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A molecular phylogenetic assessment of the genus *Gyromitra* in North America

Andrew S. Methven[1]

Department of Biological Sciences, Eastern Illinois University, Charleston, Illinois 61920

Steven E. Zelski

Department of Plant Biology, University of Illinois, Champaign, Illinois 61801

Illinois Natural History Survey, University of Illinois, Champaign, Illinois 61820

Andrew N. Miller

Illinois Natural History Survey, University of Illinois, Champaign, Illinois 61820

Abstract: *Gyromitra* is a widespread genus of macroscopic apothecial ascomycetes whose taxa may be mycorrhizal, saprophytic or parasitic. Nuclear ribosomal 28S large subunit sequence data from 35 specimens from North America, along with sequences available in GenBank, were used in maximum-parsimony, maximum-likelihood and Bayesian analyses to reconstruct a phylogeny of *Gyromitra* in North America. *Gyromitra* sensu lato forms a monophyletic group within the Discinaceae composed of five distinct subgenera and 11 well supported clades that include *Discina*, *Hydnotrya* and *Pseudorhizina*. A new subgenus is proposed to accommodate *G. californica* and *G. sphaerospora*.

Key words: Discinaceae, false morel, Helvellaceae, large subunit, Morchellaceae, Rhizinaceae, systematics

Introduction

Gyromitra Fr. is a widespread genus of macroscopic apothecial ascomycetes whose taxa may be mycorrhizal, saprophytic or parasitic (Egger and Paden 1986, Hansen and Pfister 2006). Linnaeus (1753) proposed the genus *Elvela* (= *Helvella*, orth. var.) to house *Elvella mitra* (= *Helvella crispa* Fr.). Fries (1823) established the Elvellaceae, which soon after was recognized as the Helvellaceae (Corda 1842). The Discinaceae subsequently was proposed by Benedix (1961) to accommodate the genera *Discina* Fr. and *Maublancomyces* Hert. (= *Gyromitra* Fr. = *Neogyromitra* Imai.). Eckblad (1968) considered *Neogyromitra* and *Discina* to be congeneric and placed *Gyromitra*, *Pseudorhizina* Jacz. and *Rhizina* Fr. in the Rhizinaceae. Abbott and Currah (1997) included *Gyromitra* in the Helvellaceae along with *Balsamia* Vittad., *Barssia* Gilkey, *Choiromyces* Vittad., *Gymnohydnotrya* B.C. Zhang & Minter, *Helvella* Fr., *Hydnotrya* Berk. & Broome, *Pseudorhizina*, *Rhizina* and *Underwoodia* Peck and thought that *Discina* was a subgenus within *Gyromitra*. O'Donnell et al. (1997) transferred *Gyromitra* to the Discinaceae Benedix emend. N.S. Weber, Trappe and O'Donnell where it resides today.

Fries (1849) considered *Discina* (based on *Discina perlata* [Fr.] Fr.), *Gyromitra* (based on *G. esculenta* [Pers.] Fr.) and *Helvella* (based on *Helvella crispa* [Scop.] Fr.) to be segregate genera. Based on an examination of *Neogyromitra gigas* (Krombh.) Imai and *N. caroliniana* (Bosc.) Imai, Harmaja (1969) considered differences in the perispore of species of *Gyromitra* to be significant only at the infrageneric level and transferred these two species to *Gyromitra*. Harmaja (1969) also proposed the new combinations *G. perlata* and *G. leucoxantha* for *D. perlata* Fr. and *D. leucoxantha* Bres. respectively, stating that ''[f]ortunately the name *Gyromitra* is older than the name *Discina*.'' Harmaja (1973) later transferred *D. macrospora* Bubák to *Gyromitra* as well. Within these putative taxonomic groupings, traditional characters that have been used to segregate species have included ecology, biogeography, gross morphology (ascomata size, shape, color), microscopic features (ascospore size, shape, staining reactions) and scanning electron microscopy (Abbott and Currah 1997).

With the exception of a restriction enzyme analysis of 28S large subunit (LSU) by Bunyard et al. (1995), which targeted species with affinities to *Morchella* Dill. ex Pers., *Disciotis* Boud., and *Verpa* Sw., taxonomic relationships among these taxa were inferred solely on morphological characters. Utilizing 18S small subunit (SSU) nrDNA, Gargas and Taylor (1995) were the first to identify a monophyletic *Gyromitra-Morchella* clade that Landvik et al. (1997) later expanded to include *Hydnotrya*. O'Donnell et al. (1997) published a molecular study based on SSU and LSU which showed that the Helvellaceae and Tuberaceae formed a sister clade to a group comprising the Discinaceae and Morchellaceae. This split was supported by Hansen and Pfister (2006), Spatafora et al. (2006) and Tedersoo et al. (2006) who placed *Gyromitra* in the Discinaceae. These studies, as well as the study of Medel (2005), all stressed the need to perform more comprehensive molecular phylogenetic analyses on *Gyromitra* and closely related taxa.

