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„Gyromitra species in Central Europe, species delimitation and
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Index

Index	3
Kurzfassung	6
Abstract	7
Acknowledgments	8
Introduction	9
Overview of <i>Gyromitra</i> taxa represented in the present study	10
Material and Methods	14
Fungal Material	14
Sample Preparations	18
DNA extraction	18
DNA marker and Primer Selection	18
PCR (Polymerase chain reaction)	19
Agarose Gel electrophoresis	20
Purification of PCR products	20
Sanger Sequencing (chain termination sequencing, cycle sequencing)	21
Data processing and Phylogenetic analysis	23
Data Processing	23
Assembling and editing	23
Additional Sequence Data	23
Microscopic observations	24
Phylogenetic analysis	24
Results	26
<i>Gyromitra perlata</i> (Fr.) Harmaja, Karstenia 9: 11 (1969)	26
Sample no. 4_ <i>Gyromitra perlata</i> _WU_0031240	26
Sample no. 5_ <i>Gyromitra perlata</i> _WU_0026583	27
<i>Gyromitra apiculatula</i> (McKnight) Berthet [as 'apiculata'], Trav. du Laboratoire de 'La Jaysinia' a Samoëns, Haute-Savoie 4: 104 (1972)	28
Sample no. 12_ <i>Gyromitra apiculatula</i> _WU_0025874	28
Sample no.13_ <i>Gyromitra apiculatula</i> _WU_0020233	29
<i>Gyromitra leucoxantha</i> (Bres.) Harmaja, Karstenia 9: 11 (1969)	30
Sample no. 17_ <i>Gyromitra leucoxantha</i> _WU_0004453	30
<i>Gyromitra parma</i> (J. Breitenb. & Maas Geest.) Kotl. & Pouzar, Česká Mykol. 28(2): 91 (1974)	31
Sample no. 18_ <i>Gyromitra parma</i> _WU_0035109	31
Sample no. 19_ <i>Gyromitra parma</i> _WU_0024817	32
20_ <i>Gyromitra parma</i> _WU_0036232	32

<i>Gyromitra esculenta</i> Pers. ex Fr., Summa veg. Scand., Sectio Post. (Stockholm): 346 (1849)	33
Sample no. 21_ <i>Gyromitra esculenta</i> _WU_0043890	33
Sample no. 25_ <i>Gyromitra esculenta</i> _WU_0041117	34
Sample no. 63_ <i>Gyromitra esculenta</i> _WU_001620	34
<i>Gyromitra gigas</i> (Krombh.) Quél., Mém. Soc. Émul. Montbéliard, Sér. 2 5: 338 (1873)	35
Sample no. 8_ <i>Gyromitra gigas</i> _WU_0009567	35
Sample no. 28_ <i>Gyromitra gigas</i> _WU_0010578	36
Sample no. 30_ <i>Gyromitra gigas</i> _WU_0011622	38
Sample no. 34_ <i>Gyromitra gigas</i> _WU_0009596	39
Sample no. 35_ <i>Gyromitra fastigiata</i> _WU_0024806	40
Sample no. 39_ <i>Gyromitra fastigiata</i> _WU_0021318	41
Sample no. 40_ <i>Gyromitra fastigiata</i> _WU_0008756	42
Sample no. 41_ <i>Gyromitra fastigiata</i> _WU_0013814	43
Sample no. 45_ <i>Gyromitra fastigiata</i> _WU_0040483	44
<i>Gyromitra inflata</i> Cooke, Mycogr., Vol. 1. Discom. (London)(no. 6): 248 (1879)	45
Sample no. 42_ <i>Gyromitra inflata</i> _WU_0032695	45
Sample no. 43_ <i>Gyromitra inflata</i> _WU_0044303	46
Sample no.44_ <i>Gyromitra inflata</i> _WU_0043891	46
<i>Gyromitra infula</i> (Schaeff.) Quél., Enchir. fung. (Paris): 272 (1886)	47
Sample no. 54_ <i>Gyromitra infula</i> _WU_0010139.....	47
Sample no. 61_ <i>Gyromitra infula</i> _WU_0027935.....	48
<i>Gyromitra ticiniana</i> Littini, Pagine Botaniche 12: 19 (1988).....	49
Sample no. 68_ <i>Gyromitra ticiniana</i> _WU_0008714.....	49
Sample no. 69_ <i>Gyromitra ticiniana</i> _WU_0039781.....	50
<i>Gyromitra longipes</i> Harmaja, Acta bot. fenn. 16(3): 159 (1979)	51
Sample no. 71_ <i>Gyromitra longipes</i> _WU_0037177	51
Genbank blast results	53
Reference Sequences.....	54
Phylogenetic analysis.....	58
ITS marker region.....	58
LSU marker region.....	60
TEF region marker	62
LSU and TEF marker regions concatenated	64
Discussion	67
Species identification.....	67
Microscopic characters.....	67

Phylogenetic analysis	72
Conclusion	74
References	75
Appendix	80

Kurzfassung

Die Gattung *Gyromitra* in der Ordnung *Pezizales* umfasst viele verschiedene Arten, die für viele geografische Standorte unterschiedlich sind. *Gyromitra*-Arten sind als "falsche Morcheln" mit zerebriformen oder scheibenförmigen Apothezien, bekannt. *Gyromitra*-Arten sind in der Regel nur schwer anhand morphologischer Merkmale zu unterscheiden, wodurch DNA-Barcoding zu einem nützlichen Werkzeug wird.

In dieser Masterarbeit wurden verschiedene DNA-Regionen 72 *Gyromitra*-Herbarbelege für phylogenetische Analysen sequenziert. Die ausgewählten Regionen waren die Internal Transcribed Spacer Region (ITS), die Large Subunit Region (LSU) und die Translational Elongation Factor Region (TEF). Vor allem die ITS-LSU Regionen sind aufgrund ihrer hohen Mutationsrate und ihres Vorhandenseins in vielen Kopien optimal für intra- und interspezifische taxonomische Vergleiche und Differenzierungen. TEF ist eine single copy Region. Diese Masterarbeit konzentriert sich auf die Artabgrenzung für Mitteleuropa.

Die Analysen ergaben einige gut etablierte Verwandtschaftsgruppen, aber es zeigten sich auch etliche Unsicherheiten, wo weitere Analysen der *Gyromitra*-Verwandtschaft nötig sind. Zusammen mit den mikroskopischen Merkmalen zeigte sich, dass drei aller untersuchten Arten nicht richtig identifiziert wurden. Einige der Arten sind phylogenetisch in einer anderen Artengruppe angesiedelt, schienen aber aufgrund der mikroskopischen Merkmale richtig identifiziert worden zu sein.

Zusammenfassend lässt sich sagen, dass die molekulargenetischen Sequenzdaten unterschiedliche Artencluster zeigten, und die zuverlässigen phylogenetischen Stammbäume weisen eine kongruente taxonomische Anordnung mit Literaturbefunden auf.

Abstract

The genus *Gyromitra* in the order *Pezizales* comprises many distinct species, which are different for many geographical locations. *Gyromitra* species are known as “false morels” having cerebriform or discoid apothecia. *Gyromitra* species are difficult to distinguish via morphological characteristics alone, with that DNA-Barcoding becomes a useful tool.

In this master thesis, different DNA regions of 72 *Gyromitra* herbarium specimens have been sequenced for phylogenetic analysis. The chosen regions were the Internal Transcribed Spacer region (ITS), the Large Subunit region (LSU) and Translational Elongation factor region (TEF). ITS-LSU regions are optimal for intra- and interspecific taxonomic comparison and differentiation, because of their high mutation rate and presence in many copies. TEF is a single copy region. This master work focuses on the species delimitation for Central Europe.

The results showed some well-established relationships between species, but there are still some uncertainties. Also, together with the microscopical characteristics it was shown that three of all the species looked at were not correctly identified. Some of the species are situated phylogenetically in another species group, but from the microscopical characteristic seemed to have been correctly identified.

To sum up molecular genetic sequence data showed distinct clusters of species and the reliable phylogenetic trees have congruent taxonomical arrangement with literature findings.

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Introduction

The analysis of molecular genetic sequencing data is a modern technique (Sanger et al. 1977), which revolutionized the phylogenetic understanding. Until 1977 the taxonomic classification was based almost exclusively on morphological characteristics. Now it is well known that every organism or group of organisms has its own specific barcode, like a fingerprint, of a specified marker region. To genetically identify those regions sequences of the DNA with slow mutation rates are needed. Individuals of the same species who are directly related to each other, show a low variability of their barcodes. It can be said, the higher the differences between marker regions, the less related individuals are assumed (Heather et al. 2016).

Fungi are especially hard to identify morphologically because cryptic species are more relevant. There are many macro- and micro-morphological characteristics for identification of fungi. But the intra- and interspecific variations are high and often not easy to distinguish. For that molecular genetic sequencing analysis becomes valuable for fungal identification.

For fungi the most used marker is the ITS (internal transcribed spacer) region. The eukaryotic nuclear rRNA cistron is made up off 18S, 5.8S and 28S rRNA genes transcribed as a unit by Polymerase I (Schoch et al. 2012). Those two spacers including the 5.8S gene, are normally referred to as ITS (Schoch et al. 2012). The ITS1 is located between 18S and 5.8S region, followed by the ITS2, which is located between the 5.8S and 28S region. Mostly for fungi barcoding the ITS1, 5.8S and ITS2 is used. This non coding region is quite variable, which makes it a perfect fit for molecular genetic analysis and comparison. In most cases it exhibits a higher interspecific than intraspecific variation, thereby revealing a good barcode gap. ITS was proposed by Schoch et al. (2012) as universal fungal barcode region. In some groups it has to be supplemented with a second or third barcode marker. And nowadays it is also practice to use more marker regions for more information. The other two highly variable marker regions with high mutation rates are the LSU region (Large subunit) and TEF region (translation elongation factor). In recent studies LSU and TEF were additional included to ITS (Methven et al. 2013, Wang & Zhuang 2019, Miller et al 2020). The best way for analysis is in turn combining those markers together into a concatenated phylogenetic tree to obtain the optimal taxonomic resolution.

The genus *Gyromitra* belongs to the phylum *Ascomycota* (Fig.1.), subphylum *Pezizomycotina*, order *Pezizales*, family *Discinaceae* (Fig.2.). It comprises more than 70 taxa, which are commonly known as “false morels”. The species are mainly distributed through the north temperate and boreal regions of the northern hemisphere. They form sessile to stipitate ascomata with discoid to cerebriform hymenophores and occur mostly during spring (Miller et al. 2020). It is hard to distinguish the individual species solely by morphological characteristics (the primarily distinguishing feature is the shape and proportion of the pileus and the stipe), therefore often microscopic traits such as ascospores (length, width, form and apiculi) and paraphyses or molecular genetic traits are considered.

Gyromitra is situated in the family Discinaceae and forms a clade within the order Pezizales together with its sister group Morchellaceae. Taxonomically, *Discina* was reduced to a subgenus of *Gyromitra* (Harmaja 1973), because of ascospore features and due to the name *Gyromitra* being older. This change was generally accepted in scientific literature (Abbot & Currah 1997, Van Vooren & Moreau 2009c, Methven et al. 2013). *Gyromitra* has a long history of taxonomic transfers among various genera (e.g. *Discina*, *Helvella*, *Maublancomyces*, *Pseudorhizina*, *Neogyromitra*, among other genera) being segregated into multiple subgenera, with splitting and combining of taxa at species level and below (Miller et al. 2020). This long history has been documented and summarized by many authors (Donadini 1984, 1986; Harmaja 1969, 1973; McKnight 1969, 1971, 1973; Van Vooren and Moreau 2009a, 2009b, 2009c).

Overview of *Gyromitra* taxa represented in the present study

The different taxa may be mycorrhizal, saprotrophic or parasitic (Egger and Paden 1986; Hansen and Pfister 2006). Some species can cause poisoning, which can result even in death of humans, through the compound gyromitrin (Michelot & Toth 1991). Some of the most poisonous species of *Gyromitra* with a high gyromitrin content are *G. esculenta* (Persoon) Fries 1849, *G. fastigiata* (Krombholz) Rehm 1895, *G. gigas* (Krombholz) Quélet 1834 and *G. infula* (Schaeffer) Quélet 1886.

Gyromitra esculenta s.l. is one of the most well-known *Gyromitra* species being closely related to *G. infula*, which is shown in the phylogenetic tree by Methven et al. (2013). Furthermore, in the *G. esculenta* species complex the position of *G. inflata* Cooke is still uncertain (see Klofac & Krisai-Greilhuber 2019). This relationship is investigated since 1940, since then a lot of collections of the *G. esculenta* species complex were found, which resulted, e.g. in the re-introduction of *G. inflata* as a species of the *G. esculenta* complex (Klofac & Krisai-Greilhuber 2019). Also *G. longipes* Harmaja was revealed as an overlooked species due to the high similarities in seasonal and macroscopic characteristics to *G. esculenta* s. str. (Harmaja 1979, Carlsen & Stensrud 2003). For *G. apiculatula* (McKnight) Berthet, the phylogenetic relationship is not resolved, but seems to somehow be closely related to the *G. esculenta* species complex (Harmaja 1973).

One of the richest species complexes of the genus *Gyromitra* is the *Gyromitra gigas* complex. Many phylogenetic studies have been conducted (Methven et al. 2013, Miller et al. 2015, Krisai-Greilhuber et al. 2017, Carbone et al. 2018, Wang & Zhuang 2019), which resulted in six taxa being included in this species complex, namely *G. gigas*, *G. khanspurensis* Jabeen & Khalid (Jabeen & Khalid 2017), *G. leucoxantha* (Bres.) Harmaja (Harmaja 1969), *G. ticiniana* Littini (Littini 1988), *G. korfii* (Raitv.) Harmaja (Harmaja 1973), *G. montana* Harmaja (Harmaja 1973), *G. pseudogigas* X. C. Wang & W.Y. Zhuang (Wang & Zhuang 2018). *Gyromitra gigas* ss. str. is a common, widespread taxon in Europe, first described by Krombholtz (1834) as *Helvella gigas* based on a specimen collected in the Czech Republic. The same name *Gyromitra gigas* has been frequently used for north American specimens, but

two other names have been proposed for those specimens that of *Gyromitra korfii* (eastern areas of north America) and *Gyromitra montana* (western/northern areas of north America).

With *G. fastigiata* (Krombholz) Rehm 1895, there was a historical grouping together with *G. gigas*, but this was resolved by McKnight (1971). But the taxonomic status is not fully clear yet and there are further taxa, which have not been looked at completely and of which the taxonomic situation is not clear, too. It is indicated that *G. brunnea* Underwood, which is a North American species, is the same species as *G. fastigiata* (McKnight 1987), which is a European species. In a recent study by Dizkirici et al. (2018) they were segregated as separate species. Two more closely related *Gyromitra* species are *G. parma* (J. Breitenb. & Maas Geest.) Kotl. & Pouzar and *G. perlata* (Fries) Harmaja.

This master thesis focuses on central European species of the genus *Gyromitra*. *Gyromitra* specimens were assessed molecular genetically with three different marker regions (ITS, LSU and TEF). In addition, microscopical characteristics for all species involved were obtained to check if they were correctly identified. Furthermore, the microscopical characteristics were taken into account for the purpose of unclear species identifications or relationships to be resolved through additional information. The overall aim was to establish phylogenetic trees with the maximum-likelihood method to see the relationships between all the *Gyromitra* species investigated.

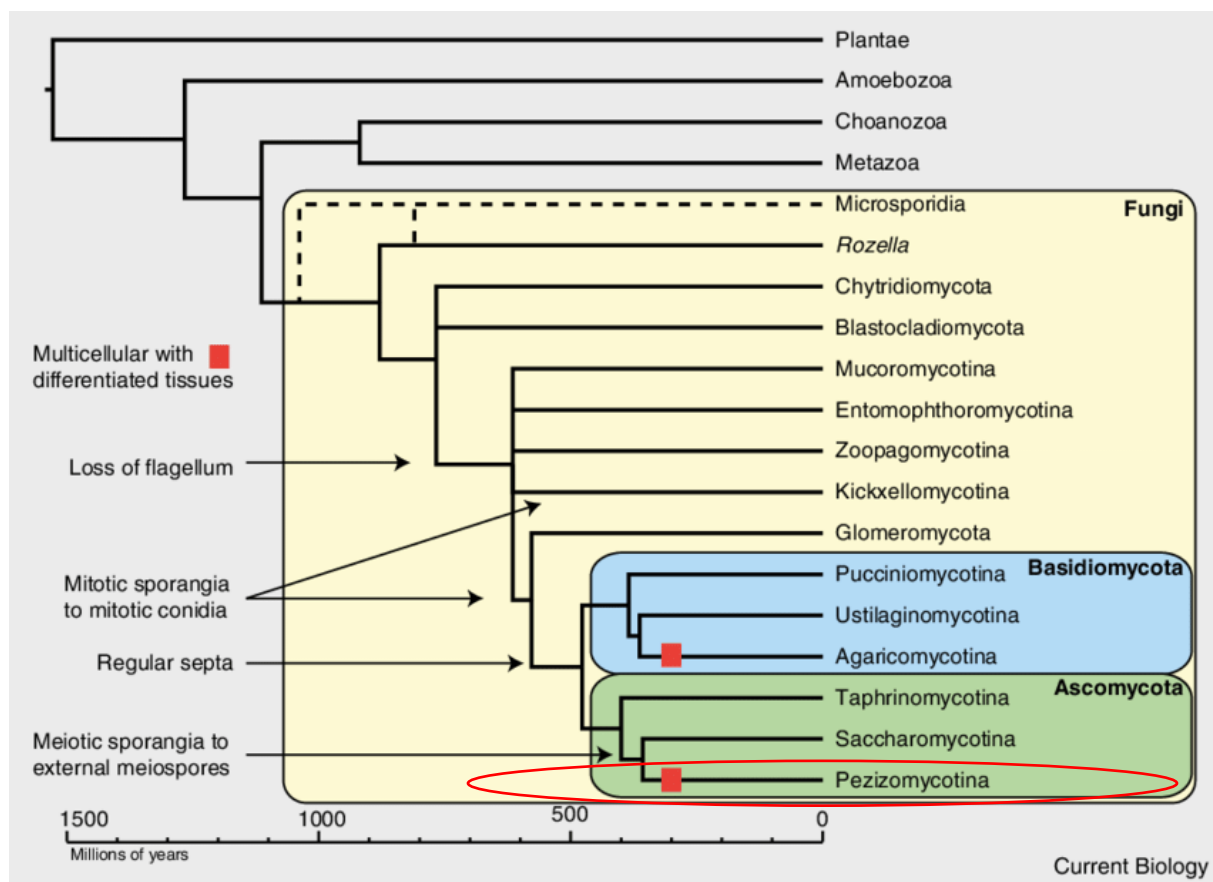


Fig. 1. Phylogenetic tree influenced by the fungal tree of life project, with the focus on the fungi showing many fungal lineage relationships fitting to geologic time using program r8s (Stajich et al. 2009). Pezizomycotina highlighted in red.

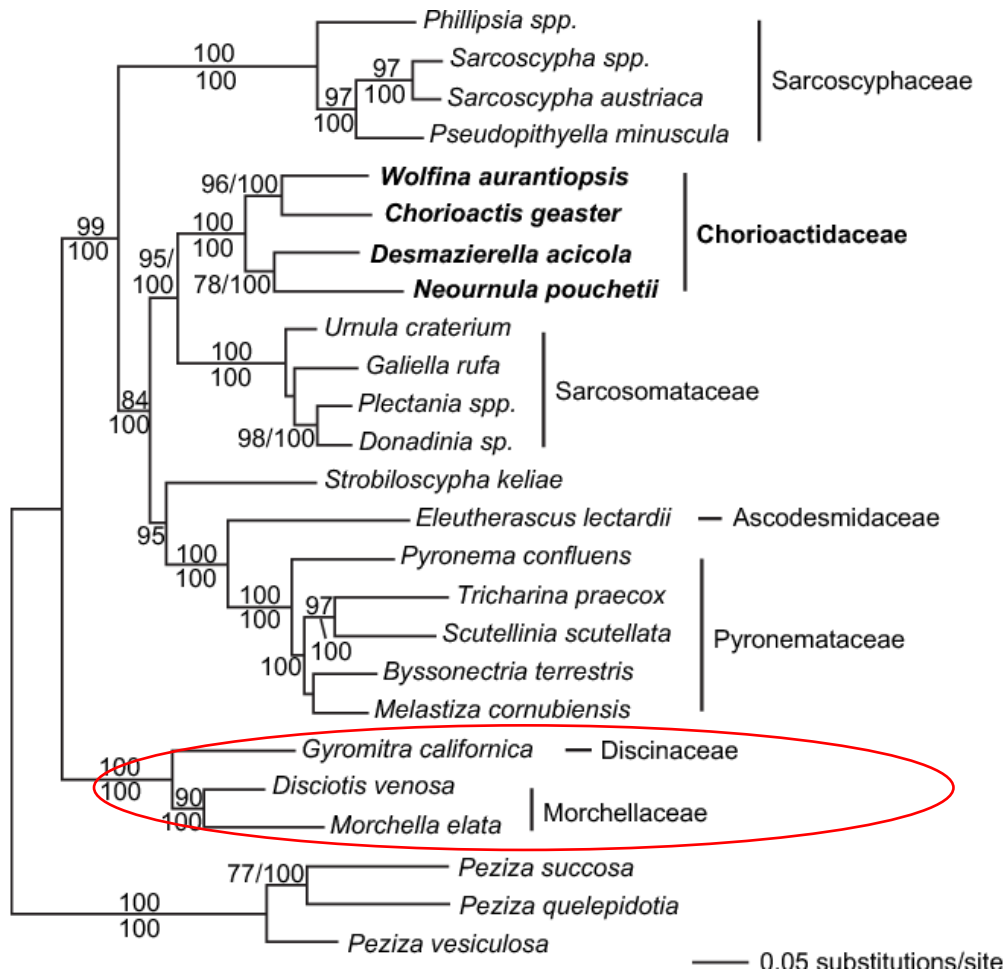


Fig. 2. Maximum likelihood phylogenetic tree of the *Pezizales* taken from Pfister et al. (2008). *Discinaceae* and *Morchellaceae* highlighted in red.

Material and Methods

Fungal Material

Fungal (ascomata) material from the Herbarium WU-Mycologicum (<https://sweetgum.nybg.org/science/ih/>) was used (Fig.1.). The samples were air dried and stored in cardboard boxes (Fig. 3). They were mostly collected in Austria, one sample in Germany and one in France (Tab.1).

Nomenclature of the species was taken from the database of Mycobank (<https://www.mycobank.org/page/Simple%20names%20search>). Following Mycobank all sequences and species of the genus *Discina* were included in genus *Gyromitra*.



Fig.3. Fungal (ascomata) samples stored in the herbarium WU-Myc.

Tab.1. Sample number, species, voucher no., collection date, collection site and collectors of the material used.

Nr.	Species	WU-Myc Number	Collection Date	Collection Site	Descriptor/Collector
1	<i>Disciotis venosa</i>	WU0039564	06.05.2017	Austria, NÖ, Donau-Auen	Krisai-Greilhuber I.
2	<i>Disciotis venosa</i>	WU0040868	24.03.2008	Germany, Bayern, Windach	Hahn C.
3	<i>Gyromitra ancilis</i>	WU0026716	06.05.2005	Austria, Stmk, Mariazell: Erlaufsee	Klofac W.
4	<i>Gyromitra ancilis</i>	WU0031240	10.05.2007	Austria, Stmk, Mariazell: Grünau	Klofac W.
5	<i>Gyromitra melaleuca</i>	WU0037339	20.03.2016	Austria, NÖ, Bad Fischau, Brunn	Klofac W.
6	<i>Gyromitra melaleuca</i>	WU0025389	08.05.2004	Austria, Voralberg, Bregenz	Oswald W.
7	<i>Gyromitra gigas</i>	WU0009570	30.05.1991	Austria, NÖ, Retz	Hausknecht A.
8	<i>Gyromitra gigas</i>	WU0009567	02.05.1991	Austria, NÖ, Hardegg	Hausknecht A. & Klofac W.
9	<i>Gyromitra gigas</i>	WU0011767	29.04.1993	Austria, NÖ, Hohenberg	Krisai-Greilhuber I.
10	<i>Gyromitra gigas</i>	WU0019288	22.05.1999	Austria, NÖ, Muckendorf	Klofac W.
11	<i>Gyromitra apiculatula</i>	WU0032700	26.04.2013	Austria, Stmk, Mariazell, Grünau	Klofac W.
12	<i>Gyromitra apiculatula</i>	WU0025874	30.04.2005	Austria, Stmk, Mariazell, Grünau	Klofac W.
13	<i>Gyromitra apiculatula</i>	WU0020233	29.04.2000	Austria, Stmk, Mariazell, Grünau	Klofac W.
14	<i>Gyromitra geogenia</i>	WU0020248	14.05.2000	Austria, NÖ, Gr. Kesselbachgraben	Bardorf Th.
15	<i>Gyromitra perlata</i>	WU0026583	05.05.2006	Austria, Stmk, Mariazell, Erlaufsee	Klofac W.
16	<i>Gyromitra parma</i>	WU0039802	14.04.2017	Austria, OÖ, Braunau am Inn, Ortsgebiet	Krisai-Greilhuber I.
17	<i>Gyromitra leucoxantha</i>	WU004453	01.05.1985	Austria, Bgld, Leithagegebirge	Klofac W.
18	<i>Gyromitra parma</i>	WU0035109	26.04.2014	Austria, NÖ, Lichtenwörth	Koller G.
19	<i>Gyromitra parma</i>	WU0024817	23.04.2004	Austria, NÖ, Großenzersdorf	Klofac W.
20	<i>Gyromitra parma</i>	WU0036232	23.04.2014	Austria, Wien, Moosgraben	Bardorf T.
21	<i>Gyromitra esculenta</i>	WU0043890	15.05.2021	Austria, NÖ, Neunkirchen, St. Corona	Apschner C.
22	<i>Gyromitra esculenta</i>	WU0025851	05.05.2005	Austria, NÖ, Baden	Till W.
23	<i>Gyromitra esculenta</i>	WU0027475	06.04.2007	Austria, NÖ, Ober-Wölbling	Klofac W.
24	<i>Gyromitra esculenta</i>	WU0041939	23.04.1992	Austria, NÖ, Gloggnitz: Weißenbach, Eichberg	Klofac W.
25	<i>Gyromitra esculenta</i>	WU0041117	24.05.2019	Austria, NÖ, Scheiblingkirchen-Thernberg, Kreuth	Klofac W. & Brandstätter R.

