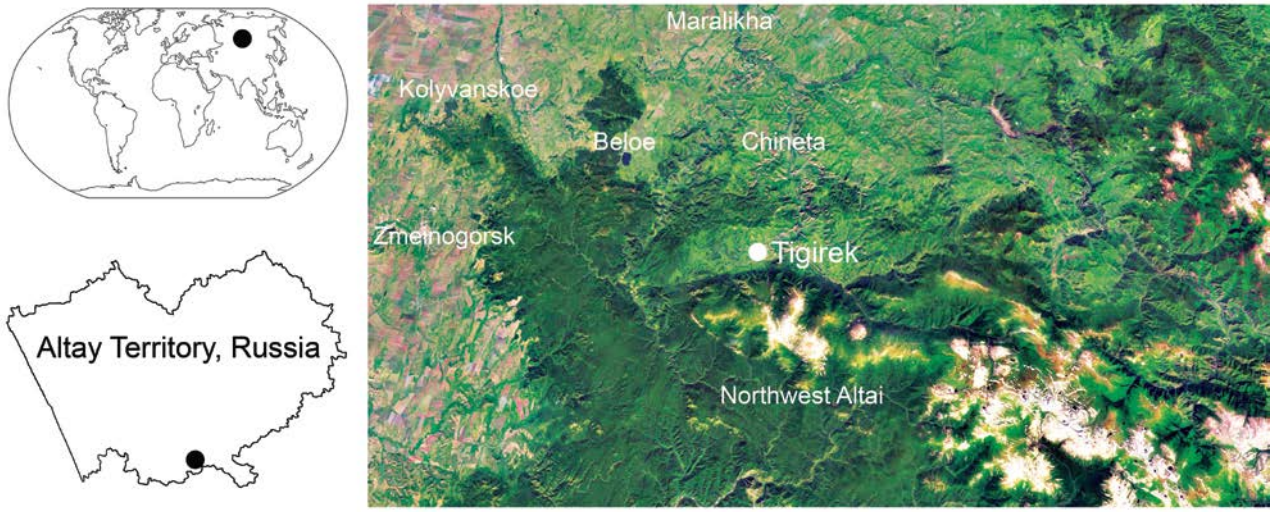

Fig. 1 – Map of the Altai Territory with the locality details of *Disciseda hyalothrix* (color drawing Google Earth Pro V 7.3 2019).

The Tigirek State Nature Reserve was established in 1999 to preserve the biodiversity of the natural complexes in the middle mountains of Western Altai. The reserve is located in the south-

western part of the Altai Territory, i.e., in the western part of the Altai Mountains on the left bank of the Upper Charysh basin. The total area of the reserve is 40,693 hectares, the conservation zone of the reserve in total covers 26,257 hectares (Davydov et al. 2011).

A basidiocarp of *D. hyalothrix* was found in "Dragunskaya" cave (or "Pasechnaya"). It was described by P.S. Pallas during an expedition in 1771. The cave is located in the Krasnoschekovsky district of the Altai Territory, at Altai northern foothills, 3 km west of the Tigirek village, on the eastern edge of "Dragunsky" ravine, which starts in the vicinity of "Shlyapnaya" Mountain. It is a small erosive limestone cave shaped as a horizontal gallery, with a flat bottom, vertical walls, and a lancet arch. The cave has a small slope in the direction from the end towards the entrance. The bottom of the cave is soil, on which 1 old fruiting bodies of *D. hyalothrix* was found.

We do not have reliable data to show the fruiting bodies were growing on soil in a cave. In addition to soil and seeping rainwater, the gallery of the cave is partially illuminated by sunlight. The aggregate factors were conducive for the appearance and growth of the fungus. Possibility that the fruiting bodies of *D. hyalothrix* could be deposited into the cave by wind, thawing or rainwater from the surrounding area of the cave cannot be excluded.

The terrain in the fungus area is typical for low mountains and is located in the mountain-forest-steppe belt, with a combination of grass, mixed grass petrophytic and meadow steppes (Fig. 2). Shrub communities are well developed. They are widely represented in the steppes, and form a separate belt with thickets of *Sibiraea laevigata*, *Caragana arborescens*, *Lonicera tatarica*, *Crataegus sanguinea*, *Sambucus sibirica* along the pristine slopes and logs. Forest vegetation is represented by larch, birch-larch or pine forests on the tops of the northern slopes of the hills.