The limited molecular phylogenetic studies that have included species in the Discinaceae and, more

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[1] Corresponding author. E-mail: asmethven@eiu.edu

specifically, members of the genus *Gyromitra* (O'Donnell et al. 1997, Hansen and Pfister 2006, Spatafora et al. 2006, Tedersoo et al. 2006, Kellner et al. 2007) are reflected in the paucity of sequence data available from GenBank. Only 11 LSU sequences are deposited in GenBank for the Discinaceae including seven of *Gyromitra* species. Because the phylogenetic relationships of *Gyromitra* are poorly resolved, we generated 35 nrDNA. LSU sequences from North American specimens to test phylogenetic relationships within *Gyromitra*. The aims of this study were to: (i) determine whether North American species of *Gyromitra* represent a monophyletic group; (ii) assess evolutionary relationships between *Gyromitra* and related species in North America; and (iii) evaluate previous subgeneric classifications.

MATERIALS AND METHODS

Taxon sampling.—29 LSU sequences obtained from GenBank (TABLE I) were used in the analysis; an additional 35 LSU sequences were generated during this study. *Rhizina undulata* Fr. was used as outgroup taxon based on analyses of O'Donnell et al. (1997) and Hansen and Pfister (2006).

DNA extraction, PCR amplification, sequencing and sequence alignment.—DNA extraction was performed from 1 cm^3 pieces excised from dried ascomata with the aid of a DNeasy Plant Mini Kit (QIAGEN, Valencia, California) according to the manufacturer's specifications except fungal tissue first was rehydrated in 50 μL AP1 buffer solution and frozen overnight at −80 C. After extraction, genomic DNA was viewed on a 1% TBE agarose gel containing ethidium bromide. PCR amplification was performed with PuReTaq Ready-To-Go™ PCR beads (GE Healthcare, Piscataway, New Jersey) and the primers LROR and LR6 (Vilgalys and Hester 1990, Rehner and Samuels 1994). Each 25 μL PCR reaction contained 1 μL of each primer, 2.5 μL DMSO, 3 μL DNA and 17.5 μL PCR-grade H_2O. Reactions were run on a PTC–200 thermo-cycler (MJ Research, St Bruno, Quebec) with these cycling parameters: initial denaturation at 95 C for 5 min, followed by 40 cycles of 95 C for 30 s, 52 C for 15 s and 72 C for 1 min, with a final extension step of 72 C for 10 min. PCR products were viewed on a 1% TBE agarose gel containing ethidium bromide and purified with the QIAquick PCR Purification Kit (QIAGEN). Sequencing reactions using the primers LROR, LR3 (Vilgalys and Hester 1990), LR3R (Rehner and Samuels 1995) and LR6 were carried out with the BigDye® Terminator 3.1 Cycle Sequencing kit (Applied Biosystems, Foster City, California). Sanger sequencing was performed on an AB3730xl DNA Analyzer at the WM Keck Center for Comparative and Functional Genomics at the University of Illinois at Urbana-Champaign. Sequences were assembled and initially aligned in Sequencher 4.9 (Gene Codes Corp., Ann Arbor, Michigan). Further alignment was performed with MUSCLE 3.6 (Edgar 2004) followed by manual correction.

Phylogenetic analyses.—Maximum-parsimony (MP), Maximum-likelihood (ML) and Bayesian analyses were performed on the LSU dataset. Maximum-parsimony bootstrap values and Bayesian posterior probabilities were used to assess nodal support. Characters at the 5′ and 3′ ends were excluded in all analyses due to missing data. For MP analyses, three ambiguous regions, representing 38 characters, were delimited and recoded with INAASE 2.3b (Lutzoni et al. 2000) and added to the dataset as three additional characters. STMatrix 2.2 (François Lutzoni and Stefan Zoller, Department of Biology, Duke University) was used to model nucleotide changes in the unambiguously aligned characters. The resulting 953 bp alignment was analyzed in PAUP 4.0b10 (Swofford 2002). Branch support for the analysis was estimated by performing 100 bootstrap replicates (Felsenstein 1985) with a heuristic search consisting of a simple stepwise addition and a random starting tree. The number of trees saved for each bootstrap replicate was limited to 10, and the subtree pruning and regrafting (SPR) model was incorporated.

