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Distribution and phylogeography of the genus *Mattirolomyces* with a focus on the Asian *M. terfezioides* haplotypes

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Mattirolomyces is an edible commercial sequestrate genus that is globally distributed. Of the genus's five described species, *Mattirolomyces terfezioides* is the most common in Asia. Recent attempts to locate *M. terfezioides* outside its current distribution area in China documented the first records in areas of poplar trees with the lowest known temperature and precipitation averages for this species. This peculiar ecology was not reflected in the species' morphological features or phylogenetic positioning in the genus. The first attempt at applying the phylogenetic network approach in *Mattirolomyces* revealed that its geographic origin was in Asia-Pacific areas prior to frequent long-distance migration events. Based on recent areas of study, we found that the collections from Inner Mongolia and the Shanxi province were similar to European collections. Other Asian haplotypes were more basal, supporting the idea that *M. terfezioides* originated from this area in China and was subsequently transported to Europe. Exploring *M. terfezioides'* ecology and potential to grow with poplars also increases its potential for cultivation and consumption in central and eastern China, a currently underexploited opportunity that deserves further ethnomycological investigations.

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ABSTRACT

Mattirolomyces is an edible commercial sequestrate genus that is globally distributed. Of the genus's five described species, *Mattirolomyces terfezioides* is the most common in Asia. Recent attempts to locate *M. terfezioides* outside its current distribution area in China documented the first records in areas of poplar trees with the lowest known temperature and precipitation averages for this species. This peculiar ecology was not reflected in the species' morphological features or phylogenetic positioning in the genus. The first attempt at applying the phylogenetic network approach in *Mattirolomyces* revealed that its geographic origin was in Asia-Pacific areas prior to frequent long-distance migration events. Based on recent areas of study, we found that the collections from Inner Mongolia and the Shanxi province were similar to European collections. Other Asian haplotypes were more basal, supporting the idea that *M. terfezioides* originated from this area in China and was subsequently transported to Europe. Exploring *M. terfezioides'* ecology and potential to grow with poplars also increases its potential for cultivation and consumption in central and eastern China, a currently underexploited opportunity that deserves further ethnomycological investigations.

Keywords: *Mattirolomyces terfezioides*, desert truffle, Inner Mongolia, phylogeograph

INTRODUCTION

The genus *Mattirolomyces* (Tuberaceae, Pezizales) was called the "Mattirolo fungus" by Fisher in 1938, since Mattirolo was the first to describe the species, which he called *Choiromyces terfezioides*, back in 1887 (*Fisher, 1938*). The type specimen was first collected from clay agricultural soils in a non-typical ecological location in Piemonte, northern Italy, and was at that time considered a potential partner of *Prunus avium* (*Mattirolo, 1887*). *Mattirolomyces* is an ascomycetous genus and all known species of the genus form sequestrate to hypogeous sporocarps (*Fisher, 1938*). Currently, five species in the genus *Mattirolomyces* have been shown to have an intriguing geographical distribution. The genus type species *Mattirolomyces terfezioides* (Mattir.) E. Fisch was recorded in Europe and Asia; *M. spinosus* (Harkn.) Kovács, Trappe & Alsheikh in North America and Pakistan; *M. mulpu* Kovács, Trappe & Claridge in Australia; *M. austroafricanus* (Marasas & Trappe) Kovács in South Africa; and *M. mexicanus* Kovács, Trappe & Claridge in Mexico. The distribution of these five species suggest a wide geographical (presumably global) distribution of the genus (*Kagan-Zur et al., 2014*) with most records coming from eastern and southeastern Europe (*Glejdura & Kunca, 2012; Kagan-Zur et al., 2014; Assyov & Slavova, 2016*).

The desert regions of the southern hemisphere have low and variable average rainfall and high summer temperatures. *Mattirolomyces* spp. and other desert truffles have broad geographical, botanical, and cultural attributes that have made them regularly hunted and harvested for food since prehistoric times (*Trappe et al., 2010*). In Europe and Asia, however, this species is rarely recognized as a significant commercial species (*Boa, 2004*). *Mattirolomyces* spp. sporocarps are traditionally collected, sold, and consumed under the name *Terfezia terfezioides* (Mattir.) Trappe, a synonym of *Mattirolomyces terfezioides*. Despite its value as a mycorrhizal species and culinary delicacy, this species is not well-known in the northern hemisphere (*Kovács et al., 2007*). Most available collections of *M. terfezioides* are from Hungary (Europe) and northeastern China, namely Beijing, Hebei Province, and Shanxi Province. Most of the Chinese collections date several decades back with the most recent being from 1986 (*Wang et al., 2017*). The economic and culinary value of the Chinese of the of the Chinese collections have not yet been evaluated. Furthermore, this species has been considered long-lost in China by many mycologists.

We were motivated by a recently discovered *M. terfezioides* collection from the desert areas of Inner Mongolia, China to revive the study of the Chinese genus, characterize the current ecological span of the species, and prepare a morphology-based description of the Chinese collection. Due to a low number of records and available nucleotide sequences, no phylogeographic insight into the genus is currently available. Therefore, we aimed to position the Chinese collections of *M. terfezioides* in a phylogenetic network of the whole genus, focusing on the relationship between the Chinese collections and the collections from other areas worldwide, in order to ultimately hypothesize the genus's geographic origin.

MATERIALS AND METHODS

Study site and sampling

The most temperate continental part of China, Inner Mongolia, has a cold semi-arid (BSk) to cold desert (BWk) climate (*Peel et al., 2007*). Although the occurrence of *Mattirolomyces* was not previously recorded in this area, the genus was found in most of its neighboring provinces, which indicated its potential for fruiting in Inner Mongolia as well. Sporocarps were primarily sought for in ecosystems that were suitable for *Mattirolomyces* (*Kagan-Zur & Akyuz, 2014*). When selecting sampling microlocations, we targeted the known ectomycorrhizal partner sites with pines and black locusts (*Kagan-Zur & Akyuz, 2014*), as well as young plantations of *Populus alba* ~~L. var.~~ *pyramidalis* Bunge at various locations in the Baotou area, Inner Mongolia, China, between September and October 2018 and 2019.