26	<i>Gyromitra parma</i>	WU0043889	21.04.2021	Austrial, Wien Donaustadt 22., Lobau, Mittelwasser	Apschner C. & Brandstätter R.
27	<i>Gyromitra gigas</i>	WU0008758	20.04.1990	Austria, NÖ, Wolkersdorf	Klofac W.
28	<i>Gyromitra gigas</i>	WU0010578	04.04.1992	Austria, Oberpullendorf, Groß Warasdorf	Klofac W.
29	<i>Gyromitra gigas</i>	WU0010603	04.05.1991	Austria, NÖ, Lichtenau, Brunn am Walde	Mader A. & K.
30	<i>Gyromitra gigas</i>	WU0011622	23.04.1993	Austria, NÖ, Gloggnitz, Eichberg	Klofac W.
31	<i>Gyromitra gigas</i>	WU0011629	01.05.1993	Austria, NÖ, Waldviertel, Manhartsberg	Reisinger H.
32	<i>Gyromitra gigas</i>	WU0011653	23.05.1993	Austria, Stmk, Rettenegg	Klofac W.
33	<i>Gyromitra fastigiata</i>	WU0017225	13.04.1997	Austria, Wien, Kahlenberg	Jaklitsch W.
34	<i>Gyromitra gigas</i>	WU0009596	27.04.1991	Austria, NÖ, Großweikersdorf, Wischatal	Hausknecht A.
35	<i>Gyromitra fastigiata</i>	WU0024806	22.04.2004	Austria, NÖ, Bruck an der Leitha: Arbesthal, Ellender Wald	Reisinger H.
36	<i>Gyromitra fastigiata</i>	WU0014300	13.04.1995	Austria, Burgenland, Buchkogel	Klofac W.
37	<i>Gyromitra fastigiata</i>	WU0008721	18.04.1990	Austria, NÖ, Bisamberg	Klofac W.
38	<i>Gyromitra fastigiata</i>	WU0037368	10.04.2016	Austria, NÖ, Kottes-Purk, Münichreith "Schmaleben"	Krisai-Greilhuber I.
39	<i>Gyromitra fastigiata</i>	WU0021318	24.04.2001	Austria, NÖ, Hainburg, Wolfskogel	Klofac W.
40	<i>Gyromitra fastigiata</i>	WU0008756	13.04.1990	Austria, NÖ, Edelsthal, Spitzerberg	Klofac W.
41	<i>Gyromitra fastigiata</i>	WU0013814	05.05.1995	Austria, NÖ, Gföhl, Dobrasperre	Hausknecht A.
42	<i>Gyromitra inflata</i>	WU0032695	10.05.2013	Austria, NÖ, Wiesfleck	Klofac W.
43	<i>Gyromitra inflata</i>	WU0044303	26.05.2001	Austria, NÖ, Neunkirchen: Thomasberg: Wiesfleck	Klofac W.
44	<i>Gyromitra inflata</i>	WU0043891	15.05.2021	Austria, NÖ, Neunkirchen, St. Corona am Wechsel	Apschner C.
45	<i>Gyromitra fastigiata</i>	WU0040483	11.05.2018	Austria, NÖ, Rothwald, NSG Großer Urwald	Yatsiuk I. & Y.
46	<i>Gyromitra infula</i>	WU0036456	09.10.2014	Austria, NÖ, Zwettl, Großwolfgers	Filler J.
47	<i>Gyromitra infula</i>	WU0016830	29.10.1996	Austria, NÖ, Kirchberg	Lauermann H.
48	<i>Gyromitra infula</i>	WU0025425	30.09.2004	Austria, Stmk, Bruck an der Leitha, Halltal	Urban A.
49	<i>Gyromitra infula</i>	WU0022433	15.09.2002	Austria, K, St. Margareten im Rosenthal	Jaklitsch W.
50	<i>Gyromitra infula</i>	WU0014858	13.10.1995	Austria, NÖ, Merckensteinertal, Haidlhof	Halbritter H.
51	<i>Gyromitra infula</i>	WU0009809	24.01.1991	Austria, Burgenland, Neckenmarkt	Klofac W.
52	<i>Gyromitra infula</i>	WU0018774	18.10.1998	Austria, NÖ, Zwettl, Schloß Waldreichs	N. N.
53	<i>Gyromitra infula</i>	WU0037665	15.09.2016	Austria, OÖ, NP Kalkalpen: Reichraming, Anlaufalm	Klofac W.
54	<i>Gyromitra infula</i>	WU0010139	19.10.1991	Austria, NÖ, Horn, Etzelsreith	Mader A.

55	<i>Gyromitra esculenta</i>	WU0010541	23.04.1992	Austria, NÖ, Gloggnitz, Eichberg	Klofac W.
56	<i>Gyromitra esculenta</i>	WU0019289	15.05.1999	Austria, NÖ, östl. Roßburg	Dworak M.
57	<i>Gyromitra infula</i>	WU0010141	20.09.1991	Austria, NÖ, Hohenberg, Lahnsattel	Hausknecht A.
58	<i>Gyromitra infula</i>	WU0024728	23.09.2002	Austria, NÖ, Neunkirchen: Mariensee, Feistritz Schwaig	Koller G.
59	<i>Gyromitra infula</i>	WU0024735	21.10.2002	Austria, NÖ, Drosendorf	Koller G.
60	<i>Gyromitra infula</i>	WU0009957	27.09.1991	Austria, NÖ, Puchberg	Hausknecht A. & Klofac W.
61	<i>Gyromitra infula</i>	WU0027935	10.10.2007	Austria, NÖ, Rastendorf	Hausknecht A. & Klofac W.
62	<i>Gyromitra infula</i>	WU0042248	15.11.2019	Austria, Bgld, Oberpullendorf: Draßmarkt, Kogelfangwald	Koller G.
63	<i>Gyromitra esculenta</i>	WU0010620	28.04.1992	Austria, NÖ, Ottenstein, Siebenwirth	Hausknecht A. & Klofac W.
64	<i>Gyromitra infula</i>	WU0016844	29.10.1990	Austria, NÖ, Kirchberg	Lauermann H.
65	<i>Gyromitra infula</i>	WU0017556	03.09.1997	Austria, K, Wolfsberg, Weinebene	Klofac W.
66	<i>Gyromitra ticiniana</i>	WU0039784	25.04.1985	Austria, NÖ, Korneuburg, Bisamberg	Klofac W.
67	<i>Gyromitra ticiniana</i>	WU0005255	30.04.1986	Austria, NÖ, Hainburg, Wolfsthal	Hausknecht I.
68	<i>Gyromitra ticiniana</i>	WU0008714	11.04.1990	Austria, NÖ, Hainburg, Wolfsthal	Klofac W.
69	<i>Gyromitra ticiniana</i>	WU0039781	23.04.2017	Austria, NÖ, Zwettl, Eisenreith, südl. des Steinbruches	Krisai-Greilhuber I.
70	<i>Gyromitra perlata</i>	WU0040478	06.04.2008	Austria, NÖ, Bad Fischau-Brunn, Kürassier	Klofac W.
71	<i>Gyromitra longipes</i>	WU0037177	06.05.2016	Austria, NÖ, Eichberg	Klofac W. & Hacker A.
72	<i>Gyromitra esculenta</i>	MG846990	01.04.2015	Frankreich	Wang & Zhuang 2019

Sample Preparations

The material was taken from the cap and the hymenial layer of the dried fungi with tweezers; 5mg with a Sartorius Basic weigh were put into 2-ml Eppendorf-tubes with 5 glass beads. The tubes were Placed in a grinder (QIAGEN, Tissuelyzer II), with the basic settings of a frequency: of 30 Hz for 5 mins.

DNA extraction

The DNeasy Plant Minikit (QIAGEN) was used for DNA extraction, the usage was done as instructed by the producer with only slight variations. The protocol with the information of how to use this Kit is obtainable at <https://www.qiagen.com/us>

Primer Selection

In fungi sequencing the ITS (Internal transcribed spacer) region is normally used for species identification, nowadays the LSU region (Large Subunit) and TEF region (Translation elongation factor) are also used, for extra Information.

For this purpose universal Primers were used, which were ITS4, ITS5, LR5, LROR, TEF1-1567R and TEF1-INTF. Those Primers were diluted into 500 µM concentrations.

Tab. 2. Primer sequences from the ITS, LSU and TEF-region.

Primer	Direction	Sequence (5'-3')	Reference
ITS 5	forward	5'-GGAAGTAAAAGTCGTAACAAGG-3'	White et al. 1990
ITS 4	reverse	5'-TCCTCCGCTTATTGATATGC-3'	White et al. 1990
LROR	forward	5'-ACCCGCTGAACTTAAGC-3'	Vilgalys and Hester 1990
LR5	reverse	5'-TCCTGAGGGAACTTCG-3'	Vilgalys and Hester 1990
TEF1-INTF	forward	5'-CCGTGAYTTCATCAAGAACATG-3'	Liu et al. 1999
TEF1-1567R	forward	5'-ACHGTRCCRATACCACCRATCTT-3'	Liu et al. 1999

PCR (Polymerase chain reaction)

The PCR protocol has been developed and optimized in house by Michael H. J. Barfuss described in his protocol. To prepare our samples for the PCR, we mixed the master mix like described in Tab. 3., the loading dye was already inside. The primers used for the reaction can be seen in Tab. 2. We calculated the amount of mixture needed for the 15 μl reaction, we took 14 μl mixture and 1 μl DNA-extract. The PCR settings were as mentioned in the PCR program down below (Tab 4.). PCR was conducted with the GeneAmp PCR-system 9700 (Applied Biosystems).

Tab. 3. Shows the concentrations needed for PCR preparations of the samples.

	11.1 μl reaction
1. PCR Master Mix (1.1x)	9 μl
2. Primer forward (50 μM)	0.5 μl
3. Primer reverse (50 μM)	0.5 μl
4. BSA (Bovine Serum albumine) (20 $\mu\text{g}/\mu\text{l}$)	0.1 μl
5. DNA template (extract)	1 μl
Total volume	11.1 μl

Tab. 4. Shows the settings of the PCR machine varying slightly between the Primers. Step 2,3 and 4 were repeated for 40 cycles.

	ITS	LSU	TEF
1.Initial heating	95°C for 2 min	95°C for 2 min	95°C for 2 min
2.Denaturation Step	95°C for 25 sec	95°C for 25 sec	95°C for 25 sec
3.Annealing Step	50°C for 35 sec	53°C for 35 sec	55°C for 35 sec
4.Extension Step	72°C for 1 min	72°C for 1 min	72°C for 1 min
5.Final Extension	72°C for 5 min	72°C for 5 min	72°C for 5 min
Hold	10°C for ∞	10°C for ∞	10°C for ∞

Agarose Gel electrophoresis

Gel electrophoresis was conducted with the GelDoc 2000 by BIORAD to verify and check the quality of the extracted DNA-samples after the PCR was performed. Was done like described in the protocol of Michael H. J. Barfuss. The 1,5% agarose gel was made up off 0.45g agarose, 30ml buffer, 0.6 μ l GelRed. In Fig.5. the DNA ladder which is hanging in the laboratory can be observed, from this the weight of the DNA can be gathered. In Fig. 4 is an example picture depicted of the results from the Agarose Gel electrophoresis.

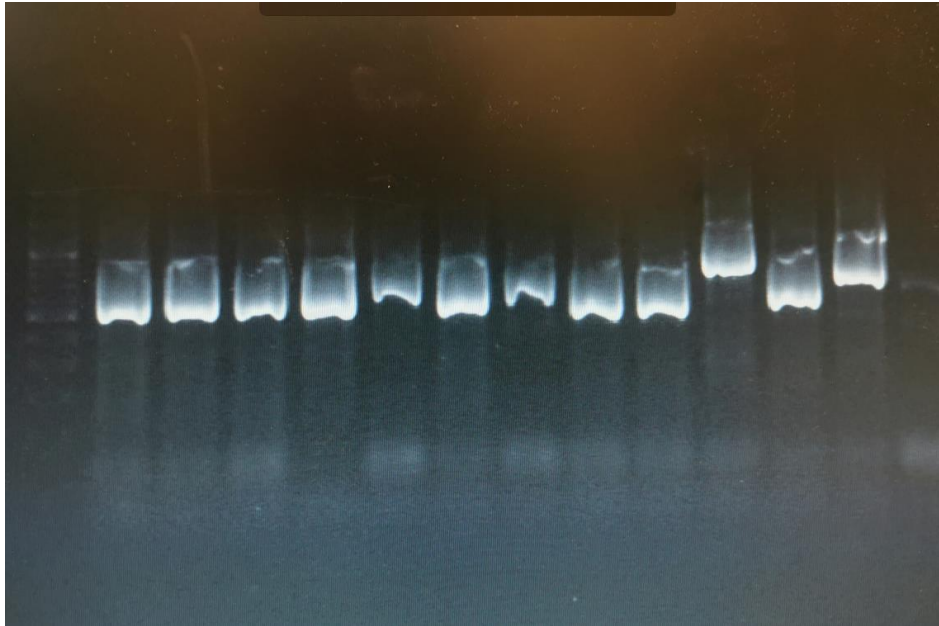


Fig.4. Depicts an example of the gel electrophoresis with the control samples on the outside of both ends. The samples range from 1-14 (left to right).

Purification of PCR products

For the purification of the PCR products an enzyme mix was used, which is a enzyme digestion of the remaining PCR components, such as unused dNTPs and primers

Was conducted like described in the protocol of Michael H. J. Barfuss, with the Exonuclease I and FastAP (Fast Alkaline Phosphatase). The resulting solution had to be spun down and incubated at 37°C for 30 min. Following that step enzymes are denaturised with 85°C for 15 min, this program was finished on the PCR machine with fixed settings.

Sanger Sequencing (chain termination sequencing, cycle sequencing)

Was conducted like described in the protocol of Michael H. J. Barfuss. Same primers like for the PCR were used (Tab.2.). To prepare our 16 samples for the cycle sequencing, we calculated the master mix and added the mixture, like depicted in Tab. 5. The cycle sequencing was conducted after the program depicted in Tab. 6, with the practical settings being applied. For analyzation of the sequences the 3730 DNA Analyzer was used.

Tab. 5. Shows the concentrations needed for preparations of samples for the cycle sequencing.

	Practical (1x)
1. Trehalose (1 M)	2 μ l
2. Sequencing buffer (5x)	1.8 μ l
3. Primer (5 μ M) only one !!!	0.5 μ l
4. H ₂ O, PCR grade (DANN/RNA free)	3.3 μ l
5. BigDye Terminator v3.1 (2.5x)	0.4 μ l
6. Cleaned PCR product (>20 ng)	2 μ l
Total volume	10 μl

Tab. 6. Depicts the cycle sequencing conditions practical use.

	Practical
1. Initial hold	96°C for 1 min
2. Denaturation	96°C for 10 s
3. Annealing	50 °C for 5 s
4. Extension	60°C for 3 min
	No. of cycles: 35
5. Final hold	4°C for ∞

BigDye terminator imprints and prepares the DNA for Sanger Sequencing. In this technique both sides the dNTPs (deoxynucleotide triphosphates) and ddNTPs (dideoxynucleotide phosphates) are utilized. Both ddNTPs are labeled with a fluorescent dye tuned to the four nucleotides present in the DNA.

Further the DNA-samples are amplified through the help of the thermocycler, in this process there are arbitrary fragments created, with varying sizes. Those varying fragments of ddNTP will bring up certain information eventually in the sequencing process. PCR primers are used to sequence the correlating PCR product.

Sephadex purification of each sequencing reaction and the 48- capillary Sequencer (AB 3730 DNA Analyzer, Applied Biosystems) maneuvering, was performed by the laboratory manager of the Department of Botany and Biodiversity Research (Mag. Dr. Michael H. J. Barfuss), who also has the position of co-supervisor in this Masterwork.

The Cycle Sequencer apparatus uncovers, DNAs of concern with varying lengths of ddNTPs fragments, those uncovered flow through the capillaries and are picked up by a detector. Different wavelengths of light are the crucial distinguishing factor for the detector to identify, what kind of ddNTPs are picked up. Furthermore, an electropherogram is constructed by the sequencer, this is identified by the computer and displayed. Through the information obtained from the electropherogram a program will create the highest correlating sequence.

Data processing and Phylogenetic analysis

Data Processing

The programs used to handle the data obtained were; Seqman, Bioedit, RAxML, MAFFT online version, Genbank and text editor for data management.

Assembling and editing

SeqMan® Version 12.0. DNASTAR. Madison, WI. was used for the contig assembly of the samples. The program provides several useful tools for simplifying the process of assembling data from a sequencing project. Particularly in performing tasks of removing extraneous data from the sequence, which was done for all the samples. We cut our sample strands most of the time, at the beginning and end, where the primer sequences are located. Furthermore the errors were corrected, which the program visualized, when wrong bases were present in one strand.

Additional Sequence Data

To see, which species the samples were similar to, in regards to the ITS, LSU and TEF barcoding region we used the online databank GenBank®. The samples were matched to sequences with the highest similarity to get information on what our species could be. Then downloaded references from the genus *Gyromitra* and *Disciotis*. GenBank® is the NIH genetic sequence database, an annotated collection of all publicly available DNA sequences (Nucleic Acids Research, 2013). Those sequences mostly were already used for other published papers.

Bioedit et al. 1999 was used to observe sequences, correct minor errors at base level and delete sequences which didn't fit in.

Then for alignment of the sample sequences and reference sequences MAFFT Version 7 (Katoh and Standley 2013, Zmasek et al. 2013) was used. The datasets were aligned with auto alignment (FFT-NS-1, FFT-NS-2, FFT-NS-i or L-INS-i; depends on data size) and kept the same base as on the input.

The Text editor was used to correct and put in new sequences or change any other text information related to the files.

Excel was used to make tables for the whole species collection, the species which changed through GenBank evaluation and references obtained through GenBank

Microscopic observations

Additional observations have been taken into account through microscopy for the species clarification. The characteristics taken into account were spore size, ornamentation, apiculi and paraphyse. Two specimens per species were observed if more than one specimen was in the herbarium.

The microscopy was done with a Zeiss Axio Imager.A1 at various magnifications (100×, 1000×, 4000×). The samples were taken out of the herbarium, which were dried specimens of the different *Gyromitra* species. For preparation small parts of the dried specimens of the hymenial layer were broken off and soaked in a 3% Ammonium-solution for some minutes to regain the structure. In two specimens cotton blue staining for identifying the correct species was used. Then the part was placed between a glass slide and a cover glass. For the highest magnification the immersion-oil technique was used. All the pictures were taken at the highest magnification.

For the ascospore measurement the Axio Imager measurement program was used. The ascospore length and width were noted, without taking the apiculi into account. From those parameters the mean, median, minimum, maximum, the standard deviation, and the quotient (length divided with width) were calculated in a pre-made excel sheet. All measurements were in micrometres.

Phylogenetic analysis

To generate the maximum likelihood tree with the obtained data for the markers ITS, LSU and TEF the program RAxML GUI (Stamatakis a. 2014) was used. The settings used can be seen in Fig., were RAxML first ran an modeltest to find the best fitting model and afterwards generated the phylogenetic tree. In this the bootstrap values were calculated with at least 50% for sufficient values, all the lower values were cut out. With 50-74% weakly supported, 75-89% moderately supported and 90-100% highly supported. For all the generated trees *Disciotis venosa* was used as the outgroup to the root trees.

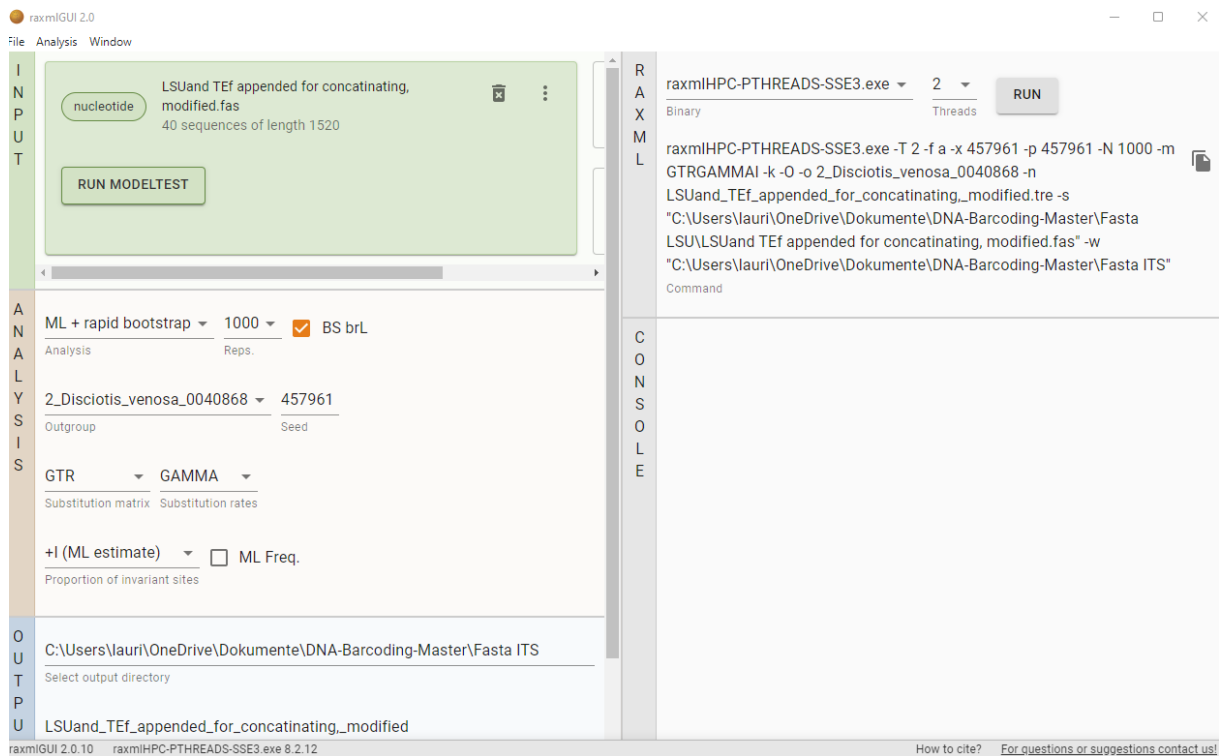


Fig. 5. Settings used in RAXMLGUI for generating the Maximum likelihood tree.

For every marker there was a matrices created resulting in a ITS-matrices, LSU-matrices and TEF matrices. Furthermore there was a concatenating matrices for the combining of LSU and TEF conducted. From the matrices the analysis was conducted resulting in the generation of the maximum likelihood phylogenetic trees.

We used FigTree (<http://tree.bio.ed.ac.uk/software/figtree/>, 2021) for visualization and further to observe the progress of our phylogenetic tree, which was generated with RAXML GUI and to see what has to be changed.

Inkscape was used to enlarge or delete the species names and the bootstrap values (<50%) of the maximum likelihood phylogenetic tree, change the font design to better read the names and cut out unnecessary information.

Results

Gyromitra perlata (Fr.) Harmaja, Karstenia 9: 11 (1969)

Sample no. 4_*Gyromitra perlata*_WU_0031240

Microscopic characteristics: Ascospores: ellipsoid, hyaline; ornamentation fine netted verrucous, pointed apiculi on both ends, when mature; mature mostly uniguttulate (some bi- or triguttulate); (21.7-23.7-27(-28.3) μm long, (8.6-)9.8-12(-13.1) μm wide, quotient (1.9-)2.1-2.6(-2.9) (n=21). Paraphyses: cylindrical, septate, with slightly clavate apex, at apex 9 μm , cylindrical part 6 μm .

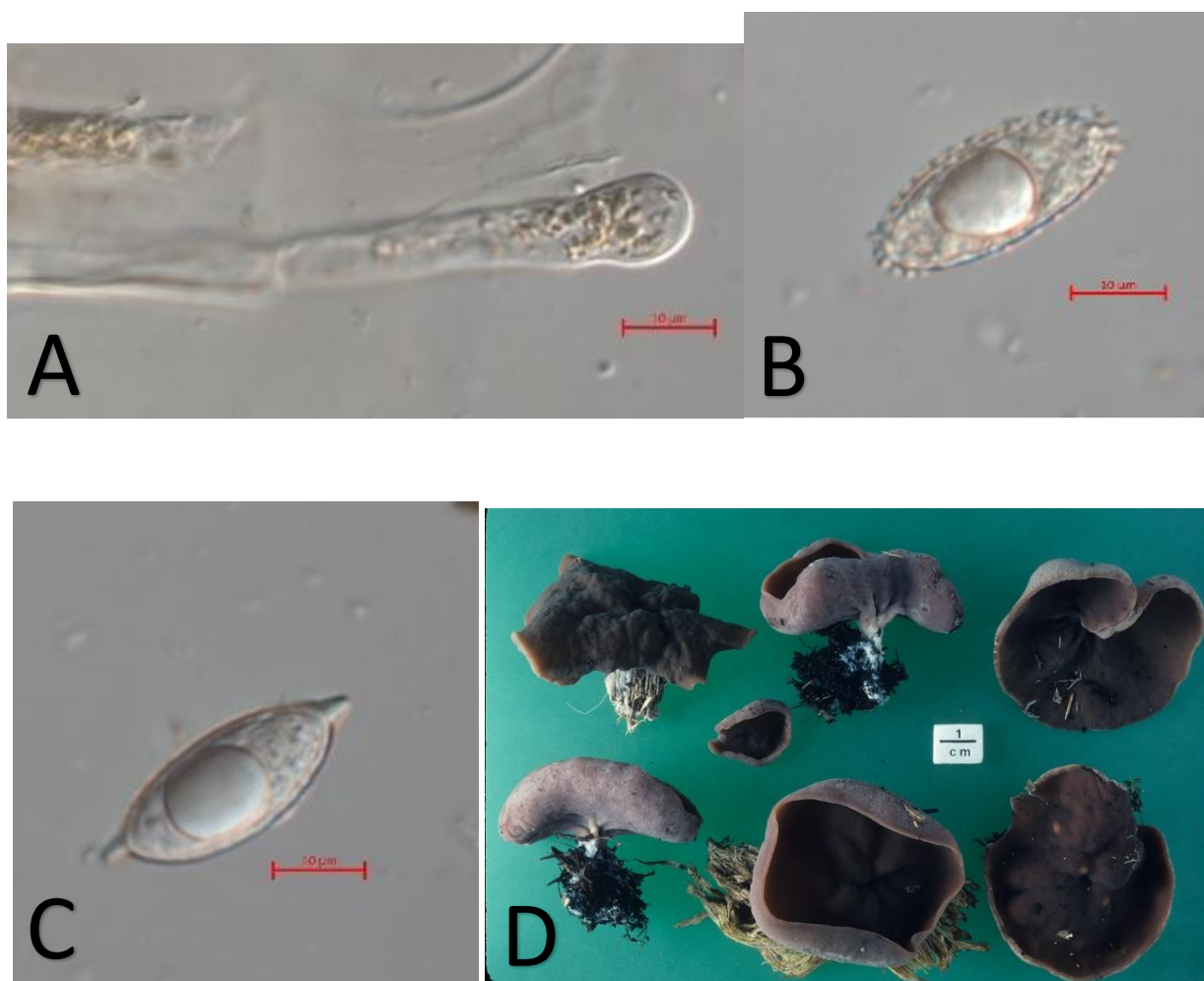


Fig. 6. *Gyromitra perlata*, WU-Myc 0031240. A. Paraphysis. B. Verrucose ascospore. C. Apiculate ascospore. D. Ascomata (<https://pilzdaten-austria.eu/#tax/378431>), phot.: I. Krisai-Greilhuber. - Scale bars: A-C 10 μm , D 1 cm.

Sample no. 15_*Gyromitra perlata*_WU_0026583

Microscopic characteristics: Ascospores: ellipsoid, hyaline, ornamentation fine verrucous, mature with fine net-shaped ornaments, pointed apiculi on both ends; uni- and triguttulate; (26.6-)27.1-32.3(-33.9) μm long, (8.9-)9.1-11.2(-11.2) μm wide, quotient (2.7-)2.8-3.1(-3.1) (n=6). Paraphyses: cylindrical, septate, with slightly clavate apex; at apex 7.3 μm , cylindrical part 7 μm .

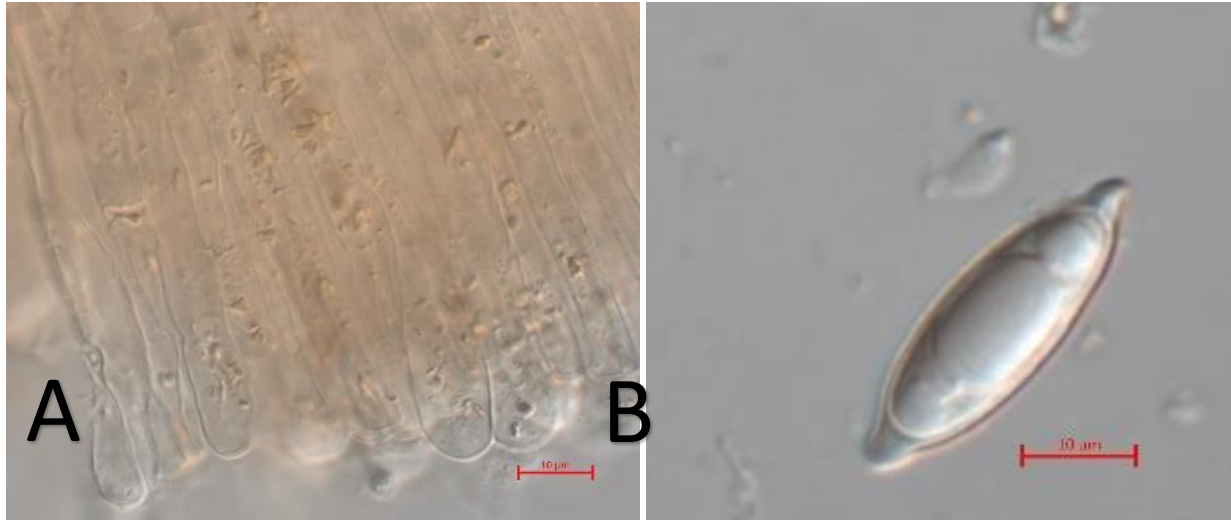


Fig. 7. *Gyromitra perlata* WU-Myc 0026583. A. Paraphyses and immature asci. B. Submature ascospore.
- Scale bars: 10 μm .