For ML analysis, ModelTest 3.7 (Posada and Crandall 1998) was used to determine the best-fit model of nucleotide evolution for the dataset. A variation of the GTR model (TrNef+I+G) was selected based on both the AIC and the hLRT. Base pair frequencies were: freqA = 0.2485, freqC = 0.2302, freqG = 0.2987, and freqT = 0.2226. A rate matrix was incorporated into the model based on the following rates of transitions and transversions: r[AC] = 1, r[AG] = 2.0541, r[AT] = 1, r[CG] = 1, r[CT] = 5.5620, and r[GT] = 1. Invariable sites comprised 0.4963 of the dataset, and the gamma-shape parameter was 0.6441. One hundred bootstrap replicates were performed with PHYML (Guindon and Gascuel 2003) incorporating a combined nearest neighbor interchange (NNI) and SPR search tree option.

Bayesian analysis was conducted with MrBayes 3.1.2 under default settings (Huelsenbeck and Ronquist 2001, Ronquist and Huelsenbeck 2003). A total of 10 000 000 generations were run with trees sampled every 1000 generations, resulting in a total of 10 000 trees. The above TrNef+I+G model was implemented. The first 1000 trees were discarded because the burn-in had reached stationarity at that point, and the remaining 9000 trees were used to calculate the posterior probability (PP). The consensus tree was viewed in PAUP* 4.0b10 (Swofford 2002).

RESULTS

Maximum-likelihood PHYML analysis produced a single most likely tree (FIG. 1). *Gyromitra* sensu lato forms a monophyletic group within the Discinaceae and is composed of five distinct subgenera and 11 well supported clades that include *Discina macrospora* (hereafter treated as a synonym of *G. perlata* [Fr.] Harmaja), *Pseudorhizina californica* (W. Phillips) Harmaja (hereafter recognized as *G. californica* [W. Phillips] Raitv.) and *Hydnotrya*. The single most parsimonious tree produced in MP analysis did not differ significantly from the tree topologies generated

TABLE I. Species used in this study with LSU sequences retrieved from GenBank and with newly generated LSU sequences

Species	Reference	GenBank accession no.
Discina macrospora	O'Donnell et al. 1997	U42678
Disciotis venosa	Spatafora et al. 2006	AY544667
Disciotis venosa	O'Donnell et al. 1997	U42670
Disciotis venosa	Kellner et al. 2007	AJ698472
Fischerula subcaulis	O'Donnell et al. 1997	U42673
Gyromitra californica	Spatafora et al. 2006	AY544673
Gyromitra esculenta	O'Donnell et al. 1997	U42675
Gyromitra esculenta	Kellner et al. 2007	AJ544208
Gyromitra infula	Kellner et al. 2007	AJ698473
Gyromitra melaleucoides	O'Donnell et al. 1997	U42680
Gyromitra melaleucoides	Direct submission	AY544663
Gyromitra montana	O'Donnell et al. 1997	U42679
Helvella solitaria	Kellner et al. 2007	AM397273
Hydnotrya cerebriformis	O'Donnell et al. 1997	U42676
Hydnotrya cubispora	Tedersoo et al. 2006	DQ200845
Leucangium carthusianum	O'Donnell et al. 1997	U42674
Morchella conica	Kellner et al. 2007	AJ698468
Morchella elata	O'Donnell et al. 1997	U42667
Morchella esculenta	Bhattacharya et al. 2000	AF279398
Pseudorhizina californica	O'Donnell et al. 1997	U42677
Rhizina undulata	Spatafora et al. 2006	DQ470961
Rhizina undulata	Perry et al. 2007	DQ220410
Rhizina undulata	O'Donnell et al. 1997	U42691
Tuber gibbosum	O'Donnell et al. 1997	U42690
Verpa bohemica	Kellner et al. 2007	AJ698471
Verpa bohemica	O'Donnell et al. 1997	U42672
Verpa conica	O'Donnell et al. 1997	U42671
Verpa conica	Kellner et al. 2007	AJ698470
Verpa conica	Direct submission	AY544666