Fig. 2 – Habitats of *Disciseda hyalothrix*. A Dragunsky ravine. B Rocky (petrophytic) steppe on a hillside above the cave. Photos by V. Vlasenko.

Since *D. hyalothrix* is a species growing in arid habitats, its growth is possible in habitats entering the petrophytic steppe plant communities on the hilltops above the cave, which are formed by *Sedum hybridum*, *Thymus petraeus*, *Aster alpinus*, *Dianthus versicolor*, *Bupleurum multinerve*, *Caragana pygmaea*, *Spiraea trilobata*, *Juniperus sabina*. Habitats of *D. hyalothrix* in the Tigirek Reserve will be preserved, which in turn contributes to conservation of the species in nature, like many other species (Davydov et al. 2018).

Morphological examination

Initial morphological examination was performed using a Carl Zeiss Stemi DV4 stereomicroscope, a Carl Zeiss Axiolab E re light microscope and a Carl Zeiss Axioskop-40 light microscope. The examination of microstructures under the light microscope was made after boiling the preparation for a short time in polyvinyl lactophenol cotton blue. Specimens were prepared for

scanning electron microscopy using traditional SEM techniques, summarized as follows. The specimens were viewed and photographed using a Carl Zeiss EVO MA 10 scanning electron microscope. For photographing fruiting bodies in transmitted light, we used a Panasonic-Lumix DFC-XZ7 camera. Voucher specimens of the species are stored in the MG Popopv Herbarium (NSK), Novosibirsk, Russia. The species description is based on the literature data (Jeppson 2018, Moreno et al. 2003, 2007, Silva & Baseia 2014) and original observations by Yu. Rebriev.

DNA extraction and sequencing

Specimens of *D. hyalothrix* (NSK 1014099) was used for molecular analyses. A piece of fungal fruiting body (50 μg) was homogenized in 300 μl lysis buffer and extract the DNA with NucleoSpin Plant II kit was used. The ITS1-5.8S-ITS2 region of the rDNA were amplified by PCR with the primers ITS1F and ITS4B. For PCR, HS Taq DNA Polymerase (Evrogen, Moscow) was used. PCR reactions were performed in an C1000 Thermal Cycler (Bio-Rad, USA). PCR results were checked at Gel Doc XR+ Imager (Bio-Rad, USA). DAN amplicons sequencing performed in SB RAS Genomics Core Facilities (Novosibirsk, Russia).

Phylogenetic analyses

Additional 6 ITS sequences of other *Disciseda* and *Bovista* species based on BLAST results and 3 ITS sequences of other species were retrieved from GenBank (http://www.ncbi.nlm.nih.gov/Genbank/). *Mycenastrum corium* was used as an outgroup (Larsson & Jeppson 2008). We first generated a new sequence for ITS1-5.8S-ITS2 region and partly for large subunit rDNA (28S rDNA) for *D. hyalothrix* (GenBank No: MN151399). The final dataset consisted of 10 ITS sequences. An overview of all taxa and on sequences used for tree reconstruction, shows the species names, herbarium vouchers/strain and Genbank accession numbers given in Table 1.

Table 1 Sequences used in the phylogenetic analyses.

Species	**Herbarium voucher/strain**	**Genbank accession number**
Bovista aestivalis	MJ1122	DQ112620
Bovista pusilliformis	CBS 397.74	MH860864
Bovista promontorii	MJ7070	DQ112621
Bovista polymorpha	DA-29	AJ237613
Disciseda bovista	MJ5078	DQ112627
Disciseda candida	STB304	EU833654
Disciseda hyalothrix	**NSK 1014099**	**MN151399**
Lycoperdon perlatum	MJ4684	DQ112630
Mycenastrum corium	KM162954	GQ981488
Vascellum pratense	MJ4864	DQ112554

Sequences were align using ClustalW methods (Thompson et al. 1994). The ITS sequences were aligned in MEGA 7 (Kumar et al. 2016). The evolutionary history was inferred using the Neighbor-Joining method (Saitou & Nei 1987). The optimal tree with the sum of branch length = 0,016 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches (Felsenstein 1985). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura et al. 2004) and are in the units of the number of base substitutions per site. The differences in the composition bias among sequences were considered in evolutionary comparisons (Tamura & Kumar 2002). The analysis involved 10 nucleotide

sequences. All positions containing gaps and missing data were eliminated. There were a total of 630 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