Gyromitra apiculatula (McKnight) Berthet [as 'apiculata'], Trav. du Laboratoire de 'La Jaysinia' a Samoëns, Haute-Savoie 4: 104 (1972)

Sample no. 12_*Gyromitra apiculatula*_WU_0025874

Microscopic characteristics: Ascospores: ellipsoid; ornamentation smooth too faintly verrucous-reticulate, mature with broadly rounded to truncate apiculi on both ends; mostly uniguttulate; (27.8-)27.7-27.9(-27.9) μm long, (11.1-)10.9-12.3(-12.1) μm wide, quotient (2.3-)2.3-2.5(-2.5) (n=2); immature spores (21.5-)22.9-25.6(-26.7) μm long, (9.7-)10.5-12.1(-12.4) μm wide, quotient (1.9-)2-2.3(-2.5) (n=17). Paraphyses: cylindrical, at apex 8 μm .

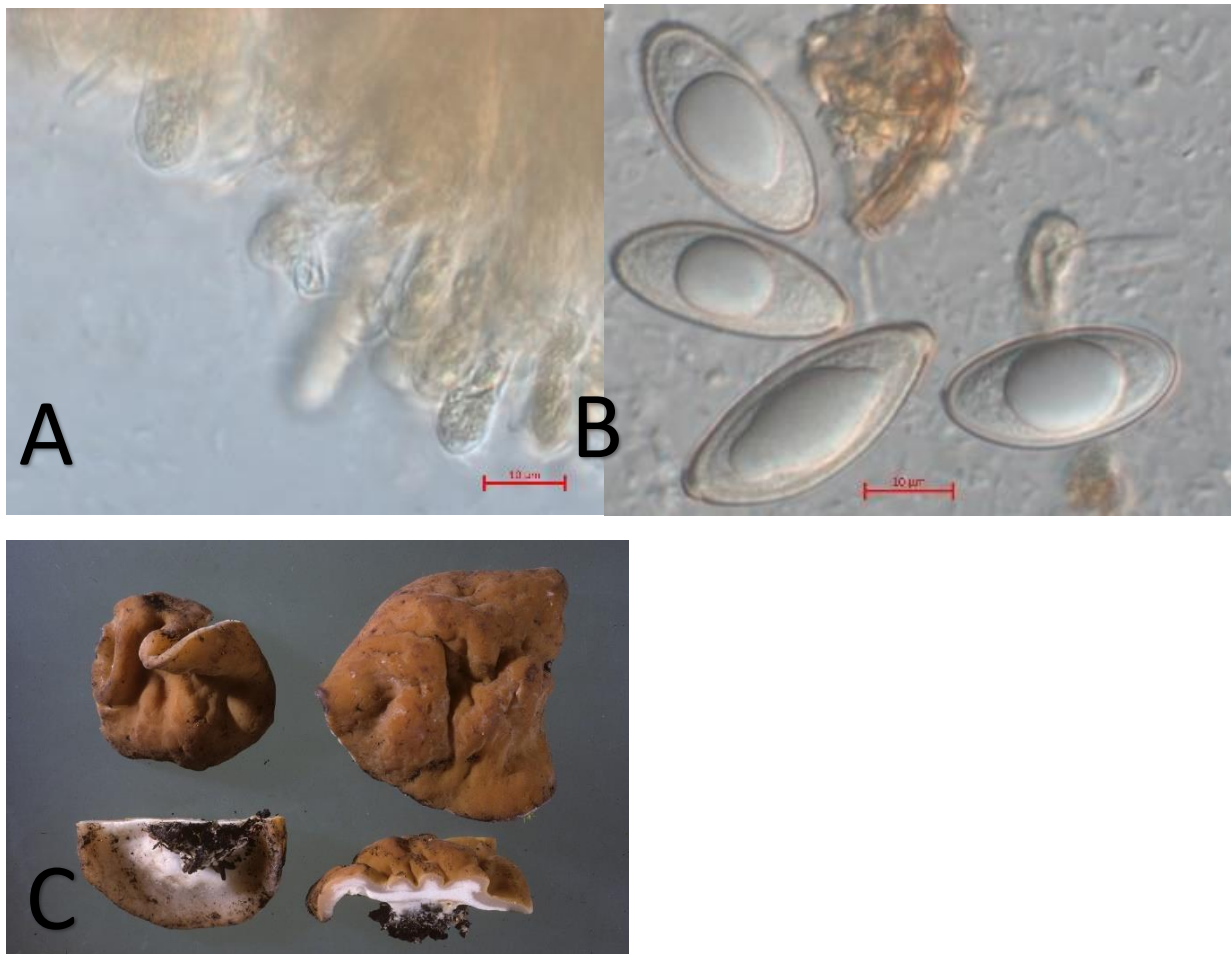


Fig. 8. *Gyromitra apiculatula* WU-Myc 0025874. A. Paraphyses and immature asci. B. immature and mature ascospores. C. Ascomata (<https://pilzdaten-austria.eu/#tax/378428>), phot. A. Hausknecht. - Scale bars: A, B 10 μm .

Sample no.13_*Gyromitra apiculatula*_WU_0020233

Microscopic characteristics: Ascospores: ellipsoid; ornamentation smooth faintly verrucous-reticulated; mature with broadly rounded to truncate apiculi on both ends; uniguttulate, one mature specimen triguttulate (25.6-)26.8-28.9(-29.5) (n=10) μm long, (9.1-)10.2-12.2(-12.2) (n=10) μm wide, quotient (2.3-)2.2-2.7(-3.1) (n=10) μm . Paraphyses: cylindrical, at apex 9 μm .



Fig. 9. *Gyromitra apiculatula* WU-Myc 0020233. A. Paraphysis. B. Ascospores, one of them mature. - Scale bars: A, B 10 μm .

Gyromitra leucoxantha (Bres.) Harmaja, Karstenia 9: 11 (1969)

Sample no. 17_*Gyromitra leucoxantha*_WU_0004453

Microscopic characteristics: Ascospores: navicular; ornamentation verrucous to incomplete reticulate, with truncate-concave apiculi on both ends; uniguttulate; (26.4-)28-32.4(-33.8) (n=17) μm long, (9.7-)10.4-12.1(-12.7) (n=17) μm wide, quotient (2.4-)2.5-2.9(-3) (n=17) μm . Paraphyses: septate, at apex 6.4 μm , hyphal part under apex 5.2 μm .

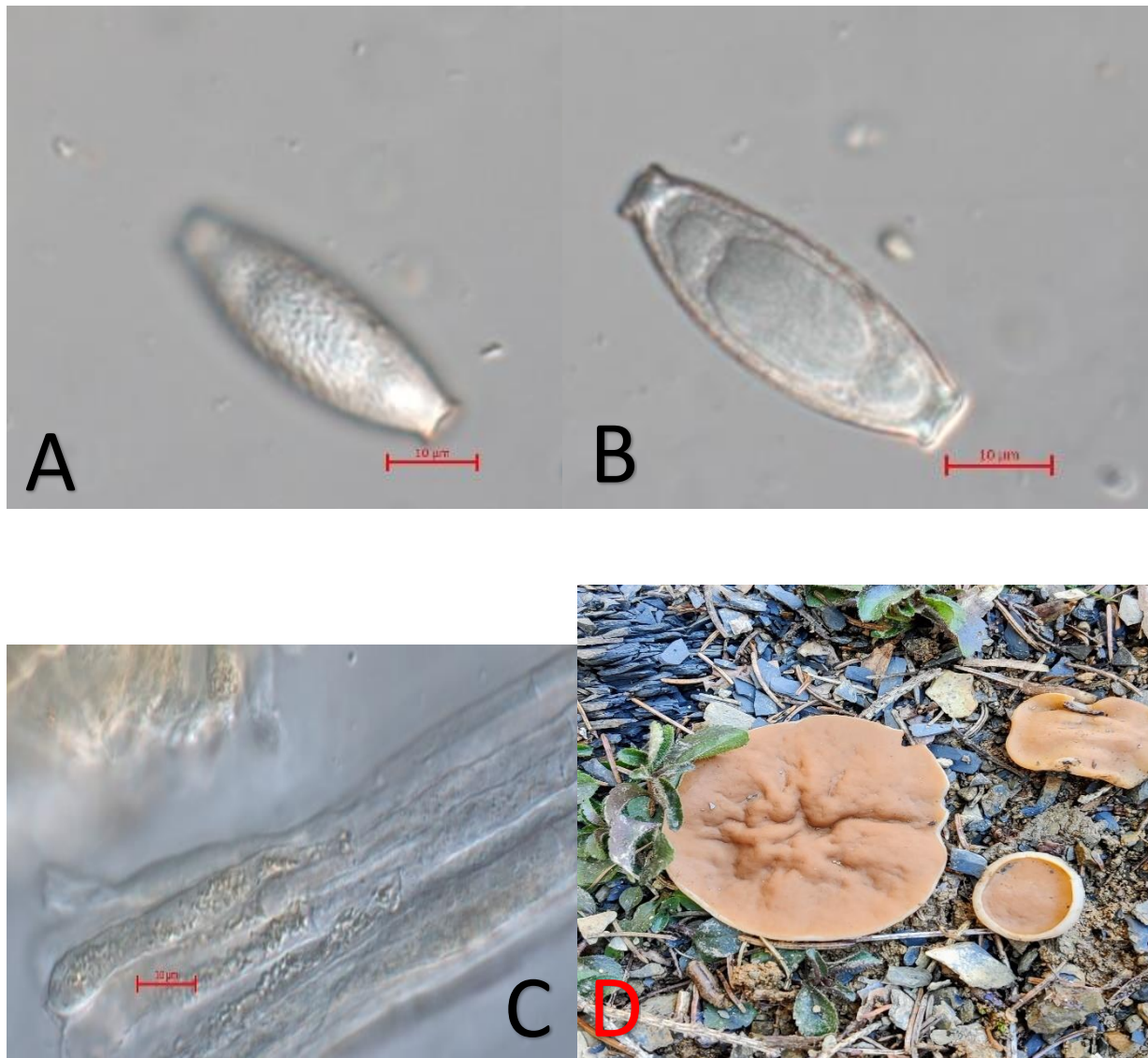


Fig. 10. *Gyromitra apiculatula* WU-Myc-0004453. A. Ascospore with ornamentation. B. Ascospore with apiculi. C. Paraphyses. D. Ascomata (<https://pilzdaten-austria.eu/#tax/378415>), phot. Schneeweiß Rebecca, specimen from May 3rd, 2021. Scale bars A-C 10 μm .

Gyromitra parma (J. Breitenb. & Maas Geest.) Kotl. & Pouzar, Česká Mykol.
28(2): 91 (1974)

Sample no. 18_*Gyromitra parma*_WU_0035109

Microscopic characteristics: Ascospores: ellipsoid to ellipsoid-fusoid; ornamentation warty and reticulate, many small pointed apiculi on both ends; uniguttulate; (19.1-)21.4-25.9(-28) (n=18) μm long, (9.3-)10.2-13.2(-13.9) (n=18) μm wide, quotient (1.7-)1.8-2.3(-2.6) (n=18) μm ; Paraphyses: septate, at apex 8.2 μm , hyphal part under apex 6 μm .

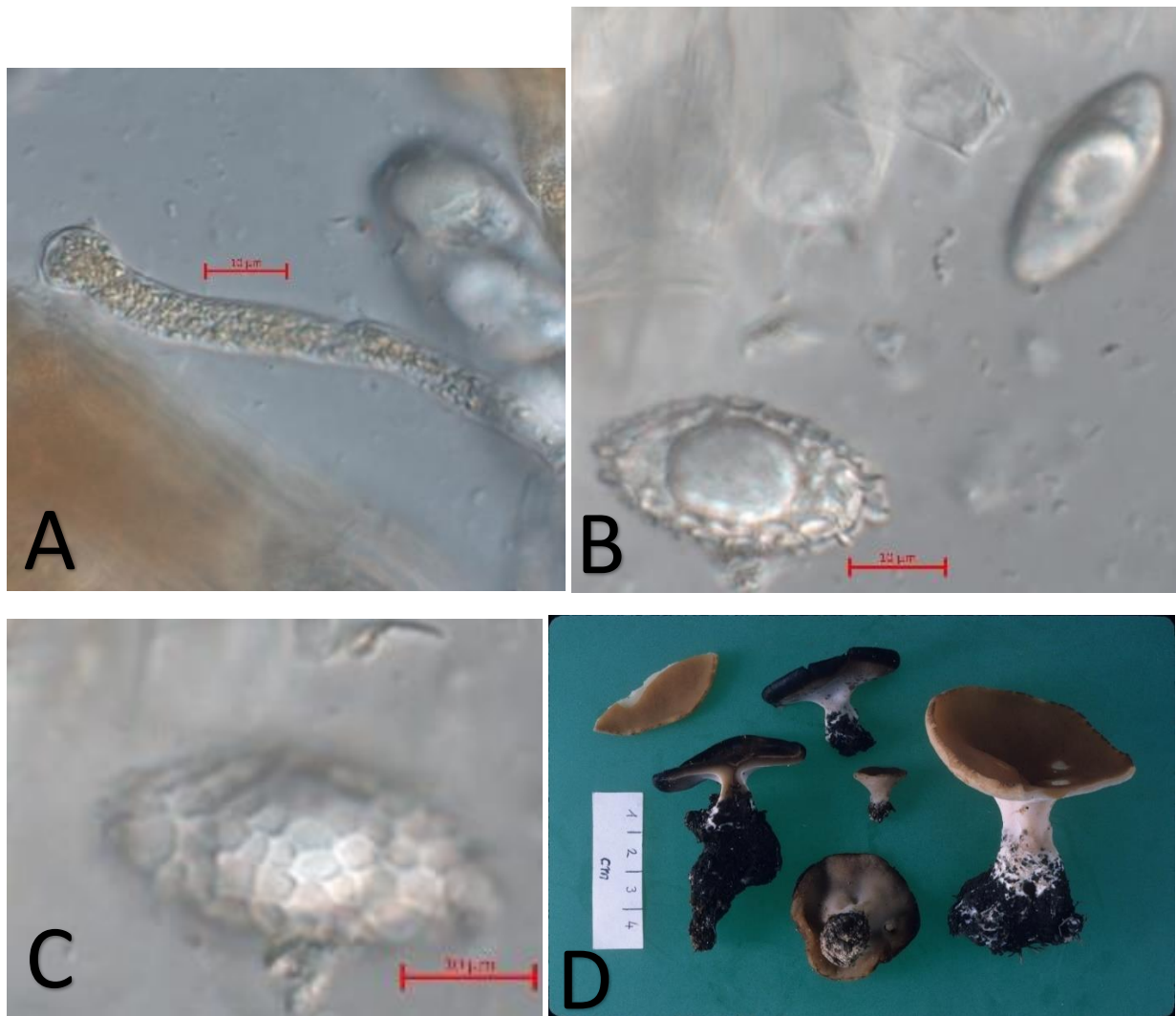


Fig. 11. *Gyromitra parma* WU-Myc-0035109. A. Paraphyses. B. Ascospore with ornamentation. C. Ascospore with ornamentation. D. Ascomata (<https://pilzdaten-austria.eu/> "l" "tax/378429"), phot. Krisai-Greilhuber Irmgard. Scale bars A-C 10 μm , D 4 μm .

Sample no. 19_ *Gyromitra parma* WU_0024817

Microscopic characteristics: Ascospores: ellipsoid to ellipsoidfusoid: ornamentation warty and rough reticulate, many small pointed apiculi on both ends; uniguttulate; (21.3-)22.5-25.1(-26.7) (n=16) μm long, (10.1-)10.8-12.4(-13.1) (n=16) μm wide, quotient (1.8-)1.9-2.2(-2.3) (n=16) μm ; Paraphyses: septate. at apex 7.3 μm .

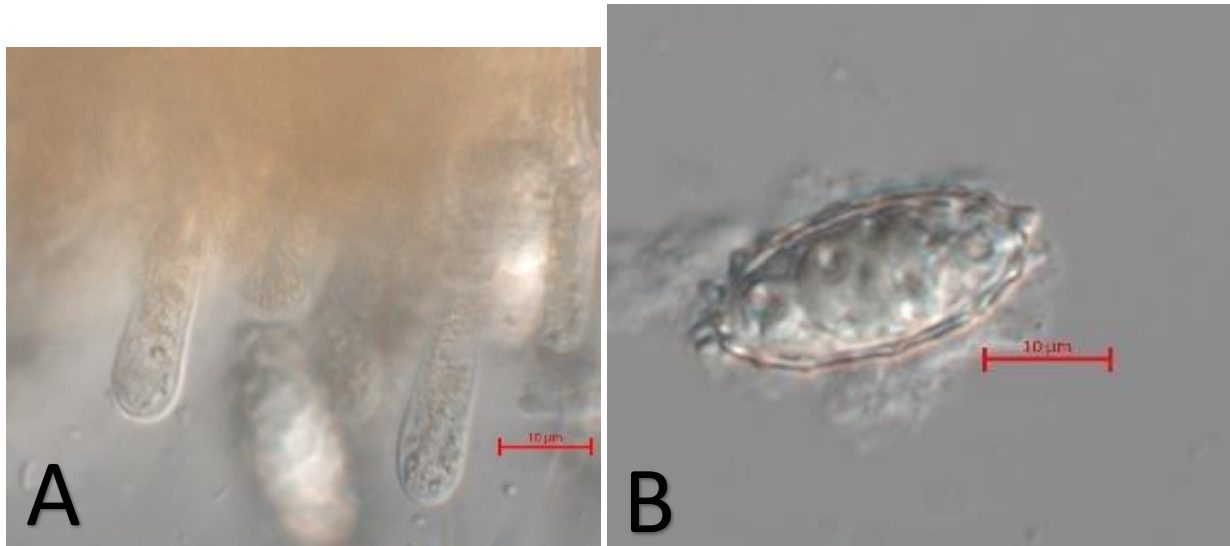


Fig. 12. *Gyromitra parma* WU-Myc-0024817. A. Paraphyses and ornamented ascospore. B. Ascospore with ornamentation. Scale bars A, B 10 μm .

20_ *Gyromitra parma* WU_0036232

Microscopic characteristics: Ascospores: ellipsoid to ellipsoidfusoid; ornamentation warty and are rough reticulate, many small pointed apiculi on both ends; immature triguttulate, mature uniguttulate; (24.9-)25.4-27.7(-27.9) (n=7) μm long, (11.6-)11.6-13.1(-13.6) (n=7) μm wide, quotient (2-)2.1-2.2(-2.3) (n=7) μm . Paraphyses at apex 8 μm .

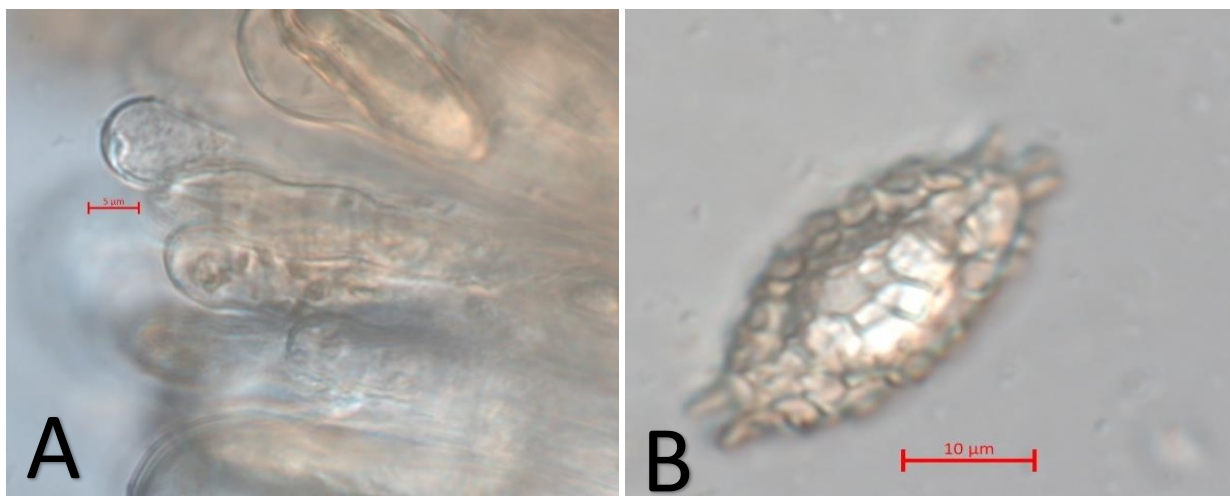


Fig. 13. *Gyromitra parma* WU-Myc-0036232. A. Paraphyses. B. Ascospore with ornamentation. Scale bars A, B 10 μm .

Gyromitra esculenta Pers. ex Fr., Summa veg. Scand., Sectio Post. (Stockholm):
346 (1849)

Sample no. 21_*Gyromitra esculenta*_WU_0043890

Microscopic characteristics: Ascospores: ellipsoid, hyaline; ornamentation smooth, no apiculi; biguttulate; (17-)18,1-20,9(-22,2) (n=26) μm long, (8,5-)8,9-10,8(-11,9) (n=26) μm wide, quotient (1,8-)1,9-2,1(-2,2) (n=26) μm . Paraphyses: cylindrical, septate, at apex 5 μm and hyphal part under apex 2,6 μm .

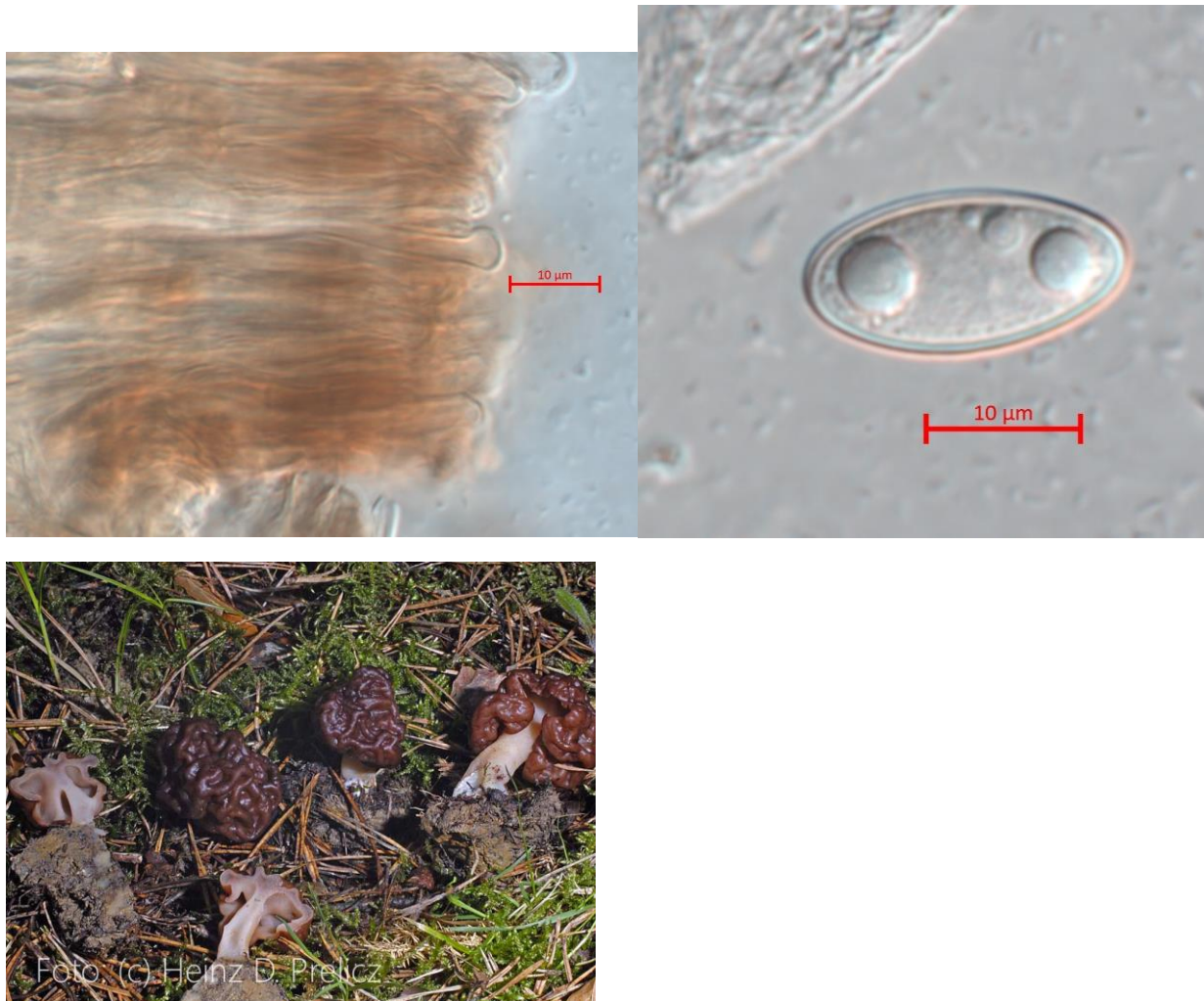


Fig. 14. *Gyromitra esculenta* WU-Myc-0043890. A. Paraphyses. B. Ascospore. C. Ascomata
(<https://pilzdaten-austria.eu/#tax/378389>) phot. Heinz D. Prelicz. Scale bars A, B 10 μm .

Sample no. 25_ *Gyromitra esculenta* _WU_0041117

Microscopic characteristics: Ascospores: ellipsoid and hyaline; ornamentation smooth, no apiculi; biguttulate. (19,3-)20,9-23,6(-25,9) (n=28) μm long, (8,1-)9,2-10,5(-10,8) (n=28) μm wide, quotient (2,1-)2,1-2,4(-2,6) (n=28) μm . Paraphyses: not measurable.



Fig. 15. *Gyromitra esculenta* WU-Myc-0041117. A. Paraphyses. B. Ascospores. Scale bars A, B 10 μm .

Sample no. 63_ *Gyromitra esculenta* _WU_001620

Microscopic characteristics: Ascospores: ellipsoid, hyaline; ornamentation smooth, no apiculi; biguttulate. (18,3-)19,1-21,4(-21,7) (n=7) μm long, (9,2-)9,3-10,6(-11,2) (n=7) μm wide, quotient (1,9-)2-2,1(-2,2) (n=7) μm . Paraphyses: at apex 8 μm .

