Preliminary phylogenetic and morphological studies in the *Gyromitra gigas* lineage (*Pezizales*): Epitypification of *Gyromitra gigas* and *G. ticiniana*

Matteo CARBONE
Nicolas VAN VOOREN
Vavřinec KLENER
Pablo ALVARADO

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Abstract: *Gyromitra gigas* and *G. ticiniana* are epitypified with modern collections with known genetic data. The taxonomy of both species is revisited on the basis of new morphological studies and phylogenetic reconstruction based on molecular data. Results suggest that *Gyromitra korfii* could be a prioritary name for *G. ticiniana*. The replacement of the name *Gyromitra littiniana* with *G. ticiniana* is also discussed. Color images of fresh material and microscopical features are provided.
Keywords: Ascomycota, *Discina*, *Discinaceae*, ITS, LSU rDNA, phylogeny, *Pseudogyromitrae*, taxonomy.

Résumé : *Gyromitra gigas* et *G. ticiniana* sont épitypifiés par des récoltes récentes, accompagnées de données génétiques. La taxinomie des deux espèces est revisitée sur la base de nouvelles études morphologiques et d'une reconstruction phylogénétique basée sur des données moléculaires. Les résultats suggèrent que *G. korfii* pourrait être un nom prioritaire pour *G. ticiniana*. L'abandon du nom *G. littiniana* au profit de *G. ticiniana* est également discuté. Des images en couleur du matériel frais et des caractères microscopiques sont fournies.

Riassunto: *Gyromitra gigas* e *G. ticiniana* vengono tipificate con raccolte recenti. La loro tassonomia viene revisionata sia morfologicamente che filogeneticamente. I risultati preliminari suggeriscono che *Gyromitra korfii* potrebbe essere sinonimo di *G. ticiniana*. Viene proposta anche una discussione sull'abbandono di *Gyromitra littiniana* in favore di *G. ticiniana*. Vengono formite foto a colori di materiale fresco e dei caratteri microscopici.

Abstrakt: *Gyromitra gigas* (ucháč obrovský) a *G. ticiniana* jsou epitypifikovány na základě recentních sběrů se zjištěním genetických dat. Taxonomická pozice obou druhů je revidována na podkladě nových morfologických studií a fylogenetické rekonstrukce opírající se o molekuární data. Výsledky naznačují, že prioritním jménem pro *Gyromitra ticiniana* může být *G. korfii*. Diskutováno je rovněž upuštění od užívání jména *Gyromitra littiniana* ve prospěch *G. ticiniana*. Zahrnuta jsou barevná vyobrazení čerstvého materiálu i mikroskopických znaků.

Introduction

The history and taxonomy of the genus *Gyromitra* Fr. were treated in detail by Van Vooren & Moreau (2009a). *Gyromitra* subgen. *Discina* (Fr.) Harmaja contains species with discoid to convex, sessile to subsessile apothecia (sect. *Discina*), but also cerebriform and definitely stalked ascomata (sect. *Pseudogyromitrae* Van Vooren). All species possess apiculate, elliptical (sub)fusoid ascospores (Van Vooren & Moreau, 2009b). Methven *et al.* (2013) were the first to conduct a phylogenetic study focused on *Gyromitra s.l.*, and suggested that subgenus *Discina sensu* Abbott & Currah (1997) is paraphyletic in its original sense, although a significantly narrower monophyletic clade can be accepted.

Gyromitra gigas (Krombh.) Cooke, the type species of *Gyromitra* sect. *Pseudogyromitrae*, is a well-known and widespread European species fruiting in spring. This species was described (as *Helvella gigas*) and perfectly depicted in a color drawing by Krombholz (1834) from a mossy forest near Prague (Czech Republic). Probably due to the good description and drawing, its identity has never been in doubt and it has been treated in many publications [for a list of references see Van Vooren & Moreau (2009b: 10–11)].

The epithet *gigas* was also used for all the North American collections fitting Krombholz's concept until Raitviir (1970) found narrower spores in some American collections and created a new species *Discina korfii* Raitv. This taxon was transferred into *Gyromitra* by Harmaja (1973), after studying the holotype and two paratypes. He concluded that ascospores were a bit shorter than those in *G. gigas*, and had slightly more delicate ornamentation and broader paraphysis apices.

In the same work, Harmaja (1973) described from Wyoming (USA) *Gyromitra montana* Harmaja based on *Gyromitra gigas sensu* McKnight (1971). Harmaja reported that it differed from *G. gigas* because of its "*slightly more ellipsoid, less fusiform spores with somewhat broader ends, the inconstancy of the presence of the spore apiculi, the variable and often irregular shape and smaller size of the latter when discernible, the slightly more delicate ornamentation of the perispore, the thicker tips of the paraphyses which may even be capitate and attain a breadth of ca.* 13 µm", and from *G. korfii* "*through similar differences as from G. gigas as regards the spore shape, features of the spore apiculi, and the characters of occurrence, but also because of the longer and broader spores of G. montana*". Abbott & Currah (1997) reviewed these three species and concluded that differences in spore features were not taxonomically relevant, and so they considered them synonyms under *G. gigas*. Methven *et al.* (2013) published a phylogenetic analysis of North American species of *Gyromitra* and their closest relatives. These authors avoided the use of the name *G. gigas* because it lacks a proper type collection, and therefore considered *G. montana* a synonym of *G. korfii*. Miller *et al.* (2015) added some sequences from European collections of *G. gigas* and found three distinct clades among them, stating that this "*raises the possibility that the three species, postulated by Raitviir and Harmaja, have been confirmed by phylogenetic analysis*".

Gyromitra ticiniana Littini was described from Italy by Littini (1988) based on collections found in a broadleaved forest (*Castanea*, *Quercus*, *Cornus*, *Carpinus*, *Corylus*). Although *G. ticiniana* was found to be very similar to *G. gigas* (and *G. curtipes* Fr.), Littini did not discuss the differences between them because he regarded *G. gigas* as a species growing strictly on old stumps in alpine conifer forests. He stated also that the unique microscopical characters of *G. ticiniana* were enough to discriminate this species from *G. gigas*. His discussion was focused instead on the differences with *G. fastigiata* (Krombh.) Rehm another species known to grow in broadleaved forests. Riva (1998; 2010) amended the original description of *G. ticiniana* (as *Gyromitra littiniana* Riva) with a more accurate microscopical study and concluded that *G. ticiniana* differed only from *G. gigas* because of its habitat and narrower ascospores showing a finer ornamentation.

Gyromitra khanspurensis Jabeen & Khalid (in Krisai-Greilhuber *et al.*, 2017) has been recently described from Pakistan, and reported to be phylogenetically different from *G. gigas* because of its ITS rDNA profile, as well as morphologically distinct because of the smaller

smooth ascospores showing very short (or absent) apiculi at their poles. With regard to the other species in the *G. gigas* clade that have ornamented ascospores, we suggest that the specimens may have been studied immature.