Species	Herbarium	Collection no.	GenBank accession no.
Disciotis venosa	NY	01293394	KC751497
Gyromitra brunnea	NY	01293396	KC751520
Gyromitra brunnea	NY	01293397	KC751521
Gyromitra brunnea	NY	01293398	KC751522
Gyromitra brunnea	NY	01797001	KC751523
Gyromitra brunnea	NY	01293399	KC751524
Gyromitra brunnea	NY	01293400	KC751498
Gyromitra brunnea	MICH	NSW 4500	KC751505
Gyromitra caroliniana	NY	01797002	KC751528
Gyromitra caroliniana	NY	01797003	KC751500
Gyromitra caroliniana	MICH	NSW 6105	KC751501
Gyromitra esculenta	NY	01797004	KC751504
Gyromitra esculenta	NY	01797005	KC751502
Gyromitra esculenta	BPI	566942	KC751503
Gyromitra infula	ILLS	54774	KC751507
Gyromitra infula	NY	01797006	KC751508
Gyromitra infula	NY	01797007	KC751509
Gyromitra korfii	NY	01797008	KC751512
Gyromitra korfii	NY	01797013	KC751510
Gyromitra korfii	NY	01797010	KC751511
Gyromitra korfii	NY	01797012	KC751506
Gyromitra korfii	ILLS	63481	KC751518
Gyromitra korfii	NY	01797011	KC751513
Gyromitra korfii	NY	01293395	KC751499

TABLE I. Continued

Species	Herbarium	Collection no.	GenBank accession no.
Gyromitra korfii	NY	01797009	KC751519
Gyromitra leucoxantha	NY	01797015	KC751515
Gyromitra leucoxantha	NY	01797014	KC751516
Gyromitra perlata	NY	01797016	KC751514
Gyromitra melaleucoides	MICH	NSW 4520	KC751517
Gyromitra sphaerospora	MICH	NSW 2443	KC751525
Gyromitra sphaerospora	MICH	JA 4214	KC751526
Gyromitra sphaerospora	MICH	JA 4213	KC751527
Morchella cf. esculenta	F	4090504	KC751529
Verpa bohemica	NY	01797017	KC751530
Verpa conica	NY	01797018	KC751531

in the ML and Bayesian analyses (data not shown). Morphological features corresponding to those assessed in traditional systematic treatments (e.g. Weber 1988, Abbott and Currah 1997), including ascomata and ascospore morphology, can be used to distinguish phylogenetic clades and assign taxa to subgenera.

Clades I (*G. brunnea* Underw.) and II (*G. caroliniana* [Bosc] Fr.) correspond to *Gyromitra* subgenus *Caroliniana* S.P. Abbott, a well supported monophyletic clade characterized by large ascomata consisting of a stipitate apical hymenophore and reticulate ascospores with multiple apiculi at each end. Clades III (*G. perlata*), IV (*G. korfii* [Raitv.] Harmaja) and V (*G. leucoxantha* [Bres.] Harmaja) correspond to a paraphyletic clade, *Gyromitra* subgenus *Discina* (Fr.) Harmaja, which is characterized by ascomata that are cup- to disk-shaped and sessile or substipitate to stipitate with an irregularly lobed hymenophore and ascospores with solitary apiculi at each end. Clades VI (*G. californica*) and VII (*G. sphaerospora* [Peck] Sacc.) form a well supported monophyletic clade that corresponds to *Gyromitra* subgenus *Pseudorhizina* (designated below), which is characterized by ascomata consisting of a stipitate apical hymenophore, smooth to finely rugose, nonapiculate ascospores with an acyanophilic perispore and aseptate, brown setae. Clades VIII (*G. esculenta* [Pers.] Fr.) and IX (*G. infula* [Schaeff.] Quel.) correspond to *Gyromitra* subgenus *Gyromitra* (Pers.) Fr., which is a paraphyletic clade characterized by ascomata consisting of a stipitate apical hymenophore and smooth to finely rugose ascospores that are either nonapiculate or feature a broadly rounded apiculus at each end. Clade X (*G. melaleucoides* [Seaver] Pfister) corresponds to *Gyromitra* subgenus *Melaleucoides* S.P. Abbott and is a well supported monophyletic clade characterized by cup- to disk-shaped, substipitate to stipitate ascomata and nonapiculate, verrucose ascospores. Clade XI, which corresponds to two species in the genus *Hydnotrya*, is a monophyletic clade nested within *Gyromitra*.

TAXONOMY

Gyromitra Subgenus *Caroliniana* S.P. Abbott, 1997, Mycotaxon 62:19.

