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ORIGINAL ARTICLE

Studies in *Gyromitra* III: the *Gyromitra brunnea* lineage including *G. japonica* sp. nov.

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Abstract

The *Gyromitra brunnea* lineage contains ten described species that occur throughout Asia, Europe, and North America and includes *G. brunnea*, *G. caroliniana*, *G. costata*, *G. fastigiata*, *G. grandis*, *G. krombholzii*, *G. martinii*, *G. parma, G. pratensis*, and *G. slonevskii*. ITS1, ITS2, LSU, and *TEF-1* sequences from 107 specimens, including type specimens for seven of the ten taxa, were included in phylogenetic analyses to establish species boundaries and resolve species relationships. Sequence similarity comparisons were also conducted for the ITS1, ITS2, LSU, and *TEF-1*. Although ITS1, ITS2, and *TEF-1* exhibited sufficient variability to discriminate among species in the *G. brunnea* lineage, LSU displayed little variability and should not be used as a molecular marker for separating taxa in this group. No voucher specimens exist for *G. krombholzii* or *G. slonevskii*. The holotype of *G. pratensis* is preserved in formaldehyde and Illumina sequencing was unsuccessful. A lectotype, which invalidates the current neotype, and an epitype are designated for *G. caroliniana*. A neotype and isoneotype are designated for *G. costata*. Molecular data from the ITS2 region is provided for the first time for the lectotype and isolectotype of *G. brunnea*, the former neotype and epitype of *G. caroliniana*, the neotype and isoneotype of *G. costata*, the isoepitype of *G. grandis,* the isolectotype of *G. martini*, and the holotype of *G. parma* and from the entire ITS for the isoepitype of *G. fastigiata*. *Gyromitra costata* is confirmed to be a synonym of *G. caroliniana* based on molecular data. *Gyromitra fastigiata* and *G. slonevskii* are confirmed to be synonyms of *G. grandis* based on molecular data. The North American *G. brunnea* and European *G. parma* occur as moderately supported sister species, as do the North American *G. caroliniana* and Asian/European *G. grandis*. The European *G. martinii* occurs as a separate lineage. A well-supported clade containing specimens from Japan that are morphologically similar to *G. parma* is described as a new species. A key to species based on morphology and geography is provided.

Keywords *Ascomycota* · Fungi · Type barcoding · ITS sequences · *Pezizales* · Molecular systematics · 4 new typifications · 1 new species

Introduction

The genus *Gyromitra* Fr. contains over 75 taxonomic names with many taxa plagued by a long, complex taxonomic history including numerous transfers among various antiquated genera (e.g., *Discina*, *Elvela*, *Fastigiella*, *Helvella*, *Maublancomyces*, *Neogyromitra*, *Pseudorhizina*, etc.). Species are distributed primarily throughout north temperate and boreal regions of the Northern Hemisphere and form sessile to stipitate ascomata with discoid to cerebriform or saddle-shaped apothecia mostly during the spring. Based on historical literature (McKnight 1971, 1973; Abbott and Currah 1997; Van Vooren and Moreau 2009) and recent phylogenetic analyses (Methven et al. 2013; Miller et al. 2015; Carbone

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et al. 2018; Van Vooren and Carbone 2019), four primary taxa occur in the *G. brunnea* lineage: *G. brunnea* Underw., *G. caroliniana* (Bosc) Fr., *G. grandis* (U. Cumino) Van Vooren & M. Carbone, and *G. parma* (J. Breitenb. & Maas Geest.) Kotl. & Pouzar. Five other taxa are believed to be synonyms: *G. costata* (Schwein.) Cooke, *G. fastigiata* (Krombh.) Rehm., *G. krombholzii* Bezděk, *G. pratensis* Velen., and *G. slonevskii* V.P. Heluta, and a sixth taxon, *G. martinii* Donadini & Astier, was thought to be closely related to *G. parma* based on morphology (Baute 2006).

Gyromitra subgenus *Caroliniana* was established to accommodate taxa with coarsely reticulate ascospores that possess multiple apiculi (Abbott and Currah 1997). Later, it was divided into two sections based on whether the ascomata were stipitate or sessile: *Gyromitra* section *Caroliniana*, which contains the lobed to cerebriform and stipitate *G. brunnea*, *G. caroliniana*, and *G. grandis*; and *Gyromitra* section *Parmae*, which contains the cupulate and sessile to substipitate *G. martinii*, *G. microspora* (Donadini & Bozonnet) Harmaja, *G. parma* (J. Breitenb. & Maas Geest.) Kotl. & Pouzar, and *G. spinosospora* (Lucchini & Pelland.) A. Koch (Van Vooren and Moreau 2009). Molecular data from the ITS and LSU suggest that *G. microspora* and *G. spinosospora* are divergent and do not occur within the *G. brunnea* lineage (Miller unpublished data).

Gyromitra brunnea was described in 1894 (Underwood 1894) based on a specimen collected in 1892 from Indiana, USA. Raitviir (1970) designated a lectotype based on this material deposited at NY and characterized the taxon based on the irregularly lobed, plicate ascoma with anastomosing ridges and a massive, fluted stipe. The folds of the lobes do not fuse at the margins in *G. brunnea*, which helps distinguish it from *G. caroliniana* with its cerebriform ascoma (McKnight 1973).

Gyromitra caroliniana is based on *Morchella caroliniana* Bosc (Bosc 1811), which was later sanctioned by Fries (1822). McKnight (1973) designated a neotype (BPI 566695) based on a specimen collected in 1942 from Virginia, USA. It is distinguished by its almost round, folded, furrowed, and cerebriform ascoma on a massive, furrowed stipe.

Gyromitra costata was originally described from North Carolina, USA, as *Helvella costata* Schwein. (Schweinitz 1822). Its protologue describes a taxon having a yellow–brown ascoma with an inrolled, free margin, ribbed lower surface, and a white, lacunose, powdery stipe. Cooke (1878) examined Schweinitz's specimen and later added microscopic characters such as clavate, septate paraphyses, cylindrical asci, and obtuse-fusoid, roughened ascospores that were 32–35 × 12 µm. He also provided a watercolor illustration, which McKnight (1973) believed was based on a specimen (Curtis 2247) in the Berkeley Herbarium at Kew. While some (e.g., Kavina 1924) consider *G. costata* to be similar to *G. brunnea*, others (e.g., Seaver 1928; McKnight 1973) believe it to be a synonym of *G. caroliniana*. Van Vooren and Moreau (2009) consider *G. costata* to be a *nomen dubium* based on Schweinitz's ambiguous description and the fact that Cooke's ascospore measurements overlap with *G. caroliniana*, whereas his illustration resembles *G. brunnea*.

Gyromitra fastigiata was described as *Helvella fastigiata* and illustrated by Krombholz (1834) based on a collection near Prague, Czech Republic. The illustration was designated as the lectotype by Van Vooren and Carbone (2019) who also designated an epitype from a Czech collection that was sequenced for LSU. This species can be distinguished by its reddish brown, lobed ascomata, large bulbous stipe, and broadly fusiform ascospores with one to five well-developed apiculi (McKnight 1971; VanVooren 2014; Van Vooren and Carbone 2019).

Gyromitra grandis was originally described in 1806 as *Helvella grandis* U. Cumino from material collected in Italy (Cumino 1806). Fries (1822) considered it a synonym of *Helvella monachella* (Scop.) Fr. so the name was forgotten until Van Vooren and Carbone (2019) rediscovered it. They transferred it into *Gyromitra*, designated Cumino's illustration as the lectotype, established an epitype from the same general locality in Italy, and proposed *G. grandis* to be the older name for *G. fastigiata* (Van Vooren and Carbone 2019).

Gyromitra krombholzii was introduced in 1901 by Bezděk when he proposed to replace the name *Helvella suspecta* Krombh., which is a synonym of *G. esculenta* (Velenovský 1934), because Bezděk considered "suspecta" to be an inappropriate epithet (Bezděk 1901). No specimens exist of *G. krombholzii*, but Luňáček (1908) provided an illustration (pl. 49) that resembles *G. fastigiata*.