Finally, *Gyromitra slonevskii* V.P. Heluta (Heluta, 2001) is another species close or identical to *G. fastigiata* due to its ascospores with "digitate" apiculi. Jabeen & Khalid (in Krisai-Greilhuber *et al.*, 2017) published a phylogenetic tree based on ITS rDNA data where *G. slonevskii* significantly clusters with *G. gigas* and *G. khanspurensis*, although no sequences of *G. fastigiata* or of any species belonging to *Gyromitra* subgen. *Caroliniana* were included. In the present work, we provide ITS rDNA sequences of *Gyromitra fastigiata* from two Italian collections to evaluate its position with regards to *G. slonevskii*.

The main aims of the work are: i) to propose the most suitable taxonomic status of *G. gigas* and *G. ticiniana* based on genetic data and stabilize both names through epitypification; and, ii) to evaluate the relationship between *G. ticiniana* and *G. korfii*.

Material and methods

Morphological study. — The microscopical studies were based on both fresh and dried specimens. Two optical microscopes were used: Olympus CX31 and Olympus CX41 trinocular with plan-achromatic objectives 10×, 40×, 60×, 100× oil immersion. The following main reagents were used: Melzer's reagent, cotton blue (lactophenol and acid lactic), Congo red, 5% KOH. Water mounts were used for the observation of the pigmentation and measurements. At least 25–30 ascospores naturally discharged from the asci were measured from each mature collection.

Phylogenetic study. — Total DNA was extracted from dried specimens employing a modified protocol based on Murray & Thompson (1980). PCR amplification was performed with the primers ITS1F and ITS4 (White *et al.*, 1990; Gardes & Bruns, 1993) for ITS region, while LR0R and LR5 (Vilgalys & Hester, 1990; Rehner & Samuels, 1995) were used to amplify the LSU rDNA region. PCR reactions were performed under a program consisting of a hot start at 95 °C for 5 min, followed by 35 cycles at 94 °C, 54 °C and 72 °C (45, 30 and 45 s respectively) and a final 10 min step at 72 °C. PCR products were checked in 1% agarose gels, and positive reactions were sequenced with one or both PCR primers. Chromatograms were checked in MEGA 5.0 software (Tamura *et al.*, 2011) software searching for putative reading errors, and these were corrected. Two independent alignments, one for ITS rDNA and another for LSU rDNA regions were built. BLAST (Altschul *et al*., 1997) was used to select the most closely related sequences from INSD public databases. Sequences first were aligned in MEGA 5.0 with its Clustal W application and then corrected manually. GBlocks (Castresana, 2000) was employed to remove 242/519 and 8/514 ambiguous sites from ITS and LSU alignments, respectively. The final alignment included 59/217 (ITS rDNA) and 84/506 (LSU rDNA) variable sites. The aligned loci were loaded in PAUP* 4.0b10 (Swofford, 2002) and subjected to MrModeltest 2.3 (Nylander, 2004) in PAUP* 4.0b10. Model GTR+G+I was selected and implemented in MrBayes 3.1 (Ronquist & Huelsenbeck, 2003), where a Bayesian analysis was performed (data partitioned, two simultaneous runs, six chains, temperature set to 0.2, sampling every 100th generation) until convergence parameters were met after 0.52M (ITS rDNA) and 2.58M (LSU rDNA) generations, standard deviation having fell below 0.01. Finally, a full search for the best-scoring maximum likelihood tree was performed in RAxML (Stamatakis, 2006) using the standard search algorithm (data partitioned, GTRMIX model, 2000 bootstrap replications). Significance threshold was set above 0.95 for posterior probability (PP) and 70% bootstrap proportions (BP).

Studied collections

***Gyromitra fastigiata*.** Italy. Piemonte, Valdieri (CN), San Giovanni, alt. 760 m a.s.l., along the stream Gesso, on sandy soil, under deciduous trees (*Corylus*, *Fraxinus*, *Alnus*, *Salix*), 25.IV.2000, *leg*. V. Pepino (pers. herb. V. Somà 00033). Piemonte, Ormea (CN), Ponte di Nava, under broadleaved trees, 23.IV.2001, *leg*. P. Fabbri (pers. herb. V. Somà 01057).

***Gyromitra gigas*.** Czech Republic. Central Bohemia, Těptín near Kamenice u Prahy, edge of the broad-leaved forest, on the base of old stumps of broad-leaved trees and in direct proximity, under *Carpinus betulus*, *Betula pendula*, *Populus tremula*, 460 m a.s.l., 49°53'35.014" N 14°33'4.509" E, 27.IV.2018, *leg*. V. Klener. (TUR-A 208088, **epitype**). France. Isère, Lans-en-Vercors, combe de Servagnet, 1210 m a.s.l., 45.122477° N 5.559077° E, 12.V.2005, under conifers, *leg*. E. Mazet (LY NV 2005.05.12). Pyrénées-Orientales, Belcaire, clos de la Plaine, 980 m a.s.l., 42.841328° N 1.954536° E, 30.IV.2007, under *Picea abies*, *leg*. J.-P. Vidonne (LY NV 2007.04.20). Alpes-de-Haute-Provence, Verdaches, Haut-Bès, 1100 m a.s.l., 44.26884° N 6.31389° E, 02.V.2010, in a mixed woodland, *leg*. G. Doublet (LY NV 2010.05.17 – immature). Savoie, Méribel-les-Allues, near the Altiport, 1760 a.s.l., 45.4104851° N 6.578843° E, 23.V.2010, close to dead branches of conifers, *leg*. E. Armada, not kept. Alpes-de-Haute-Provence, Colmars, Ratery, alt. 1700 m, 44.1897° N 6.66071° E, 08.VI.2010, in a mixed woodland, *leg*. N. Van Vooren (LY NV 2010.06.22). Alpes-de-Haute-Provence, Colmars, col des Champs, 2000 m a.s.l., 44.178141° N 6.700972° E, 08.VI.2010, under *Larix de-*

Table 1 – Samples newly generated for this study, and GenBank accession numbers.