Ascomata consisting of an apical hymenophore and stipe; hymenophore irregularly lobed; hymenium rugose, orange-brown to red-brown. Ascospores ellipsoid to subfusoid, with multiple, prominent, blunt apiculi at each end; surface a coarse, regular, widely spaced reticulum at maturity; perispore cyanophilic; contents uniguttulate to triguttulate.

Because *G. fastigiata* (Krombh.) Rehm, a European taxon, has been interpreted variously due to confusion regarding the original description by Krombholtz (1834) and type material for this taxon is lacking we chose a North American species, *G. brunnea*, which has type material (McKnight 1973), as the epithet that should be applied to Clade I. *Gyromitra brunnea* (FIG. 2A) is characterized by an apical hymenophore that often has 2–5 lobes with thick margins, ''seams'' joining the lobes and a whitish undersurface that is partially exposed, while *G. caroliniana* (FIG. 2B) has a brain-like apical hymenophore that is irregularly pitted to wrinkled, lacks the ''seams'' and is fused to the stipe so that the undersurface is not exposed.

Gyromitra subgenus *Discina* (Fr.) Harmaja, 1973, Karstenia 13:56.

Ascomata cup- to disk-shaped and sessile to substipitate or stipitate with an irregularly lobed, apical hymenophore; hymenium rugose to wrinkled, yellow-brown, orange-brown, red-brown or dark brown. Ascospores ellipsoid to fusoid, with solitary, knob-like to pointed apiculi at each end; surface with