The main climatic characteristics of the broader area where *M. terfezioides* was repeatedly collected were: an elevation of about 1,070 m in the surveyed area, average temperature of around 8.5℃, lowest temperature of minus 27.6℃, and highest temperature of 40.4℃. The average annual rainfall in this area over the last 18 years is 301.6 mm (the minimum rainfall was 175.9 mm in 2005, and the maximum rainfall was 465.2 mm in 2003). The average annual rainfall in 2018 and 2019 was 364.8 mm and 327.6 mm, respectively, according to data from Inner Mongolia Meteorology. The soils are sandy, hyphal aggregates that connected the roots with above ground parts of the plants, and the sporocarps of this fungus developed from the hyphal aggregates.

Sporocarps were collected by racking the soils where surface cracks indicated their potential presence, generally following Castellano *et al.*'s (2004) procedure. All sporocarps were photographed *in situ* with a Canon EOS 60D camera (Canon, Tokyo Japan), then dried in a forced-air dryer and kept in the Herbarium and Fungarium of Baotou Teachers's College under accession numbers Fan0273.

Morphological observation

Macro- and microscopic characters were analyzed using a stereomicroscope (Motic K400, Motic Asia, Hong Kong) and light microscope (Motic BA410E with a Moticam2506 camera, Motic Asia, Hong Kong) following the list of characters found in Kovács *et al*. (2011). For comparison, we used Melzer's reagent and Cotton Blue chemical reactions. Scanning electron microscopy of spores was done on a desiccated and platinum-palladium coated material using Hitachi E-1010 (Tokyo, Japan), and examined and photographed with a Hitachi S-530 (Tokyo, Japan).

DNA extraction, PCR amplification, and sequencing

DNA extraction, PCR, and sequencing were carried out according to Wang *et al*. (2017), except PCR amplification was performed using Taq PCR Master Mix (Biobasic, Ontario, Canada). PCR products were purified and sequenced at the Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu, Sichuan, China. Sequences were deposited in GenBank with the accession numbers listed in Table 1.

Phylogenetic analyses

Available and compete nuclear rDNA ITS sequences from the genus *Mattirolomyces* were retrieved from GenBank (*Benson et al., 2013*) and UNITE databases (*Kõljalg et al., 2013*) on December 12, 2019. We conducted a nucleotide search using the basic local alignment search tool (BLAST) with our representative sequence to trace additional sequences that were potentially misnamed in the searched databases. A local *Mattirolomyces* spp. nuclear rDNA ITS sequence database was built based on available and reliable sequences, and we selected environmental parameters from papers where sequences were published or directly from online databases (Table 1), with meteorological data from the latest FLUXNET synthesis dataset, the FLUXNET2015 database (http://fluxnet.fluxdata.org/data/fluxnet2015-dataset/data-processing/).

DNA sequences were assembled in BioEdit v5.0.9 (https://bioedit.software.informer.com). MEGA7 (*Kumar et al., ~~2015~~*) was used for manipulation of sequences, and internal MEGA7 plug-ins were used for sequence alignment (ClustalW), testing for the best nucleotide substitution model (ModelTest), maximum likelihood phylogenetic analysis (ML phylogenetic analysis), and construction of the phylogenetic tree. Kimura's 2-parametric model was selected as the best for a distance calculation of a given dataset. In order to evaluate the stability of the ML evolutionary tree topology, 1,000 bootstrap repetitions were run. Using the Bayesian method, we calculated Bayesian inference with MrBayes v. 3.1.2 (*Ronquist & Huelsenbeck, 2003*) and an HKY+G model. Four Markov chains were run for two runs from random starting trees for 1 million generations, until the split deviation frequency value < 0.01. Every 100th generation was sampled. The Bayesian inference tree was visualized in FigTree 1.4.2. Branches that received bootstrap from ML≥ 60 % and Bayesian posterior probabilities (BPP) ≥ 0.95 were considered significantly supported.

For the phylogenetic network analysis, the same nucleotide dataset was realigned in MAFFT v. 7.304b (*Katoh & Standley, 2013*) and analyzed with a Median joining approach in Network5 (*Bandelt et al., 1999*). The phylogenetic network constructed in Network5 was modified and annotated in the GNU General Public License program Inkscape 0.91 (https://inkscape.org/release/inkscape-0.91/).

Determining soil physical and chemical properties

The soil samples were taken from the immediate vicinity of the fruiting sporocarps to a depth of 10 cm. Soil pH was measured in 1M KCl (1;5 w/v). Organic C and total N were analyzed using the CHNS-analyzer system (Elementar Analysen systeme GmbH, Hanau, Germany) with the burning method at 450 and 1,250 °C, respectively (*Liu et al., 2012*). We determined total organic matter, available phosphorus content, and available potassium as well as the total content of water soluble salts following the standardized operation procedures (*Pansu & Gautheyrou, 1965*).

RESULTS

Taxonomy

Over two years of hunting for hypogeous fungi in *Mattirolomyces*-like habitats, we collected two independent collections made up of a total of 32 sporocarps, all from *Populus alba* ~~var.~~ *pyramidalis* plantations.

A morphological description of the collections from Inner Mongolia resemble

Mattirolomyces terfezioides (Mattir.) E. Fisch., as described by Fischer (1938). *Ascomata* (fresh specimens, Figure 1A) were hypogeous or subepigeous, 4-5 cm in diam., subglobose to irregular massy, white, surface smooth to scabrous, lobed and furrowed; *gleba* solid, spongy with minute pockets, white with narrow white veins (Figure 1C). *Taste and odor* were strongly sweet when fresh. *Sclerotia* as hyphal aggregates, attached with the base of the sporocarps, dark brown, 0.5-1.0 cm in diam. (Figure 1B). *Paraphyses* were absent.

Microscopic features: *peridium* thin with 120-280 mm thickness, no clear differentiation from the gleba, composed of inflated hyphae and irregular, hyaline cells; *gleba* composed of interwoven septate hyphae 7.5-11(20) μm broad, with some free hyphal ends; *asci* randomly arranged in gleba, 8- or 10-spored, hyaline, globose to ellipsoid, pockety, saccate, cylindrical or clavate, (55) 65-95(117) × (26) 35-45 (60) μm, sessile or occasionally sub-stipitate with a short stalk, disintegrating with age, thin walled, readily separable from gleba hyphae, nonamyloid (Figure 1D and Figure 1E); *ascospores* hyaline to pale yellow, globose, (12) 14-19 (22) μm in diam. excluding the ornamentation (Figure 1D and Figure 1E); ornamentation of blunt spines connected in an irregular alveolate reticulum, 1-4 μm high, mostly have a de Bary bubble and are uniguttulate, walls 1.5-2 μm thick (Figure 1F). *Sclerotia*: attached to the ascoma and the surface of the roots. *Aroma* of mature sporocarps was pleasantly sweet.