Results

Disciseda hyalothrix (Cooke et Massee) Hollós, 1902, Növényt. Közlem. 1: 107. Fig. 3

Description – Basidiocarps subglobose to depressed-globose, 1–2.8 cm in diameter. Exoperidium thin, whitish to grayish-brown, fragile, often completely falling away or remaining in the form of a shell-encrusted "case" encrusted with remnants of the substrate with the endoperidium lying in it. Endoperidium is coriaceous, hard, gray to dark brown, smooth, with a fibrillose pore of irregular shape. Gleba is brown when maturing. Threads of capillitium rarely branched, smooth, curved, light brown, easily breakable into short fragments, 2.3–2.7 μm. Spores globose, grossly verrucose, brown, (6) 6.5–7.5 μm in diameter without ornamentation, without pedicels or rarely with a colorless pedicels 4–6 (up to 10) μm. In the electron microscope, ornamentation in the form of powerful pyramidal tufts to 1–1.3 μm consisting of thin spines pressed together.

Habitat – steppes and deserts.

Known distribution – Europe, Asia, North and South America, Australia. In Russia, currently known only from Rostov Region (Rebriev 2009).

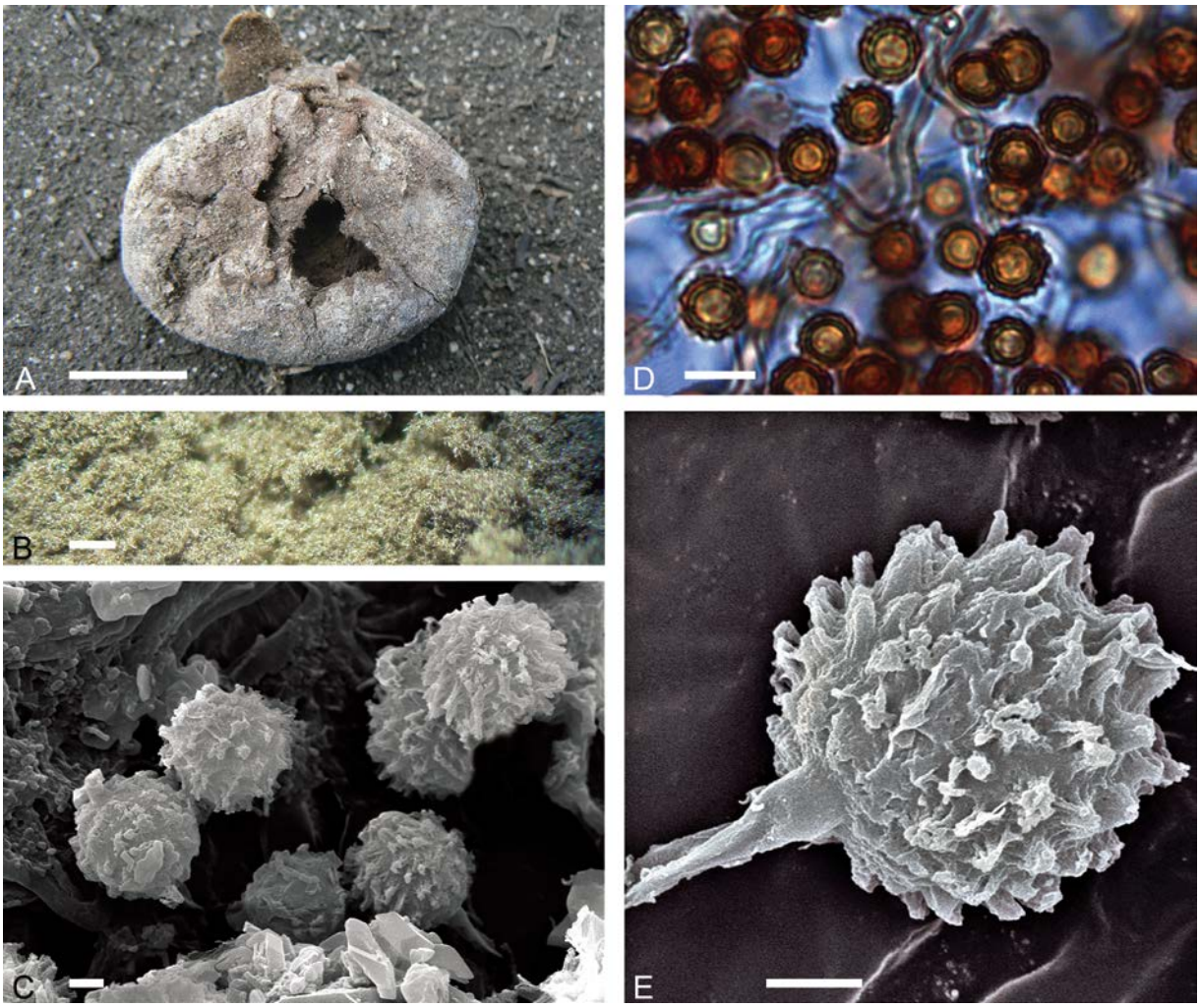

Fig. 3 – *Disciseda hyalothrix*. A Fruiting body in the collection site. B Gleba (color in RL). C Spores (SEM). D Spores (in TL, polyvinyl lactophenol). E Spore (SEM). Scale bars: A = 1 cm. B = 1 mm. C = 2 μm. D = 10 μm. E = 2 μm. Photos by: A, B, D – V. Vlasenko. C, E – A. Vlasenko.

Material examined – Russia, Altay Territory, Krasnoschekovsky district, Tigirek Reserve, 3 km west of the Tigirek village, on the eastern edge of “Dragunsky” ravine, “Dragunskaya” cave, on soil in a cave, 51° 09' 195" N, 82° 58' 798" E, 05 July 2018, V.A. Vlasenko, NSK 1014099.

Discussion

The main diagnostic feature of *Disciseda* species are the size of the spores and the nature of the ornamentation (Table 2).

Table 2 Morphological comparison of *D. hyalothrix* and other species found in Eurasia.

	D. cervina	***D. candida***	***D. bovista***	***D. verrucosa***	***D. hyalothrix***
Spore size (with ornamentation), μm	4–5.5	3.5–5.5	5.5–7 (8)	8–12	8.5–13
Spore ornamentation	smooth to asperulate	finely verrucose	verrucose	strongly verrucose	strongly verrucose*