Taxon	Voucher	Country	ITS	LSU nrDNA
Gyromitra fastigiata	V. Somà 01057	Italy	—	MH938321
Gyromitra fastigiata	V. Somà 00033	Italy	MH938675	MH938320
Gyromitra gigas	LK 95.04.08	Hungary	MH938664	MH938310
Gyromitra gigas	LY NV 2007.04.20	France	MH938665	MH938311
Gyromitra gigas	TUR-A 208088	Czech Republic	MH938663	MH938309
Gyromitra gigas	TUR-A 208089	Italy	MH938666	—
Gyromitra gigas	TUR-A 208091	Italy	MH938667	MH938312
Gyromitra gigas	TUR-A 208092	Italy	MH938668	MH938313
Gyromitra gigas	TUR-A 208093	Italy	MH938669	MH938314
Gyromitra ticiniana	LY NV 2004.05.03	France	MH938670	MH938315
Gyromitra ticiniana	TUR-A 208094	Italy	MH938671	MH938316
Gyromitra ticiniana	TUR-A 208095	Italy	MH938672	MH938317
Gyromitra ticiniana	TUR-A 208096	Italy	MH938673	MH938318
Gyromitra ticiniana	TUR-A 208097	Italy	MH938674	MH938319

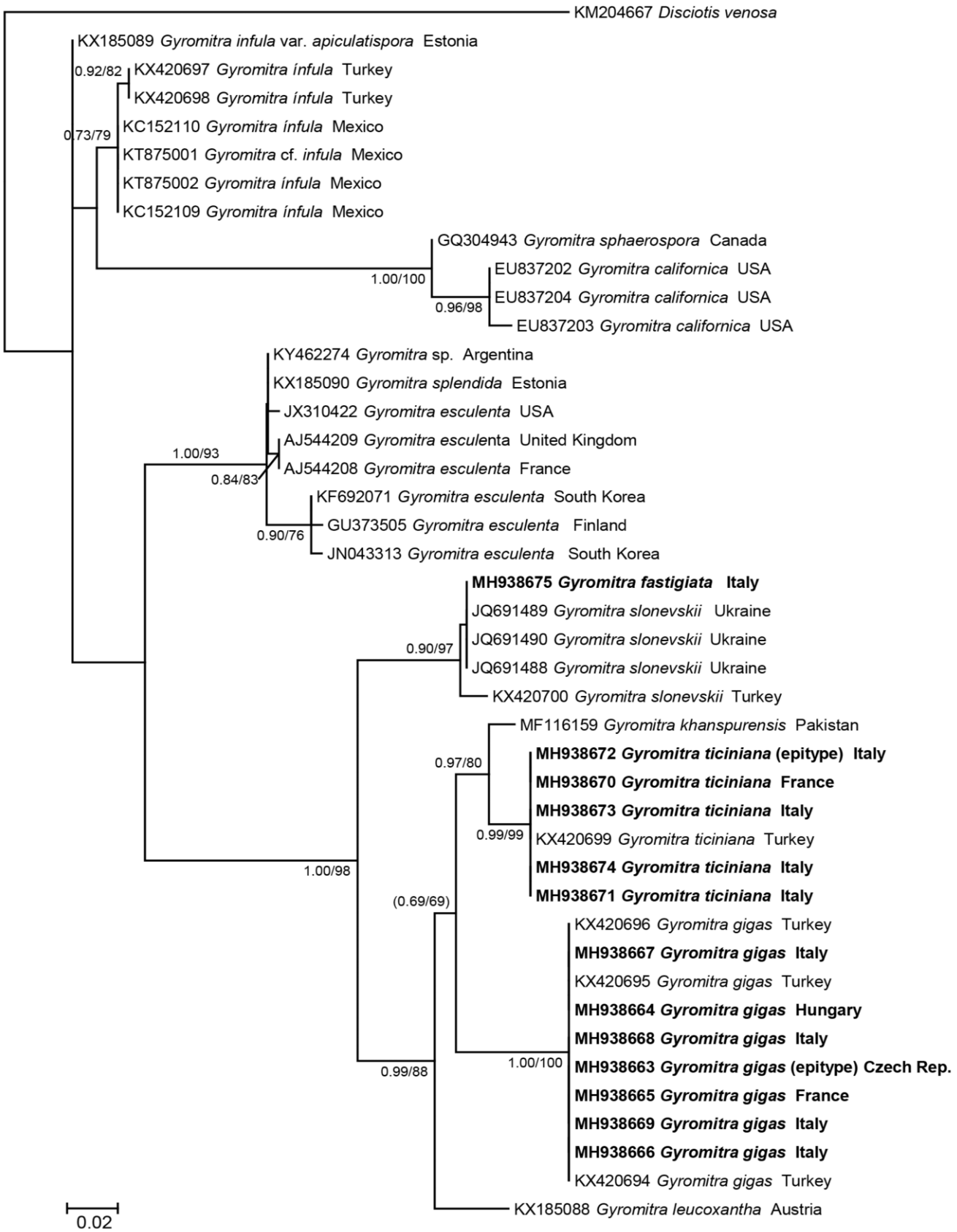


Fig. 1 – Consensus phylogram obtained in RAxML after the Maximum-Likelihood (ML) analysis of ITS rDNA sequences of *Gyromitra*. Nodes were annotated if supported by >0.95 Bayesian PP (left) or >70% ML BP (right), although lower support was sometimes annotated in parentheses.