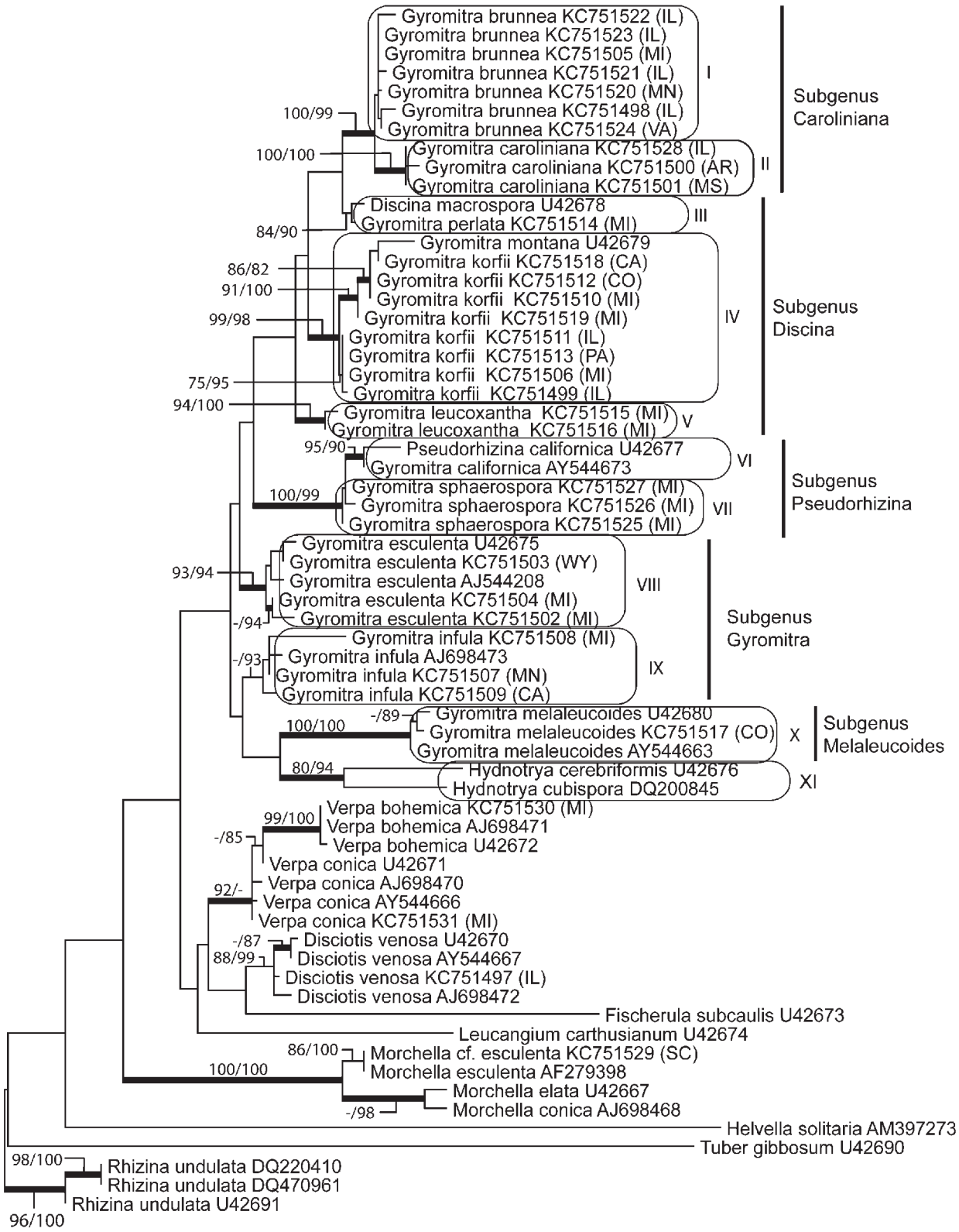

FIG. 1. Phylogram of the most likely tree (−lnL = −4853.3271) from a PHYML analysis of 64 taxa based on LSU nrDNA (950 bp). Thickened branches indicate significant Bayesian posterior probabilities ≥ 95%; numbers refer to PhyML ML/MP bootstrap support values ≥ 70% based on 100 replicates. *Rhizina undulata* was used as outgroup.