In terms of *ecology*, all collections were found in the vicinity of *Populus alba* ~~L. var.~~ *pyramidalis*. Soils were sandy to finely sandy with a history of extensive management practices. Soils have relatively high water soluble salt content (1.29 g kg^{-1}), slightly basic pH (7.34), 1.49 g kg^{-1} of total nitrogen, 46.4 mg kg^{-1} of available phosphorus, and 29.82 g kg^{-1} of organic matter.

The average annual precipitation for collections from Table 1 indicate that the collections from the desert regions of Inner Mongolia were more similar to European collections, Mediterranean collections from several countries, and Continental collections from Hungary, than to other Asian collections. The Inner Mongolian collections were from sites with larger winter/summer temperature differences and lower winter averages when compared to the other collections included in this study (Figure 2).

Phylogenetic analysis

Bayesian and ML phylogenetic analyses resulted in strongly-supported, topologically identical phylogenetic trees with clusters containing collections from Inner Mongolia together with other *M. terfezioides* from China, Hungary, Italy, and South Africa (Figure 3).

A phylogenetic network analysis (Figure 4) separated all five recognized taxa in the genus *Mattirolomyces* with an expected higher diversity in *M. terfezioides*. The high distance from the outgroup (*Elderia arenivaga*) to the genus *Mattirolomyces* indicated a poor selection, yet the most optimal one regarding the available recent taxa and their sequences. At the base of the *Mattirolomyces* cluster, three lineages were disclosed. The first lineage led to three clusters: one directed to South Africa with *M. austroafricanus*, the second with a more basal position of *M. spinosus* from south Asia (the collection was from Pakistan), and a phylogenetically close collection from the United States, with a distinct sub-cluster of closely-related *M. mexicanus* from Mexico and distantly-related (based on the comparison of the number of mutated sites) *M. mulpu* from Australia. For all four mentioned recent taxa, the number of available nucleotide sequences was low. The third lineage formed a cluster of *M. terfezioides* that showed higher intraspecific diversity and a recognizable geographic pattern with more basal haplotypes from China, followed by collections from S. Korea. At this point of evolution, there was a jump of haplotypes from Asia (China) to Europe (Hungary, Italy, France), and no supported intra-Europe geographic pattern was recognized.

DISCUSSION

Mattirolomyces terfezioides is a commonly-collected edible hypogeous fungus best known for its traditional use in the desert regions of the southern hemisphere (*Trappe et al., 2010*). Although it has been used for culinary purposes as far back as in ancient Persia (*ibid.*), its recent distribution outside its optimal ecology has not been explored. There are two well-recognized areas of distribution: the Mediterranean and Pannonian basin in Europe (*Kagan-Zur et al., 2014; Kovács et al., 2001*) and Beijing, along with neighboring regions in China. The taxonomic characteristics of the Inner Mongolian collections fit well in the concept of the *Mattirolomyces terfezioides* morphological species (*Fischer, 1938; Mattirolo, 1887*) and also the phylogenetic species (*Díez et al., 2002*).

We present the first example of a global phylogenetic network study of the genus *Mattirolomyces* and the significant distribution pattern in *M. terfezioloides*. Phylogenetic networks are known to give a better insight into species ecology and distribution (Figure 4), an approach frequently used for its ability to visualize evolutionary relationships between nucleotide sequences and depict microevolution events such as the geographical distribution of populations (*Huson & Bryant, 2006*). The geographic distribution of *Mattirolomyces* indicate that the origin of the genus was the current Asia-Pacific areas prior to frequent long-distance migration events, one of which brought *M. terfezioloides* to eastern and northeastern China. Based on the ecology of recently studied areas, the collections from Inner Mongolia and the Shanxi province were both similar to all European collections and also shared the same node in the phylogenetic networks. This supported the idea that climatic conditions were an important evolutionary drive in this species, and that *M. terfezioides* in Europe originated from this area in China, Asia. *M. terfezioloides* haplotypes from Europe appeared to from more terminal leaves on the network, indicating their more recent arrival to this area, which is additionally supported by the unresolved haplotype distribution between two main suitable habitats: Mediterranean areas and the Pannonian basin (*Kovács et al., 2001*). The observed diversification and lack of any further geographic or ecological microevolutionary structure in the European haplotypes in the more terminal leaves of the network additionally support the theory of *M. terfezioloides'* recent arrival to this area and/or lack of evolutionary pressure.

Our collections originated from the continental steppe areas of Inner Mongolia, China, an area classified as BSk according to the Köppen-Geiger climate classification, and where climate-related indicators point towards a severe spatial desertification risk (*Spinoni et al., ~~2014~~*). Presently, the area still experiences little rainfall with a low average yearly precipitation and lower average yearly temperatures and winter extremes (>5 degrees lower) compared to any other known *Mattirolomyces terfezioides* area. Our findings indicated that this species survives in dryer and colder conditions, at least outside its fruiting period, than previously reported

(*Gógán Csorbainé et al., 2009*), and are becoming further endangered due to projected future climate changes (*He et al., 2019*). The same collections also showed ecological discrepancies from other currently known species' ecologies. *Mattirolomyces terfezioloides* is usually found under *Robinia pseudoacacia* L., but rarely under artificially planted *Diospyros kaki* Thunb. or *Prunus avium* (L.) L. in diverse families of Leguminosae, Ebenaceae, and Rosaceae in southern and central Europe and northern China (*Fischer, 1938; Bratek et al., 1996; Wang et al., 2017*). *R. pseudoacacia* is native to the Southern Appalachian and Ozark Mountains of the United States (*Huntley, 1990*), and was introduced to Europe, Asia, Australia, South America, and Africa mainly as an ornamental plant, or was cultivated to revegetate disturbed sites or for agricultural and commercial uses in recent centuries (*Kereszet, 1988*). Its mycorrhiza with *Mattirolomyces terfezioloides* is most likely secondary since phylogeographically basal haplotypes of *M. terfezioloides* originated from Asia, not North America. As far as we know, this is the first study that shows that *M. terfezioloides* associates with *Populus alba,* a tree species in the family of Salicaceae with a wide distribution in Europe and central Asia (*Palancean et al., 2018*). This is another example of a host plant of *M. terfezioides* forming a dual mycorrhiza with the ratio between ectomycorrhiza and arbuscular mycorrhiza depending on specific soil conditions (*Neville et al., ~~2019~~*). Asian *Populus* spp. are most likely the primary mycorrhizal host for *M. terfezioloides*. Since the European poplar species are most closely related to the Asian species (*Cervera et al., 2005*), they are a potential source of *M. terfezioloides* inoculum for Europe.