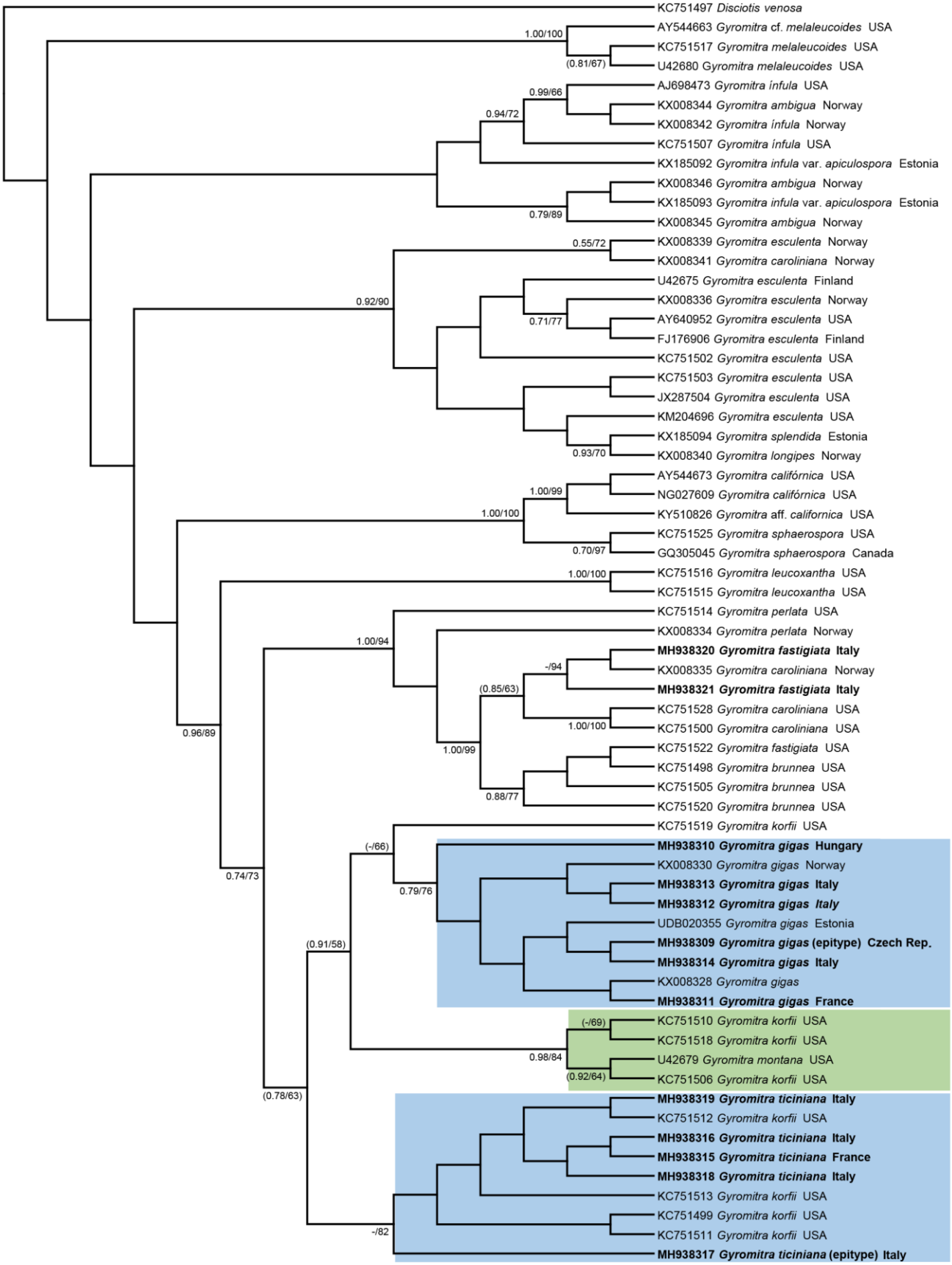


Fig. 2 – Consensus cladogram obtained in RAxML after the Maximum-Likelihood (ML) analysis of LSU rDNA sequences of *Gyromitra*. Nodes were annotated if supported by >0.95 Bayesian PP (left) or >70% ML BP (right), although lower support was sometimes annotated in parentheses.

cidua, *leg*. Y. Mourgues, not kept. Ain, Hauteville-Lompnes, col de la Berche, 865 m a.s.l., 45.9953207° N 5.5523437° E, 03.V.2013, under *Abies alba*, *leg*. D. Bouveret (LY NV 2013.05.02). Savoie, Ugine, la Mollette, 1000 m a.s.l., 45.7473077° N 6.4664716° E, 03.V.2013, under *Picea abies*, *leg*. O. Lussiana (LY NV 2013.05.03). Ain, Innimond, plaine du Bief, 900 m a.s.l., 45.810637° N 5.55577° E, under *Picea abies*, 07.V.2016, *leg*. F. Armada & A. Bidaud (LY NV 2016.05.01). Hungary. Budapest, Csúcs-hegy, 180 m a.s.l., 47.580719° N 18.988154° E, 08.IV.1995, in a mixed woodland (*Prunus avium*, *Quercus robur*, *Q. petraea*, *Fraxinus excelsior*, *Acer* sp., *Pinus nigra*), near a dead trunk of *Pinus nigra*, *leg*. Z. Lukács (pers. herb. LK 95.04.08). Italy. Fiuli-Venezia-Giulia, Paularo (UD), 1300 m a.s.l., under *Picea abies*, 22.V.2006, *leg*. G. Dose (TUR-A 208089). Valle d'Aosta, Saint Rhemy en Bosses (AO), 1700 m a.s.l., under *Picea abies* and *Larix decidua*, 08.V.2008, *leg*. M. Carbone (TUR-A 208090). Valle d'Aosta, Morgex (AO), Fraz. Arpy, 1600 m a.s.l., under *Picea abies* and *Larix decidua*, 31.V.2009, *leg*. F. Calledda, G. Boerio & M. Carbone (TUR-A 208091). Abruzzo, Prato Selva, Fano Adriano (TE), 1450 m a.s.l., under conifers, 22.IV.2018, *leg*. B. De Ruvo (TUR-A 208092). Abruzzo, Colle dell'Asino, Pietracarmela (TE), 1400 m a.s.l., in the soil under *Fagus*, 25.IV.2018, *leg*. B. De Ruvo (TUR-A 208093).

***Gyromitra ticiniana*.** France. Savoie, Saint-Pierre-d'Albigny, col du Frêne, 950 m a.s.l., 45.582929° N 6.138482° E, under *Fagus sylvatica*, 16.V.2004, *leg*. Riondet (LY NV 2004.05.03). Italy. Lombardia, Motta Visconti (MI), 1979, ex coll. Littini number 48/G.A [TUR-A 208104, **holotype**]. Lombardia, Motta Visconti (MI), 100 m a.s.l., under *Quercus robur* and other broadleaves trees, IV.2010, *leg*. R. Galli & E. Rigoni (TUR-A 208097). Marche, Propezzano, Montegallo (AP), ca. 900 m a.s.l., under *Fagus sylvatica* and *Quercus cerris*, 11.IV.2010, *leg*. E. Carassai (TUR-A 208094). Piemonte, Vignole Borbera (AL), Variano Inferiore, 250 m a.s.l., 44.718583° N 8.936888° E, under *Quercus pubescens* but also in presence of *Castanea sativa* and *Carpinus* sp., 05.IV.2010, *leg*. M. Carbone (TUR-A 208095, **epitype**). Liguria, Sassello (SV), 400 m a.s.l., under *Quercus robur* and *Ostrya carpinifolia*, 08.IV.2018, *leg*. M. Carbone & F. Boccardo (TUR-A 208096).

Phylogenetic results

Phylogenetic inference based on ITS rDNA (Fig. 1) and LSU rDNA (Fig. 2) confirmed that *Gyromitra gigas* and *G. ticiniana* represent two distinct species, in agreement with genetic data already present in public databases (Gungor *et al*., unpub.). Both species have a low intraspecific ITS rDNA variability (0–0.5% ITS rDNA) and a large interspecific gap (11%). ITS rDNA sequences of *Gyromitra ticiniana* are significantly similar to those of *G. khanspurensis*, and also *G. gigas*

Pl. 1 – *Gyromitra gigas*, lectotype, adapted from Krombholz (1834).

and *G. leucoxantha* (Fig. 1). In turn, LSU rDNA data of *G. ticiniana* match some sequences of *G. korfii* produced by Methven *et al*. (2013), while *G. gigas* seems close to the clade including *G. montana* U42679 (Fig. 2). Miller *et al.* (2015) suggested the existence of three clades in this group: one exclusively European (probably matching *G. gigas*), another found in northern USA and Canada (which includes the only known sequence of *G. montana*), and one found in temperate areas of eastern North America, which is here shown to match *G. ticiniana*. Miller *et al.* (2015) suggest that an ITS rDNA or multigenic approach should be adopted to solve the taxonomic identity of these clades.