Gyromitra martinii was described in 1974 from France (Donadini and Astier 1974). It is distinguished by its large, wrinkled, disc-shaped ascomata, large, warty ascospores with multiple, small, spiny apiculi, and habitat mostly on rotten beech wood (Van Vooren 2015). Baute (2006) suggested that this species may be the same as *G. parma*.

Gyromitra parma was described in 1973 as *Discina parma* J. Breitenb. & Maas Geest. from a collection made in Switzerland (Breitenbach and Maas Geesteranus 1973). It is characterized by its reddish-brown to yellowish-brown, disc-shaped ascomata, short stipe, and smaller, ellipsoid, reticulate ascospores with multiple, spiny apiculi.

Gyromitra pratensis was described from a collection made in 1923 near Zdice, Czech Republic (Velenovský 1934). Van Vooren and Carbone (2019) designated the only known specimen, which is preserved in formaldehyde, as the lectotype. It was distinguished from *G. gigas* by its exceptional ascomata (Velenovský 1934), but was later synonymized under *G. fastigiata* after careful examination of the lectotype specimen (Svrček 1978).

Gyromitra slonevskii was described in 2001 from the Cherkasty region southeast of Kyiv, Ukraine (Heluta 2001). It was distinguished from *G. fastigiata* by its "larger ascocarps, colour of cap, length of ascospores, smell, and a different habitat (mostly on rotten wood)" (Heluta 2001), but none of these characters significantly differ from *G. fastigiata* (Van Vooren and Carbone 2019). Carbone et al. (2018) showed that ITS sequences of *G. slonevskii* appear phylogenetically identical to *G. fastigiata*, which later resulted in the synonymy of this species under *G. fastigiata* (Van Vooren and Carbone 2019).

Although previous studies included one or more of these taxa in molecular phylogenetic analyses (Methven et al. 2013; Miller et al. 2015; Krisai-Greilhuber et al. 2017; Acar et al. 2018; Carbone et al. 2018; Van Vooren and Carbone 2019; Wang and Zhuang 2019; Hai-Jiao et al. 2020), no study to date has comprehensively analyzed all of the available taxa in the *G. brunnea* lineage or assessed the ITS, LSU, and *TEF-1* as barcode markers for identifying these taxa. The goals of this study were to sample and sequence multiple representatives, including available type specimens, for all taxa in the *G. brunnea* lineage to establish species boundaries, delimit biogeographical ranges, resolve species relationships, and assess the potential of ITS, LSU, and *TEF-1* as barcode makers.

Materials and methods

Specimens examined

Entire dried ascomata or small portions of the fertile layer of ascomata were obtained as loans or gifts. Sequences generated during this study were obtained from DNA extracted directly from these dried ascomata, which were deposited at ILLS or are available at their home institution (BPI, C, CSU, CUP, F, FH, FLAS, GLM, ILLS, ISC, K, L, LE, LJF, LY, MICH, NY, NYS, O, TENN, TNS, and VPI). Fungarium acronyms follow Index Herbariorum (Thiers 2013). Efforts were made to generate sequences from the type specimens for *G. brunnea*, *G. caroliniana*, *G. costata*, *G. fastigiata*, *G. grandis*, *G. martinii*, *G. parma*, and *G. pratensis*, even though the type specimens of *G. costata* and *G. brunnea* date from 1879 and 1892, respectively.

Micromorphological structures were studied and measurements made of material revived in 95% ethanol and distilled water, sectioned, and then mounted in distilled water (McKnight 1968) or lactophenol cotton blue. Sections were examined with a Nikon Alphaphot 2 YS2 light microscope at 400× and 1000×. A minimum of 30 ascospores and paraphyses were measured for each collection. In addition to assembling a range of ascospore lengths and widths for each specimen, the mean length (L_m), mean width (W_m), length–width ratio (Q), and mean length–width ratio (Q_m) were calculated for each specimen. The following specimens were examined and annotated:

G. brunnea (FLAS-F-67979, ILLS00105045, ILLS00105052, ILLS00105056, ILLS00122746, ILLS00122748, NY00270185, NY00270186), *G. caroliniana* (BPI 566695 (immature), BPI 566998, ILLS00105047, ILLS00165085, ISC-F-0069176, ISC-F-0069195, ISC-F-0069884), *G. grandis* (O-76103, TNS-F80830), *G. japonica* (TNS-F15628, TNS-F17968, TNS-F18001, TNS-F66301 (holotype), TNS-F66525, ILLS00122881 (immature), ILLS00122882 (immature)), *G. martinii* (CUP 60609 (isolectotype), LJF 7784, MICH 346002), *G. parma* (BPI 570017, L0054078 (holotype), MICH 345134, MICH 345138).

Voucher specimen number, date of collection, locality, GenBank accession numbers, and source for all taxa included in the ITS, LSU, and *TEF-1* analyses are shown in Table 1.

Because *Gyromitra* ascomata develop and mature over an extended period of time, ascospores vary widely in length and width as does the development of surface ornamentation and the apiculi. For the purposes of this study, apiculi are included in the measurements of ascospore length. However, it is unclear in the literature whether or not the apiculi have been consistently included in the measurements of ascospore lengths. Ascospore maturity and inclusion/exclusion of the apiculi in the ascospore length may account for the diverse ranges in ascospore lengths and widths reported in the literature.

Molecular data

DNA was extracted directly from dried ascomata using a modified NaOH extraction method (Osmundson et al. 2013) following manufacturer's instructions. For NaOH extraction, 200 µL 0.5 M NaOH was added to ~ 75 mg of dried tissue, subjected to at least three freeze–thaw cycles, ground with a micropestle, centrifuged at 14,000 RPM for 2 min, and 5 µL of the resulting supernatant added to 45 µL 100 mM Tris–HCl buffered with NaOH to pH 8.5–8.9 (Tris–HCl–DNA extraction solution). The complete ITS region, the first 600 bp of the 5′ end of LSU, and a 600-bp region of *TEF-1* were amplified separately using the following primers: ITS: ITS1F/ITS4 (Gardes and Bruns 1993; White et al. 1990) or LR1 (Vilgalys and Hester 1990), LSU: LROR/LR3 (Rehner and Samuels 1995; Vilgalys and Hester 1990), and *TEF-1*: TEF983F/TEF1567R (Rehner and Buckley 2005). PCR amplification using GoTaq Green Master mix (Promega, Madison, Wisconsin, USA) consisted of the following: 12.5 µL GoTaq Green Master mix, 2.5 µL BSA, 2.5 µL 50% DMSO, 1.5 µL of each 10 µM primer, and 3–10 µL DNA. PCR

Table 1 Specimens used in this study including type status, previous misidentifications, voucher specimen number (with year of collection in parentheses), locality, GenBank accession numbers and source of sequences. Specimens in which only the ITS2 region of ITS was sequenced are indicated by an asterisk