In addition to climate change, recent industry, urbanization, and other land use conversion factors are threatening the survival of *M. terfezioloides* in the wild, and maybe the reason for its long-lost status in China. *Populus* spp. have only recently become the dominant species in northern China and are mainly used for the restoration of degraded arid and semi-arid landscapes, combating desertification, and drought resilience strategies (*FAO, 2016*). All *Populus*-planted areas are sites where *M. terfezioides* has potential to grow. These areas may also serve to protect and preserve this rare desert fungus, as long as suitable agricultural practices for its cultivation are developed and supported, especially in rural, arid, and semiarid areas. However, *M. terfezioides* in China, especially among the Mongol people, remains underexploited and would require further ethnomycological investigation of its history in order to fully develop agricultural practices and consumption habits. *M. terfezioloides* could become an excellent model not only to develop local economy in rural areas, but also to highlight the importance of non-timber forest-related products in otherwise industrial forest tree plantations.

CONCLUSION

The first record of *M. terfezioides* showed its distribution in Inner Mongolia outside its current distribution area in China with the lowest known temperature and precipitation averages for this species. Our first attempt at phylogenetic network analysis in the genus *Mattirolomyces* revealed its geographic origin was in Asia-Pacific areas prior to frequent long-distance migration events. *M. terfezioides* originated from Inner Mongolia and the Shanxi province of China, Asia and was subsequently transported to Europe. Exploring *M. terfezioides'* ecology and potential to grow with poplars also increases its potential for cultivation and consumption in central and eastern China. This is a completely underexploited possibility among Mongols in China, and it deserves further ethnomycological investigation. Ecological investigations of *M. terfezioides* in arid areas of China should be carried out in the near future.

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Figure legend

Fig. 1. A) Sporocarps of *M. terfezioloides*; B) Sclerotium attached with sporocarps of *M. terfezioloides*; C) Gleba of sporocarps; D, E) Asci and spores; F) Spores with blunt spines.

Fig. 2. Average monthly precipitation (left) and average monthly temperature (right) for the Chinese (Baotou) (black solid line), other Asian (dark grey), and European (light gray) collections of *Mattirolomyces terfezioloides,* as listed in Table 1.

Fig. 3. Phylogram of *Mattirolomyces* based on the sequence dataset of the complete ITS region. Bootstrap values (ML) / posterior probabilities (from Bayesian inference) are shown above or beneath individual branches. Only bootstrap values larger than 60 and posterior possibilities over 0.95 are shown.

Fig. 4. A rooted phylogenetic network of *Mattirolomyces* based on the sequence dataset of the complete ITS region. Black dots represent recent taxa, gray dots represent ancestral stages / nodes. Values on mutation vectors represent the number of mutations between two nodes. Names of existing taxa and their geographic origin (country of collections) are given. Phylogenetic network was constructed and tested with a Median joining approach.

Fig. 5. A rooted phylogenetic network of *Mattirolomyces* based on the sequence dataset of the complete ITS region. A rooted phylogenetic network of *Mattirolomyces* based on the sequence dataset of the complete ITS region. Black dots represent recent taxa, gray dots represent ancestral stages / nodes. Values on mutation vectors represent the number of mutations between two nodes. Names of existing taxa and their geographic origin (country of collections) are given.

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Table 1. The list of obtained nuclear rDNA ITS sequences from the genus *Mattirolomyces* and location of the collection, climate, vegetation, and other site data. The list of obtained nuclear rDNA ITS sequences from the genus *Mattirolomyces*, retrieved from GenBank or UNITE database, are included in the study. Species name and GenBank accession numbers were supplemented with location of the collection, climate, vegetation, and other site data, if available.

Figure 1

The sporocarps morphology, spore structure and scanning electron microscope picture of spore

A Sporocarps of *M. terfezioloides*; B Sclerotium attached with sporocarps of *M. terfezioloides*; C Gleba of sporocarps; D, E Asci and spores; F Spores with blunt spines.

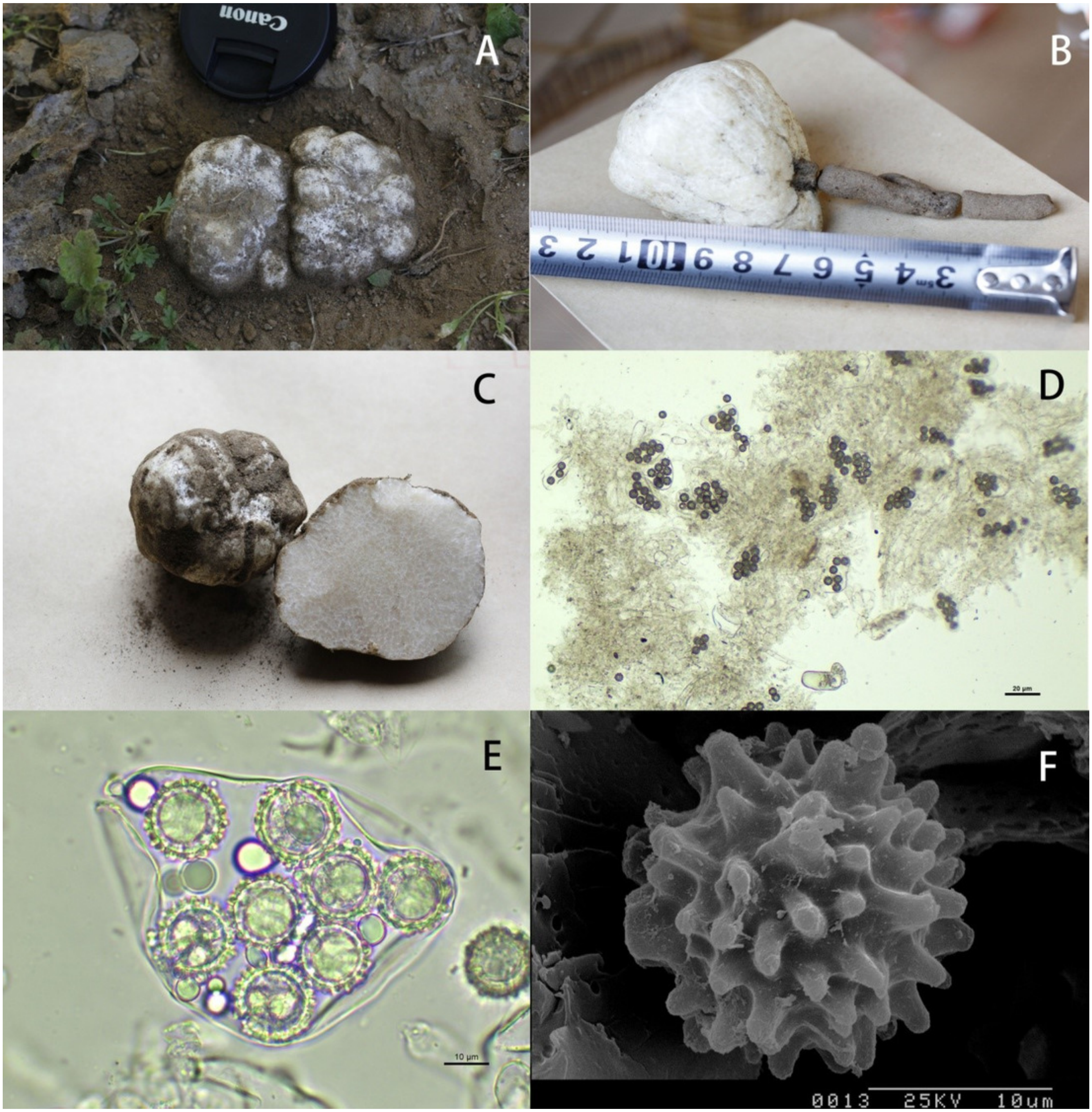
A
Canon
B
C
D
20 µm
E
10 µm
F
0013 25KV 10um