ITS rDNA obtained from the samples identified as *G. fastigiata* matched the sequences of *G. slonevskii* available in databases produced by Barseghyan *et al.* (2012), while LSU rDNA of *G. fastigiata* was significantly similar to some sequences of *G. caroliniana* (Bosc) Fr. produced by Methven *et al*. (2013). Both species were in turn significantly related with *G. brunnea* Underw., in agreement with these authors, and to a lesser extent with *G. perlata* (Fr.) Harmaja [= *G. ancilis* (Pers.) Kreisel, in Miller *et al*., 2015].

Taxonomy

Gyromitra gigas (Krombh.) Quél., *Mém. Soc. émul. Montbeliard, sér. 2*, 5: 338 (1873).

Basionym:
Helvella gigas Krombh., *Naturgetr. Abbild. Schwamme*, 3: 28 (1834).

Original diagnosis:
Helv. pileo magno, lobato, undulato, plicato vel crispo, pallido, albido vel ochraceo: lobis stipiti subadnatis adpressis subundulatis; stipite crasso, celluloso, ceraceo, albido, extus lacunoso, sub glabro; ascis majusculis; sporis magnis, ovalibus; mycelio ceraceo-tomentoso, crasso, effuso.

Homotypic synonyms:
≡ *Mitrophora gigas* (Krombh.) Lév., *Ann. sci. nat., sér. 3, botanique*, 5: 250 (1846).
≡ *Neogyromitra gigas* (Krombh.) S. Imai, *Bot. Mag. (Tokyo)*, 52: 358 (1938).
≡ *Maublancomyces gigas* Herter, *Rev. Sudam. bot*., 8 (5): 161 (1950).
≡ *Discina gigas* (Krombh.) Eckblad, *Nytt Mag. Bot*., 15 (1-2): 99 (1968).

Other synonyms:
= *Gyromitra gigas* var. *pumila* Velen., *Monogr. Disc. Bohem*.: 389 (1934).
= *Gyromitra curtipes* Fr., *Atl. Sw*., 34, pl. 56 (1861); *Maublancomyces curtipes* (Fr.) Herter, *Rev. Sudam. bot*., 10 (1): 17 (1951).
= *Gyromitra ussuriensis* Lj. N. Vassiljeva, *Notulae Syst. Sect. Crypt. Inst. Bot. Acad. Sci. U.S.S.R*., 6 (7-12): 189 (1950), *fide* Raitviir (1970); *Neogyromitra ussuriensis* (Lj. N. Vassiljeva) Raitv., *Soobsch. Akad. Nauk. Soyuza SSR, Sibirsk. Otdel. Dal'nevost Fil*., 23: 53 (1964), inval.

Typification:
Lectotype selected here: Krombholz, *Naturgetr. Abbild. Beschr. Schwämme*, 3: Tab. 20, fig. 1-5 (1834). MBT 383599.
Epitype selected here: Czech Republic, Central Bohemia, Těptín near Kamenice u Prahy, 27.IV.2018, *leg.* V. Klener, TUR-A 208088 (Isoepitype in ILLS); Genbank: MH938663, MH938309). MBT 383600.

Macroscopical features (Pl. 2 and 4):
Ascomata stipitate, 5–12 cm high. **Pileus** 4–11 (13) cm wide, 5–6.5 cm high, inflated, irregular-shaped, in some cases mildly flattened or divided into several short and blunt lobes, distinctly wrinkled by intestine-shaped, contorted, round and tumid folds, multiply attached to stipe, but with margins generally loose. **Hymenium** finely tuberculate or veined, glabrous; initially honey-yellow coloured, even ivory-whitish close to pileus margin, then ochre, buff to rust-brown. **Stipe** robust and thick, rather short, 4–6 × 3–5 cm, occasionally partially buried into substrate, often nearly entirely covered by beetling pileus margin, irregular, distortedly and roundly grooved, hollow, ivory, yellowish to yellowish-gray. **Flesh** thin, waxy, quite fragile, white to yellowish-gray, with inconspicuously pleasant taste and smell. **Spore print** white.

Microscopical features (Pl. 3):
Asci cylindrical, 290–330 × 19–21 µm, 8-spored, operculate, pleurorynchous, inamyloid. **Paraphyses** cylindrical, septate, enlarged at the top, up to 11 µm, filled by a brown pigment in the upper part. **Ascospores** ellipsoid to subfusoid, sometimes inequilateral, (25–) 27–32 (–34.5) × (11.5–) 12–13 (–14) µm on free spores [the most frequent 27–30 × 12–13 µm], Q= (2.1–) 2.2–2.5 (–2.75) [n>50], walls up 0.8–1 µm thick, hyaline, ornamented by low but well defined crests (also visible without Cotton blue at 100× oil immersion) which mostly form an incomplete reticulum, containing one large central oil drop, 9–10 µm diam. and two smaller ones, up to 3 µm diam., at the poles, with blunt, sometimes truncated and not decurrent apiculi at each pole, up to 2.5 µm high. **Medullary excipulum** of *textura intricata*, composed of hyaline cylindrical hyphae, septate, 7–12 µm wide, but sometimes inflated up to 25 µm, av. 0.8 µm thick-walled. **Ectal excipulum** of *textura globulosa* to *subglobulosa/angularis* composed of hyaline cells, up to 35 µm diam. or more elongated up to 40 × 55 µm; external part with cylindrical to clavate, thin-walled, hyaline hyphae.