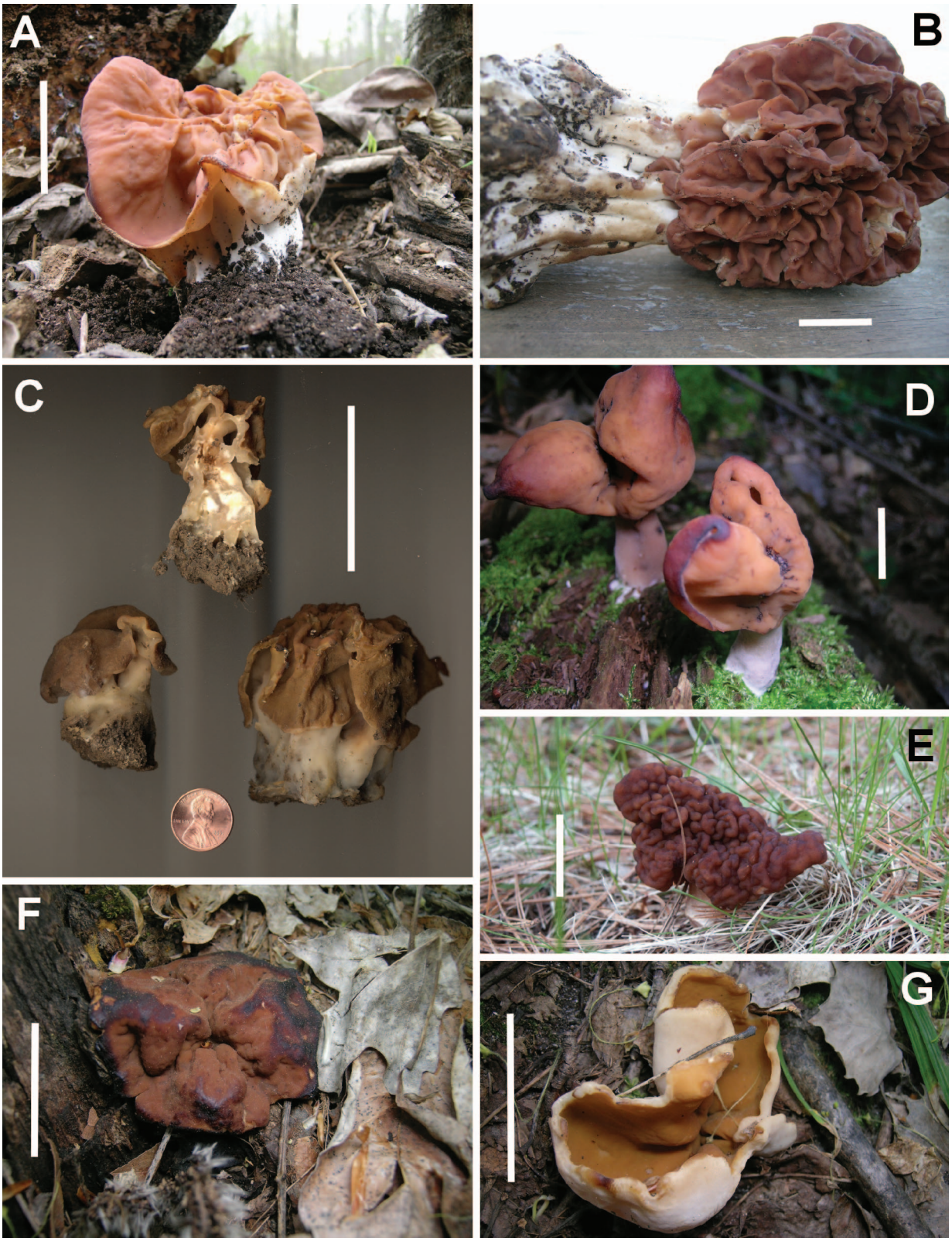

FIG. 2. Ascomata of *Gyromitra*. A. *Gyromitra brunnea* (NY 01293397). B. *Gyromitra caroliniana* (NY 01797002). C. *Gyromitra korfii* (NY 01797010). D. *Gyromitra infula* (NY 01797006). E. *Gyromitra esculenta* (NY 01797004). F. *Gyromitra perlata* (NY 01797016). G. *Gyromitra leucoxantha* (NY 01797014). Bars = 5 cm.

rounded ridges, wrinkles or a reticulum at maturity; perispore cyanophilic; contents uniguttulate to triguttulate.

Due to ongoing confusion regarding the concept of *G. gigas* (e.g. size of ascospores), a European taxon, and the lack of type material, we have chosen to recognize a North American species, *G. korfii*, which is represented by a type collection, as the epithet for Clade IV. Based on the results of this study, *Gyromitra montana* Harmaja is considered to be synonymous with *G. korfii*. *Gyromitra korfii* (FIG. 2C) has an irregularly wrinkled or convoluted hymenophore borne on a well developed, ribbed stipe, while *G. leucoxantha* and *G. perlata* have a cup- to disk-shaped hymenophore that becomes repand at maturity and a rudimentary stipe that often arises from a mass of mycelium and soil. *Gyromitra leucoxantha* (FIG. 2G) has a yellow to yellow-brown hymenium and the tips of the apiculus on the ascospores are blunt and depressed, while the hymenium of *G. perlata* (FIG. 2F) is reddish brown and the tips of the apiculi are pointed rather than blunt and depressed.

Gyromitra subgenus Pseudorhizina Methven, Zelski, and A.N. Mill., subg. nov.
MycoBank MB804623

Apothecium stipitate, convex to saddle-shaped to irregularly lobed, hymenium gray-brown, ascospores globose to ellipsoid, biguttulate, nonapiculate, smooth to finely rugose, perispore acyanophilic. *Type species*: *Gyromitra sphaerospora* (Peck) Saccardo, 1889, Sylloge fungorum omnium hucusque cognitarum 8:16.

Ascomata consisting of an apical hymenophore and stipe; hymenophore irregularly convex, saddle-shaped or irregularly lobed, margin reflexed; hymenium brown to gray-brown or blackish brown; stipe ribbed with ribs extending onto lobes. Ascospores globose to ellipsoid, nonapiculate; surface smooth to finely rugose; perispore acyanophilic; contents biguttulate. Setae aseptate, brown.

Species in *Gyromitra* subg. *Pseudorhizina* have acyanophilic ascospores and brown, aseptate setae. The hymenium of *G. californica* is brown to dark brown with ellipsoid ascospores, 16–20 × 8.5–10.5 µm, with a smooth to rugose surface, while *G. sphaerospora* has a grayish brown hymenium and globose ascospores, 8.5–12 µm, with a smooth surface.

Gyromitra Subgenus *Gyromitra* (Pers.) Fr., 1849, Summa Vegetabilium Scandinaviae 2:346.

Ascomata consisting of an apical hymenophore and stipe; hymenophore irregularly convex to irregularly lobed; hymenium rugose to convoluted, orange-brown to dark red-brown. Ascospores ellipsoid to subfusoid, nonapiculate or with a slightly inflated, broadly rounded apiculus at each end; surface smooth to finely rugose; perispore cyanophilic; contents biguttulate.

The hymenophore of *G. infula* (FIG. 2D) is saddle-shaped with two prominent lobes and ascospores that are nonapiculate, while *G. esculenta* (FIG. 2E) features an irregularly lobed to brain-like hymenophore and ascospores with a broadly rounded apiculus at each end.

Gyromitra subgenus *Melaleucoides* S.P. Abbott, 1997, Mycotaxon 62:34.

Ascomata cup- to disk-shaped, subsessile to stipitate; hymenium rugose, dark gray-brown to brown. Ascospores ellipsoid, nonapiculate; surface with isolated, rounded warts; perispore cyanophilic; contents biguttulate. *Gyromitra melaleucoides* exhibits a cupulate hymenophore that is borne on a well developed stipe and nonapiculate ascospores with rounded warts on the surface.