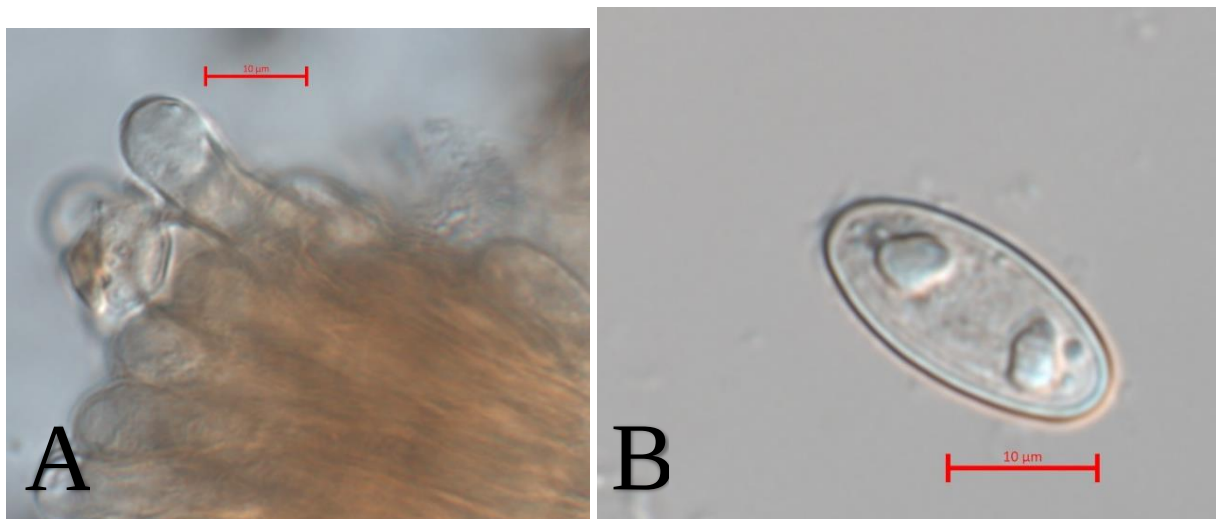


Fig. 16. *Gyromitra esculenta* WU-Myc-001620. A. Paraphyses. B. Ascospore. Scale bars A,B 10 μm .

Gyromitra gigas (Krombh.) Quél., Mém. Soc. Émul. Montbéliard, Sér. 2 5: 338 (1873)

Sample no. 8_*Gyromitra gigas*_WU_0009567

Microscopic characteristics: Ascospores: ellipsoid to subfusoid, hyaline; ornamentation with incomplete reticulum, truncate apiculi on both ends; guttula number not measurable. (21,1-)22,5-25,3(-25,3) (n=8) μm long, (9,5-)10,7-12,7(-13) (n=8) μm wide, quotient (1,9-)2-2,2(-2,2) (n=8) μm .

Paraphyses: club shaped, at apex 9,1 μm .

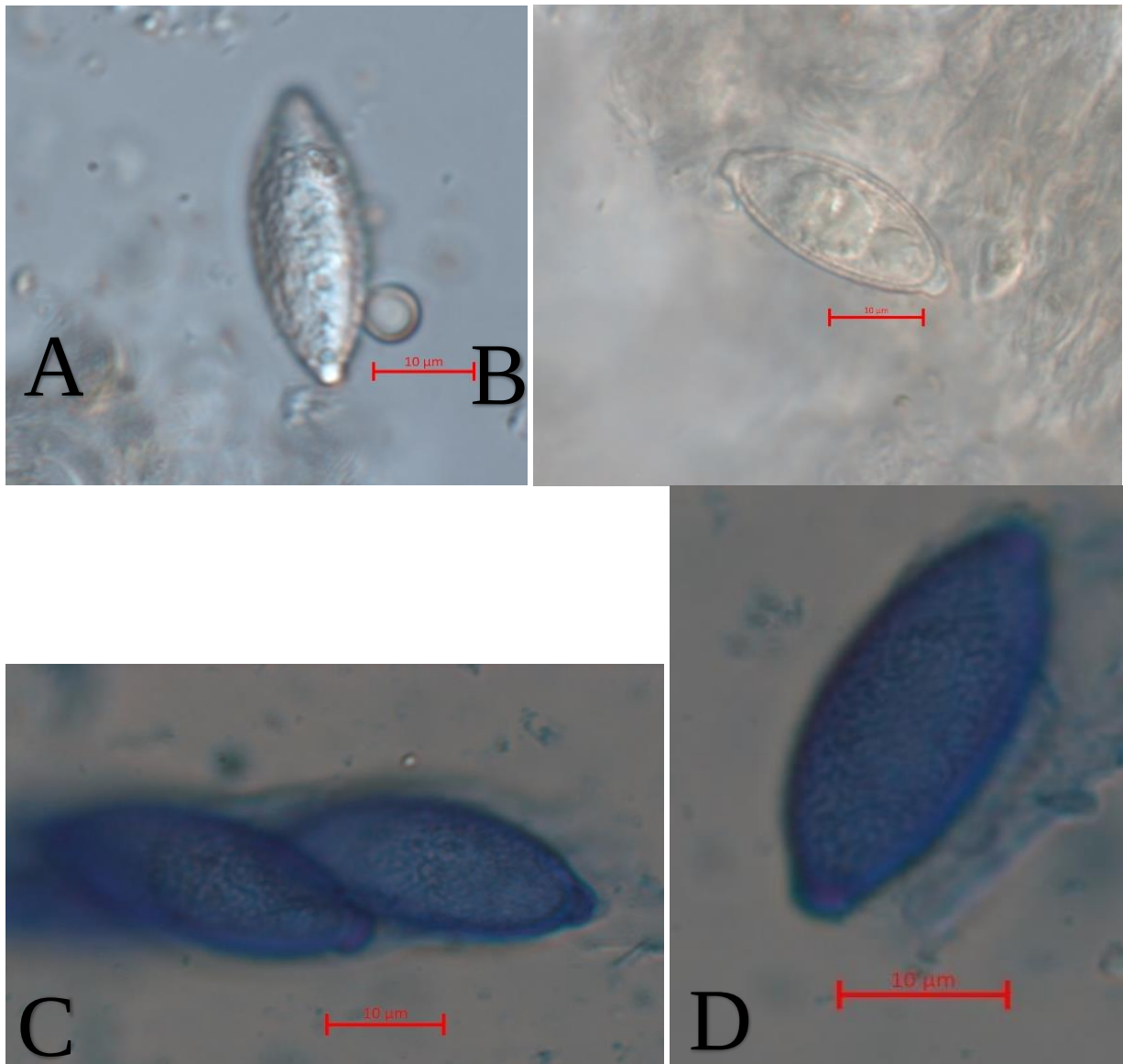
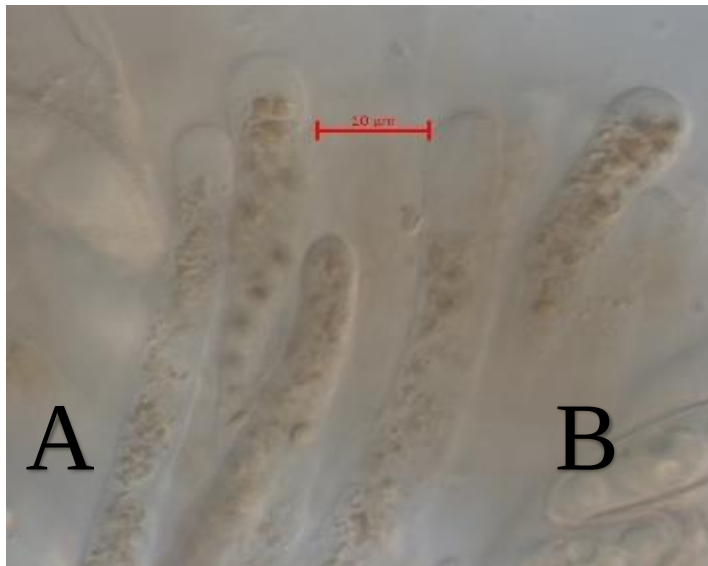




Fig. 17. *Gyromitra gigas* WU-Myc-0009567. A. Ascospore with ornamentation. B. Ascospore. C. Cotton blue stained ascospores. D. Cotton blue stained ascospore. E. Ascomata (<https://pilzdaten-austria.eu/#tax/378403>) phot. A. Hausknecht. Scale bars A-D 10 μ m.

Sample no. 28_*Gyromitra gigas*_WU_0010578

Microscopic characteristics: Ascospores: ellipsoid to subfusoid, hyaline; ornamentation with incomplete reticulum, with truncate apiculi on both ends; uniguttulate or triguttulate, mostly triguttulate. (20,8)20,1-27(-34,6) (n=18) μ m long, (7,7-)8,5-10,1(-11,3) (n=18) μ m wide, quotient (2,3-)2,3-2,8(-3,3) (n=18). Paraphyses: cylindrical, septate, apex club-shaped, at apex 8,2 μ m and hyphal part under apex 6 μ m.



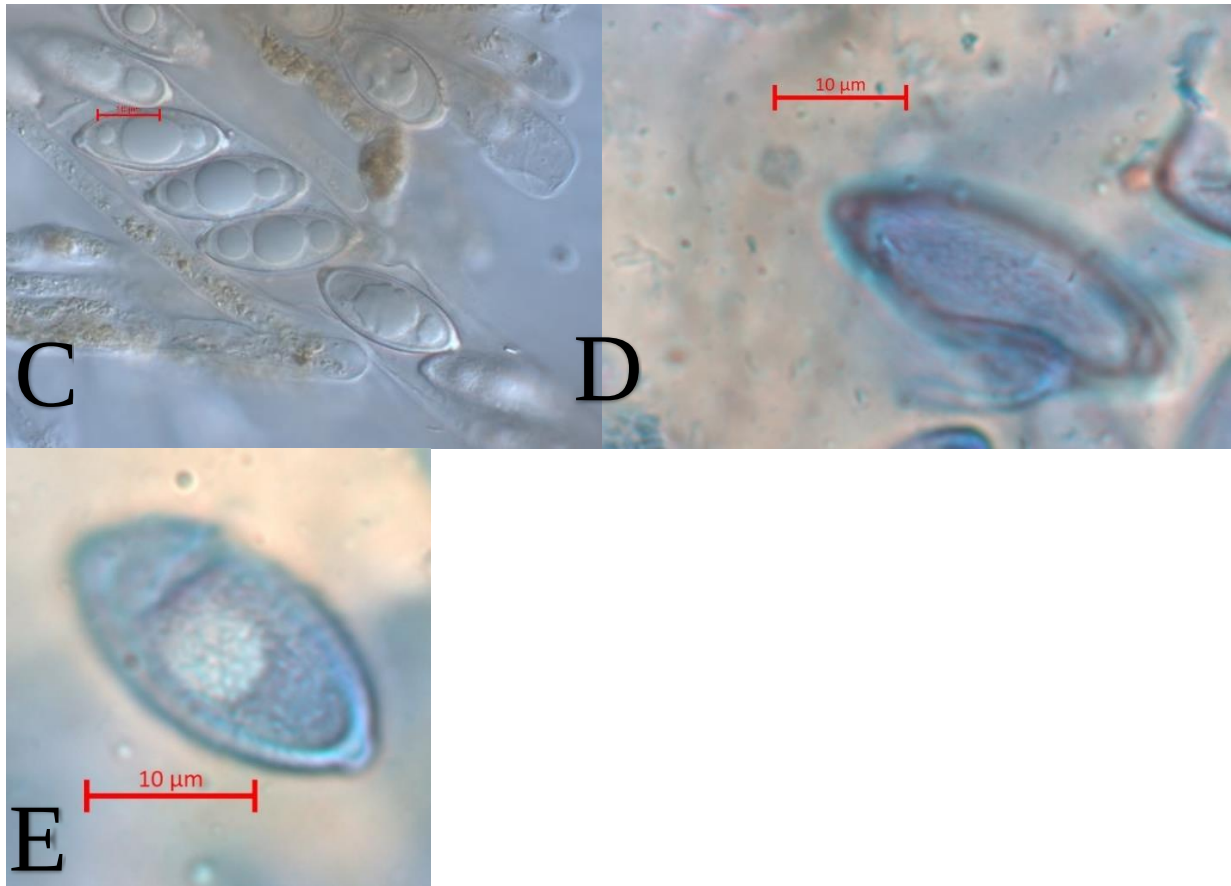


Fig. 18. *Gyromitra gigas* WU-Myc-0010578. A. Paraphyses B. Ascospore. C. Cotton blue stained ascospores in basidia, lowest ascospore light ornamentation. D. Cotton blue stained ascospore. E. Cotton blue stained ascospore. Scale bars A-E 10 µm.

Sample no. 30_*Gyromitra_gigas*_WU_0011622

Microscopic characteristics: Ascospores: ellipsoid to subfusiform, hyaline; ornamentation with incomplete reticulum, truncate apiculi on both ends; uniguttulate or triguttulate, mostly triguttulate. (26,3-27,2-30,2(-31,6) (n=16) μm long, (9,8-)10,8-12,8(-13) (n=16) μm wide, quotient (2,1-)2,3-2,6(-2,9) (n=16) μm . Paraphyses: cylindrical, septate, apex club-shaped, apex not measurable due clustering (Fig A.).

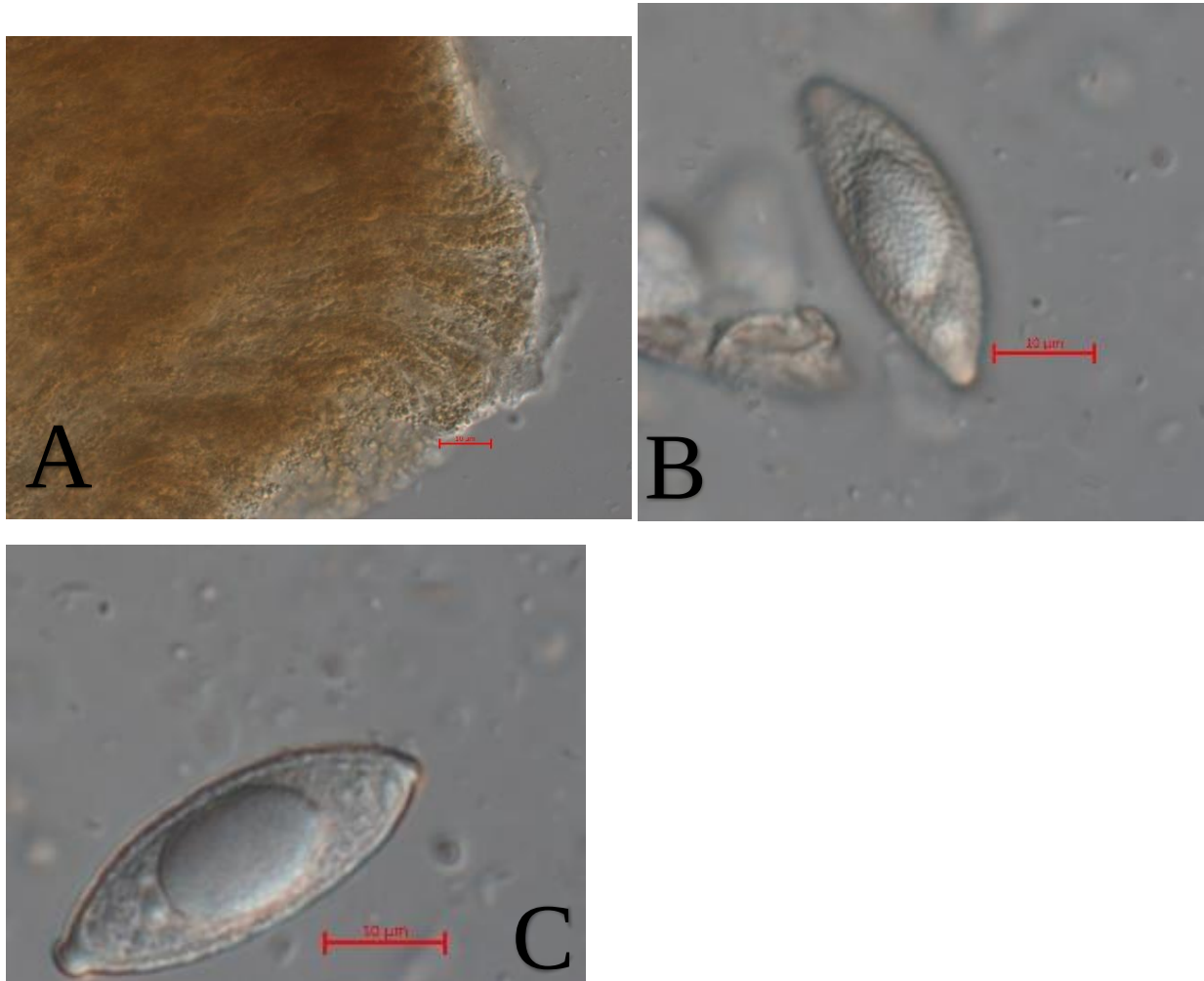


Fig. 19. *Gyromitra gigas* WU-Myc-0011622. A. Paraphyses clustered together. B. Ascospore with ornamentation. C. Ascospore. Scale bars A-C 10 μm .

Sample no. 34_*Gyromitra_gigas*_WU_0009596

Microscopic characteristics: Ascospores: ellipsoid to subfusiform, hyaline; ornamentation with incomplete reticulum, truncate apiculi on both ends; uniguttulate or triguttulate. (22,4-)23,4-28,4(-28,9) (n=8) μm long, (10,7-)11-12,7(-13,1) (n=8) μm wide, quotient (1,9-)2-2,4(-2,4) (n=8). Paraphyses: cylindrical, septate, apex club-shaped, at apex 6,2 μm .

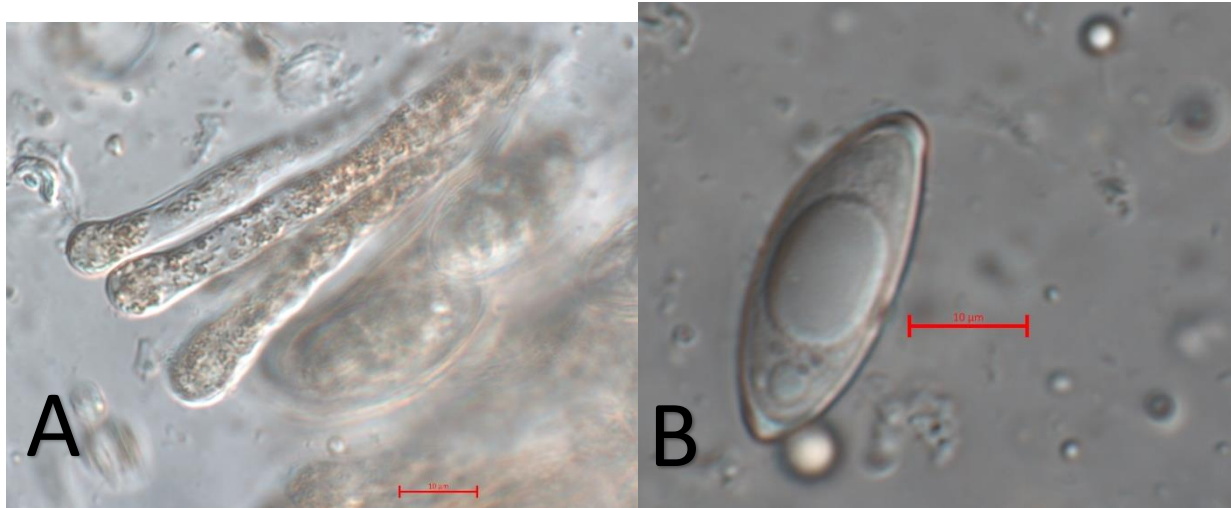


Fig. 20. *Gyromitra gigas* WU-Myc-0009596. A. Paraphyses. B. Ascospore with apiculi. Scale bars 10 μm .

Gyromitra fastigiata (Krombh.) Rehm, Rabenh. Krypt.-Fl., Edn 2 (Leipzig)
1.3(lief. 53): 1194 (1895) [1896]

Sample no. 35_*Gyromitra fastigiata*_WU_0024806

Microscopic characteristics: Ascospores: fusiform to ellipsoid; immatures look smooth but reticulate ornamentation, truncate to round small apiculi on both ends; uniguttulate. (23,7-)25,5-28,8(-31) (n=30) μm long, (10,4-)11,7-13(-13,6) (n=30) μm wide, quotient (1,9-)2-2,4(-2,7) (n=30) μm . Paraphyses: capitate to subcapitate, at apex 6,7 μm .

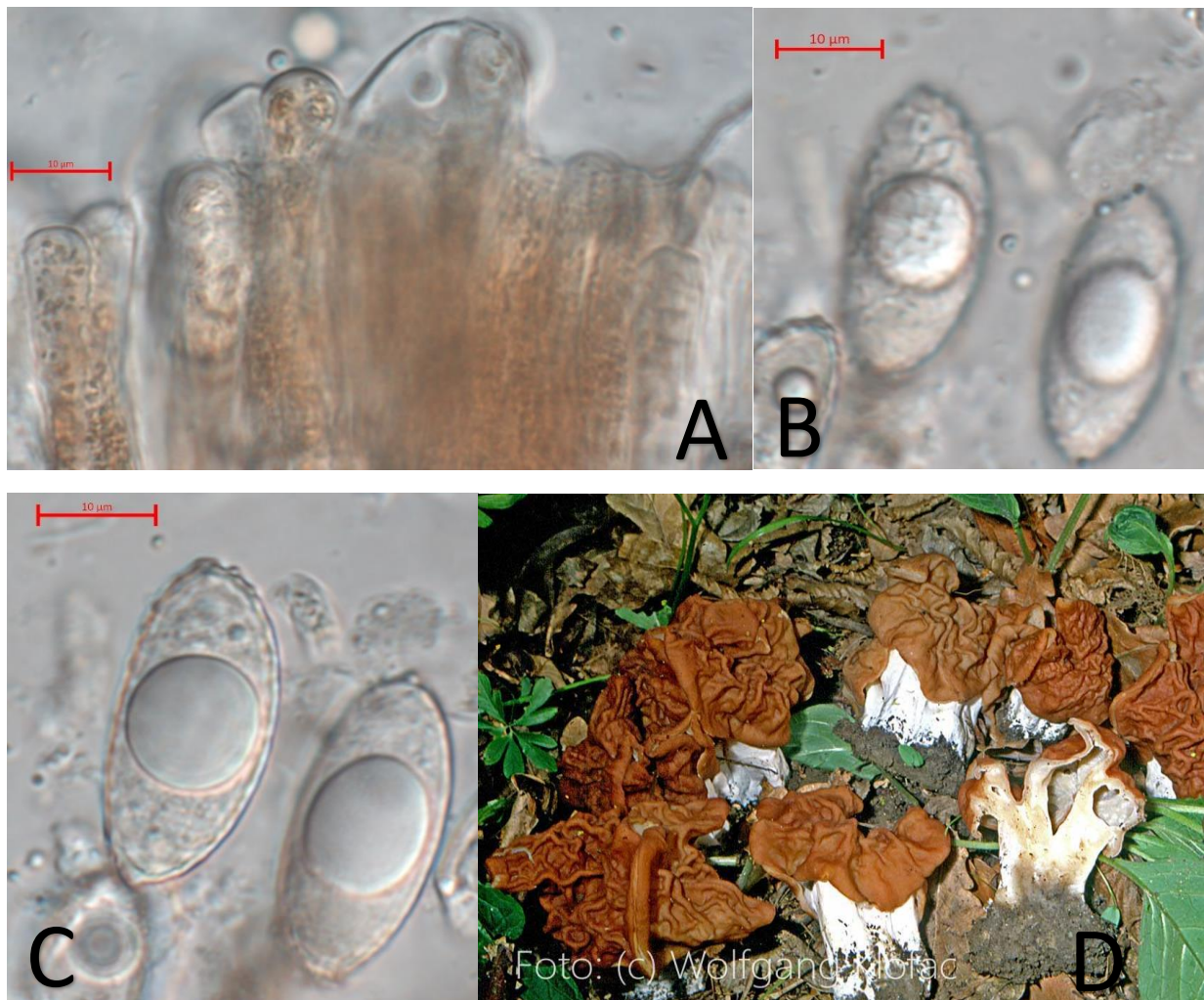


Fig. 21. *Gyromitra fastigiata* WU-Myc-0024806. A. Paraphyses. B. Ascospore with ornamentation. C. Ascospore without ornamentation. D. Ascomata (<https://pilzdaten-austria.eu/#tax/378396>) phot. Wolfgang Klofac. Scale bars A-C 10 μm .

Sample no. 39_ *Gyromitra fastigiata* _WU_0021318

Microscopic characteristics: Ascospores: fusiform to ellipsoid, immatures look smooth but reticulate ornamentation, truncate to round small apiculi on both ends; uniguttulate. (22,6-)23,9-27(-27,9) (n=16) μm long, (9,8-)10,4-12,2(-12,7) (n=16) μm wide, quotient (2-)2,1-2,5(-2,6) (n=16) μm . Paraphyses: capitate to subcapitate, at apex 8,3 μm .

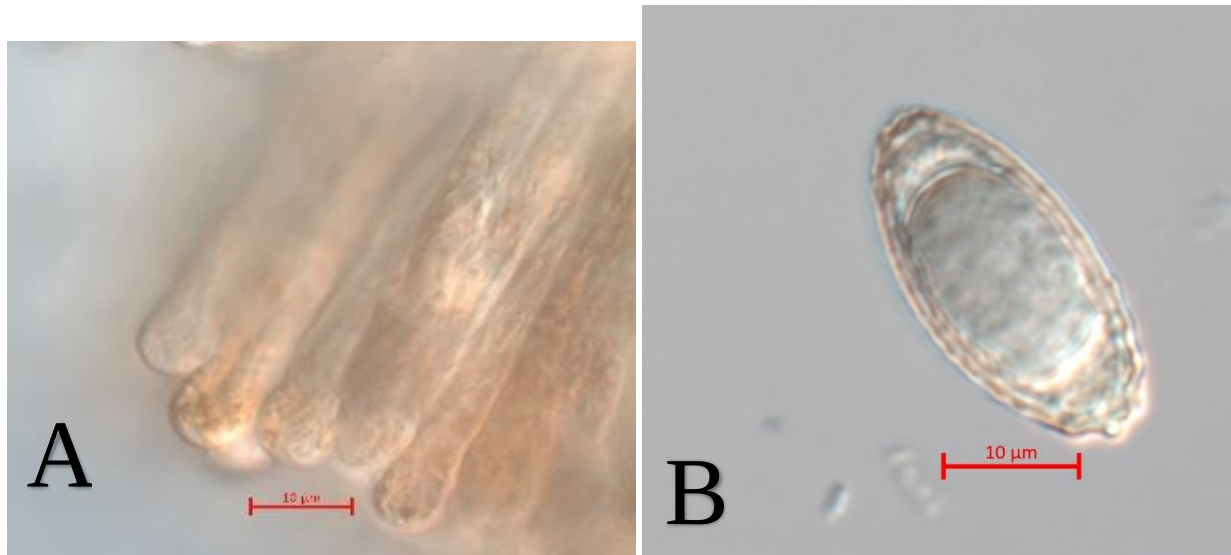


Fig. 22. *Gyromitra fastigiata* WU-Myc-0021318. A. Paraphyses. B. Ascospore with ornamentation. Scale bars A, B 10 μm .

Sample no. 40_*Gyromitra fastigiata*_WU_0008756

Microscopic characteristics: Ascospores: fusiform to ellipsoid, immatures look smooth but reticulate ornamentation, truncate to round small apiculi on both ends; uniguttulate. (23-)23,7-26,5(-27,6) (n=29) μm long, (10,1-)10,8-12,3(-13) (n=29) μm wide, quotient (1,9-)2-2,3(-2,5) (n=29) μm . Paraphyses: not measurable.

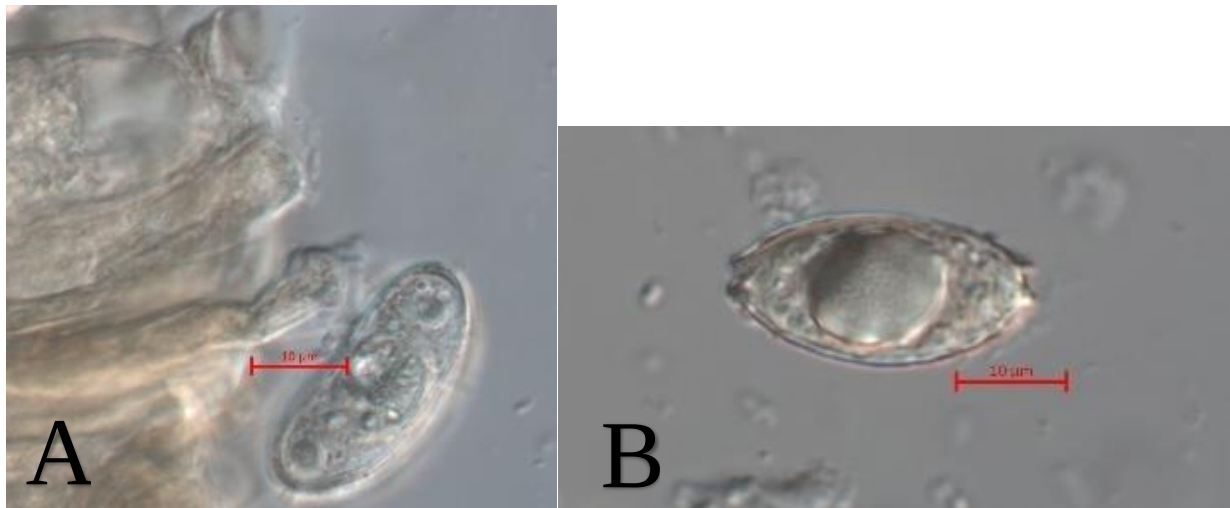


Fig. 23. *Gyromitra fastigiata* WU-Myc-0008756. A. Paraphyses with ascospore. B. Ascospore with ornamentation. Scale bars A, B 10 μm .