*Spores often with a pedicel 4–6 (up to 15) μm.

The studied specimen is characterized by relatively small spores, 6.5–7.5 μm. Another significant difference from the typical description is absence of pedicels in most spores, if any, small sizes of 4–6 μm. This was noted in specimens from Mexico, which can be explained by the degree of maturity of the fruiting bodies, its preservation and/or growth conditions (Moreno et al. 2007).

Small smooth or verrucose spores up to 5.5 μm in diameter are found in two species in Russia – *D. cervina* and *D. candida*. In the remaining species, spores are large, more than 5.5 μm in diameter, with well-developed ornamentation. Several species of this group are found in Eurasia. *Disciseda bovista* is the most common form with well-ornamented spores. It is distinguished by a smaller size of spores. The spores are large verrucose 5.5–7 (8) μm in diameter with ornamentation. Pedicels up to 3.5 μm are present or absent. Ornamentation of the spores of *D. verrucosa* G. Cunn. (=*D. arida* Velen.) – finger-shaped spines often up to 2 μm high. The spores are 8–12 μm in diameter, taking into account the ornamentation.

Another species described from Europe, *D. nigra*, has verrucose spores 7.5–8.5 μm in diameter with ornamentation about 1.8 μm (Dörfeld & Nowak 2002). The species description is invalid, the type is not specified. According to the obtained molecular data, the species should be classified as *Geastrum* genus (http://www.indexfungorum.org).

Disciseda ochrochalcea Kreisel was described from Nepal, the Himalayas Mountains, where it was collected in a dry Alpine meadow at an altitude of 4,700–4,800 m (Kreisel 1976), and is nowhere to be found. It is characterized by grossly verrucose olive-brown to almost black spores of 8.2–10.5 μm in diameter, taking into account the ornamentation. Pedicels up to 4.7 μm. In the description of the species only the drawings are presented and there is no detailed information on the ornamentation of the spores. Of all the listed species, *D. hyalothrix* is clearly distinguished by the ornamentation of its spores in the form of pinned thin spines, assembled into powerful pyramidal tufts.