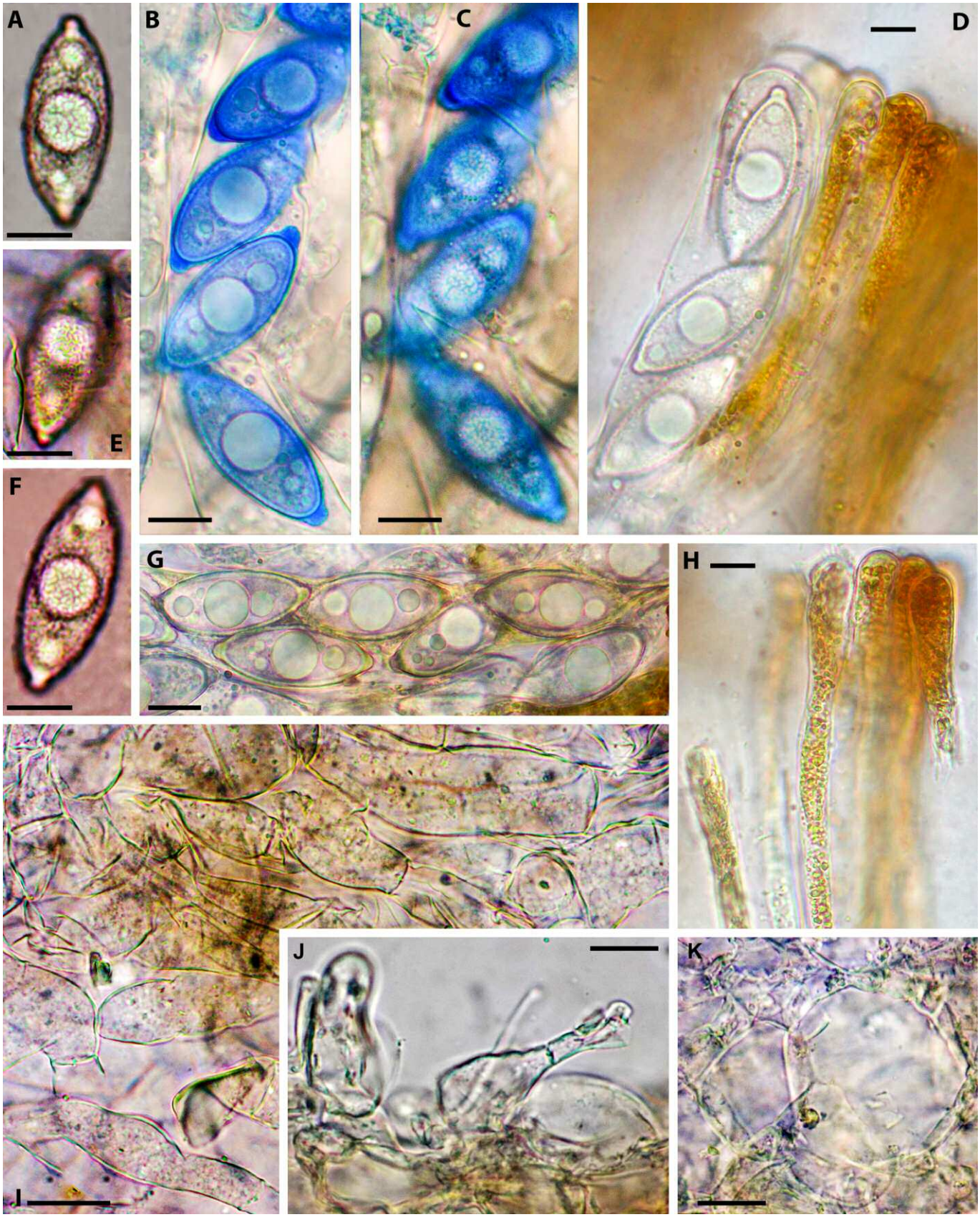
Ecology and phenology:
Gyromitra gigas appears in hardwoods as well as in coniferous forests or in forest clearings, prevalently in close proximity of (or directly from) old stumps, rotten logs or other decayed wood. In central Europe it grows with preference in woods of birch (*Betula*) or aspen (*Populus tremula*), but also in association with spruce (*Picea*), linden (*Tilia*), hornbeam (*Carpinus*) or oak (*Quercus*), occasionally with other trees. *Gyromitra gigas* occurs from submontane to montane level, both on alkaline (e.g. calcareous or basaltic) and acidic (e.g. plutonic) substrates. Ascomata appear from the second half of March until the middle of April and persist until the beginning of May. Growing solitary to gregariously. Generally not very common, however, in some regions and some years more abundant.

In western and southern Europe it is mostly collected in coniferous forests (mainly *Picea abies* but also *Abies alba*) at medium to high altitude, although collections under *Fagus* are frequently reported. The period of fruiting is the same as above. In the Alps, at high elevation or after a good snow winter, it can also be found until the first half of June.

Gyromitra ticiniana Littini, *Pagine Bot*., 12: 19 (1988).

Original diagnosis:
Omnis ascocarpus firmitate cerea; hymenium cerebriforme plus minusve incavatum 5–15 *cm. latum, globosum, primitus gilvum sufflavum, postea badioargillaceum. Stipes brevis profunde sulcatus ut composites, alte intus mitra posiys, albogriseus, levis sed basi pilosus concolor; intus quasi cavus,* 3–8 *cm. longus. Asci cylindracei* 230–250 × 28–30 *mmm., jodo haud tincti; paraphises subtiles, apice clavato* 5–6 *mmm (heteroparaphises per corruptionem). Sporidia apiculata sed truncate apice, leva, crasse triguttulata* 24–28 × 8–10 *mmm. In silvis mixtis: Castanea, Quercus, Cornus, Carpinus, Corylus; praecox, ab initio usque ad finem mensis martii ad truncos marcidos, interdum, lignicola. Loco*: Motta Visconti.

Pl. 2 – *Gyromitra gigas*. Epitype collection in different stage of maturation. Photos V. Klener



Pl. 3 – *Gyromitra gigas* (epitype). A, B, C, E, F, G: Ascospores; D: Ascus tip with ascospores and paraphyses; H: Upper part of paraphyses; I: Hyphae of medullary excipulum; J: External part of ectal excipulum; K: Globose cell of the ectal excipulum. All pictures in water mounts except B and C in Lactic Cotton Blue not heated. Scale bars = 10 µm. Photos M. Carbone

Pl. 4 – *Gyromitra gigas.* A: TUR-A 208090 (M. Carbone); B: TUR-A 208091 (M. Carbone); C: TUR-A 208092 (B. De Ruvo); D: TUR-A 208089 (G. Dose); E: LY NV 2016.05.01 (F. Armada); F: Coll. from Méribel-les-Allues (France), 23.V.2010 (N. Van Vooren); G: Coll. from Colmars (France), 8.VI.2010 (N. Van Vooren).

Accepted synonym:

= *Gyromitra littiniana* A. Riva, *Schweiz. Z. Pilzk.*, 88 (6): 233 (2010), superfluous name.

Type: Coll. n. 14130 (LUG).

Putative synonym (see Discussion):

Gyromitra korfii (Raitv.) Harmaja, *Karstenia*, 13: 48 (1973).

≡ *Discina korfii* Raitv., *Botaanika-alased tood*, 9: 371 (1970).

Typification:

The holotype is coll. 48/G.A. in Littini's personal herbarium, as designated in the protologue. Littini died in 2009 and never deposited his collections in an institutional herbarium. He lived his last years not far from Emanuele Campo's house. Thanks to Emanuele and Littini's wife we have found the holotype collection in the Littini's remaining private herbarium. Unfortunately the sample is scanty and not well preserved. Even if useful for microscopical study, our attempts to obtain DNA sequences were unsuccessful and so a modern sequenced collection was then necessary to stabilize the species concept. We have decided to select as epitype an abundant collection, fitting perfectly the ecological and morphological concept, and made in the same habitat more or less 60 km from the original locality.