Species	Voucher specimen no.	Locality	ITS GenBank no.	LSU GenBank no.	*TEF-1* GenBank no.	Source
Gyromitra brunnea						
LECTOTYPE	NY00915266 (1892)	USA, Indiana	PQ350208*	PQ600918		This study
ISOLECTOTYPE	NYSf3694 (1892)	USA, Indiana	PQ350209*	– – – – – –		This study
	NY01293400 (2006)	USA, Illinois	PQ493317	KC751498		This study; Methven et al. 2013
	NY01293397 (2004)	USA, Illinois	PQ493318	KC751521		This study; Methven et al. 2013
	NY01797001 (2005)	USA, Illinois	PQ493319	KC751523		This study; Methven et al. 2013
	NY01293398 (2005)	USA, Illinois	PQ493320	KC751522		This study; Methven et al. 2013
	ILLS00105045 (2003)	USA, Illinois	PQ493321	PQ600919		This study
previously *G. fastigiata*	ILLS00105056 (1989)	USA, Illinois	PQ350210*	PQ600920		This study
previously *G. fastigiata*	ILLS00105052 (1989)	USA, Illinois	PQ350211*	– – – – – –		This study
	FC0346667F (2013)	USA, Indiana	PQ493322	– – – – – –		This study
previously *G. ambigua*	FLAS-F-65575 (2013)	USA, Iowa	PQ350212*	PQ600921		This study
	ILLS00122745 (2022)	USA, Iowa	PQ493323	PQ600922	PQ628300	This study
	ILLS00122746 (2022)	USA, Iowa	PQ493324	PQ600923	PQ628301	This study
	NY270185 (1952)	USA, Kansas	PQ350213*	– – – – – –		This study
	NY270186 (1952)	USA, Kansas	PQ350214*	– – – – – –		This study
previously *G. fastigiata*	NY01943049 (1990)	USA, Kansas	PQ493325	PQ600924	PQ628302	This study
	MICH352048 (2020) (genome sequenced)	USA, Kansas	ON693640*	ON693592	PQ628303	Dirks et al. 2023
	BPI566972 (1984)	USA, Maryland	PQ493326	– – – – – –		This study
	MICH25610 (1983)	USA, Michigan	PQ350215*	KC751505		This study; Methven et al. 2013
	MICH352054 (2020) (genome sequenced)	USA, Michigan	ON693642	ON693594	PQ628304	Dirks et al. 2023
	MICH352063 (2021)	USA, Michigan	PQ493327	PQ600925		This study
	MICH352067 (2021)	USA, Michigan	PQ493328	PQ600926		This study
	MICH346001 (2021)	USA, Michigan	PQ493329	PQ600927		This study
	NY01293396 (2004)	USA, Minnesota	PQ493330	KC751520		This study; Methven et al. 2013
	NY03817717 (2005)	USA, Missouri	PQ350216*	PQ600928		This study
previously *G. slonevskii*	ILLS00122747 (2006)	USA, Missouri	PQ493331	– – – – – –		This study

Table 1 (continued)

Species	Voucher specimen no.	Locality	ITS GenBank no.	LSU GenBank no.	*TEF-1* GenBank no.	Source
	FLAS-F-67979 (2020)	USA, New York	PQ493332	PQ600929	PQ628305	This study
	BPI566967 (1976)	USA, New York	PQ493333	– – – – – –		This study
	BPI566903 (1972)	USA, New York	PQ493334	– – – – – –		This study
	CUP-A-019740 (1906)	USA, Ohio	PQ350217*	– – – – – –		This study
previously *G. fastigiata*	MICH25597 (1979)	USA, Ohio	PQ350218*	– – – – – –		This study
	TENN-F-012567 (1940)	USA, Tennessee	PQ350219*	– – – – – –		This study
	NY01293399 (2005)	USA, Virginia	PQ493335	KC751524		This study; Methven et al. 2013
	ILLS00122748 (2021)	USA, Virginia	PQ493336	PQ600930		This study
previously *G. fastigiata*	VPI-F-0004824 (1981)	USA, Virginia	PQ493337	PQ600931		This study
	MICH352018 (2019)	USA, Wisconsin	PQ493338	PQ600932		This study
Gyromitra caroliniana						
EPITYPE	MICH352092 (2021) (genome sequenced)	USA, Missouri	ON693649	ON693601	PQ628306	Dirks et al. 2023
former NEOTYPE	BPI566695 (1942)	USA, Virginia	PQ350220*	PQ600933		This study
	NY01797003 (2005)	USA, Arkansas	PQ493339	KC751500		This study; Methven et al. 2013
	NY01797002 (2006)	USA, Illinois	PQ350221*	KC751528		This study; Methven et al. 2013
	ILLS00165085 (2005)	USA, Illinois	PQ350222*	PQ600934		This study
	FC0228128F (1995)	USA, Illinois	PQ350223*	PQ600935		This study
	MICH346460 (2020)	USA, Indiana	PQ493340	PQ600936		This study
	iNaturalist 24274575 (2019)	USA, Indiana	MZ667893	– – – – – –		Stephen D. Russell, unpub.
	ISC-F-0069197 (1992)	USA, Iowa	PQ493341	– – – – – –		This study
	ISC-F-0069195 (1992)	USA, Iowa	PQ493342	– – – – – –		This study
	ISC-F-0069176 (1992)	USA, Iowa	PQ493343	– – – – – –		This study
	ISC-F-0069886 (1991)	USA, Iowa	PQ493344	– – – – – –	PQ628307	This study
	ISC-F-0069884 (1991)	USA, Iowa	PQ350224*	– – – – – –		This study
	MICH25515 (1984) (genome sequenced)	USA, Mississippi	PP326931*	KC751501	PQ628308	Dirks et al. 2025; Methven et al. 2013
	MICH11600 (1984)	USA, Mississippi	PQ350225*	– – – – – –		This study
	MICH11601 (1977)	USA, Mississippi	PQ350226*	– – – – – –		This study
	NY03817722 (2014)	USA, Missouri	PQ493345	PQ600937	PQ628309	This study

Table 1 (continued)

Species	Voucher specimen no.	Locality	ITS GenBank no.	LSU GenBank no.	*TEF-1* GenBank no.	Source
	CSU-F-00083 (2007)	USA, Oklahoma	PQ493346	PQ600938		This study
	CSU-F-00087 (2012)	USA, Oklahoma	PQ350227*	PQ600939	PQ628310	This study
	CSU-F-00101 (1994)	USA, Oklahoma	PQ350228*	PQ600940	PQ628311	This study
	ILLS00105047 (1987)	USA, Tennessee	PQ350229*	– – – – – –		This study
	TENN-F-047264 (2005)	USA, Tennessee	PQ350230*	PQ600941	PQ628312	This study
previously *G. fastigiata*	BPI566998 (1945)	USA, Utah	PQ350231*	– – – – – –		This study
	VPI-F-0004860 (1978)	USA, Virginia	PQ350232*	PQ600942		This study
Gyromitra costata						
NEOTYPE	K-M-1434138 (1879) [Curtis 2247]	USA, North Carolina	PQ350233*	– – – – – –		This study
ISONEOTYPE	FH00601209 (1879) [Curtis 2247]	USA, North Carolina	PQ350234*	– – – – – –		This study
	K-M-1434139 (1885?)	USA, state unknown	PQ350235*	– – – – – –		This study
Gyromitra fastigiata (now *G. grandis*)						
EPITYPE	V18042206 (2018) ALV17918 LY N.V. 2018.04.10	Czech Republic	– – – – – –	MK784816		Van Vooren & Carbone 2019
ISOEPITYPE	V18042206 (2018) ALV17918 LY N.V. 2018.04.10	Czech Republic	PQ493347	PQ600943		This study
	CUP-063720 (1975)	Czech Republic	PQ350236*	PQ600944		This study
	BPI895939 (1975)	Czech Republic	PQ350237*	– – – – – –		This study
	BPI903287 (1975)	Czech Republic	PQ350238*	– – – – – –		This study
previously *G. caroliniana*	O76103 (1965)	Austria	PQ350239*	KX008335		This study
	NV2004.05.01 (2004)	France	MG846992	MG847003	MG847049	Van Vooren & Carbone 2019
	ALV17018, VS 01057 (2001)	Italy	– – – – – –	MH938321		Carbone et al. 2018
previously *G. gigas*	TNS-F80830 (2017) (genome sequenced)	Japan	PP326935	PQ600945	PQ628313	Dirks et al. 2025
	LE247912 (2011) (genome sequenced)	Russia	PP326934	PQ600946	PQ628314	Dirks et al. 2025
	LE236229 (2006)	Russia	PQ493348	– – – – – –	PQ628315	This study
	LE247724 (2011)	Russia	PQ350240*	PQ600947		This study
previously *G. brunnea*	Isolate A, G20 (2015)	Turkey	– – – – – –	MH376402		Acar et al. 2018
previously *G. brunnea*	Isolate B, G21 (2015)	Turkey	– – – – – –	MH698933		Acar et al. 2018

Table 1 (continued)