Sample no. 41_*Gyromitra fastigiata*_WU_0013814

Microscopic characteristics: Ascospores: fusiform to ellipsoid; immatures look smooth but reticulate ornamentation, truncate to round small apiculi on both ends; uni- to triguttulate. (23,6-)24,7-27,8(-28,8) (n=23) μm long, (11,4-)11,8-13,2(-14,2) (n=23) μm wide, quotient (1,8-)1,9-2,3(-2,5) (n=23) μm . Paraphyses: at apex 8,3 μm .

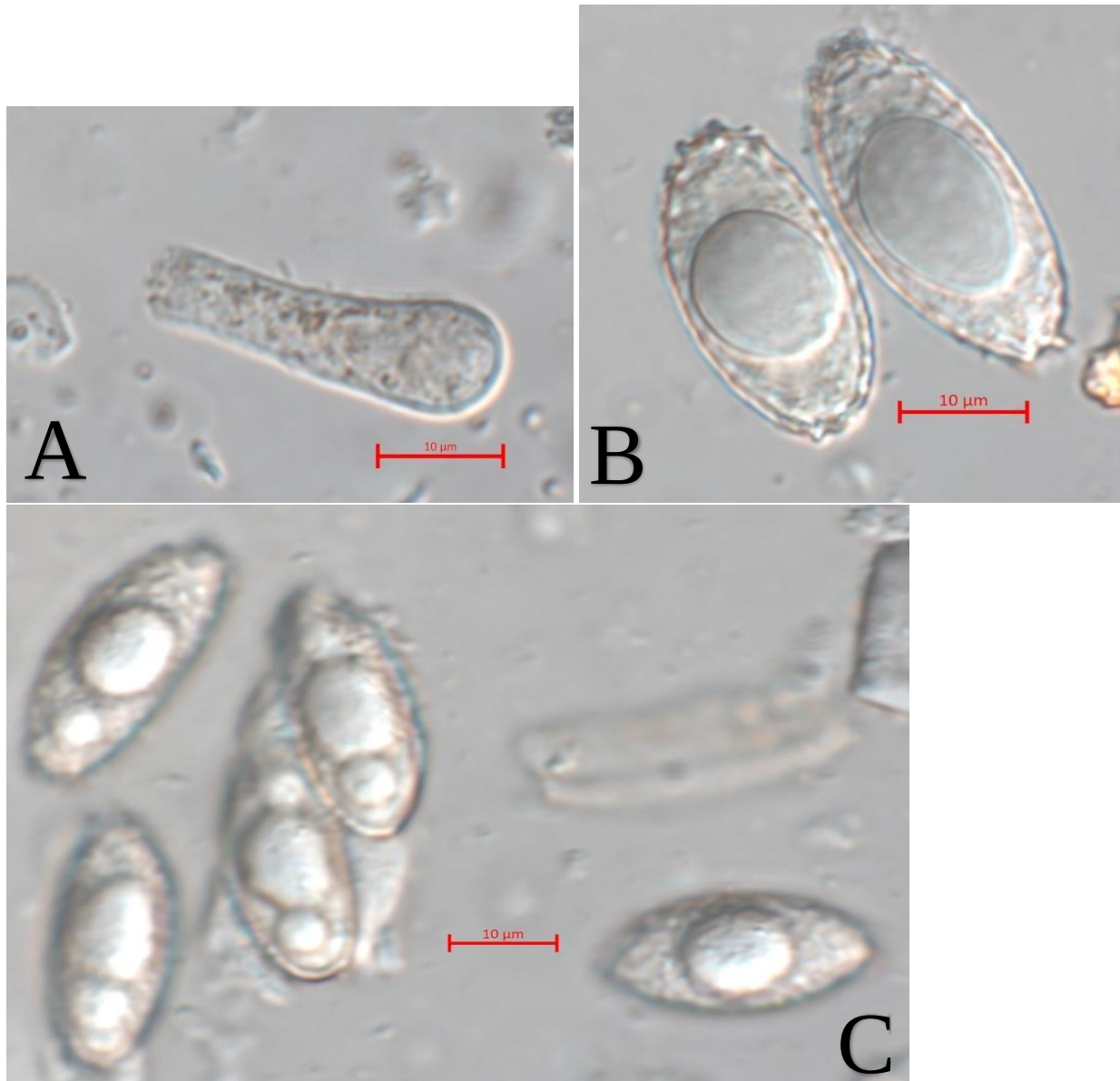


Fig. 24. *Gyromitra fastigiata* WU-Myc-0013814. A. Paraphyses. B. Ascospores with apiculi. C. Ascospores with ornamentation. Scale bars A-C 10 μm .

Sample no. 45_*Gyromitra_fastigiata*_WU_0040483

Microscopic characteristics: Ascospores: fusiform to ellipsoid; immatures look smooth but reticulate ornamentation, truncate to round small apiculi on both ends; uni- to triguttulate. (23,4-)24,1-26,4(-27,6) (n=17) μm long, (9,4-)10-11,6(-12) (n=17) μm wide, quotient (2,1-)2,2-2,5(-2,8) (n=17) μm . Paraphyses: at apex 7,6 μm and hyphal part under apex 5,9 μm .



Fig. 25. *Gyromitra fastigiata* WU-Myc-0040483. A. Paraphyses with ascospores. B. SAscospores with Paraphyses. Scale bars A, B 10 μm .

Gyromitra inflata Cooke, Mycogr., Vol. 1. Discom. (London)(no. 6): 248 (1879)

Sample no. 42_*Gyromitra inflata*_WU_0032695

Microscopic characteristics: Ascospores: ellipsoid; ornamentation smooth, thick-walled, no apiculi; biguttulate. (15,5-)16,6-18,5(-19,4) (n=15) μm long, (8,8-) 9,5-11(-11,4) (n=15) μm wide, quotient (1,6-)1,6-1,8(-1,9) (n=15) μm . Paraphyses: at apex 5,3 μm , hyphal part under apex 4,2 μm .

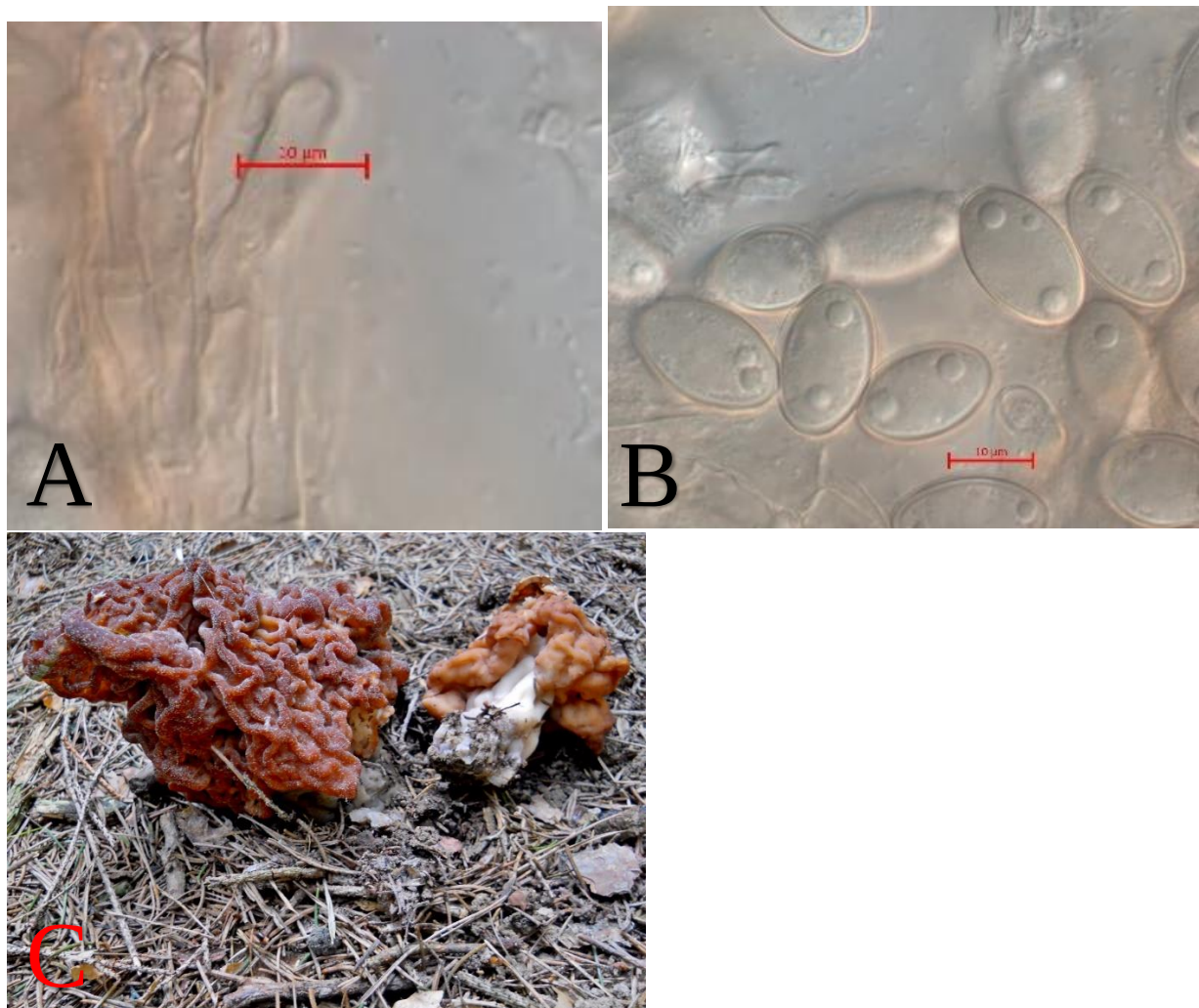


Fig. 26. *Gyromitra inflata* WU-Myc-0032695. A. Paraphyses. B. Ascospores. C. Ascomata (<https://pilzdaten-austria.eu/#tax/1000901>) phot. W. Klofac. Scale bars A,B 10 μm .

Sample no. 43_ *Gyromitra inflata* _WU_0044303

Microscopic characteristics: Ascospores: ellipsoid; ornamentation smooth, thick-walled, no apiculi; biguttulate. (16,8-)17,8-19,2(-19,4) (n=19) μm long, (9,1-)9,2-10(-10,4) (n=19) μm wide, quotient (1,7-)1,8-2(-2) (n=19) μm . Paraphyses: at apex 8 μm .

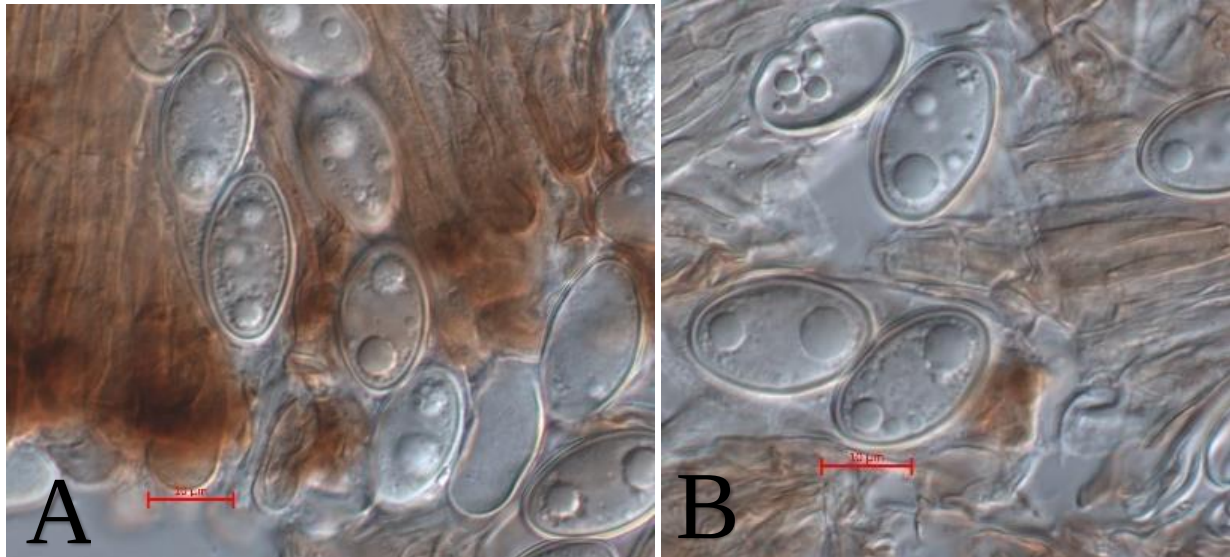


Fig. 27. *Gyromitra inflata* WU-Myc-0044303. A. Paraphyse with ascospores. B. Ascospores with Paraphyses. Scale bars A, B 10 μm .

Sample no.44_ *Gyromitra inflata* _WU_0043891

Microscopic characteristics Ascospores: ellipsoid; ornamentation smooth, thick-walled, no apiculi; biguttulate. (18-)19,3-21,9(-22,9) (n=34) μm long, (9,1-)9,7-11,4(-12) (n=34) μm wide, quotient (1,7-)1,8-2,1(-2,2) (n=34) μm . Paraphyses: at apex 6 μm .



Fig. 28. *Gyromitra inflata* WU-Myc-0043891. A. Paraphyses with ascospores. B. Ascospores. Scale bars A, B 10 μm .

Gyromitra infula (Schaeff.) Quél., Enchir. fung. (Paris): 272 (1886)

Sample no. 54_*Gyromitra infula*_WU_0010139

Microscopic characteristics: Ascospores: ellipsoid, hyalin; ornamentation smooth, no apiculi; biguttulate. (17,3-)17,5-20(-20,4) (n=4) μm long, (7,7-)7,6-8,6(-8,8) (n=4) μm wide, quotient (2,3-)2,3-2,4(-2,4) (n=4) μm . Paraphyses: not measurable due to clustering (Fig. A.).

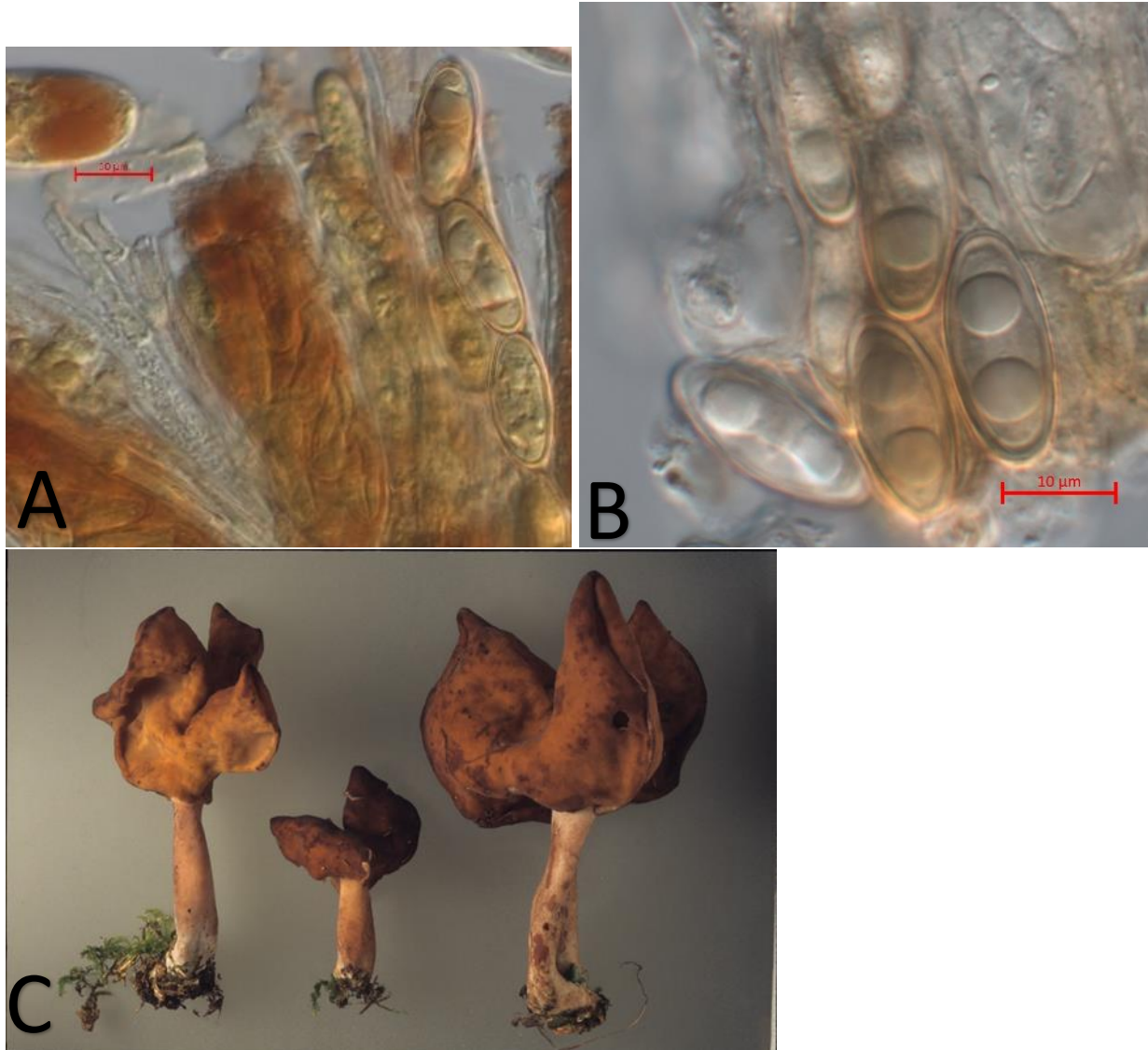


Fig. 29. *Gyromitra infula* WU-Myc-0010139. A. Paraphyses clustered. B. Ascospores. C. Ascomata (<https://pilzdaten-austria.eu/#tax/378406>), phot. I. Krisai-Greilhuber. Scale bars A, B 10 μm .

Sample no. 61_*Gyromitra infula*_WU_0027935

Microscopic characteristics: Ascospores: ellipsoid, hyalin; ornamentation smooth, no apiculi; biguttulate. (17,3-)17,3-17,9(-18,2) (n=7) μm long, (6,5-)6,5-7,1(-7,5) (n=7) μm wide, quotient (2,4-)2,5-2,7(-2,7) (n=7) μm . Paraphyses: at apex 8,3 μm , hyphal part under apex 5,9 μm .

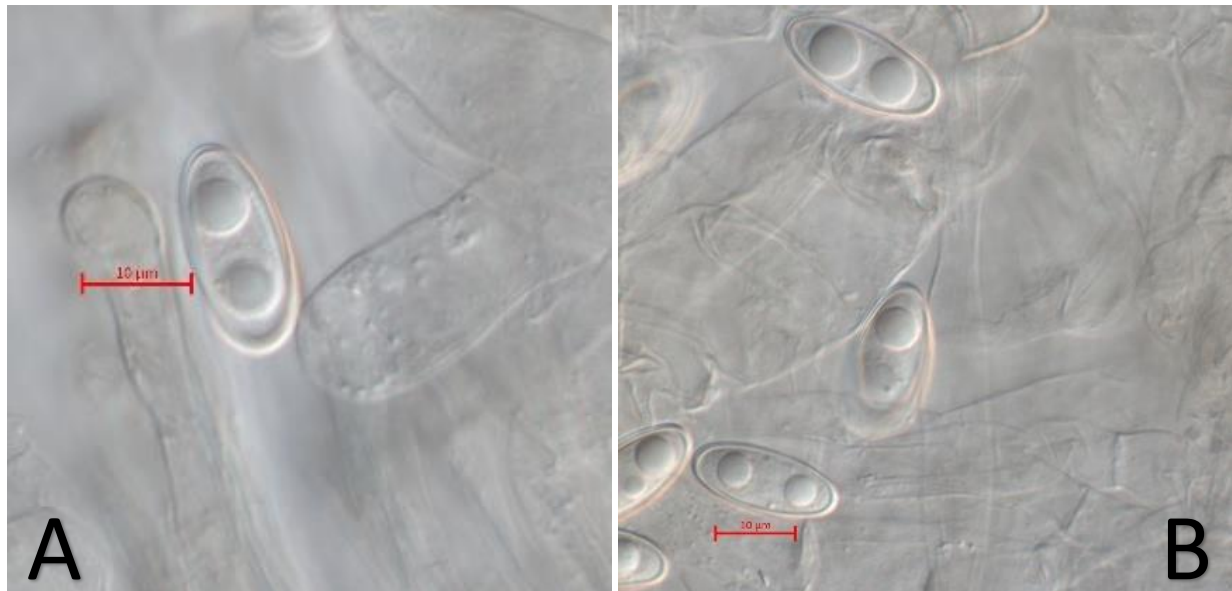


Fig. 30. *Gyromitra infula* WU-Myc-0027935. A. Paraphyses with ascospores. B. Ascospores. Scale bars A, B 10 μm .

Gyromitra ticiniana Littini, Pagine Botaniche 12: 19 (1988)

Sample no. 68_*Gyromitra ticiniana*_WU_0008714

Microscopic characteristics: Ascospores: ellipsoid to subfusoid, inequilateral, hyaline; ornamentation incomplete reticulum, blunt to truncate apiculi on both ends; triguttulate. (20,8-)21,7-24,6(-25,7) (n=28) μm long, (7,1-)8,2-9,5(-10,1) (n=28) μm wide, quotient (2,3-)2,4-2,8(-3,2) (n=28) μm . Paraphyses: cylindrical, septate, enlarged at apex, at apex 6,3 μm .

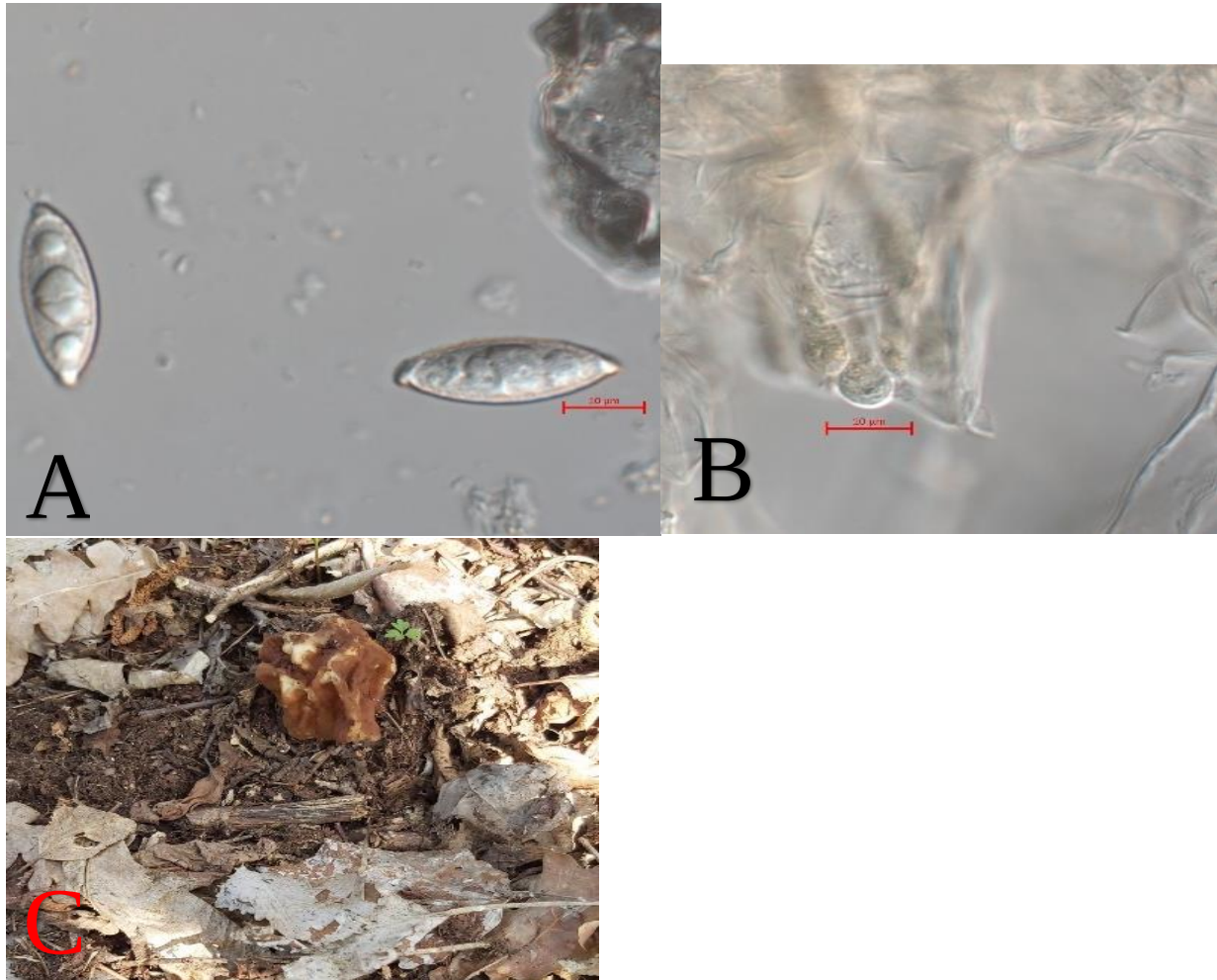


Fig. 31. *Gyromitra ticiniana* WU-Myc-0008714. A. Ascospores. B. Paraphyses. C. Ascomata (<https://pilzdaten-austria.eu/#tax/1000902>) phot. P. Kresitschnig. Scale bars A, B 10 μm .

Sample no. 69_ *Gyromitra ticiniana* WU_0039781

Microscopic characteristics: Ascospores: ellipsoid to subfusoid, sometimes inequilateral, hyaline; ornamentation incomplete reticulum, blunt to truncate apiculi on both ends; triguttulate. (24,6-)26,2-29,9(-31,6) (n=25) μm long, (10-)11,4-12,8(-13) (n=25) μm wide, quotient (1,9-)2,2-2,5(-2,6) (n=25) μm . Paraphyses: at apex 6,5 μm .