Figure 2

Average monthly precipitation for different area

Average monthly precipitation for the recent Chinese (Baotou) (black solid line), for other Asian (dark grey) and European (light gray) collections of *Mattirolomyces terfezioloides*

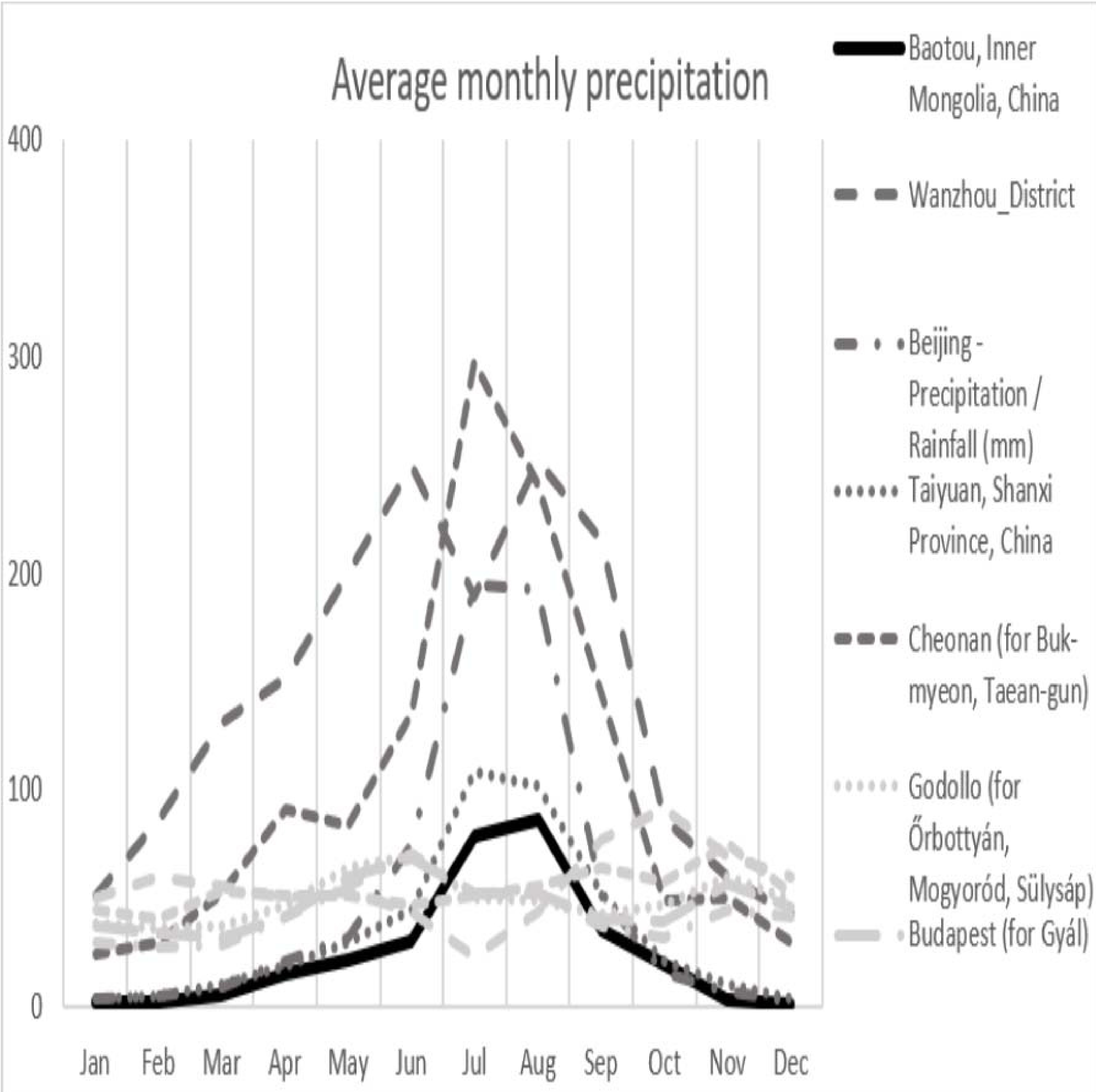
Average monthly precipitation
400
300
200
100
0
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Baotou, Inner Mongolia, China
Wanzhou_District
Beijing - Precipitation / Rainfall (mm)
Taiyuan, Shanxi Province, China
Cheonan (for Buk-myeon, Taean-gun)
Godollo (for Őrbottyán, Mogyoród, Sülysáp)
Budapest (for Gyál)

Figure 3

Average monthly temperature for different area

Average monthly temperature for the recent Chinese (Baotou) (black solid line), for other Asian (dark grey) and European (light gray) collections of *Mattirolomyces terfezioloides*