Species	Voucher specimen no.	Locality	ITS GenBank no.	LSU GenBank no.	*TEF-1* GenBank no.	Source
Gyromitra gigas (outgroup)						
	ILLS00121401 (2005)	France	MW076969	MW076969		Miller et al. 2020
	ILLS00121407 (2016)	France	MW076970	MW076970		Miller et al. 2020
Gyromitra grandis						
EPITYPE	VS00033 (2000) LY N.V. 2000.04.01	Italy	MH938675	MH938320		Van Vooren & Carbone 2019
ISOEPITYPE	VS00033 (2000) LY N.V. 2000.04.01	Italy	PQ493349	– – – – – –		This study
Gyromitra japonica						
HOLOTYPE	TNS-F66301 (2014)	Japan	PQ350241*	PQ600948	PQ628316	This study
	TNS-F15628 (2007)	Japan	PQ493350	PQ600949		This study
	TNS-F18001 (2006)	Japan	PQ493351	PQ600950	PQ628317	This study
	TNS-F17968 (2006)	Japan	PQ350242*	PQ600951	PQ628318	This study
	TNS-F66525 (2010)	Japan	PQ350243*	– – – – – –		This study
	ILLS00122881 (2024)	Japan	PQ493352	PQ600952	PQ628319	This study
	ILLS00122882 (2024)	Japan	PQ493353	PQ600953	PQ628320	This study
Gyromitra martinii						
ISOLECTOTYPE	CUP-060609 (1973)	France	PQ350244*	MT273650	PQ628321	This study
Topotype	NV2014.04.02 (2014)	France	PQ493354	PQ600954		This study
	NV2017.03.17 (2017)	France	PQ493355	PQ600955		This study
	MICH346002 (2014) (genome sequenced)	Germany	PP326942	PP326972	PQ628322	Dirks et al. 2025
previously *G. parma*	LJF7784 (2018)	Slovenia	PQ350245*	PQ600956		This study
previously *G. melaleucoides*	FH00433637 (1993)	Spain	PQ350246*	PQ600957	PQ628323	This study
	NV2013.06.09 (2013)	Spain	PQ350247*	PQ600958		This study
Gyromitra parma						
HOLOTYPE	L0054078 (1972)	Switzerland	PQ350248*	– – – – – –		This study
	C64497 (1995)	Denmark	PQ350249*	– – – – – –		This study
	C54887 (1994)	Denmark	PQ493356	– – – – – –	PQ628324	This study
	C25048 (1994)	Denmark	PQ350250*	– – – – – –	PQ628325	This study
	MICH345134 (2004) (genome sequenced)	Germany	PP326939	PP326969	PQ628326	Dirks et al. 2025
	MICH345138 (2014) (genome sequenced)	Germany	PP326941	PP326971	PQ628327	Dirks et al. 2025

Table 1 (continued)

Species	Voucher specimen no.	Locality	ITS GenBank no.	LSU GenBank no.	*TEF-1* GenBank no.	Source
	GLM-F044563 (1997)	Germany	PQ493357	PQ600959	PQ628328	This study
	GLM-F097983 (1997)	Germany	PQ350251*	– – – – – –		This study
	LJF6076 (2017)	Slovenia	PQ493358	PQ600960	PQ628329	This study
	BPI570017 (1977)	Yugoslavia (now Slovenia)	PQ493359	PQ600961		This study
Gyromitra slonevskii						
	H.561	Turkey	KX420700	– – – – – –		Gungor et al. unpublished
	HAI-D-077	Ukraine	JQ691488	– – – – – –	KF019285	Barseghyan et al. 2012
	HAI-D-078	Ukraine	JQ691490	– – – – – –	KF019286	Barseghyan et al. 2012
	HAI-D-079	Ukraine	JQ691489	– – – – – –	KF019287	Barseghyan et al. 2012

amplification was completed on a Bio-Rad C1000 thermal cycler under the following parameters: initial denaturation at 94 °C for 2 min, followed by 40 cycles of 94 °C for 30 s, 41 °C (for ITS and LSU) or 52 °C (for *TEF-1*) for 20 s, and 72 °C for 1 min with a final extension step of 72 °C for 10 min. If PCRs failed to amplify, which was typical for specimens older than 30 years, then one or both of the following salvage methods were attempted: (1) whole genomic DNA was cleaned and concentrated using a DNA Clean and Concentrator-25 kit (ZYMO Research, Irvine, California) with 20–35 µL water instead of elution buffer added in the final step, and/or (2) the PCR product was purified using a Wizard SV Gel and PCR Clean-Up System (Promega). The ITS1 region using ITS1F–ITS2 (White et al. 1990) and the ITS2 region using ITS3–ITS4 (White et al. 1990) or LR1 were then PCR amplified separately as above. Gel electrophoresis (1% TBE agarose gel stained with ethidium bromide) was used to verify the presence of a PCR product. PCR products were purified using a Wizard SV Gel and PCR Clean-Up System and template DNA was used in 10-µL sequencing reactions with BigDye Terminator v3.1 (Applied Biosystems, Foster City, California, USA) using a combination of the following primers: ITS: ITS1F, ITS2, ITS3, ITS4, LR1; LSU: LROR, LR3; *TEF-1*: TEF983F, TEF1567R. Sequences were generated on an Applied Biosystems 3730XL high-throughput capillary sequencer at the Roy J. Carver Biotechnology Center at the University of Illinois Urbana-Champaign. Consensus ITS, LSU, and *TEF-1* sequences were assembled with Sequencher 5.4 (Gene Codes Corp., Ann Arbor, Michigan, USA). In a few cases, Illumina sequencing was performed following the methods of Miller et al. (2022b).

Phylogenetic analyses

The ITS1 dataset was aligned with MUSCLE 3.8.425 as implemented in Geneious Prime 2022.1.1 (Edgar 2004; Dotmatics, Boston, Massachusetts). Ambiguous regions of the ITS1 alignment were trimmed with trimAl using the "automated1" option (Capella-Gutiérrez et al. 2009). The ITS2, LSU, and *TEF-1* datasets were individually aligned using the MUSCLE multiple alignment program as implemented in Sequencher 5.4. Portions of the 5′ and 3′ ends of the ITS2, LSU, and *TEF-1* alignments only required minor, manual trimming due to missing data in most taxa. The ITS1, ITS2, and LSU individual trees were rooted with *G. gigas* based on previous analyses (Dirks et al. 2023), and the *TEF-1* tree was rooted with *G. martinii* based on the ITS1, ITS2, and LSU trees. A fixed parameter-rich model (GTR + G + I) was used in lieu of running a test to select the most suitable evolutionary model for each dataset (Abadi et al. 2019). A maximum likelihood (ML) analysis with 1000 bootstrap replicates was performed using PhyML as implemented in Seaview 4.7 (Gouy et al. 2010) with all parameters optimized and the GTR model. A ML analysis with 1000 bootstrap replicates was also performed using RAxML-HPC2 v.8.2.12 (Stamatakis 2014) with a GTRCAT approximation using the CIPRES Science Gateway v.3.3 portal (Miller et al. 2010). Bootstrap replicates were performed under the GTR model employing GAMMA model of rate heterogeneity and the rapid bootstrapping option (Stamatakis et al. 2008). Clades with bootstrap values (BV) ≥ 70% were considered significant and strongly supported (Hillis and Bull 1993). Bayesian analyses were performed using MrBayes v 3.2.7 (Huelsenbeck and Ronquist 2001, 2005) under the above model on the CIPRES

3.3 portal. The B-MCMCMC analyses lasted until the average standard deviation of split frequencies was below 0.01. Constant characters were included and 10 million generations were run with trees sampled every 1000th generation, resulting in 10,000 total trees. The first 2500 trees were discarded as burn-in, and Bayesian posterior probabilities (BPP) were determined from a consensus tree generated from the remaining 7500 trees using PAUP* 4.0b10 (Swofford 2002). Clades with BPP ≥ 95% were considered significant and strongly supported (Alfaro et al. 2003; Larget and Simon 1999). Individual ITS1, ITS2, LSU, and *TEF-1* trees were congruent with no conflicting clades so the four datasets were combined and analyses conducted as above. The final ITS1-ITS2-LSU-*TEF-1* alignment is deposited in TreeBase (31515).