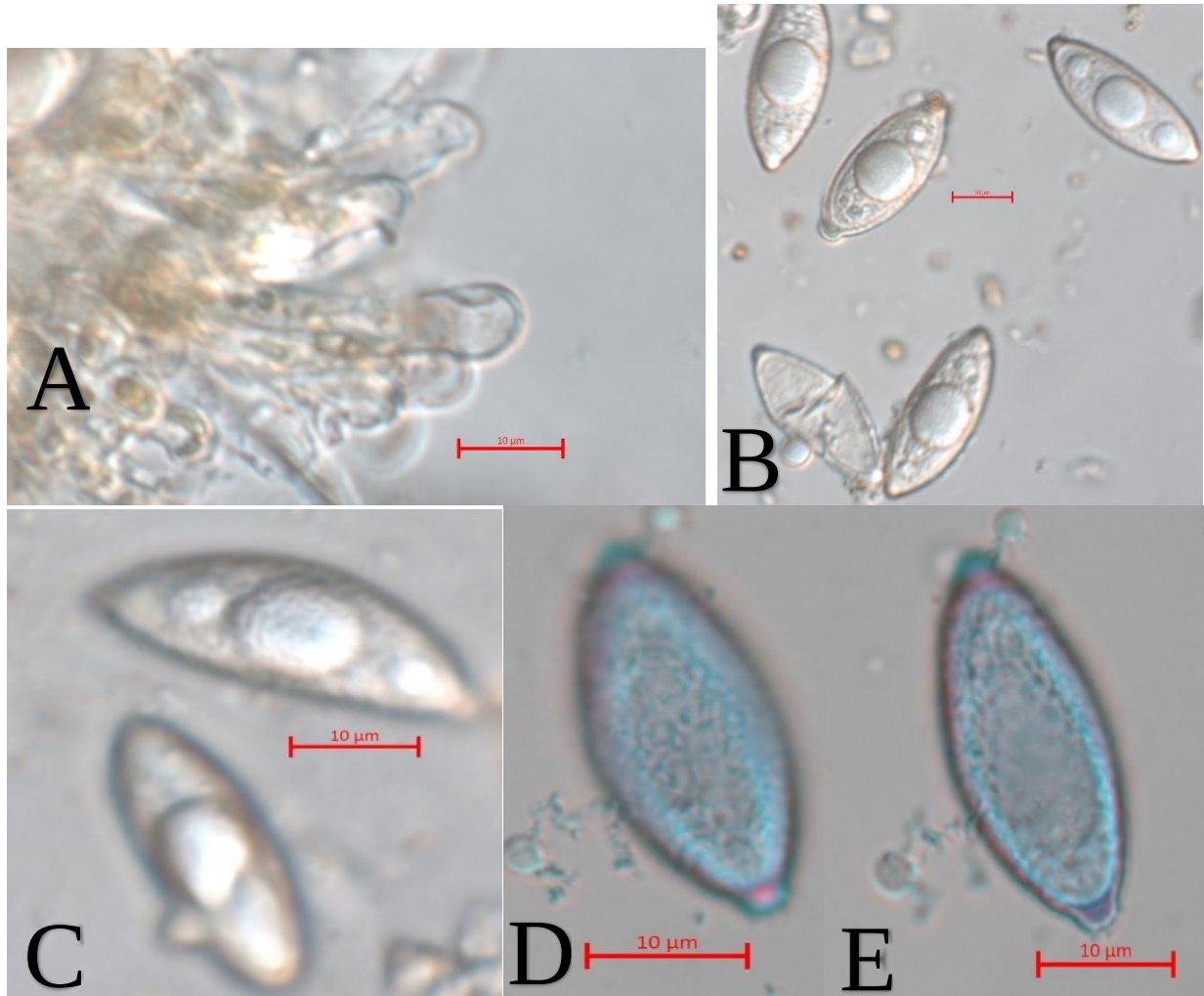


Fig. 32. *Gyromitra ticiniana* WU-Myc-0039781. A. Paraphyses. B. Ascospores. C. Ascospores with ornamentation. D. -Cotton blue stained ascospore. E. Cotton blue stained ascospore. Scale bars A-E 10 μm .

Gyromitra longipes Harmaja, Acta bot. fenn. 16(3): 159 (1979)

Sample no. 71_*Gyromitra longipes*_WU_0037177

Microscopic characteristics: Ascospores: subfusiform, hyaline; ornamentation incomplete reticulate, apiculi at both ends; triguttulate. (27,3-)27,8-29,5(-29,6) (n=6) μm long, (11,2-)11,4-12,5(-12,7) (n=6) μm wide, quotient (2,3-)2,3-2,5(-2,5) (n=6) μm . Paraphyses: not measurable due to clustering (Fig A.)

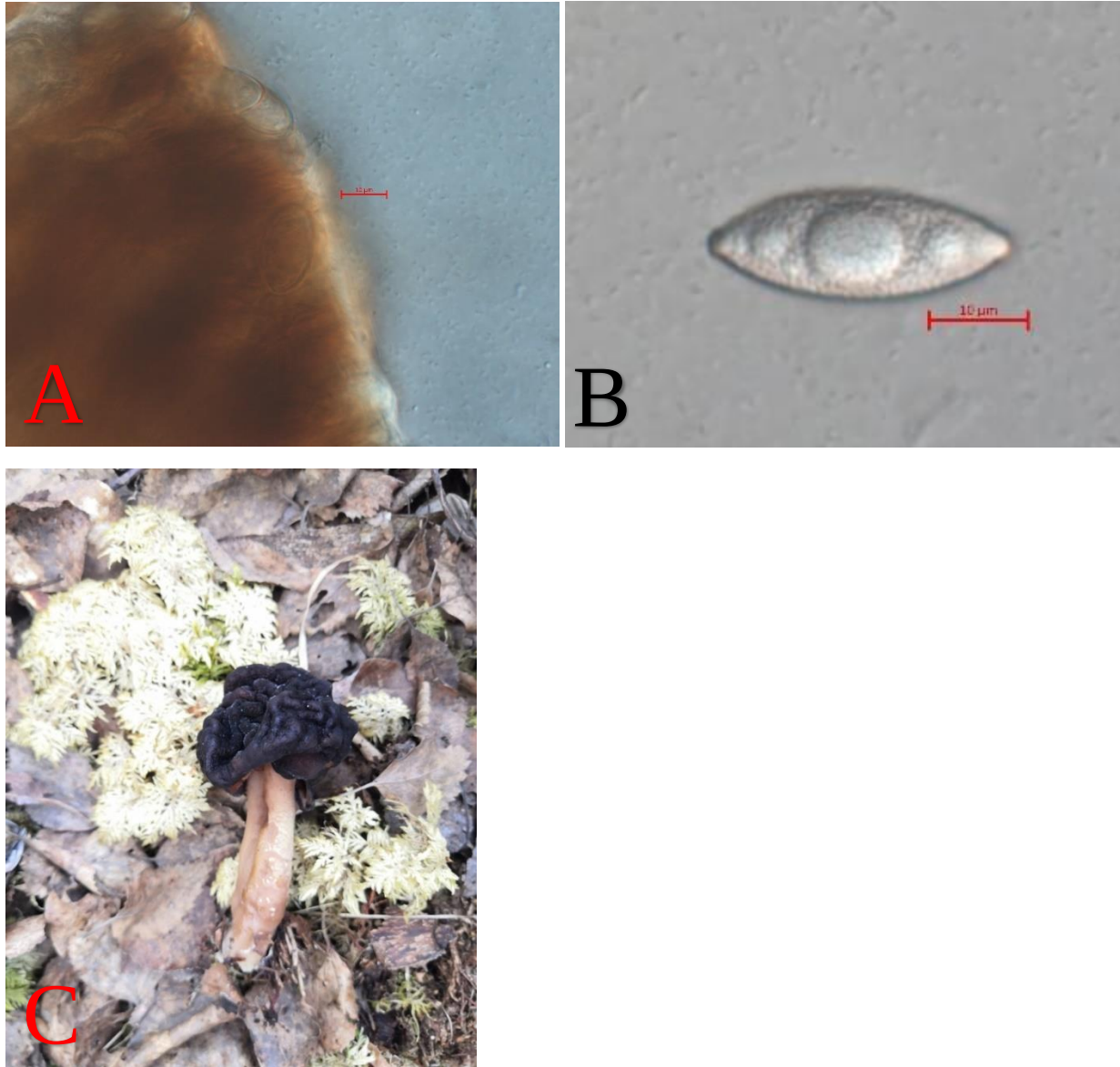


Fig. 33. *Gyromitra longipes* WU-Myc-0037177. A. Clustered Paraphyses. B. Ascospore with ornamentation. C. Ascomata (<https://www.inaturalist.org/observations/118529881>). Scale bars A, B 10 μm .

Tab. 7. Microscopical characteristics compared for all investigated species. Ascospore size range is the min and max of the samples, first size range is length and second width. Paraphyses size at apex is an average. Numeric metrics in μm .

Species	Ascospore size range	Ascospore morphology	Paraphyses at apex	Ornamentation	Apiculi	Guttula
<i>G. perlata</i>	21.7-33.9 $\times$ 8.6-13.1	ellipsoid, hyaline	8.2	fine netted, verucous	pointed	triguttulate
<i>G. apiculatula</i>	25.6-29.5 $\times$ 9.1-12.2	ellipsoid	8.5	smooth to faintly verucous- reticulate	broadly rounded to truncate	uniguttulate
<i>G. leucoxantha</i>	26.4-33.8 $\times$ 9.7-12.7	navicular	6.4	verrucous to incomplete reticulate	truncate- concave	uniguttulate
<i>G. parma</i>	19.1-28 $\times$ 9.3-13.9	ellipsoid to ellipsoidfusoid	7.8	warty, rough reticulate	many small pointed	uniguttulate
<i>G. esculenta</i>	17-25.9 $\times$ 8.5-13	ellipsoid, hyaline	6.5	smooth	no	biguttulate
<i>G. gigas</i>	20.8-34.6 $\times$ 7.7-13.1	ellipsoid to subfusoid, hyaline	7.8	incomplete reticulum	truncate	triguttulate
<i>G. fastigiata</i>	23.6-31 $\times$ 9.4-14.2	fusiform to ellipsoid	7.8	reticulate	truncate to round small	uniguttulate
<i>G. inflata</i>	15.5-22.9 $\times$ 8.8-12	ellipsoid	6.4	smooth, thick- walled	no	biguttulate
<i>G. infula</i>	17.3-20.4 $\times$ 6.5- 8.8	ellipsoid, hyaline	8.3	smooth	no	biguttulate
<i>G. ticiniana</i>	20.8-31.6 $\times$ 7.1-13	ellipsoid to subfusoid, inequilateral, hyaline	6.4	incomplete reticulum	blunt to truncate	triguttulate
<i>G. longipes</i>	27.3- 29.6 $\times$ 11.2-12.7	subfusiform, hyaline	not measurable	incomplete reticulate	yes	triguttulate

Genbank blast results

The sample sequences were blasted in GenBank to find the most similar sequences and the respective percentages of similarity (see appendix Table 9). Most of the samples matched with the original identification. Samples, which resulted in a different GenBank species name are given in Tab. 7.

Tab. 8. Sample identifications compared to resulting GenBank names of matches with the marker regions ITS, LSU and TEF.

Sample name	GenBank Species Name	Marker
8_ <i>Gyromitra gigas</i>	<i>Gyromitra ticiniana</i>	ITS
12_ <i>Gyromitra apiculatula</i>	<i>Gyromitra leucoxantha</i>	ITS
13_ <i>Gyromitra apiculatula</i>	<i>Discina accumbens</i>	LSU
15_ <i>Gyromitra geogenia</i>	<i>Discina ancilis</i>	LSU, TEF
18_ <i>Gyromitra parma</i>	<i>Discina brunnea</i>	LSU, TEF
20_ <i>Gyromitra parma</i>	<i>Discina brunnea</i>	LSU
25_ <i>Gyromitra longipes</i>	<i>Gyromitra esculenta</i>	ITS, LSU
28_ <i>Gyromitra gigas</i>	<i>Gyromitra korfii</i>	LSU
34_ <i>Gyromitra fastigiata</i>	<i>Gyromitra gigas</i>	ITS
40_ <i>Gyromitra fastigiata</i>	<i>Gyromitra brunnea</i>	LSU
41_ <i>Gyromitra fastigiata</i>	<i>Gyromitra esculenta</i>	ITS
42_ <i>Gyromitra inflata</i>	<i>Gyromitra esculenta</i>	LSU, TEF
43_ <i>Gyromitra inflata</i>	<i>Gyromitra esculenta</i>	LSU
44_ <i>Gyromitra inflata</i>	<i>Gyromitra esculenta</i>	ITS, LSU, TEF
45_ <i>Gyromitra slonevskii</i>	<i>Gyromitra fastigiata</i>	LSU, TEF
63_ <i>Gyromitra esculenta</i>	<i>Gyromitra infula</i>	TEF
69_ <i>Gyromitra ticiniana</i>	<i>Gyromitra gigas</i>	LSU, TEF
71_ <i>Gyromitra longipes</i>	<i>Gyromitra esculenta</i>	LSU, TEF

Reference Sequences

For the phylogenetic trees additional reference sequences were retrieved from GenBank (Tab. 9).

Tab.9. Sequences retrieved from GenBank for the markers ITS, LSU and TEF with GenBank numbers, voucher specimen numbers, country of origin and reference.

GenBank/Taxon	Voucher specimen no.	Country	ITS	LSU	TEF	Reference
<i>Disciotis venosa</i>	N. V.	China	JQ723149	/	/	Du et al. 2012
<i>D. venosa</i>	MICH:352035	USA: Michigan	MZ919223	/	/	Dirk A. C., unpubl.
<i>D. venosa</i>	N. V.	USA: Oregon	/	/	DQ471060	Spatafora et al. 2017
<i>D. venosa</i>	N. V.	USA: Washington State	/	/	KM034918	Carris et al. 2015
<i>D. venosa</i>	N. V.	USA	/	/	GU551575	Kerry O'Donnell et al. 2010
<i>D. venosa</i>	N. V.	USA	/	/	KM034916	Carris et al. 2015
<i>D. venosa</i>	O67664	Norway	/	KX008358.1	/	Stensrud O.
<i>D. venosa</i>	NY 01293394	USA	/	KC751497	/	Methven et al. 2013
<i>D. venosa</i>	OSC 100045	N. V.	/	AY544667	/	Spatafora et al. 2006
<i>Gyromitra perlata</i>	NV2014.05.14	China	/	MG847022.1	/	Wang & Zhuang 2018
<i>G. perlata</i>	HKAS87839	Thailand	MG871303	/	/	Ekanayaka et al. 2018
<i>G. perlata</i>	KH:KA20-0002	South Korea	MZ567192	/	/	Cho et al. 2021
<i>G. perlata</i>	HMAS 254607	France	MG846993	MG847022	MG847068	Wang & Zhuang 2018
<i>G. perlata</i>	NY 01797016	USA: Illinois	/	KC751514	/	Methven et al. 2017
<i>G. gigas</i>	TUR-A 208088	Czech Republic	MH938663	MH938309	/	Carbone et al. 2018
<i>G. gigas</i>	TUR-A 208089	Italy	MH938666	MH938312	/	Carbone et al. 2018
<i>G. gigas</i>	TUR-A 208091	Italy	MH938667	MH938313	/	Carbone et al. 2018
<i>G. gigas</i>	TAAM190163	Estonia	MW076963	MW076976	/	Miller et al. 2020
<i>G. gigas</i>	O174609	Norway	MW076964	KX008328	/	Miller et al. 2020
<i>G. gigas</i>	O174628	Norway	MW076965	KX008329	/	Miller et al. 2020
<i>G. gigas</i>	OULU-F 23717; ILLS00121402	Finland	MW076967	/	/	Miller et al. 2020
<i>G. gigas</i>	LY NV 2007.04.20	France	MH938665	MH938311	/	Carbone et al. 2018
<i>G. gigas</i>	HMAS254604; ILLS00121400	France	MG846996	MG847005	MG847051	Wang & Zhuang 2018

<i>G. gigas</i>	LK95 04 08	Hungary	MH938664	/	/	Carbone et al. 2018
<i>G. gigas</i>	HMAS89008	China: Jilin	/	MG847004	MG847050	Wang & Zhuang 2018
<i>G. gigas</i>	YSU-F-08006; ILLS00121415	Russia: Khanty-Mansiyskiy	/	MW076981	/	Miller et al. 2020
<i>G. gigas</i>	HKAS107386	China: Tibet	/	/	MT522868	Li, H. J., et al. 2020
<i>G. leucoxanta</i>	TKL395	Austria: Tyrol	/	KX185091	/	Miller et al. 2015
<i>G. leucoxanta</i>	HMAS 279665	Spain	/	MG847020	MG847066	Wang & Zhuang 2018
<i>G. leucoxanta</i>	NY 01797015	USA	/	KC751515	/	Methven et al. 2017
<i>G. leucoxanta</i>	NY 01797014	USA	/	KC751516	/	Methven et al. 2017
<i>G. ticiniana</i>	TUR-A 208095	Italy	MH938672	MH938317	/	Carbone et al. 2018
<i>G. ticiniana</i>	TUR-A 208096	Italy	MH938673	MH938318	/	Carbone et al. 2018
<i>G. ticiniana</i>	ILLS00121412	France	MH938670	MH938315	/	Carbone et al. 2018
<i>G. ticiniana</i>	ILLS00121412	France	/	MH938315	/	Carbone et al. 2018
<i>G. korfii</i>	CUP 28997	USA: North Carolina		MW078425	/	Miller et al. 2020
<i>G. korfii</i>	NY 01797011	USA: Pennsylvania		KC751513	/	Methven et al. 2013
<i>G. korfii</i>	NY 01797010	USA: Illinois	/	KC751511	/	Miller et al. 2020; Methve et al. 2013
<i>G. korfii</i>	S. D. Russell Myco Map #5452	USA: Indiana	MZ667891	/	/	Russell S. D. 2021
<i>G. korfii</i>	CUP28997	USA: North Carolina	MW075385	/	/	Miller A. N. 2021
<i>G. melaleuca</i>	HMAS 279666	France	/	MG847020	MG847066	Wang & Zhuang 2018
<i>G. esculenta</i>	HMAS 254602	France	MG846990	MG847002	MG847048	Wang & Zhuang 2018
<i>G. esculenta</i>	HMAS 39743	Bulgaria	MG846989	/	/	Wang & Zhuang 2018
<i>G. esculenta</i>	m954	UK	AJ544209	/	/	Kellner et al. 2007
<i>G. esculenta</i>	11-12-BC1-1	USA: Idaho	KM204672	KM204697	KM034920	Carris et al. 2015
<i>G. esculenta</i>	HMAS 51681	USA: Wisconsin	MG846988	MG847001	MG847047	Wang & Zhuang 2019
<i>G. esculenta</i>	NY 01797004	USA	/	KC751504	/	Methven et al. 2017
<i>G. esculenta</i>	NY 01797005	USA	/	KC751502	/	Methven et al. 2017
<i>G. esculenta</i>	BPI 566942	N.V.	/	KC751503	/	Methven et al. 2017
<i>G. esculenta</i>	KA19-0028	Pyeongchang-gun, Korea	MZ567191	MZ573190	/	Cho et al. 2021

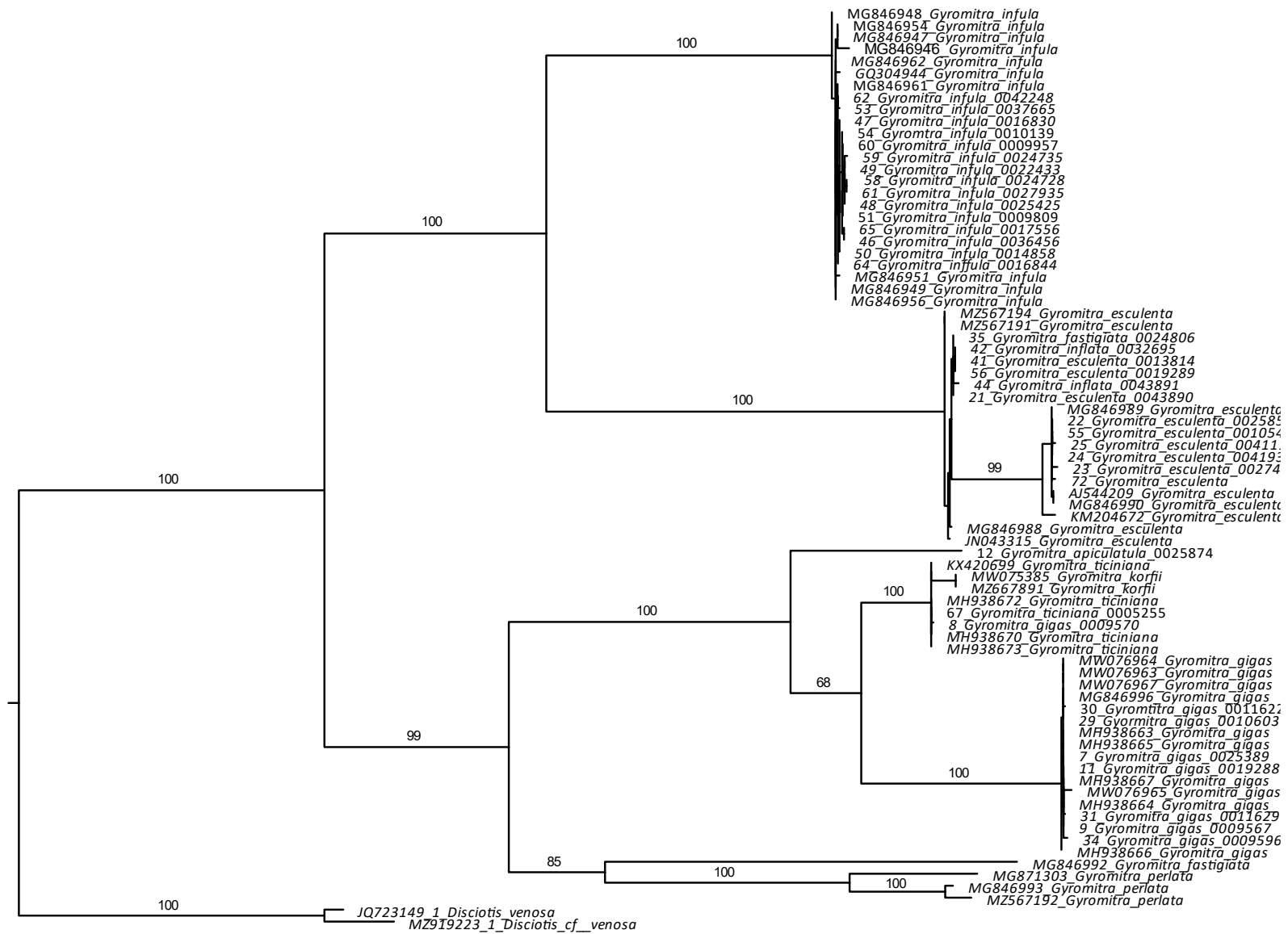
<i>G. esculenta</i>	KA21-0149	Pyeongchang-gun, Korea	MZ567194	MZ573193	/	Cho et al. 2021
<i>G. infula</i>	HMAS 51194	China: Sichuan	MG846946	/	/	Wang & Zhuang 2018
<i>G. infula</i>	HMAS 57849	China: Tibet	MG846947	MG847011	MG847057	Wang & Zhuang 2018
<i>G. infula</i>	HMAS 86049	China: Xinjiang	MG846948	MG847012	MG847058	Wang & Zhuang 2018
<i>G. infula</i>	HMAS 86054	China: Xinjiang	MG846949	MG847013	MG847059	Wang & Zhuang 2018
<i>G. infula</i>	HMAS 86055	China: Xinjiang	/	MG847014	MG847060	Wang & Zhuang 2018
<i>G. infula</i>	HMAS 86056	China: Xinjiang	MG846951	MG847015	MG847061	Wang & Zhuang 2018
<i>G. infula</i>	HMAS 99334	China: Tibet	MG846954	/	/	Wang & Zhuang 2018
<i>G. infula</i>	HMAS 250470	China: Yunnan	MG846956	MG847006	MG847052	Wang & Zhuang 2018
<i>G. infula</i>	HMAS 254605	France	MG846961	MG847018	MG847064	Wang & Zhuang 2018
<i>G. infula</i>	HMAS 254606	France	MG846962	MG847019	MG847065	Wang & Zhuang 2018
<i>G. infula</i>	QFB9249	Canada: Quebec	GQ304944	/	/	Stefani et al. 20120
<i>G. infula</i>	NY 01797006	USA	/	KC751508	/	Methven et al. 2017
<i>G. infula</i>	ILLS 54774	Thailand	/	KC751507	/	Ekanayaka et al. 2018
<i>G. infula</i>	NY 01797007	USA: Illinois	/	KC751509	/	Methven et al. 2017
<i>G. infula</i>	HKAS 83612	Thailand	/	MG871347	/	Ekanayaka et al. 2018
<i>G. brunnea</i>	NY 01293396	USA	/	KC751520	/	Methven et al. 2017
<i>G. brunnea</i>	NY 01293396	USA	/	KC751520	/	Methven et al. 2017
<i>G. brunnea</i>	NY 01293397	USA	/	KC751521	/	Methven et al. 2017
<i>G. brunnea</i>	NY 01293398	USA	/	KC751522	/	Methven et al. 2017
<i>G. brunnea</i>	NY 01797001	USA	/	KC751523	/	Methven et al. 2017
<i>G. brunnea</i>	N. V.	USA	/	ON561391.1	/	Mycoflora of Indiana, Fungal Diversity Survey (funga.org) , Russel S. D.
<i>G. fastigiata</i>	NV2004.05.01	France	MG846992	MG847003	MG847049	Wang & Zhuang 2018
<i>G. fastigiata</i>	V. Somà 01057	Italy	/	MH938321	/	Carbone et al. 2018
<i>Gyromitra accumbens</i>	N. V.	Norway	/	KX008331.1		Miller, unpubl.

Phylogenetic analysis

The ITS dataset was from all markers the one with the most gaps over the alignment, which was 1378 bp long. The phylogenetic tree for the ITS marker is a maximum likelihood tree (Fig. 34) generated with RAxMLGUI. The substitutions per site are indicated as a scale bar on the bottom. One can observe the evolutionary diversification of each *Gyromitra* group. *Disciotis venosa* was used as outgroup.

ITS marker region

The first node separates the species into two clades. The upper clade (upper on this tree configuration, which is interchangeable) having *G. infula* situated next to the clade of *G. esculenta*, both as sister groups to each other. Within the *Gyromitra esculenta* s.l. clade there are one sample of *G. fastigiata* and two of *G. inflata* in one subclade together. . The sister clade to the *G. infula-G.esculenta* s.l. clade splits in two main subclades: the *apiculata-ticiniana-korfii-gigas*-clade and a *fastigata-perlata*-clade. The *Gyromitra apiculatula* subclade is the first one to split at the node and stands alone as a sister to the *ticiniana-korfii-gigas* clade. At the next node there is a subclade (2) with *G. ticiniana* and *G. korfii*, but *G. korfii* being separated from *G. ticiniana* within it. The other subclade (3) contains *G. gigas*. These three subclades are sister to the *fastigiata-perlata* clade. Further all *G. perlata* samples are grouped together.



0.09

Fig. 34. Maximum likelihood tree for all *Gyromitra* ITS samples and reference species included in the study, calculated with raxmlGUI with bootstrap support values (>50, obtained from rapid bootstrap). Graph depicted in Figtree, names and bootstrap values enlarged and changed with Inkscape. Scale bar indicates number of substitutions per site, result in 9 base differences for every 100 bases.

LSU marker region

The LSU dataset had almost no gaps, with the alignment being 887 bp long. The phylogenetic tree for the LSU marker is a maximum likelihood tree (Fig. 35) and was generated with RAxMLGUI. The substitutions per site are indicated as a scale bar on the bottom. *Disciotis venosa* was used as outgroup. At the first node *G. infula* branches off as a sister to all others. *Gyromitra infula* is forming two subgroups with one outlier splitting off (KC751507). At the next node *G. esculenta*, *G. inflata* and 71_*G. longipes*_WU_0037177 split off. *Gyromitra inflata* and *G. longipes* are placed in two different subgroups within the *G. esculenta* clade.

At the next node *G. leucoxantha* splits off, forming two subgroups.

At the next node two clades split off. One of the *G. perlata* samples branches off as the first sister to the rest. The next node splits into several other *G. perlata* samples versus *G. brunnea* with *G. parma* and a *G. fastigiata* subclade. In the second clade a *leucoxantha-accumbens-apiculatula* clade branches off versus a *ticiana-korfii-gigas*-clade, which exhibits further a subclade of *G. ticianiana* with *G. korfii* versus a last clade consisting of two *G. gigas* subgroups (incl. a misidentified *G. ticianiana* sample).