Holotype: Italy, Motta Visconti (MI), 1979, *ex* coll. Littini number 48/G.A., deposited by us in Turku Herbarium under the accession number TUR-A 208104. MBT 383601.

Epitype selected here: Italy, Vignole Borbera (AL), 05.IV.2010, *leg.* M. Carbone, TUR-A 208095 (Isoepitype in ILLS); Genbank: MH938672, MH938317. MBT 383602.

Macroscopical features (Pl. 5):

Ascomata stipitate, up to 11 cm high. **Pileus** up to 8 cm wide (13 cm in the French collection), bi-trilobate or subglobose, irregular-shaped, gently folded to more wrinkled by deep and round folds, attached to the stipe in some spots, but with margin generally free. **Hymenium** very finely tuberculate, glabrous; yellow-ochre coloured then ochre, buff to rust-brown at maturity. **Stipe** stout and rather short, up to 6 cm high and 7(–9) cm diam., hollow, often nearly entirely covered by pileus margin, irregular; ivory, yellowish to yellowish-gray. **Flesh** thin, waxy, quite fragile, white to yellowish-gray sometimes with pale pinkish hues, with inconspicuously pleasant taste and smell. **Spore print** white.

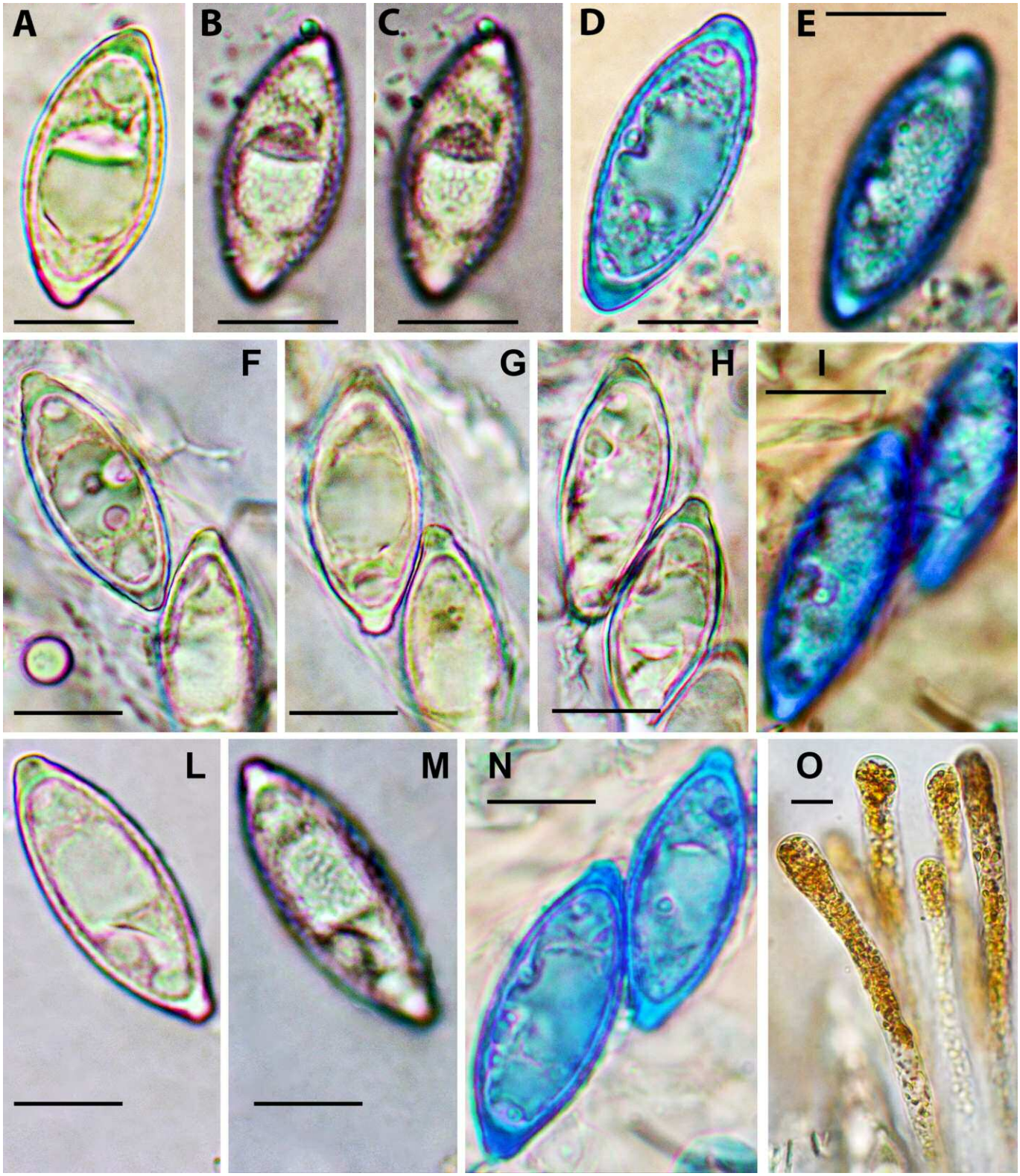
Microscopical features (Pl. 6):

Asci cylindrical, 280–300 × 18–20 µm, 8-spored, operculate, pleurorynchous, inamyloid. **Paraphyses** cylindrical, septate, enlarged at the top, up to 9.5 µm, filled by a brown pigment in the upper part. **Ascospores** ellipsoid to subfusoid, sometimes inequilateral, (22–)25–31 (–34) × (9.5–) 10.5–11 (–12) µm on free spores [the most frequent 27–29 × 11 µm], Q= (2.2–) 2.5–2.7 (–2.8) [n>50], wall up 0.8–1 µm thick, hyaline, ornamented by low crests (barely visible without Cotton blue at 100× oil immersion) which form an incomplete reticulum, containing one large central oil drop and two smaller ones at the poles, with blunt and mostly decurrent apiculi at each pole, up to 2 µm high. **Trama** composed by a medullary excipulum and an ectal excipulum not different from that in *Gyromitra gigas* (see above).

Ecology and phenology:

Gyromitra ticiniana grows in hardwoods on soil rich in woody remains, or close to (or directly from) old stumps or rotten logs. It seems to prefer *Quercus* spp. although we have a collection collected in pure beech forest (*Fagus sylvatica*). According to our knowledge *G. ticiniana* occurs from lowland to mountainous areas, up to 1000 m a.s.l., from the second half of March until May (depending on the elevation).