Sequence similarity comparisons

The ITS1 and ITS2 alignments were subjected to further analyses to assess their utility as the fungal barcode in this lineage (Schoch et al. 2012). Distance analysis was set to uncorrected "p" in PAUP v.4.0a (build 166) (Swofford 2002) to calculate the mean and range as percent differences for infraspecific and interspecific variation. Similar analyses were performed with the LSU and *TEF-1* alignments.

Results

Phylogenetic analyses

PCR amplification and Sanger sequencing of ITS2 and LSU were largely successful for most specimens, even those as old as 130 years (Table 1). The ITS2 region was successfully sequenced using Illumina Nano sequencing for all three specimens of *G. costata*, the isolectotype of *G. martinii*, and the holotype of *G. parma*. Although PCR amplification of *TEF-1* failed for most older specimens, a small sampling of all major taxa was successfully sequenced (Table 1).

The ITS1 alignment of 54 sequences consisted of 809 nucleotides after the removal of 442 nucleotides representing ambiguous regions. The ITS1 contained 520 parsimony-informative characters. The ITS2 alignment of 104 sequences consisted of 327 nucleotides after the removal of 105 nucleotides representing ambiguous regions. The ITS2 contained 76 parsimony-informative characters. The LSU alignment of 69 sequences consisted of 587 nucleotides after the removal of 48 nucleotides representing ambiguous regions. The LSU contained only 25 parsimony-informative characters and lacked sufficient phylogenetic signal to resolve relationships among the taxa. The *TEF-1* alignment of 34 sequences consisted of 622 nucleotides and 79 parsimony-informative characters. The ITS1-ITS2-LSU-*TEF-1* concatenated alignment of 107 sequences contained 2498 nucleotides and 705 parsimony-informative characters.

Analyses of the ITS1, ITS2, LSU, and *TEF-1* dataset generated identical most likely trees in both the PhyML and RAxML analyses. The RAxML tree is shown in Fig. 1. Six well-supported monophyletic clades represent distinct species: *G. brunnea*, *G. caroliniana*, *G. grandis*, *G. martinii*, *G. parma*, and the newly described species, *G. japonica*. *Gyromitra brunnea* and *G. parma* are highly supported (BV = 92, BPP = 99) sister taxa as are *G. caroliniana* and *G. grandis* (BV = 91). *Gyromitra martinii* and *G. japonica* occur at the base of the tree.

Gyromitra fastigiata and *G. slonevskii* are confirmed to be synonymous with *G. grandis* (Fig. 1) as previously shown by Van Vooren and Carbone (2019). The ITS sequences of the isoepitypes of *G. fastigiata* and *G. grandis* generated in this study had zero nucleotide differences. The three ITS sequences of *G. slonevskii* from Ukraine were nearly identical to the isoepitypes of *G. fastigiata* and *G. grandis*. *Gyromitra costata* is shown to be a later synonym of *G. caroliniana* (Fig. 1). The ITS2 sequences of all three specimens of *G. costata* generated via Illumina sequencing in this study were identical to the sequences of *G. caroliniana*.

Distribution

Each species inhabits a specific geography with some overlap among certain taxa (Fig. 2). *Gyromitra brunnea* and *G. caroliniana* occur throughout Midwestern and Eastern North America with *G. brunnea* occupying a slightly more northern range, whereas *G. caroliniana* occupies a slightly more southern range (but also a western collection from Utah). *Gyromitra martinii* and *G. parma* occur throughout Western Europe, whereas *G. grandis* has a much larger range occurring throughout Europe into Russia with a collection in Japan. *Gyromitra japonica* is only known from Japan.

Taxonomy

Gyromitra brunnea **Underw.**, *Proc. Indiana Acad. Sci.* 3: 33 (1894) [1893]

MycoBank: MB146393

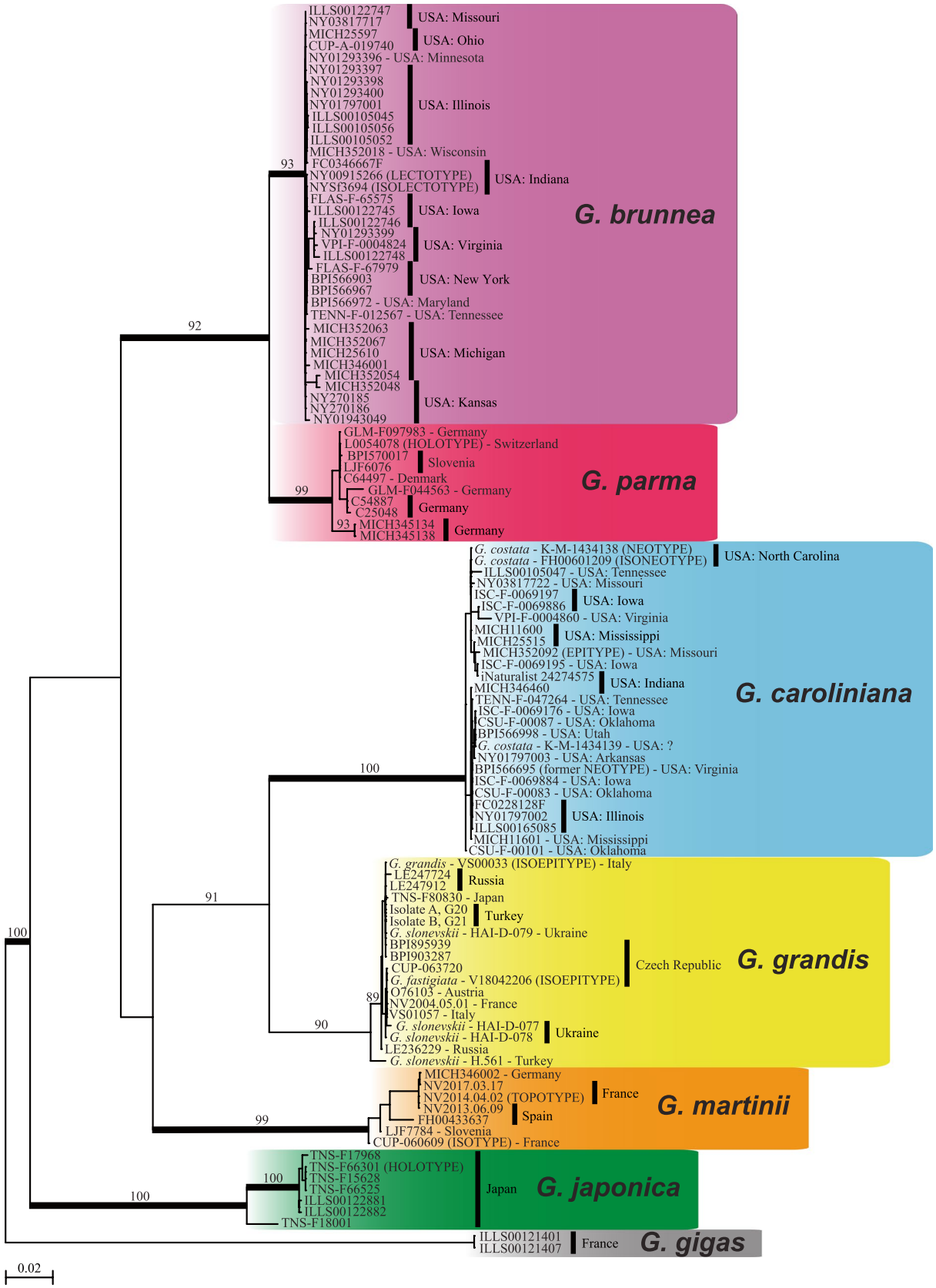
Basionym: *Gyromitra brunnea* Underw., *Proc. Indiana Acad. Sci.* 3: 33 (1894) [1893]

≡ *Neogyromitra brunnea* (Underw.) Herter, *Revista Sudamer. Bot.* 10 (1): 16 (1951)

≡ *Discina brunnea* (Underw.) Raitv., *Uchen. Zap. Tartu. Gos. Univ.* 9: 368 (1970)

= *Elvella underwoodii* Seaver, *North Am. Cup-fungi (Operculates):* 254 (1928)

Lectotype: USA, Indiana, Putnam County, Greencastle, 39.64449N, 86.864732W, 4492 m, May 1892, L.M.