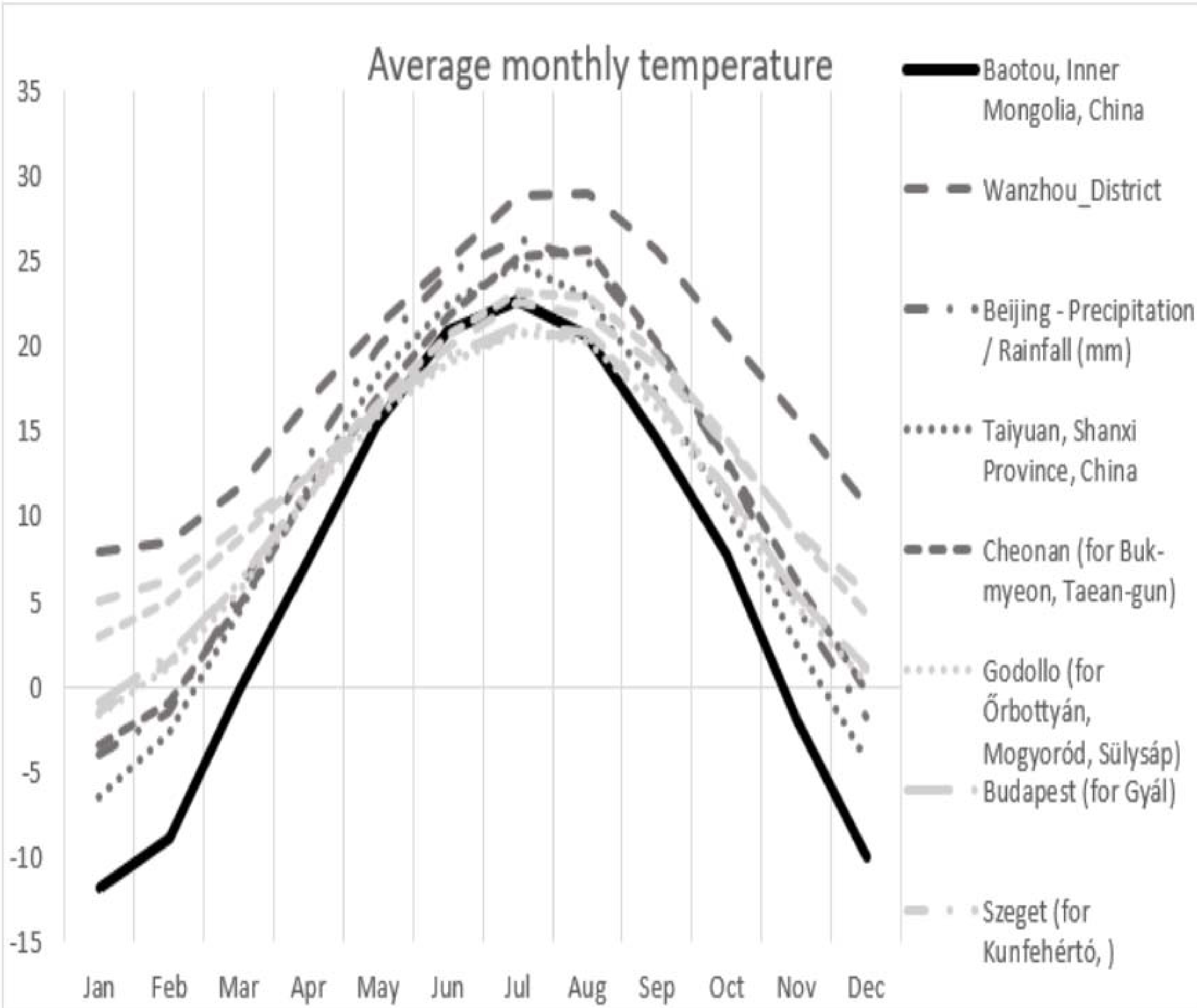
Average monthly temperature
35
30
25
20
15
10
5
0
-5
-10
-15
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Baotou, Inner Mongolia, China
Wanzhou_District
Beijing - Precipitation / Rainfall (mm)
Taiyuan, Shanxi Province, China
Cheonan (for Buk-myeon, Taean-gun)
Godollo (for Őrbottyán, Mogyoród, Sülysáp)
Budapest (for Gyál)
Szeget (for Kunfehértó,)

Figure 4

Phylogram of *Mattirolomyces* based on the sequences dataset of the complete ITS region

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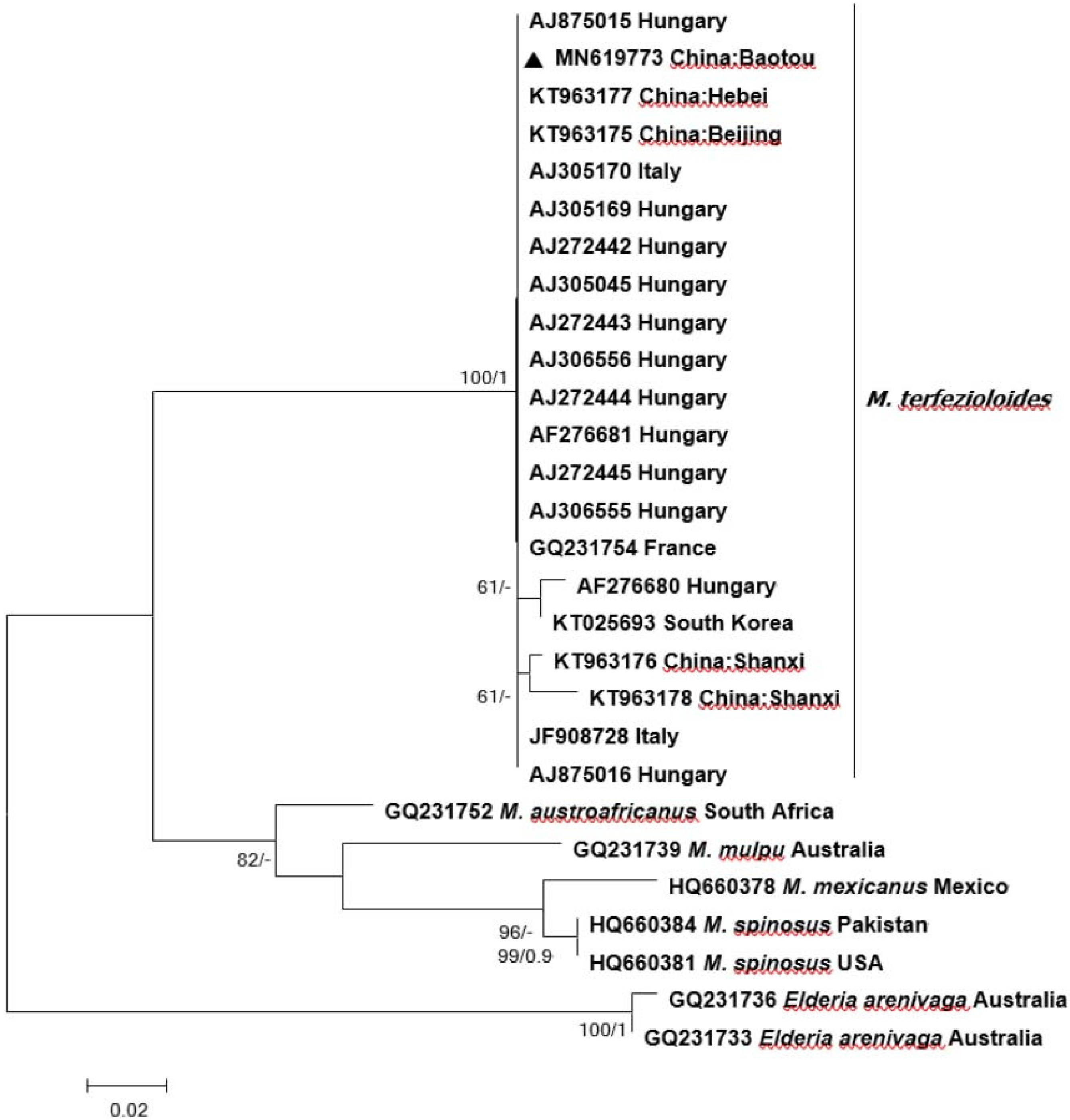