KEY TO SOME *GYROMITRA* SPECIES IN NORTH AMERICA

1. Ascomata cup- to disk-shaped; sessile, substipitate or stipitate 2
1. Ascomata not cup- to disk-shaped, consisting of an apical hymenophore and stipe; hymenophore wrinkled to lobed or saddle-shaped 4
2. Mature ascospores apiculate 3
2. Mature ascospores nonapiculate . . . *G. melaleucoides*
3. Apiculi pointed or broadly rounded . . . *G. perlata*
3. Apiculi blunt or apically depressed . . . . *G. leucoxantha*
4. Mature ascospores with multiple, blunt apiculi at each end 5
4. Mature ascospores nonapiculate or with a single apiculus at each end 6
5. Hymenophore with 2-5 distinct lobes; hymenium smooth to wrinkled . . . . . *G. brunnea*
5. Hymenophore brain-like; hymenium irregularly wrinkled to pitted . . . . . *G. caroliniana*
6. Mature ascospores nonapiculate 7
6. Mature ascospores apiculate; apiculi round to knob-like; hymenophore wrinkled or convoluted . . . . . *G. korfii*
7. Hymenophore irregularly convex to brain-like . . . 8
7. Hymenophore saddle-shaped . . . . . *G. infula*
8. Hymenophore irregularly convex 9
8. Hymenophore brain-like . . . . . *G. esculenta*
9. Ascospores ellipsoid . . . . . *G. californica*
9. Ascospores globose . . . . . *G. sphaerospora*

DISCUSSION

Traditional systematic studies based on morphology of *Gyromitra* and related taxa have offered various hypotheses about its placement in the Pezizales as well

as the inclusion or exclusion of genera such as *Discina*, *Hydnotrya* and *Pseudorhizina* (Benedix 1961; Eckblad 1968; Harmaja 1969, 1973, 1976; Abbott and Currah 1997). Molecular studies, while few and not concentrated on *Gyromitra* species, to date have shown that three of the four genera (*Hydnotrya* currently is included in the Helvellaceae) group together monophyletically in the Discinaceae (O'Donnell et al. 1997, Hansen and Pfister 2006, Tedersoo et al. 2006). This study suggests that *Hydnotrya* also might belong in the Discinaceae, although a more thorough sampling of the genus, including its type species, clearly is warranted. To accurately discern the disposition of taxa currently recognized in the genus *Hydnotrya*, additional specimens of *Hydnotrya*, including the type species of the genus, *Hydnotrya tulasnei* (Berk.) Berk. & Broome, should be sequenced and included in future phylogenetic analyses.

In addition, a more robust sampling of *Discina* is necessary in that it represents the type genus of the family (Benedix 1961). Benedix (1961) based his circumscription of the family on the concept that there was a natural progression of ascospore episporia, from apiculate with no exosporium to full-fledged episporia. From a morphological point of view this is intuitive, although the molecular evidence suggests variation rather than progression in the morphology of the epispore. The subgenera of Abbott and Currah (1997) are well supported in this analysis. Although Abbott and Currah (1997) put lesser emphasis on the episporium of the ascospores and took a more holistic approach to the delimitation of taxa at various ranks, they also thought that the Discinaceae, Helvellaceae and Rhizinaceae belonged in a single family, the Helvellaceae.

Reports of *G. caroliniana* (Clade II, FIG. 1) from Europe are unsubstantiated and apparently result from confusion over European concepts of *G. fastigiata* and *G. gigas* (Krombh.) Cooke. Considerable confusion remains regarding the disposition of *G. perlata*, *G. macrospora* (Bubák) Harmaja and *G. warnei* (Peck) Harmaja, which were accepted by McKnight (1969) and Weber (1988) based on differences in ascospore dimensions. Abbott and Currah (1997) considered *G. macrospora* and *G. warnei* synonyms of *G. perlata*, and we concur based on our results (Clade III, FIG. 1). *Discina macrospora* is treated herein as a synonym of *G. perlata*.

The lumping/splitting issues presented in this study should be addressed with a more robust sampling of *Gyromitra* and related taxa. Future studies also should include additional genes such as the internal transcribed spacer, *MCM7*, *RPB1*, *RPB2* and *TEF1α* to resolve basal nodes that were not well supported in this analysis. With further evidence the relationships of these taxa will become better resolved and the nomenclatural issues that remain can be addressed.

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