ILLS00122747
NY03817717
USA: Missouri
MICH25597
CUP-A-019740
USA: Ohio
NY01293396 - USA: Minnesota
NY01293397
NY01293398
NY01293400
NY01797001
ILLS00105045
ILLS00105056
USA: Illinois
ILLS00105052
MICH352018 - USA: Wisconsin
93
FC0346667F
NY00915266 (LECTOTYPE)
NYSf3694 (ISOLECTOTYPE)
USA: Indiana
FLAS-F-65575
ILLS00122745
USA: Iowa
G. brunnea
ILLS00122746
NY01293399
VPI-F-0004824
ILLS00122748
USA: Virginia
FLAS-F-67979
BPI566903
BPI566967
USA: New York
BPI566972 - USA: Maryland
TENN-F-012567 - USA: Tennessee
92
MICH352063
MICH352067
MICH25610
MICH346001
USA: Michigan
MICH352054
MICH352048
NY270185
NY270186
NY01943049
USA: Kansas
GLM-F097983 - Germany
L0054078 (HOLOTYPE) - Switzerland
BPI570017
Slovenia
LJF6076
C64497 - Denmark
G. parma
99
GLM-F044563 - Germany
C54887
C25048
Germany
93
MICH345134
MICH345138
Germany
G. costata - K-M-1434138 (NEOTYPE)
G. costata - FH00601209 (ISONEOTYPE)
USA: North Carolina
ILLS00105047 - USA: Tennessee
NY03817722 - USA: Missouri
ISC-F-0069197
ISC-F-0069886
USA: Iowa
VPI-F-0004860 - USA: Virginia
MICH11600
MICH25515
USA: Mississippi
MICH352092 (EPITYPE) - USA: Missouri
ISC-F-0069195 - USA: Iowa
iNaturalist 24274575
USA: Indiana
MICH346460
G. caroliniana
TENN-F-047264 - USA: Tennessee
ISC-F-0069176 - USA: Iowa
CSU-F-00087 - USA: Oklahoma
BPI566998 - USA: Utah
G. costata - K-M-1434139 - USA: ?
NY01797003 - USA: Arkansas
100
BPI566695 (former NEOTYPE) - USA: Virginia
ISC-F-0069884 - USA: Iowa
CSU-F-00083 - USA: Oklahoma
FC0228128F
NY01797002
USA: Illinois
ILLS00165085
MICH11601 - USA: Mississippi
CSU-F-00101 - USA: Oklahoma
G. grandis - VS00033 (ISOEPITYPE) - Italy
LE247724
Russia
LE247912
TNS-F80830 - Japan
Isolate A, G20
Turkey
Isolate B, G21
G. slonevskii - HAI-D-079 - Ukraine
BPI895939
BPI903287
CUP-063720
Czech Republic
G. grandis
G. fastigiata - V18042206 (ISOEPITYPE)
91
O76103 - Austria
89
NV2004.05.01 - France
VS01057 - Italy
100
G. slonevskii - HAI-D-077
90
G. slonevskii - HAI-D-078
Ukraine
LE236229 - Russia
G. slonevskii - H.561 - Turkey
MICH346002 - Germany
NV2017.03.17
NV2014.04.02 (TOPOTYPE)
France
G. martinii
NV2013.06.09
Spain
99
FH00433637
LJF7784 - Slovenia
CUP-060609 (ISOTYPE) - France
TNS-F17968
TNS-F66301 (HOLOTYPE)
100
TNS-F15628
TNS-F66525
Japan
G. japonica
100
ILLS00122881
ILLS00122882
TNS-F18001
ILLS00121401
ILLS00121407
France
G. gigas
0.02

◂**Fig. 1** RAxML phylogram inferred from ML and Bayesian analyses of 107 ITS1-ITS2-LSU-*TEF-1* sequences from type and voucher specimens in the *Gyromitra brunnea* lineage. *Gyromitra gigas* is used as an outgroup. Specimen numbers are given followed by country and state/province. Type specimens for each species are given in parentheses. RAxML bootstrap support values above 70% are shown at the nodes and Bayesian posterior probability scores above 0.95 are shown as thickened branches

Underwood, Underwood (1), NY00915266; GenBank PQ350208 (ITS2), PQ600918 (LSU).

Notes: The name *G. brunnea* was published in 1894 (Underwood 1894), and Raitviir designated a lectotype in 1970 (Raitviir 1970). There is some confusion regarding the elements associated with the lectotype. Raitviir (1970) lists four elements (1, 2, 3, 5) associated with the type, whereas McKnight (1973) lists five elements (1–5) associated with the type. These were all collected by Underwood from Indiana between 1892 and 1894. Raitviir clearly designated element (1) at NY as the lectotype (Raitviir 1970, p. 369). The lectotype is NY00915266 collected in May 1892. The specimen (NYS 3694) at NYS collected on 8 May 1892 in the same locality as the lectotype and sequenced in this study is most likely an isolectotype since Underwood sent part of the lectotype to Peck. The specimen (TAAM199312d) at TAAM that is a piece of element (1) is another isolectotype. Element (2) was collected in May 1893 and consists of a slide and a single ascoma. Element (5) was also collected in May 1893 and consists of a single ascoma. Elements (2) and (5) at NY are syntypes. The whereabouts of elements (3) and (4) at NY are unknown (Laura Briscoe, personal communication), but element (3) collected in May 1894 does exist at TAAM (TAAM199312a) and is a syntype. Element (2) (TAAM206911), which corresponds to NY00915267, and element (5) (TAAM177897), which corresponds to NY00915265, are isosyntypes. Based on our phylogenetic analyses, *G. brunnea* from North America is a separate species distinct from the North American *G. caroliniana* and the European/Asian *G. grandis* (formerly *G. fastigiata*).

***Gyromitra caroliniana* (Bosc) Fr.**, *Öfvers. K. Svensk. Vetensk.-Akad. Förhandl.* 28 (2): 173 (1871) [1872]

MycoBank: MB146867

Basionym: *Morchella caroliniana* Bosc, *Mag. Neuesten Entdeck. Gesammten Naturk. Ges. Naturf. Freunde* Berlin 5: 86 (1811), *nom. sanct.*

≡ *Discina caroliniana* (Bosc) Eckblad, *Nytt. Mag. Bot.* 15 (1–2): 100 (1968)

≡ *Helvella caroliniana* (Bosc) Nees, *Syst. Pilze (Würzburg)*: 176 (1816) [1816–1817]

≡ *Helvella caroliniana* (Bosc) Seaver, *North Am. Cup-fungi (Operculates)*: 253 (1928), superfl. comb.

≡ *Mitophora caroliniana* (Bosc) Lév., *Ann. Sci. Nat., Bot. sér. 3,* 5: 250 (1846)

≡ *Neogyromitra caroliniana* (Bosc) S. Imai, *Bot. Mag., Tokyo* 46: 174 (1932)

≡ *Fastigiella caroliniana* (Bosc) Benedix, *Kulturpflanze*: 17: 277 (1969)

= *Helvella costata* Schwein., *Schriften Naturf. Ges. Leipzig* 1: 114 (1822)

≡ *Gyromitra costata* (Schwein.) Cooke, *Mycographia, Vol. 1*. Discom. (London) (no. 5): 194 (1878)

For *G. caroliniana*:

Former Neotype (from McKnight 1973): USA, Virginia, Lorton, 38.704282N, 77.22776W, on sawdust, 24 Apr 1942, O.E. Bland, BPI566695; GenBank PQ350220 (ITS2), PQ600933 (LSU).