Pl. 5 – *Gyromitra ticiniana*. A: TUR-A 208096 (M. Carbone); B, C, E: TUR-A 208095 (M. Carbone); D, F: Same place of TUR-A 208095 a week later (M. Carbone); G, H: TUR-A 208094 (E. Carassai).



Pl. 6 – *Gyromitra ticiniana* (from the holotype). Ascospores in water mounts except D, E, H, N in Lactic Cotton Blue not heated; O: paraphyses tips in water mount. Scale bars = 10 µm. Photos M. Carbone

Note on the name *Gyromitra littiniana* Riva:

Riva (2010) considered the name *ticiniana* not valid for the following reasons (translated from Italian) "*Littini omitted to give the number of the type collection and, above all, the official herbarium where it could be traced. It is a pity, the ICNB code is unquestionable and determinant: Gyromitra ticiniana Littini is invalid*". Then, Riva (2010) wrote "*We have to inform the reader that in the descriptive part of Littini's article the author wrote* «... the type (typus) in exsiccatum and the photographic material are deposited in the personal herbarium under the code n.48/G.A.». *This herbarium now is no longer in Milan, and some researches done with Dr. Roberto Galli failed. If one day this type will be rediscovered the ICBN rules are irremovable*".

According to these two main arguments, Riva decided that *G. ticiniana* was invalid and that the only way to proceed was to re-describe it as a new species, i.e. *Gyromitra littiniana*.

Although we do not like this way to proceed because it could be validated according to Art. 9.11 ICN Shenzhen — "If no holotype was indicated by the author of a name of a species or infraspecific taxon, or when the holotype or previously designated lectotype has been lost or destroyed, or when the material designated as type is found to belong to more than one taxon, a lectotype or, if permissible (Art.

9.7), a neotype as a substitute for it may be designated" —, we must admit that the description of *Gyromitra littiniana* is not strictly contrary to the ICN and so validly published[1].

McNeill (2014), in an article about some issues on a "holotype", wrote that "*If, at any date, an author indicates a single specimen or other collection as "type", this is a holotype*".

So, if we analyze the protologue of *G. ticiniana* (Littini, 1988) we found that the author clearly indicated the holotype, its number and location in a herbarium. So the conditions of Art. 40.1, 40.2 and 40.7 of ICN are achieved. A formal (and good) Latin diagnosis was given and the species was published in a printed bulletin. There are no reasons to consider the name *Gyromitra ticiniana* to be invalid.

Concerning the deposit of the holotype, the recommendation 7A.1 ICN indicates: "It is strongly recommended that the material on which the name of a taxon is based, especially the holotype, be deposited in a public herbarium or other public collection with a policy of giving bona fide researchers access to deposited material, and that it be scrupulously conserved". As it is only a recommendation, the housing of Littini's type in his personal herbarium do not affect the achievement of Art. 40 ICN.

For all these reasons *Gyromitra ticiniana* was validly published. On the contrary *Gyromitra littiniana* was superfluously described and must be regarded as a later synonym.

Discussion

Gyromitra gigas and *G. ticiniana* are very similar in habit, size and colours, but from a microscopical point of view it seems they could be differentiated by their ascospore morphology. In fact *G. ticiniana* ascospores have a bit higher average Q, a smaller width (10–11 µm vs 12–13 µm av.) and a less coarse spore sculpturing. Regarding their ecology, at present we must underline that *G. ticiniana* seems to prefer broadleaved forests, while *G. gigas* has a wider host range including conifers. TUR-A 208093, collected under pure *Fagus* and a 100% match to *Gyromitra gigas*, has some parts with free spores 10–11 µm wide, but in other parts of the hymenium they are typically 12–13 µm wide. As already pointed out by Van Vooren (2017), *Gyromitra* species have a slow process of maturity, often requiring several weeks to provide fully mature ascospores but spontaneous sporeprints with not fully mature ascospores could exist.

ITS and LSU rDNA data produced in the present work from *G. gigas* and *G. ticiniana* agree with the overall conclusions already pointed out by Miller *et al.* (2015) about the *G. gigas* complex. There are three distinct genetic lineages, probably matching the species concepts of *G. gigas*, *G. montana* and *G. korfii* (= ? *G. ticiniana*). Present data suggest that *G. gigas* is apparently an exclusively north European taxon, while *G. montana* is exclusively an American species, and *G. korfii* is present in America and possibly in Europe (if the synonymy with *G. ticiniana* will be confirmed). The lack of ITS rDNA data from American collections in public databases makes it difficult to conclude anything about the identity of KC751519 (NY 01797009), which seems to display an intermediate position between *G. montana* and *G. gigas*. However, it would not be rare to find specimens of *G. gigas* in northeastern North America. This lack of American ITS rDNA sequences does not allow to know if *G. khanspurensis* MF116159 (LAH35074 holotype) from Pakistan should be considered part of the intraspecific variability of *G. korfii*, as some species of *Gyromitra* seem to have a significant diversity, e.g. *G. esculenta* (Pers.) Fr., *G. infula* (Schaeff.) Quél., and probably also *G. caroliniana*.

Regarding the possible synonymy between *G. ticiniana* and *G. korfii*, we have tried to obtain original material of the latter species but due to some problems the loan was delayed. A future study will clarify this unanswered question and so, for the time being we prefer to keep them separate. In our *G. ticiniana* collections we have found the same differences stressed by Harmaja (1973) for *G. korfii* vs *G. gigas* (i.e. slender and a bit shorter ascospores with a more delicate ornamentation) except the paraphyses apex width which is said to be up to 13 µm wide in *G. korfii* but it is up to 9.5 µm in *G. ticiniana* (not different at all from those found in *G. gigas*).

In the case of *G. fastigiata*, ITS rDNA data suggest that the samples analyzed match the genetic concept of *G. slonevskii* in the sense of Barseghyan *et al.* (2012). The collections sequenced by the latter authors do not come from original material but from Haifa Herbarium. On the contrary LSU rDNA suggest they could be part of the intraspecific variability of *G. caroliniana*, but distinct from *G. brunnea* (macroscopically very close to *G. fastigiata*). However, the extent of intraspecific diversity should be further explored with additional samples before drawing conclusions about these four species.

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[1] We must underline that Riva (2010) did not use the term "type" or "holotype" in the protologue and so maybe his species could also not be valid according the Art. 40.6 ICN.

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1: M. Carbone – Via Don Luigi Sturzo 173, 16148 Genova, Italy – matteocarb@hotmail.com
2: N. Van Vooren – 36 rue de la Garde, 69005 Lyon, France – nicolas@vanvooren.info
3: V. Klener – Gončarenkova 753/10, 147 00 Praha, Czech Republic – vavrinec.k@gmail.com
4: P. Alvarado – La Rochela 47, 39012 Santander, Spain – pablo.alvarado@gmail.com