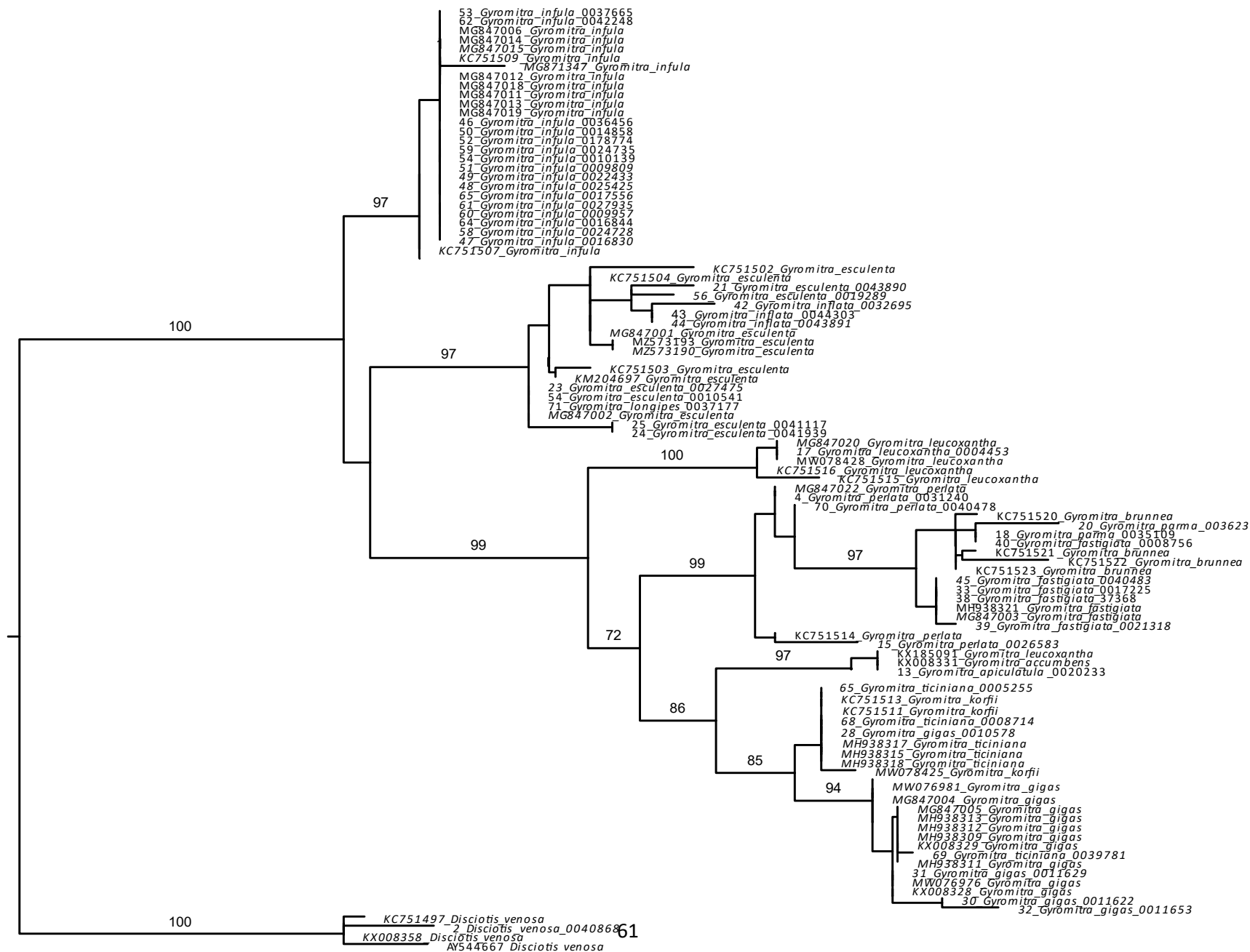
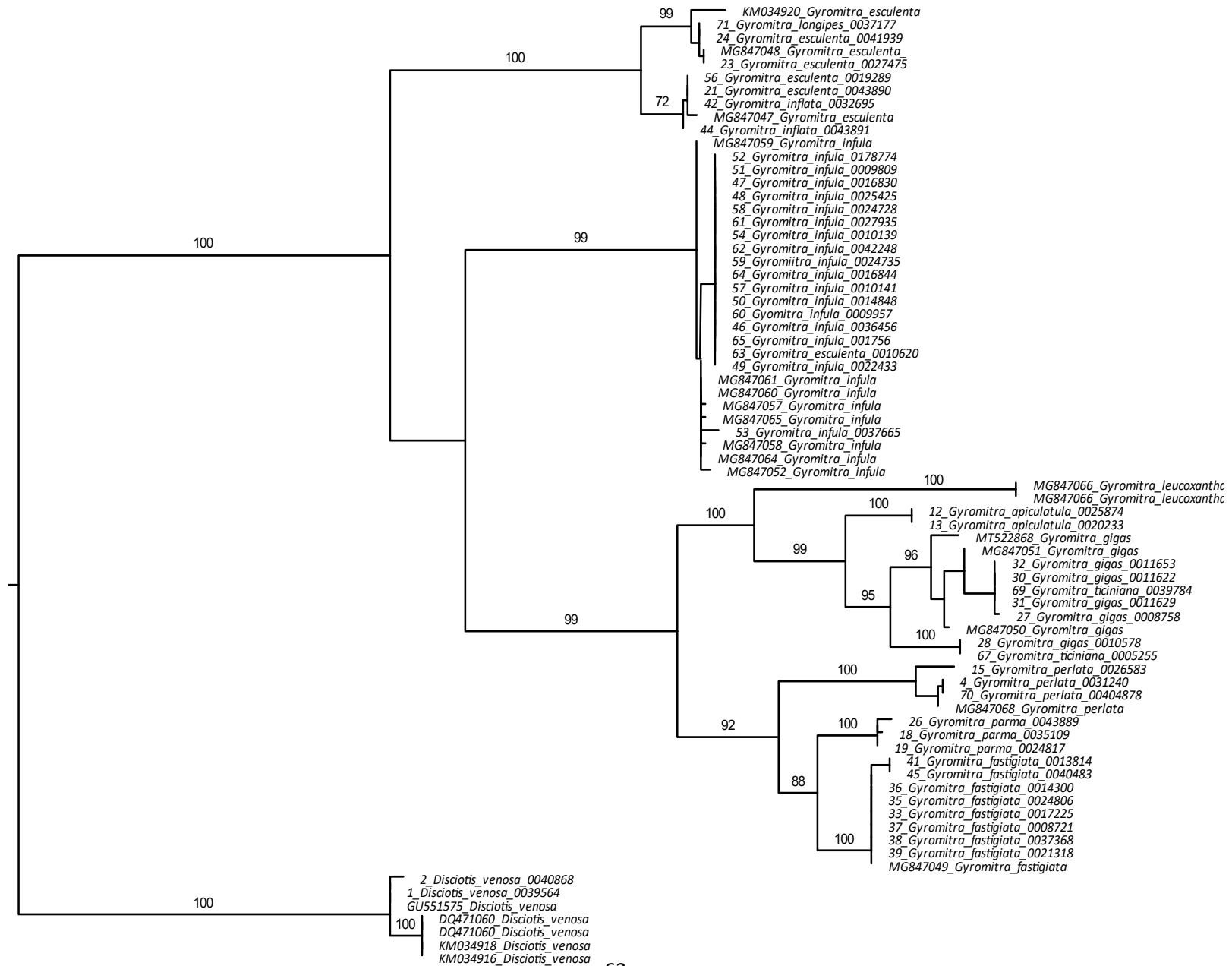


Fig. 35. Maximum likelihood tree for all LSU samples and references of this study, calculated with raxmlGUI with bootstrap support values (>50, obtained from rapid bootstrap). Graph depicted in Figtree. The graph was refined with Inkscape. Scale bar indicates number of substitutions per site, result in 0.7 base differences for every 100 bases.

TEF region marker

The TEF dataset had a few gaps, with the alignment being 693 bp long. The phylogenetic tree for the TEF marker is a maximum likelihood tree (Fig. 36) and was generated with RAxMLGUI. The substitutions per site are indicated as a scale bar on the bottom. *Disciotis* was used as outgroup. At the first node the clade *esculenta-longipes-inflata* splits off. There are two subclades, with 71_ *G. longipes_WU_0037177* and *G. inflata* being nested in one of them. The subclade of *G. infula* splits off next. *Gyromitra infula* is separated into subgroups. At the next node two clades split off. The first one being comprised of *G. leucoxantha*, which splits off first, followed by *G. apiculatula* and finally *G. gigas* being sister to *G. ticiniana*. The other clade consists of *G. perlata* as the first one to split off. Followed by *G. parma* and *G. fastigiata* as sister taxon.

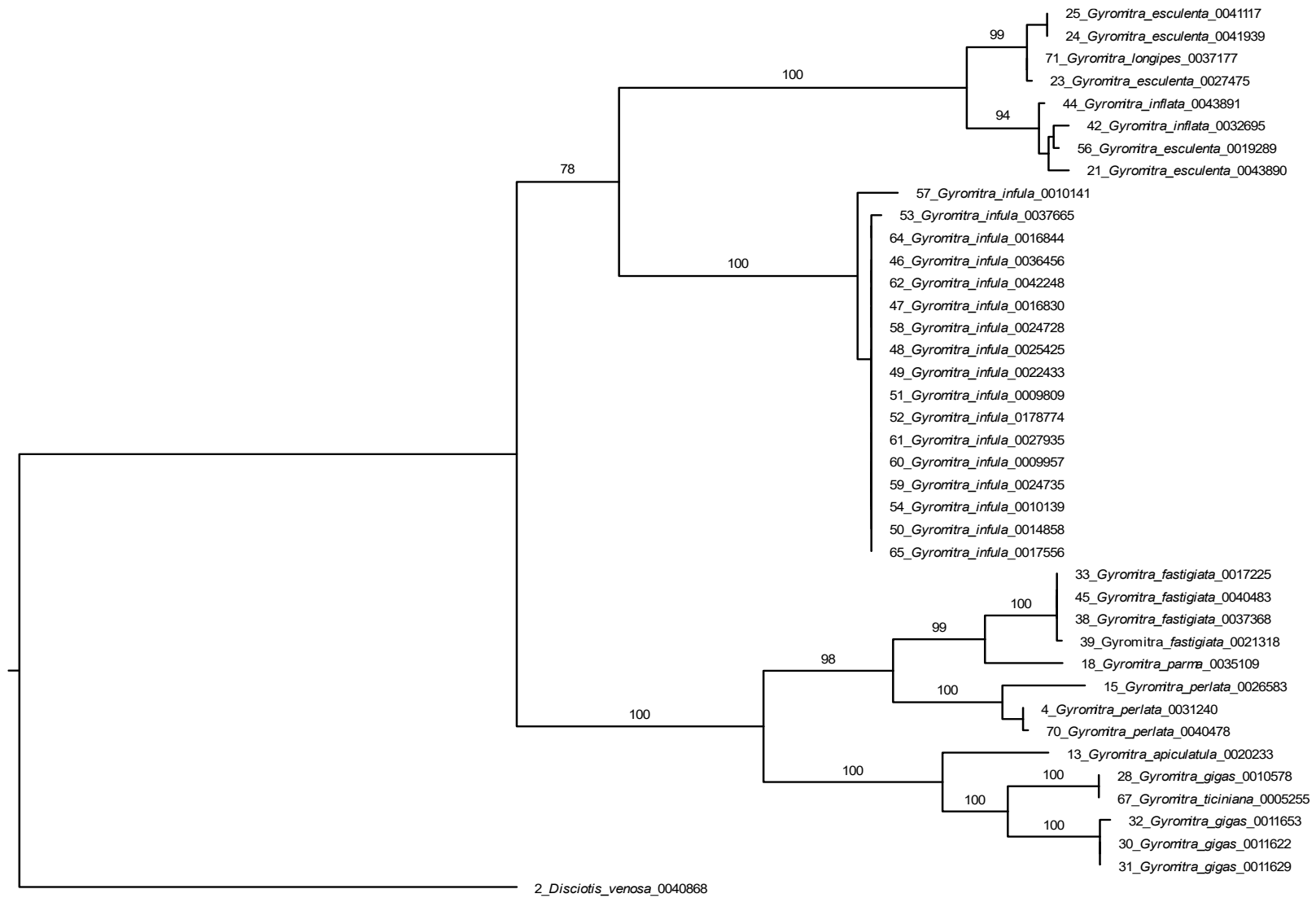


0.04

Fig. 36. Maximum likelihood phylogenetic tree for all TEF rRNA samples and reference species, calculated with raxmlGUI with bootstrap support values (>50, obtained from rapid bootstrap). Graph depicted in Figtree. The graph was refined with Inkscape. Scale bar indicates number of substitutions per site, result in 4 base differences for every 100 bases.

LSU and TEF marker regions concatenated

The concatenated LSU and TEF dataset had the least gaps overall, with the alignment being 1519 bp long. The phylogenetic tree for the LSU and TEF marker is a maximum likelihood tree (Fig. 37) and was generated with RAxMLGUI. The substitutions per site are indicated as a scale bar on the bottom. *Disciotis venosa* was used as outgroup. At the first node two clades split off. The first clade consists of two subclades. The first consisting of two subclades comprising samples originally identified as *G. esculenta* in both together with *G. longipes* and *G. inflata*. The second branch consists of *G. inflata*, with only one specimen (57_*Gyromitra inflata*_0010141) splitting off into a subgroup. The other major clade, splits off at the first node into two separate branches. With the first one consisting of *G. fastigiata* and *G. parma* being sister taxa. The second branch consisting of *G. perlata*, with one specimen being in another subgroup (15_*Gyromitra perlata*_0026583). The first species that branched off at the second clade is *G. apiculatula* as a sister to the rest. At the next node *G. ticiniana* branches off and on the other branch *G. gigas* is placed, with one subgroup species (32_*Gyromitra gigas*_0011653).



0.02

Fig. 37. Maximum likelihood tree for the concatenated matrix of LSU and TEF of all this study, calculated with raxmlGUI with bootstrap support values (>50, obtained from rapid bootstrap). Graph depicted in Figtree. The graph was refined with Inkscape. Scale bar indicates number of substitutions per site, result in 2 base differences for every 100 bases.

Discussion

Species identification

There were some difficulties with assigning the correct species to the samples for the phylogenetic analysis (see Tab. 8). To resolve this issue morphological characters, namely the microscopic characteristics were considered for all of them. For most of the species the microscopic characters showed that the specimens were originally correctly identified. Although some of them were grouped together in the phylogenetic tree with another species. A simple explanation would be that the samples have been switched in one of the many steps of the DNA-sequencing process.

Four samples have been rearranged from their originally identified species group. Due to them being situated in different species on the phylogenetic tree and, when looking at the microscopical characteristics, they matched with the newly identified species. The first one is 15_*Gyromitra geogenia*_WU_0026583, which had the ascospore characters of *Gyromitra perlata*, so this species was assigned. In the phylogenetic trees this specimen was grouped together with other *Gyromitra perlata* samples. The second one is 25_*Gyromitra longipes*_WU_0041117, which had the ascospore characters of *Gyromitra esculenta*. This specimen and the other *Gyromitra longipes* were both grouped together with *Gyromitra esculenta* ss. str. in two different closely related subclades. However, specimen number 25 was misidentified. The third one is 34_*Gyromitra fastigiata*_WU_0009596, which in the ITS phylogenetic tree was grouped together with *Gyromitra gigas* samples. When looking at the ascospore characteristics it became clear that it is a *G. gigas* specimen, due to the size and shape of the apiculi. The fourth one is 45_*Gyromitra slonevskii*_WU_0040483, which has the ascospore characters of *Gyromitra fastigiata*. In the phylogenetic trees specimen number 45 was grouped together with *Gyromitra fastigiata* species. It may be the case that for the separation of *Gyromitra slonevskii*, which clearly has different ecological needs (montane versus thermophile), from *G. fastigiata* other markers would be more useful.

Microscopic characters

The *Gyromitra perlata* specimens, when compared to Van Vooren & Moreau (2009) and Cho et al. (2021), have all the same microscopical ascospore characteristics though differing quite a bit in ascospore size ranges. 4_*Gyromitra perlata*_WU_0031240 had an ascospore size range of $21.7\text{--}28.3 \times 8.6\text{--}13.1 \mu\text{m}$, with paraphysis apical diameter of $9 \mu\text{m}$. 15_*Gyromitra perlata*_WU_0025583 had an ascospore size range of $26.6\text{--}33.9 \times 8.9\text{--}11.2 \mu\text{m}$, with paraphysis apical diameter of $.3$. Van Vooren & Moreau (2009) give an ascospore size range of $27\text{--}40 \times 12\text{--}15 \mu\text{m}$. In Cho et al. (2021) a French specimen had an ascospore size range of $40\text{--}46 \mu\text{m} \times 13\text{--}17 \mu\text{m}$ and the Korean specimen $22.5\text{--}27 \times 11\text{--}13 \mu\text{m}$, with paraphysis diameter of $6\text{--}9 \mu\text{m}$.

When comparing those metrics, the high variability of the ascospore size is quite obvious, the specimens were more similar in size to the Korean one. Summed up through the mentioned metrics and the markers used the specimens seem to be correctly identified but maybe some infraspecific taxonomical subdivision will come up due to the differing spore size ranges.

The *Gyromitra apiculatula* specimens, when compared to McKnight (1969), have consistent microscopical ascospore characters. 12_*Gyromitra_apiculatula*_WU_025874 had an ascospore size range of $27.8-27.9 \times 11.1-12.1 \mu\text{m}$, with a paraphysis apical diameter of $8 \mu\text{m}$. 13_*Gyromitra_apiculatula*_WU_0020233 had a spore size range of $25.6-29.5 \times 9.1-12.2 \mu\text{m}$, with a paraphysis apical diameter of $9 \mu\text{m}$. McKnight (1969) gives an ascospore size range of $28.5-33.4 \times 13.2-17.6 \mu\text{m}$, with paraphysis apical diameter of $3,8-7,6 \mu\text{m}$, so in conclusion our specimens were correctly identified.

Gyromitra leucoxantha ascospore microscopic characteristics are the same when compared to Van Vooren & Harmaja (1969). However, the size of the ascospores can vary greatly. The specimen 17_*Gyromitra_leucoxantha*_WU_0004453 had an ascospore size range of $26.4-33.8 \times 9.7-12.7 \mu\text{m}$ and paraphysis apical diameter of $6.4 \mu\text{m}$. Harmaja (1969) gives an ascospore size range of $52-56 \mu\text{m} \times 12-15 \mu\text{m}$ and paraphysis apical diameter of $8 \mu\text{m}$. Van Vooren & Moreau (2009) specify a spore size range of $25-35 \times 10-15 \mu\text{m}$. The comparison of ascospore length with Harmaja (1969) shows a high difference, but with Van Vooren & Moreau (2009) it was the same. So the specimen has to be considered as *G. leucoxantha* ss Van Vooren & Moreau.

The samples studied of *Gyromitra parma* showed the same overall spore characteristics as given in Peric (2005) and Breitenbach & Kränzlin (1984). The only differences were in ascospore and paraphyse size ranges. 18_*Gyromitra_parma*_WU_0035109 had an ascospore size range of $19.1-28 \times 9.3-13.9 \mu\text{m}$, with a paraphysis apical diameter of $8,2 \mu\text{m}$. 19_*Gyromitra_parma*_WU_0024817 had an ascospore size range of $21.3-26.7 \times 10.1-13.1 \mu\text{m}$, with a paraphysis apical diameter of $7.3 \mu\text{m}$. 20_*Gyromitra_parma*_WU_0036232 had an ascospore size range of $24.9-27.9 \times 11.6-13.6 \mu\text{m}$, with a paraphyse apical diameter of $8,1 \mu\text{m}$. Peric O. (2005) gives an ascospore size range of $28-32 \times 12-16 \mu\text{m}$, with a paraphysis diameter of $5-6 \mu\text{m}$. Breitenbach & Kränzlin (1984) give a spore size range of $25-32.5 \times 11-12.5 \mu\text{m}$, with a paraphysis apical diameter of $5-6 \mu\text{m}$. The ascospore size metrics are similar in comparison, so the samples were correctly identified.

Gyromitra esculenta specimens, when compared to Harmaja (1979) and Breitenbach & Kränzlin (1984), had the same microscopic characteristics of the spores. 21_*Gyromitra esculenta*_WU_0043890 had an ascospore size range of $17-22.2 \times 8.5-11.9 \mu\text{m}$, with a paraphysis apical diameter of $4.9 \mu\text{m}$. 25_*Gyromitra esculenta*_WU_0041117 had an ascospore size range of $19.3-25.9 \times 8.1-10.8 \mu\text{m}$. Harmaja (1979) gives an ascospore size range of $18-23 \times 10-12.5 \mu\text{m}$. 63_*Gyromitra esculenta*_WU_001620 had an ascospore size range of $18,3-21,7 \times 9,2-11,2 \mu\text{m}$, with a paraphysis apical diameter of $8.1 \mu\text{m}$. According to Breitenbach & Kränzlin (1984) specimens of one collection site had an ascospore size range of $16-21 \times 8-10 \mu\text{m}$ and from another $18-30 \mu\text{m} \times 9-12 \mu\text{m}$, with a paraphysis apical diameter of $6-8 \mu\text{m}$. So already in Breitenbach & Kränzlin (1984) differing spore size ranges are listed arising the suspicion that maybe several taxonomic entities could be involved. There are a lot of older taxa which have not yet been thoroughly restudied with modern approaches, e.g., *G. bubakii* Velen. which was already considered as a large-spored form of *G. esculenta*, namely as *G. esculenta* var. *bubakii* (Velen.) J. Moravec by Moravec (1986). When the specimens are compared to the literature, it shows that the spore size was in a similar range. Further, it is not easy to distinguish *G. esculenta* ss. str. microscopically from *G. infula* and *G. inflata*, due to their similar ascospore characteristics. Of course, *G. infula* has a completely different ecology and phenology, growing on wood and fructifying in autumn.

Gyromitra gigas specimens, when comparing the ascospores and paraphyses to Breitenbach & Kränzlin (1984) and Miller et al. (2020), are identical in paraphyses appearance and in shape and ornamentation of spores, but there are differences in spore size. 8_*Gyromitra gigas*_WU_0009567 had an ascospore size range of $21.1-25.3 \times 9.5-13 \mu\text{m}$, with a paraphysis apical diameter of $9 \mu\text{m}$. 28_*Gyromitra gigas*_WU_0010578 had an ascospore size range of $20.8-34.6 \times 7.7-11.3 \mu\text{m}$, with a paraphysis apical diameter of $8.2 \mu\text{m}$. 30_*Gyromitra gigas*_WU_0011622 had an ascospore size range of $26.3-31.6 \times 9.8-13 \mu\text{m}$, and paraphyses were not measurable. 34_*Gyromitra gigas*_WU_0009596 had an ascospore size range of $22.4-28.9 \times 10.7-13.1 \mu\text{m}$, with a paraphysis apical diameter of $6.2 \mu\text{m}$. Breitenbach & Kränzlin (1984) give an ascospore size range of $23-28 \mu\text{m} \times 10-12.5 \mu\text{m}$, Miller et al. (2020) of $25-34.5 \mu\text{m} \times 12-13 \mu\text{m}$, with paraphysis apical diameter of up to $11 \mu\text{m}$. The ascospore size range is similar in comparison, concluding that the specimens were correctly identified.

Gyromitra fastigiata specimens, when comparing to McKnight (1971) had similar microscopical characteristic of the ascospores and paraphysis. 35_*Gyromitra fastigiata*_WU_0024806 had an ascospore size range of $23.7-31 \mu\text{m} \times 10.4-13.6 \mu\text{m}$, with a paraphysis apical diameter of $6.7 \mu\text{m}$. 39_*Gyromitra fastigiata*_WU_0021318 had an ascospore size range of $22.6-27.9 \mu\text{m} \times 9.8-12.7 \mu\text{m}$, with a paraphyse apical diameter of $8.3 \mu\text{m}$. 40_*Gyromitra fastigiata*_WU_0008756 had an ascospore size range of $23-27.6 \mu\text{m} \times 10.1-13 \mu\text{m}$, and paraphyses were not measurable. 41_*Gyromitra fastigiata*_WU_0013814 had an ascospore size range of $23.6-28.8 \mu\text{m} \times 11.4-13.2 \mu\text{m}$, with paraphysis apical diameter of $8.3 \mu\text{m}$. 45_*Gyromitra fastigiata*_WU_0040483 had an ascospore

size range of 23.4-27.6 $\mu\text{m} \times 9.4\text{-}12\text{ }\mu\text{m}$, with a paraphysis apical diameter of 7.5 μm . McKnight (1971) gives an ascospore size range of 23.5-32 $\mu\text{m} \times 10\text{-}14\text{ }\mu\text{m}$, with paraphysis apical diameter of 4-5.5 μm . Dizkirici et al. (2018) give an ascospore size range of 28-33.5 $\mu\text{m} \times 13\text{-}15.5\text{ }\mu\text{m}$, with paraphysis apical diameter range of 5-9 μm . The ascospores of the present study were a bit shorter compared to the literature data cited, but this may be due to some immature spores measured. Taking everything into account, the specimens were correctly identified.

Gyromitra inflata, when compared to Klofac & Krisai-Greilhuber (2019), had the same microscopical characteristics. 42_*Gyromitra inflata*_WU_0032695 had an ascospore size range of 15.5-19.4 $\mu\text{m} \times 8.8\text{-}11.4\text{ }\mu\text{m}$, with a paraphysis apical diameter of 5.3 μm . 43_*Gyromitra inflata*_WU_0044303 had an ascospore size range of 16.8-19.4 $\mu\text{m} \times 9.1\text{-}10.4\text{ }\mu\text{m}$, with a paraphysis apical diameter of 7.9 μm . 44_*Gyromitra inflata*_WU_0043891 had an ascospore size range of 18-22.9 $\mu\text{m} \times 9.1\text{-}12\text{ }\mu\text{m}$, with a paraphysis apical diameter of 6 μm . Klofac & Krisai-Greilhuber (2019) had an ascospore size range of 17.5-19 (-22) $\mu\text{m} \times 10\text{-}11\text{ (-}12\text{) }\mu\text{m}$, with a paraphysis apical diameter of 4-7 μm . The ascospore size metrics are similar in comparison, it can thus be concluded that the specimens were correctly identified. However, it is hard to distinguish *G. inflata* specimens microscopically from *G. esculenta*, due to their similar ascospore characteristics.

Gyromitra infula specimen when compared to Breitenbach & Kränzlin (1984), had identical microscopic characteristics. 54_*Gyromitra infula*_WU_0010139 had an ascospore size range of 17.3-20.4 $\mu\text{m} \times 7.7\text{-}8.8\text{ }\mu\text{m}$, and paraphysis were not measurable. 61_*Gyromitra infula*_WU_0027935 had a spore size range of 17.3-18.2 $\mu\text{m} \times 6.5\text{-}7.5\text{ }\mu\text{m}$, with a paraphysis apical diameter 8.2. Breitenbach & Kränzlin (1984) give a spore size range of 19-23 $\mu\text{m} \times 7\text{-}8\text{ }\mu\text{m}$, which is a bit longer than in the specimens studied and again can result from measuring immature spores. Furthermore, it is not easy to distinguish *G. infula* from *G. esculenta* due to their similar ascospore characteristics, but of course, again, there is a bit phenological difference in appearance in autumn versus spring. Taking everything into account, the conclusion is that the specimens were correctly identified.

In *Gyromitra ticiniana* specimens the spores and paraphyses, when comparing to Van Vooren & Moreau (2009) and Miller et al. (2020), are identical in microscopic characteristics. 68_*Gyromitra ticiniana*_WU_0008714 had an ascospore size range of 20.8-25.7 $\mu\text{m} \times 7.1\text{-}10.1\text{ }\mu\text{m}$, with a paraphysis apical diameter of 6.3 μm . 69_*Gyromitra ticiniana*_WU_0039781 had an ascospore size range of 24.6-31.6 $\mu\text{m} \times 10\text{-}13\text{ }\mu\text{m}$, with a paraphyses apical diameter of 6.5 μm . Van Vooren & Moreau (2009) give an ascospore size of 24-28(30) $\mu\text{m} \times 11\text{-}14\text{ }\mu\text{m}$. Miller et al. (2020) give an ascospore size of 22-34 $\mu\text{m} \times 9.5\text{-}12\text{ }\mu\text{m}$, with a paraphysis apical diameter of 9.5 μm . The ascospore size ranges overlapped, meaning that the specimens were correctly identified.