Typification: Lectotype: Bosc's illustration, *Mag. Neuesten Entdeck. Gesammten Naturk. Ges. Naturf. Freunde* Berlin 5 (1811) (**Lectotype designated here**: Tab. V, Fig. 6; MBT10023771).

Typification: Epitype: USA, Missouri, Lawrence County, Robert E. Talbot Conservation Area, 37.178N, 93.931W, on soil, 03 Apr 2021, J. Shaffer, A.C. Dirks 0409, (**Epitype designated here** MICH352092; MBT10023772), GenBank ON693649 (ITS), ON693601 (LSU), PQ628306 (*TEF-1*).

For *G. costata*:

Typification: Neotype and Isoneotype: USA, North Carolina, on a sandy bank, 1879, Curtis 2247, (**Neotype designated here** K-M-1434138; MBT10023773), GenBank PQ350233 (ITS2), (**Isoneotype designated here** FH00601209; MBT10023774), GenBank PQ350234 (ITS2).

Notes: When *Morchella caroliniana* was described, Bosc (1811) published an illustration (Tab. V. Figure 6), which could serve as a lectotype since it is considered part of the original material (Article 9.3) sanctioned by Fries (1822). However, McKnight (1973) did not make this typification and instead designated a neotype. Here, we designate the illustration created by Bosc as the lectotype, which makes the neotype superfluous. Since the lectotype is an illustration showing only an ascomata, and is demonstrably ambiguous, we designate an epitype based on a specimen from Missouri (MICH352092) for which the whole genome has been sequenced (Dirks et al. 2025).

McKnight (1973) stated there was no material of *H. costata* at PH or among Michener's collections (apparently he had some of Schweinitz's specimens) at BPI. No Schweinitz material of *H. costata* was found at BPI (Lisa Castlebury, personal communication), FH (FH Curatorial Staff, personal communication), or K (Lee Davies, personal communication). The Curtis material (Curtis 2247) was split between K (K-M-1434138) and FH (FH00601209). The specimen at K is designated here as the neotype, which makes the material at FH an isoneotype. ITS2 sequences from these types along with a third specimen of *G. costata* at K were nearly identical to the ITS2 sequence from the epitype of *G. caroliniana*.

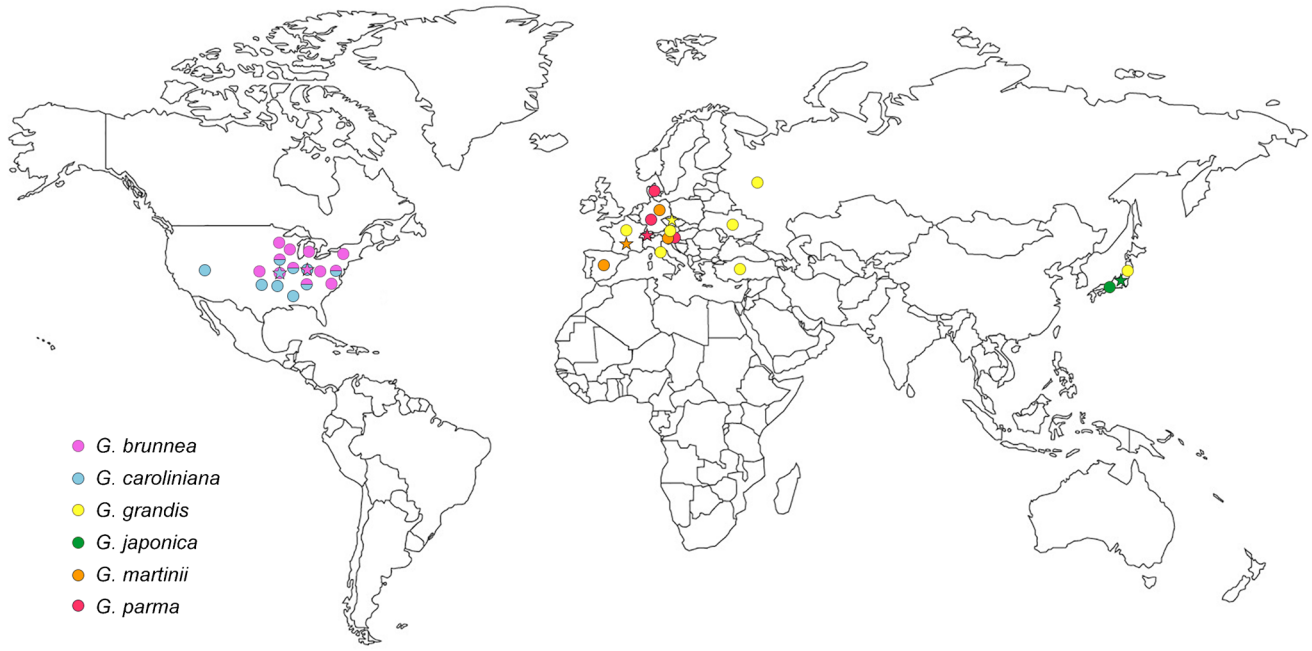


Fig. 2 Distribution map of species in the *G. brunnea* lineage for all specimens sequenced in this study. Type specimen localities for each species are shown as stars and voucher specimen localities are shown as circles. Colors for each species correspond to those used in Fig. 1

Gyromitra grandis **(U. Cumino) Van Vooren & M. Carbone**, *Ascomycete.org* 11(3): 72 (2019).

MycoBank: MB830624

Basionym: *Helvella grandis* U. Cumino, *Mém. Acad. Imp. sci., litt. beaux-arts Turin* 8: 230 (1806).

≡ *Discina grandis* (U. Cumino) M. Carbone & Van Vooren, *Ascomycete.org* 15 (5): 183 (2023)

= *Gyromitra fastigiata* (Krombh.) Rehm, *Rabenh. Krypt.-Fl.*, Ed. 2 (Leipzig), 1 (3): 1194 (1895) [1896]

≡ Helvella fastigiata Krombh., Naturgetr. Abbild. Beschr. Schwämme (Prague) 3: 32, tab. 21, figs 9–11 (1834)

≡ *Physomitra infula* var. *fastigiata* (Krombh.) Boud., *Hist. Class. Discom. Eur.* (Paris): 35 (1907)

≡ *Maublancomyces fastigiata* (Krombh.) Herter, *Revista Sudamer. Bot.* 10 (1): 17 (1951)

≡ *Neogyromitra fastigiata* (Krombh.) McKnight, *Mycologia* 60 (3): 725 (1968)

≡ *Discina fastigiata* (Krombh.) Svrček & J. Moravec, *Česká Mykol.* 26 (1): 5 (1972)

= *Gyromitra krombholzii* Bezděk, *Houby jedlé a jim podobné jedovaté*: 199 (1901)

= *Gyromitra pratensis* Velen., *Monogr. Discom. Bohem.* (Prague): 389 (1934)

≡ *Maublancomyces pratensis* (Velen.) Herter, *Revista Sudamer. Bot.* 8 (5): 161 (1950)

= *Gyromitra slonevskii* V.P. Heluta, *Ukrain. Bot. J.* 58 (1): 83 (2001)

Lectotype: Cumino, *Mém. Acad. Imp. sci., litt. beaux-arts Turin*, 8, pl. 2, fig. "*Helvella grandis*" (1806); MBT 386672.

Epitype: ITALY, Cuneo, Valdieri, San Giovanni, 44.2691 N, 7.401983 E, 760 m, along the stream Gesso, on sandy soil, under deciduous trees (*Corylus*, *Fraxinus*, *Alnus*, *Salix*), 25 Apr 2000, V. Pepino, under *G. fastigiata*, ex herb. V.S. 00033 (LY NV 2000.04.01); MBT 386671, GenBank MH938675 (ITS1), MH938320 (LSU).