The molecular phylogenetic analyses placed the specimens of *Disciseda hyalothrix* (NSK 1014099) from Northern Asia close to *Disciseda* and *Bovista* genera. Genetic distance 0.024 with bootstrap to support *D. hyalothrix* branch 70% (Fig. 4). Phylogenetic studies of species of *Disciseda* genus are poorly represented (Larsson & Jeppson 2008, Bates et al. 2009), data on the nucleotide sequences of ITS1-5.8S-ITS2 region rDNA are available for *Disciseda bovista* and *D. candida*. New sequence data for studied loci of rDNA received by us, can be used as barcodes to identify the *D. hyalothrix*. Classification of *Disciseda* genus will be updated with the advent of new sequences for members of the genus.

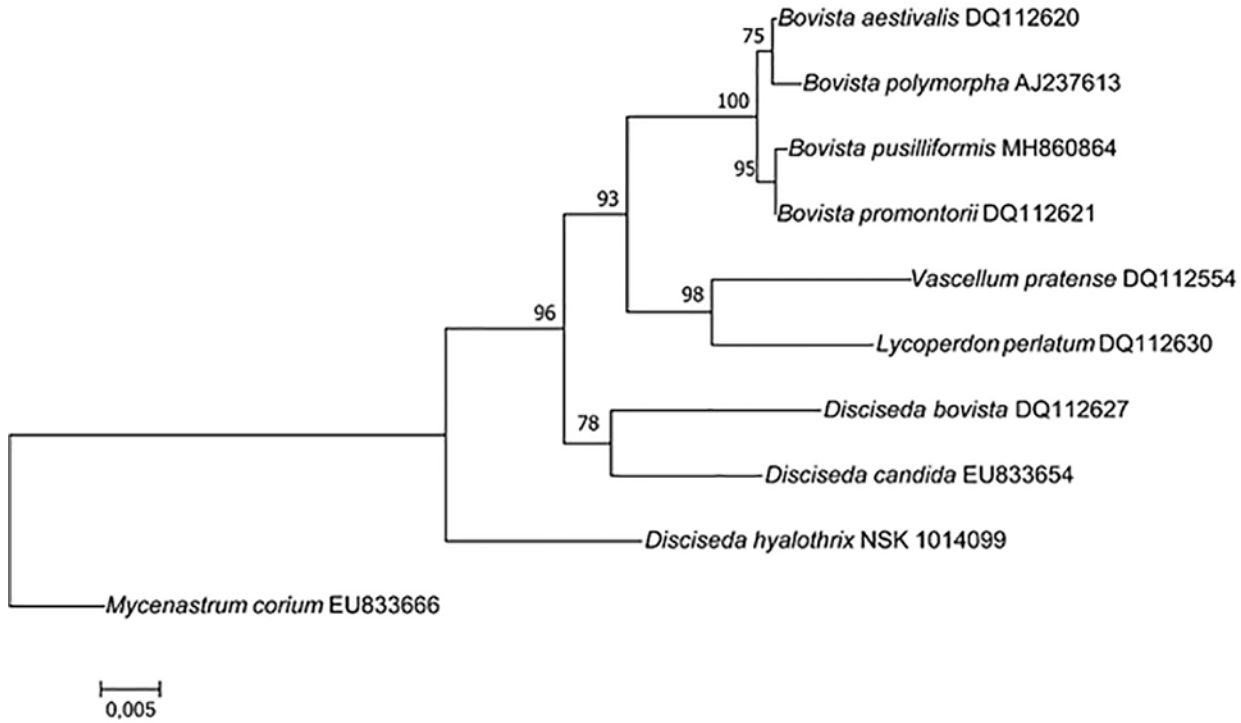

Fig. 4 – Neighbor Joining (NJ) tree showing phylogenetic relationships between the *D. hyalothrix* (NSK 1014099) from Northern Asia and other related species of Ggasteromycetes, based on the ITS rDNA sequences. *Mycenastrum corium* (GQ981488) was used as the outgroup taxon. Values on the branches represent the percentage of 1000 bootstrap replicates and bootstrap values over 75% are shown in the tree.

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