AJ875015 Hungary
MN619773 China:Baotou
KT963177 China:Hebei
KT963175 China:Beijing
AJ305170 Italy
AJ305169 Hungary
AJ272442 Hungary
AJ305045 Hungary
AJ272443 Hungary
AJ306556 Hungary
100/1
AJ272444 Hungary
M. terfezioloides
AF276681 Hungary
AJ272445 Hungary
AJ306555 Hungary
GQ231754 France
61/-
AF276680 Hungary
KT025693 South Korea
KT963176 China:Shanxi
61/-
KT963178 China:Shanxi
JF908728 Italy
AJ875016 Hungary
GQ231752 M. austroafricanus South Africa
82/-
GQ231739 M. mulpu Australia
HQ660378 M. mexicanus Mexico
96/-
HQ660384 M. spinosus Pakistan
99/0.9
HQ660381 M. spinosus USA
GQ231736 Elderia arenivaga Australia
100/1
GQ231733 Elderia arenivaga Australia
0.02

Figure 5

A rooted phylogenetic network of *Mattirolomyces* based on the sequences dataset of the complete ITS region

A rooted phylogenetic network of *Mattirolomyces* based on the sequences dataset of the complete ITS region. Black dots represent recent taxa, gray dots represent ancestral stages / nodes. Values on mutation vectors represent the number of mutations between two nodes. Names of existing taxa and their geographic origin (country of collections) are given. Phylogenetic network was constructed and tested with a Median joining approach.

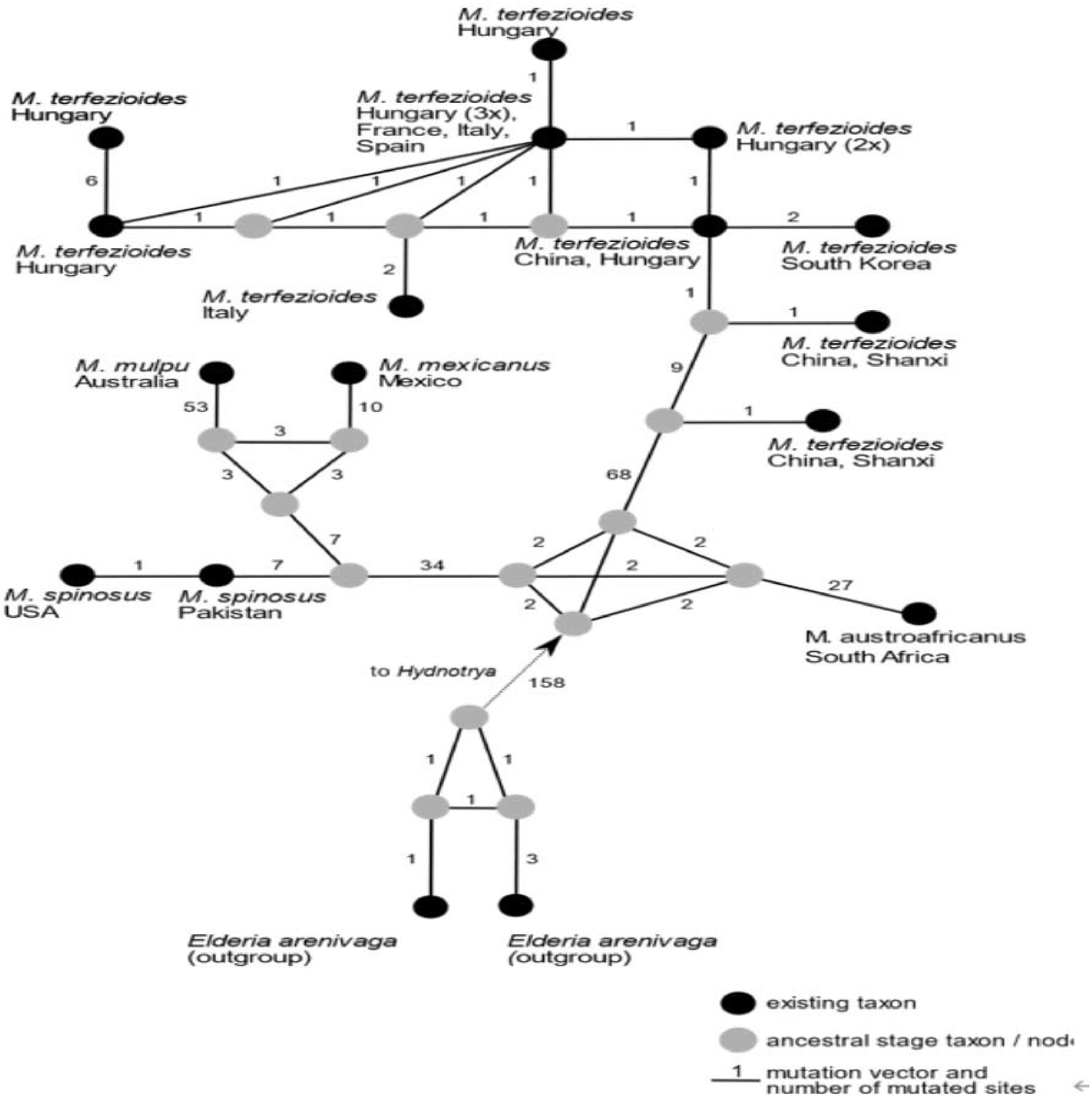
M. terfezioides
Hungary
M. terfezioides
Hungary
M. terfezioides
Hungary (3x),
France, Italy,
Spain
M. terfezioides
Hungary (2x)
M. terfezioides
Hungary
M. terfezioides
China, Hungary
M. terfezioides
South Korea
M. terfezioides
Italy
M. terfezioides
China, Shanxi
M. mulpu
Australia
M. mexicanus
Mexico
M. terfezioides
China, Shanxi
M. spinosus
USA
M. spinosus
Pakistan
M. austroafricanus
South Africa
to Hydnotrya
Elderia arenivaga
(outgroup)
Elderia arenivaga
(outgroup)
existing taxon
ancestral stage taxon / nod
mutation vector and
number of mutated sites