Our *Gyromitra longipes* specimen, when compared to Carlsen & Stensrud (2003) and Medel (2005), has ascospores similar in size and shape. The major difference was that our specimen had mostly triguttulate ascospores, compared to biguttulate ones in literature cited. 71_*Gyromitra longipes*_WU_0037177 had an ascospore size of $27.3\text{-}29.6\text{ }\mu\text{m} \times 11.2\text{-}12.7\text{ }\mu\text{m}$. Carlsen & Stensrud (2003) give an ascospore size range of $22\text{-}32\text{ }\mu\text{m} \times 10\text{-}14\text{ }\mu\text{m}$, with a paraphysis apical diameter of $10\text{-}15\text{ }\mu\text{m}$. Medel (2005) gives an ascospore size of $20\text{-}25\text{ }\mu\text{m} \times 9\text{-}10\text{ }\mu\text{m}$. The ascospore size metrics are overlapping with the measurements of Carlsen & Stensrud (2003) but not with Medel (2005). When taken everything into account the conclusion is that behind the name *G. longipes* there are obviously several species opinions and the classification can only be done in the sense of Carlsen & Stensrud for instance.

Phylogenetic analysis

Phylogenies of *Gyromitra* inferred from the ITS, LSU, TEF, and concatenated LSU and TEF dataset (Figs. 34-37.) revealed some clades, which are congruent with literature findings. There are differences and similarities between the phylogenetic trees among the species and their taxonomic situation. The ITS phylogenetic tree (Fig. 34) is from all the trees the one, which is the least reliable, due to the many gaps in the alignment, but was kept in for comparison purposes. It also is similar in many distributions to the most reliable of the ML-trees, the LSU and TEF concatenated phylogenetic tree (Fig. 37). Some of the reference sequences, could also be misidentified, which should be considered in looking at the phylogenetic tree. Furthermore, all the species used in this study were classified as *Gyromitra*, following Mycobank. Formerly, some of them were classified as *Discina*, but comparing the relatedness it is obvious that they all should be considered as *Gyromitra* species.

In the ITS phylogenetic tree *Gyromitra infula* was in the same clade with *G. esculenta*, this was the same for the LSU and TEF phylogenetic tree. The clade comprising *G. esculenta* and *G. infula* is congruent with the findings from Methven et al. (2013) and Wang & Zhuang (2019). In the LSU phylogenetic tree (Fig. 35) *G. infula* is the first species to branch off and after that *G. esculenta*. In Methven et al. (2013) *G. infula* was also the first to branch off followed by *G. esculenta*, when considering only the same species. In the TEF phylogenetic tree (Fig. 36) *G. esculenta* branched off first and after that *G. infula*. This was not congruent with any other study. From the microscopic ascospore characteristics both species are similar. Specimen 63_*Gyromitra esculenta*_WU_0010620 grouped within *G. infula*, which is probably due to an error in sequencing, because both species are quite different in macromorphology and phenology and thus cannot be confused.

In the ITS phylogenetic tree there was the specimen 35_*Gyromitra fastigiata*_WU_0024806 mixed in with the *G. esculenta* specimens. Clearly, the macroscopical appearance, *id est* multilobed, and the microscopical characteristics point towards *G. fastigiata* and there is not enough evidence to consider it as an *G. esculenta* specimen. Furthermore, the *G. esculenta* clade has two more species grouped together, *G. longipes* and *G. inflata*. Those separate into two major subgroups, which is consistent over all the trees. *Gyromitra longipes* and *G. inflata* are exactly situated in the two subgroups in opposition to each other together with *G. esculenta* species. Obviously, they are very closely related to each other. It was already shown by Klofac & Krisai-Greilhuber (2019) that *G. inflata* is closely related to *G. esculenta*, so close that they were situated in a subgroup of *G. esculenta*. This is supported by the very similar appearance of the microscopic characteristics of the spores for *G. inflata*. In *G. esculenta* and *G. inflata* both are biguttulate, smooth, ellipsoid and with no apiculi present. When looking at the microscopic characteristic of *G. longipes*, the difference to the others is apparent. The spores are subfusiform, triguttulate, with ornamentation and apiculi present. Still in every phylogenetic tree the relation between *G. longipes* und *G. esculenta* is the same.

Gyromitra fastigiata is placed in a clade together with *G. parma*, showing their close relatedness to each other. This relationship was true for the concatenated LSU and TEF and the TEF tree. In the LSU tree a similar relation was revealed and *G. brunnea* was also grouped together with *G. parma* as a sister taxon to *G. fastigiata*. McKnight (1987) considered *G. fastigiata* and *G. brunnea* as synonymous, but in the present phylogenetic trees these two species were separated like in Dizkirici et al. (2018). The relationship of *G. fastigiata* with *G. parma* becomes also apparent when looking at the microscopical characteristics of the ascospore, which are similar in shape and type of apiculi present on both ends. *Gyromitra brunnea* was grouped next to *G. parma*, but there were only reference sequences from USA available, because *G. brunnea* has a North American distribution. Furthermore *G. perlata* seems to be a sister taxon to the taxa mentioned before, which is revealed in every phylogenetic tree. The young *G. perlata* specimens had spore characters similar to *G. fastigiata* and *G. parma*, due to the many small apiculi and ornamentation. When maturing the apiculi of the ascospores of *G. perlata* are much larger.

The next clade separating is composed of *G. apiculatula*, *G. ticiniana*, *G. korfii* and *G. gigas*. The first one to branch off is *G. apiculatula*, in every phylogenetic tree. *Gyromitra apiculatula* is microscopically similar to *G. gigas* and *G. ticiniana*, due to the similar shape and size and faint ornamentation of the ascospores, but has a different shape of the apiculi. Next, *G. ticiniana* and the North American *G. korfii* cluster together and are closely related to each other according to all phylogenetic trees. This relationship is also apparent in Miller et al. (2020), where *G. ticiniana* and *G. korfii* are grouped together. The last one to branch off is *G. gigas*, which is congruent over all phylogenetic trees, confirming the results of Miller et al. (2020). There was one specimen of *G. ticiniana* grouped with *G. gigas* and vice versa. From the microscopical characteristics in comparison they look very similar, they differ only in ornamentation and form of the ascospore apiculi. But telling from the microscopical characteristics, they seem to have been identified correctly so there was probably some sequencing error.

Gyromitra leucoxantha could only be assessed for the TEF and LSU regions. In the TEF phylogenetic tree it was placed as a sister to the *G. gigas* clade and branched off as the first species in this clade. In the LSU tree *G. leucoxantha* branched off as the third species as sister to the other *Gyromitra* species. From the microscopical characteristics *G. leucoxantha* has similar ascospore shape with ornamentation, when compared to the *G. gigas* species complex, but the apiculi are flat truncate and differ significantly. There must be more data gathered for a conclusive taxonomic arrangement of this species.

With some specimens it happened that they were phylogenetically placed in another species group than in the one they were identified morphologically. But most of the time when looking at the microscopical characteristics the original identification was the correct one. An explanation for that could be that the sequences for those species were switched accidentally.

Conclusion

To conclude there has been a high overlap in the taxonomic relationships of the *Gyromitra* specimens presently studied to the literature findings. Although the clade concepts tended to vary compared to literature in some regards. The relationships between the species were congruent in the most reliable trees. For the microscopic characteristics it seems that from the specimens observed, all of them but four were correctly identified. For a formal taxonomic rearrangement of the genus more intensive research with increased sampling and further analysis of the same and different gene regions must be done.

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Appendix

Tab. 9. Shows all the results of the species ITS sequence similarity hits on the GenBank, when being blasted.

Sample no.	GenBank Species Name	Percent identity	Query Cover	Identities	Gaps	Accession
7_Discina_melaleuca	<i>Gyromitra gigas</i>	99,73%	100%	734/736(99%)	0/736(0%)	KX420696.1
8_Gyromitra_gigas	<i>Gyromitra ticiniana</i>	100%	96%	668/668(100%)	0/668(0%)	MH938670.1
9_Gyromitra_gigas	<i>Gyromitra gigas</i>	100%	98%	722/722(100%)	0/722(0%)	MH938667.1
11_Gyromitra_gigas	<i>Gyromitra gigas</i>	100%	97%	722/722(100%)	0/722(0%)	MH938667.1
12_Discina_apiculata	<i>Gyromitra leucoxantha</i>	99,02%	73%	504/509(99%)	1/509(0%)	KX185088.1
21_Gyromitra_esculenta	<i>Gyromitra esculenta</i>	99.62%	97%	788/791(99%)	0/791(0%)	MG846988.1
22_Gyromitra_esculenta	<i>Gyromitra esculenta</i>	100.00%	99%	774/774(100%)	0/774(0%)	MG846989.1
23_Gyromitra_esculenta	<i>Gyromitra esculenta</i>	99.74%	99%	772/774(99%)	1/774(0%)	MG846989.1
23_Gyromitra_esculenta	<i>Gyromitra esculenta</i>	99.74%	98%	772/774(99%)	1/774(0%)	MW413338.1
24_Gyromitra_esculenta	<i>Gyromitra esculenta</i>	99.87%	97%	764/765(99%)	1/765(0%)	MW413338.1
25_Gyromitra_longipes	<i>Gyromitra esculenta</i>	100.00%	93%	750/750(100%)	0/750(0%)	KX420693.1
26_Gyromitra_parma	<i>Gyromitra brunnea</i>	92,44%	19%	220/238(92%)	5/238(2%)	ON245325.1
29_Gyromitra_gigas	<i>Gyromitra gigas</i>	100%	97%	765/765(100%)	0/765(0%)	KX420696.1
30_Gyromitra_gigas	<i>Gyromitra gigas</i>	99,87%	97%	753/754(99%)	0/754(0%)	KX420696.1
31_Gyromitra_gigas	<i>Gyromitra gigas</i>	99,87%	97%	749/750(99%)	1/750(0%)	KX420696.1
34_Discina_fastigiata	<i>Gyromitra gigas</i>	99,87%	97%	742/743(99%)	1/743(0%)	KX420696.1
35_Gyromitra_infula	<i>Maublancomyces inflatus</i>	99.12%	100%	785/792(99%)	1/792(0%)	MW413336.1
41_Discina_fastigiata	<i>Gyromitra esculenta</i>	99.23%	98%	777/783(99%)	2/783(0%)	GU373505.1
42_Gyromitra_inflata	<i>Maublancomyces inflatus</i>	99.62%	98%	782/785(99%)	1/785(0%)	MW413336.1
44_Gyromitra_inflata	<i>Gyromitra esculenta</i>	99,25%	98%	799/805(99%)	3/805(0%)	GU373505.1
46_Gyromitra_infula	<i>Gyromitra infula</i>	99,74%	53%	391/392(99%)	0/392(0%)	MH220222.1
47_Gyromitra_infula	<i>Gyromitra infula</i>	100%	50%	392/392(100%)	0/392(0%)	MH220222.1
48_Gyromitra_infula	<i>Gyromitra infula</i>	100%	53%	392/392(100%)	0/392(0%)	MH220222.1

49_Gyromitra_infula	<i>Gyromitra infula</i>	100%	51%	392/392(100%)	0/392(0%)	MH220222.1
49_Gyromitra_infula	<i>Gyromitra infula</i>	99,69%	84%	642/644(99%)	0/644(0%)	MZ567199.1
50_Gyromitra_infula	<i>Gyromitra infula</i>	100%	53%	714/714(100%)	0/714(0%)	MH220222.1
51_Gyromitra_infula	<i>Gyromitra infula</i>	100%	53%	392/392(100%)	0/392(0%)	MH220222.1
53_Gyromitra_infula	<i>Gyromitra infula</i>	100%	99%	735/735(100%)	0/735(0%)	MG846961.1
54_Gyromitra_infula	<i>Gyromitra infula</i>	98.82%	99%	751/760(99%)	1/760(0%)	MG871296.1
55_Gyromitra_esculenta	<i>Gyromitra esculenta</i>	99,87%	94%	764/765(99%)	0/765(0%)	MW413338.1
56_Gyromitra_esculenta	<i>Gyromitra esculenta</i>	99,22%	99%	763/769(99%)	2/769(0%)	GU373505.1
58_Gyromitra_infula	<i>Gyromitra infula</i>	99,74%	51%	391/392(99%)	0/392(0%)	MH220222.1
59_Gyromitra_infula	<i>Gyromitra infula</i>	100%	97%	715/715(100%)	0/715(0%)	MZ091681.1
60_Gyromitra_infula	<i>Gyromitra infula</i>	100%	51%	392/392(100%)	0/392(0%)	MH220222.1
61_Gyromitra_infula	<i>Gyromitra infula</i>	100%	53%	392/392(100%)	0/392(0%)	MH220222.1
62_Gyromitra_infula	<i>Gyromitra infula</i>	100%	97%	392/392(100%)	0/392(0%)	MH220222.1
64_Gyromitra_infula	<i>Gyromitra infula</i>	99,74%	53%	391/392(99%)	0/392(0%)	MH220222.1
65_Gyromitra_infula	<i>Gyromitra infula</i>	99,98%	51%	390/394(99%)	2/394(0%)	MH220222.1
67_Gyromitra_ticiniana	<i>Gyromitra ticiniana</i>	99,85%	96%	667/668(99%)	0/668(0%)	MH938671.1
72_Gyromitra_esculenta	<i>Gyromitra esculenta</i>	99,35%	88%	759/764(99%)	4/764(0%)	MG846989.1

Tab. 10. Shows all the results of the species LSU sequence similarity hits on the GenBank, when being blasted.

Sample no.	Genbank Species Name	Percent identity	Query Cover	Identities	Gaps	Accession
2_Disciotis_venosa	<i>Disciotis venosa</i>	99.65%	99%	843/846(99%)	0/846(0%)	KX008358.1
4_Discina_ancilis	<i>Discina ancilis</i>	100%	96%	854/854(100%)	0/854(0%)	MG847022.1
13_Discina_apiculata	<i>Discina accumbens</i>	100%	92%	832/832(100%)	0/832(0%)	KX008331.1
15_Discina_geogenia	<i>Discina ancilis</i>	99.89%	99%	875/876(99%)	0/876(0%)	KC751514.1
17_Discina_leucoxantha	<i>Gyromitra leucoxantha</i>	100%	100%	886/886(100%)	0/886(0%)	MW078428.1
18_Gyromitra_parrma	<i>Gyromitra brunnea</i>	99.78%	100%	894/896(99%)	0/896(0%)	KC751523.1
20_Gyromitra_parma	<i>Gyromitra brunnea</i>	99.66%	99%	876/879(99%)	0/879(0%)	KC751523.1
21_Gyromitra_esculenta	<i>Gyromitra esculenta</i>	99.88%	93%	827/828(99%)	0/828(0%)	AY640952.1
23_Gyromitra_esculenta	<i>Gyromitra esculenta</i>	99.88%	96%	846/847(99%)	1/847(0%)	MG847002.1
24_Gyromitra_esculenta	<i>Gyromitra esculenta</i>	100%	96%	849/849(100%)	0/849(0%)	MG847002.1
25_Gyromitra_longipes	<i>Gyromitra esculenta</i>	100%	97%	848/848(100%)	0/848(0%)	MG847002.1
29_Gyromitra_gigas	<i>Gyromitra korfii</i>	99.89%	100%	885/886(99%)	1/886(0%)	KC751499.1
30_Gyromitra_gigas	<i>Gyromitra gigas</i>	100%	97%	859/859(100%)	0/859(0%)	MG847005.1
31_Gyromitra_gigas	<i>Gyromitra gigas</i>	100%	100%	886/886(100%)	0/886(0%)	MW076974.1
32_Gyromitra_gigas	<i>Gyromitra gigas</i>	99.88%	99%	856/857(99%)	0/857(0%)	MW076974.1
33_Discina_fastigiata	<i>Discina fastigiata</i>	99.88%	95%	842/843(99%)	0/843(0%)	MH938321.1
38_Discina_fastigiata	<i>Discina fastigiata</i>	100%	95%	851/851(100%)	0/851(0%)	MH938321.1
39_Discina_fastigiata	<i>Discina fastigiata</i>	100%	99%	883/883(100%)	0/883(0%)	MK784816.1
40_Discina_fastigiata	<i>Gyromitra brunnea</i>	99.44%	100%	882/887(99%)	0/887(0%)	KC751523.1
42_Maublancomyces_inflatus	<i>Gyromitra esculenta</i>	100%	93%	819/819(100%)	0/819(0%)	AY640952.1
43_Maublancomyces_inflatus	<i>Gyromitra esculenta</i>	99.88%	94%	852/853(99%)	1/853(0%)	KX008337.1
44_Maublancomyces_inflatus	<i>Gyromitra esculenta</i>	99.88%	94%	851/852(99%)	0/852(0%)	KX008337.1
45_Gyromitra_slovenskii	<i>Discina fastigiata</i>	100%	93%	857/857(100%)	0/857(0%)	MH938321.1
46_Gyromitra_infula	<i>Gyromitra infula</i>	100%	95%	852/852(100%)	0/852(0%)	KX008342.1
47_Gyromitra_infula	<i>Gyromitra infula</i>	100%	94%	860/860(100%)	0/860(0%)	KX008342.1

48_Gyromitra_infula	<i>Gyromitra infula</i>	100%	94%	860/860(100%)	0/860(0%)	KX008342.1
49_Gyromitra_infula	<i>Gyromitra infula</i>	100%	97%	860/860(100%)	0/860(0%)	KX008342.1
50_Gyromitra_infula	<i>Gyromitra infula</i>	100%	91%	835/835(100%)	0/835(0%)	MH298889.1
51_Gyromitra_infula	<i>Gyromitra infula</i>	100%	94%	860/860(100%)	0/860(0%)	KX008342.1
52_Gyromitra_infula	<i>Gyromitra infula</i>	100%	94%	857/857(100%)	0/857(0%)	KX008342.1
53_Gyromitra_infula	<i>Gyromitra infula</i>	100%	96%	860/860(100%)	0/860(0%)	KX008342.1
54_Gyromitra_infula	<i>Gyromitra infula</i>	100%	96%	860/860(100%)	0/860(0%)	KX008342.1
55_Gyromitra_esculenta	<i>Gyromitra esculenta</i>	100%	95%	872/872(100%)	0/872(0%)	MG847002.1
56_Gyromitra_esculenta	<i>Gyromitra esculenta</i>	100%	93%	840/840(100%)	0/840(0%)	AY640952.1
57_Gyromitra_infula	<i>Gyromitra esculenta</i>	98.11%	87%	570/581(98%)	1/581(0%)	KM204697.1
58_Gyromitra_infula	<i>Gyromitra infula</i>	100%	94%	860/860(100%)	0/860(0%)	KX008342.1
59_Gyromitra_infula	<i>Gyromitra infula</i>	100%	94%	860/860(100%)	0/860(0%)	KX008342.2
60_Gyromitra_infula	<i>Gyromitra infula</i>	99.88%	94%	859/860(99%)	0/860(0%)	KX008342.1
61_Gyromitra_infula	<i>Gyromitra infula</i>	100%	95%	872/872(100%)	0/872(0%)	MG847006.1
62_Gyromitra_infula	<i>Gyromitra infula</i>	100%	98%	865/865(100%)	0/865(0%)	MG871330.1
63_Gyromitra_esculenta	<i>Gyromitra infula</i>	97.9%	95%	514/525(98%)	0/525(0%)	MG847012.1
64_Gyromitra_infula	<i>Gyromitra infula</i>	100%	97%	860/860(100%)	0/860(0%)	KX008342.1
65_Gyromitra_infula	<i>Gyromitra infula</i>	100%	97%	860/860(100%)	0/860(0%)	KX008342.1
67_Gyromitra_ticiniana	<i>Gyromitra ticiniana</i>	100%	93%	855/855(100%)	0/855(0%)	MH938319.1
68_Gyromitra_ticiniana	<i>Gyromitra ticiniana</i>	100%	93%	855/855(100%)	0/855(0%)	MH938319.1
69_Gyromitra_ticiniana	<i>Gyromitra gigas</i>	100%	92%	876/876(100%)	0/876(0%)	MH938314.1
70_Discina_ancilis	<i>Discina ancilis</i>	98.87%	100%	875/885(99%)	4/885(0%)	KX008334.1
71_Gyromitra_longipes	<i>Gyromitra esculenta</i>	100%	94%	864/864(100%)	0/864(0%)	KX008341.1

Tab. 11. Shows all the results of the species TEF sequences similarity hits on the GenBank, when being blasted.

Sample no.	Blasted Species	Percent identity	Query Cover	Identities	Gaps	Accession
1_Disciotis_venosa	<i>Disciotis venosa</i>	99.83%	100%	577/578(99%)	0/578(0%)	GU551575.1
2_Disciotis_venosa	<i>Disciotis venosa</i>	99.6%	100%	581/583(99%)	2/583(0%)	GU551575.1
4_Discina_ancilis	<i>Discina ancilis</i>	99.82%	97%	547/548(99%)	0/548(0%)	MG847068.1
12_Discina_apiculata	<i>Gyromitra pseudogigas</i>	94.56%	96%	521/551(95%)	8/551(1%)	MG847069.1
13_Discina_apiculata	<i>Gyromitra pseudogigas</i>	94.56%	96%	521/551(95%)	8/551(1%)	MG847069.2
15_Discina_geogenia	<i>Discina ancilis</i>	97.29%	97%	538/553(97%)	3/553(0%)	MG847068.1
18_Gyromitra_parma	<i>Gyromitra parma</i>	95.67%	97%	530/554(96%)	2/554(0%)	MG847049.1
19_Gyromitra_parma	<i>Discina fastigiata</i>	95.87%	97%	534/557(96%)	2/557(0%)	MG847049.1
21_Gyromitra_esculenta	<i>Gyromitra esculenta</i>	100%	85%	492/492(100%)	0/492(0%)	FJ238419.1
23_Gyromitra_esculenta	<i>Gyromitra esculenta</i>	100%	97%	560/560(100%)	0/560(0%)	MG847048.1
24_Gyromitra_esculenta	<i>Gyromitra esculenta</i>	99.82%	97%	559/560(99%)	0/560(0%)	MG847048.1
25_Gyromitra_longipes	<i>Gyromitra esculenta</i>	99.82%	95%	559/560(99%)	0/560(0%)	MG847048.1
26_Gyromitra_parma	<i>Discina fastigiata</i>	95.69%	97%	533/557(96%)	3/557(0%)	MG847049.1
27_Gyromitra_gigas	<i>Gyromitra gigas</i>	99.28%	97%	551/555(99%)	3/555(0%)	MG847051.1
28_Gyromitra_gigas	<i>Gyromitra pseudogigas</i>	95.89%	97%	537/560(96%)	4/560(0%)	MG847069.1
28_Gyromitra_gigas	<i>Gyromitra gigas</i>	95.71%	97%	536/560(96%)	4/560(0%)	MG847050.1
30_Gyromitra_gigas	<i>Gyromitra gigas</i>	99.82%	97%	554/555(99%)	1/555(0%)	MG847051.1
31_Gyromitra_gigas	<i>Gyromitra gigas</i>	99.64%	97%	558/560(99%)	2/560(0%)	MG847051.1
32_Gyromitra_gigas	<i>Gyromitra gigas</i>	99.82%	97%	554/555(99%)	1/555(0%)	MG847051.1
33_Discina_fastigiata	<i>Discina fastigiata</i>	100%	97%	552/552(100%)	0/552(0%)	MG847049.1
35_Discina_fastigiata	<i>Discina fastigiata</i>	100%	97%	552/552(100%)	0/552(0%)	MG847049.1
36_Discina_fastigiata	<i>Discina fastigiata</i>	100%	97%	553/553(100%)	0/553(0%)	MG847049.1
37_Discina_fastigiata	<i>Discina fastigiata</i>	100%	97%	552/552(100%)	0/552(0%)	MG847049.1
38_Discina_fastigiata	<i>Discina fastigiata</i>	100%	97%	552/552(100%)	0/552(0%)	MG847049.1
39_Discina_fastigiata	<i>Discina fastigiata</i>	100%	97%	552/552(100%)	0/552(0%)	MG847049.1

41_Discina_fastigiata	<i>Discina fastigiata</i>	100%	97%	553/553(100%)	0/553(0%)	MG847049.1
42_Gyromitra_inflata	<i>Gyromitra esculenta</i>	99.64%	97%	558/560(99%)	0/560(0%)	MG847047.1
44_Gyromitra_inflata	<i>Gyromitra esculenta</i>	99.59%	85%	485/487(99%)	2/487(0%)	FJ238419.1
45_Gyromitra_slovenskii	<i>Discina fastigiata</i>	99.29%	97%	559/563(99%)	2/563(0%)	MG847049.1
46_Gyromitra_infula	<i>Gyromitra infula</i>	99.64%	97%	561/563(99%)	2/563(0%)	MG847060.1
47_Gyromitra_infula	<i>Gyromitra infula</i>	99.82%	97%	562/563(99%)	1/563(0%)	MG847060.1
48_Gyromitra_infula	<i>Gyromitra infula</i>	99.82%	97%	562/563(99%)	1/563(0%)	MG847060.1
49_Gyromitra_infula	<i>Gyromitra infula</i>	100%	97%	558/558(100%)	0/558(0%)	MG847060.1
50_Gyromitra_infula	<i>Gyromitra infula</i>	99.64%	97%	561/563(99%)	2/563(0%)	MG847060.1
51_Gyromitra_infula	<i>Gyromitra infula</i>	100%	97%	558/558(100%)	0/558(0%)	MG847060.1
52_Gyromitra_infula	<i>Gyromitra infula</i>	100%	97%	558/558(100%)	0/558(0%)	MG847060.1
53_Gyromitra_infula	<i>Gyromitra infula</i>	99.82%	99%	558/559(99%)	0/559(0%)	MG847060.1
54_Gyromitra_infula	<i>Gyromitra infula</i>	99.82%	99%	562/563(99%)	1/563(0%)	MG847060.1
56_Gyromitra_esculenta	<i>Gyromitra esculenta</i>	100%	85%	489/489(100%)	0/489(0%)	FJ238419.1
57_Gyromitra_infula	<i>Gyromitra infula</i>	99.82%	97%	562/563(99%)	1/563(0%)	MG847060.1
58_Gyromitra_infula	<i>Gyromitra infula</i>	99.82%	97%	562/563(99%)	1/563(0%)	MG847060.1
59_Gyromitra_infula	<i>Gyromitra infula</i>	99.82%	97%	562/563(99%)	1/563(0%)	MG847060.1
60_Gyromitra_infula	<i>Gyromitra infula</i>	99.82%	97%	562/563(99%)	1/563(0%)	MG847060.1
61_Gyromitra_infula	<i>Gyromitra infula</i>	99.64%	97%	561/563(99%)	2/563(0%)	MG847060.1
62_Gyromitra_infula	<i>Gyromitra infula</i>	99.64%	97%	561/563(99%)	2/563(0%)	MG847060.1
63_Gyromitra_esculenta	<i>Gyromitra infula</i>	100%	97%	558/558(100%)	0/558(0%)	MG847060.1
64_Gyromitra_infula	<i>Gyromitra infula</i>	99.82%	97%	562/563(99%)	1/563(0%)	MG847060.1
65_Gyromitra_infula	<i>Gyromitra infula</i>	99.65%	94%	567/569(99%)	1/569(0%)	MG847060.1
67_Gyromitra_ticiniana	<i>Gyromitra pseudogigas</i>	99.68%	97%	531/555(96%)	5/555(0%)	MG847069.1
68_Gyromitra_ticiniana	<i>Gyromitra pseudogigas</i>	99.89%	93%	537/560(96%)	4/560(0%)	MG847069.1
69_Gyromitra_ticiniana	<i>Gyromitra gigas</i>	99.64%	97%	558/560(99%)	2/560(0%)	MG847051.1
70_Discina_ancilis	<i>Discina ancilis</i>	99.82%	97%	547/548(99%)	0/548(0%)	MG847068.1
71_Gyromitra_longipes	<i>Gyromitra esculenta</i>	99.82%	97%	554/555(99%)	0/555(0%)	MG847048.1