Table 1(on next page)

The list of obtained nuclear rDNA ITS sequences from the genus *Mattirolomyces* and location of the collection, climate, vegetation and other site data

The list of obtained nuclear rDNA ITS sequences from the genus *Mattirolomyces*, retrieved from the GenBank or UNITE database and included in the study. Species name and the GenBank accession numbers were supplemented with location of the collection, climate, vegetation and other site data, if available.

Table 1: The list of obtained nuclear rDNA ITS sequences from the genus *Mattirolomyces*, retrieved from the GenBank or UNITE database and included in the study. Species name and the GenBank accession numbers were supplemented with location of the collection, climate, vegetation and other site data, if available.

Species	GenBank reference code	Country and area or origin	Climate (Köppen-Geiger climate classification)	Potential(*) symbiotic partners	Sequence reference
Mattirolomyces terfezioides	**KT963177**	China, Hebei Province, Wanxian	Dwa	*Robinia pseudoacacia*	Wang et al. 2017
Mattirolomyces terfezioides	**KT963175**	China, Beijing province	Dwa	*Robinia pseudoacacia*	Wang et al. 2017
Mattirolomyces terfezioides	**AJ305170**	Italy, Ravenna	Cfa	n/a	Kovács et al. 2001
Mattirolomyces terfezioides	**AJ305169**	Hungary, Great Hungarian Plain, Kunfehértó	Cfb	n/a	Kovács et al. 2001
Mattirolomyces terfezioides	**AJ272442**	Hungary, Great Hungarian Plain, Őrbottyán	Dfa/Dfb	n/a	Kovács et al. 2001
Mattirolomyces terfezioides	**AJ305045**	Hungary, Great Hungarian Plain, Mogyoród	Dfa/Dfb	n/a	Kovács et al. 2001
Mattirolomyces terfezioides	**AJ272443**	Hungary, Great Hungarian Plain, Gyál	Dfa/Dfb	n/a	Kovács et al. 2001
Mattirolomyces terfezioides	**AJ306556**	Hungary, Great Hungarian Plain, Kunfehértó	Cfb	n/a	Kovács et al. 2001
Mattirolomyces terfezioides	**AJ272444**	Hungary, Great Hungarian Plain, Őrbottyán	Dfa/Dfb	n/a	Kovács et al. 2001
Mattirolomyces terfezioides	**AF276681**	Hungary, Surány	Dfa	n/a	Díez et al. 2002
Mattirolomyces terfezioides	**AJ272445**	Hungary, Sülysáp	Dfa/Dfb	n/a	Kovács et al. 2001
Mattirolomyces terfezioides	**GQ231754**	France, Provence-Alpes-Côte d'Azur, Le Thor	Csa	n/a	Trappe et al. 2010
Mattirolomyces terfezioides	**AJ306555**	Hungary, Great Hungarian Plain, Kunfehértó	Cfb	n/a	Kovács et al. 2001
Mattirolomyces terfezioides	**AF276680**	Hungary, Csomád	Dfa	n/a	Kovács et al. 2001
Mattirolomyces terfezioides	**KT025693**	South Korea, Buk-myeon, Taean-gun	Dwa	*Robinia pseudoacacia*	Ka et al. 2015
Mattirolomyces terfezioides	**KT963176**	China, Shanxi Province, Taiyuan	BSk	*Robinia pseudoacacia*	Wang et al. 2017
Mattirolomyces terfezioides	**JF908728**	Italy	n/a	n/a	Osmundson et al. 2013
Mattirolomyces terfezioides	**AJ875015**	Hungary	Dfa/Dfb	*Robinia pseudoacacia*	Bratek et al. 1996

Mattirolomyces terfezioides	**KT963178**	China, Shanxi Province, Taiyuan	BSk	*Robinia pseudoacacia*	Wang et al. 2017
Mattirolomyces terfezioides	**AJ875016**	Hungary	Dfa/Dfb	*Robinia pseudoacacia*	Bratek et al. 1996
Mattirolomyces terfezioides	**MN619773**	China, Inner Mongolia, Baotou	BSk	*Populus alba* ~~var. pyramidalis~~	this study
Mattirolomyces spinosus	**HQ660384**	Pakistan, Punjab, Sheikhupura	BSh	n/a	Kovács et al. 2011
Mattirolomyces mexicanus	**HQ660378**	Mexico, Nuevo Leon, Guadalupe	BSh	n/a	Kovács et al. 2011
Mattirolomyces spinosus	**HQ660381**	USA, Louisiana, Natchitoches	Cfa	n/a	Kovács et al. 2011
Mattirolomyces austroafricanus	**GQ231752**	South Africa, Northern Cape province, Barkly West	BSh	n/a	Trappe et al. 2010
Mattirolomyces mulpu	**GQ231739**	Australia, Northern Territory	Bwh	n/a	Trappe et al. 2010
Elderia arenivaga (outgroup)	**GQ231736**	Australia, Northern Territory, Alice Springs Desert Park	Bwh	n/a	Trappe et al. 2010
Elderia arenivaga (outgroup)	**GQ231733**	Australia, South Australia, Great Victoria Desert	Bwh/Bwk	n/a	Trappe et al. 2010

4 n/a not available.