Notes: A lectotype and an epitype for *G. grandis* have been designated by Van Vooren and Carbone (2019). Although they sequenced only the ITS1 region for the epitype of *G. grandis*, the complete ITS has been sequenced for isoepitypes of *G. grandis* and *G. fastigiata* during this study. These ITS sequences are nearly identical and *G. grandis* is the accepted name since it has priority.

Gyromitra krombholzii appears to be a synonym of *G. fastigiata* based on the illustration by Luňáček (1908, pl. 49) (Van Vooren and Carbone 2019). Bezděk did not save any specimens (Jan Holec, personal communication) to confirm the identity of *G. krombholzii*. The name is invalid (Art. 51) and is a later synonym of *G. fastigiata*.

The lectotype of *Gyromitra pratensis* is preserved in formaldehyde and our attempts to sequence this specimen via Illumina technology resulted in only contaminate sequences. Svrček (1978) examined the specimen and placed it in synonymy under *G. fastigiata*.

The holotype specimen of *G. slonevskii* at KW-M, which was never sequenced, no longer exists due to insect damage (Mariia Zykova, Curator of the Mycological collection, personal communication). The three specimens deposited at HAI (Barseghyan et al. 2012) no longer exist because

the fungal herbarium at HAI has been disbanded and the whereabouts of the specimens are unknown (Solomon Wasser, personal communication). *Gyromitra slonevskii* is a later synonym of *G. grandis* as suggested by Van Vooren and Carbone (2019) and corroborated with ITS data by Carbone et al. (2018) and this study.

***Gyromitra japonica* A.N. Mill. & Methven** sp. nov. Figure 3

MycoBank: MB856951

Type: JAPAN, Fukushima Prefecture, Yotsukuramachi, Umegaoka, Iwaki, 37.10731 N, 140.98247 E, 09 May 2014, Holotype (TNS-F66301), GenBank PQ350241 (ITS2), PQ600948 (LSU), PQ628316 (*TEF-1*).

Fig. 3 *Gyromitra japonica* (TNS-F66301, holotype; ILLS00122881, ILLS00122882). **a**–**d** Single ascocarp (bar = 1 cm), **e** transverse section showing asci and paraphyses in the hymenial layer, subhymenium, medullary excipulum, and ectal excipulum composed of *textura intricata* (bar = 100 µm) (BF), **f** asci (bar = 50 µm) (DIC), **g** paraphyses with apices staining blue in lactophenol in cotton blue (bar = 50 µm) (DIC), **h**–**j** ascospores (DIC), **h**–**i** ascospores stained with lactophenol in cotton blue (bar = 20 µm)

Etymology: Named for the country where it is presently known.

Description: Ascomata consisting of an apical hymenophore and stipe. Apothecium (1–2) 3–6 cm diam., cupulate to repand, margin incurved to recurved, surface smooth to wrinkled or rugulose, dark brown. Stipe 5–10 (–20) mm long, 5–10 mm diam., smooth, pubescent to tomentose, cream color. Excipulum one-layered, of *textura intricata*, hyaline. Paraphyses 5–7 µm diam., cylindric, thin-walled, hyaline, septate, unbranched, apices clavate, inflated up to 7.5–10 µm diam. Asci 250–400 × 17.5–22.5 µm, cylindric, operculate, thin-walled, hyaline, eight-spored. Ascospores uniseriate, 27.5–33.75 × 12.5–15 µm, (L_m = 30.6 µm; W_m = 13.7 µm; Q = 2.0–2.4; Q_m = 2.2), ellipsoid to fusoid, perispore 1–2 µm thick, ornamentation reticulate, cyanophilic; multiple apiculi at both ends, 2.5–3 µm high; contents triguttulate, with one large central oil droplet and two smaller polar oil droplets, hyaline. Ascospores yellowish white (4A2) to orange white (5A2) in mass.

Ecology and distribution: Solitary to scattered or in caespitose clusters on soil and wood in temperate forests from April to June. Known only from Japan.

Notes: This new species is morphologically similar to *G. parma* and *G. martinii* and would not have been discovered without molecular data. The apothecia of *G. martinii* were described as being 10–15 cm diam. with a purple hymenophore; those of *G. parma* as 7–10 cm diam. with a yellow–brown hymenophore; and those of *G. japonica* as 3–6 cm diam. with a dark brown hymenophore. The ascospores vary slightly among the three taxa based on the collections we examined. First, the shape of the ascospores of *G. parma* are fusoid while those of *G. japonica* are ellipsoid to fusoid and those of *G. martini* are narrowly ellipsoid. Second, the length and width of the ascospores varies slightly; 27.5–33.75 × 12.5–15 µm (L_m = 30.6; W_m = 13.7; Q_m = 2.2) in *G. japonica*, (27.5–) 28.75–35 (–37.5) × (11.25–) 12.5–15 (–16.75) µm (L_m = 32.0; W_m = 14.1; Q_m = 2.3) in *G. martinii*, and 27.5–35 × 11.25–13.75 (–15) µm (L_m = 31.0; W_m = 12.7; Q_m = 2.4) in *G. parma*. Third, the length of the apiculi at either end of the ascospores varies among the three taxa; up to 3 µm in *G. japonica*, up to 2.5 µm in *G. martinii*, and up to 4.5 µm in *G. parma*. While the ornamentation on the surface of the ascospores of *G. japonica* and *G. parma* forms a well-defined, clean reticulum, the ornamentation on the surface of the ascospores of *G. martinii* is often verrucose to subreticulate, lower in height and not as "clean." While *G. parma* and *G. martinii* occur in Europe, *G. japonica* is restricted to Japan. Finally, *G. martinii* and *G. parma* are apparently mainly associated with beech, whereas the forest associates of *G. japonica* were not recorded.

Additional specimens examined: JAPAN, Gunma, 36.572 N, 139.38292 E, 3 June 2007, Takashi Suda (TNS-F15628); Ibaraki, 36.10239 N, 140.11363 E, 23 April 2006, Tsuyoshi Hosoya (TNS-F17968); Hiroshima, 34.66442 N, 132.91235 E, 1 May 2006, Machiko Yamate (TNS-F18001); Tokyo, 35.74406 N, 139.47394 E, 19 April 2010, collector unknown (TNS-F66525); Kanagawa Prefecture, Midori-ku, Niiharu Citizens' Forest, 35.512967 N, 139.515725 E, on soil, 7 April 2024, Atsushi Nakajima (ILLS00122882) (immature); Kanagawa Prefecture, Midori-ku, Niiharu Citizens' Forest, 35.514322 N, 139.514178 E, on wood, 7 April 2024, Atsushi Nakajima (ILLS00122881) (immature).

***Gyromitra martinii* Donadini & Astier**, *Bull. Trimestriel Soc. Mycol. France* 90(3): 193 (1974).

MycoBank: MB314833

Basionym: *Gyromitra martinii* Donadini & Astier, *Bull. Trimestriel Soc. Mycol. France* 90(3): 193 (1974).

≡ *Discina martinii* (Donadini & Astier) Donadini & Astier, *in* Donadini, *Mycol. helv.* 1(4): 254 (1986).

Notes: This species was described by Donadini and Astier in 1974. A holotype was deposited in the personal herbarium of J.C. Donadini as J.C.D. 176–73, now housed at MPU. As explained in Van Vooren & Mauruc (2020), this type also contains specimens of *G. perlata*. A lectotype (MPU J.C.D. 176-73b), containing a specimen in conformity with the protologue, was designated by the authors and an isolectotype was sent to CUP (CUP060609). The isolectotype was sequenced for the ITS2, LSU, and *TEF-1* during this study and phylogenetic analyses clearly show *G. martinii* to be a distinct species.

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

MycoBank: MB314837

Basionym: *Discina parma* J. Breitenb. & Maas Geest., *Proc. Kon. Ned. Akad. Wetensch. C,* 76(1): 103 (1973).

≡ *Gyromitra parma* (J. Breitenb. & Mass Geest.) Bozonnet, *Bull. Ann. Fed. Centre-est Hist. Nat. et Mycol.* 1: 9 (1980), superfl. comb.

Notes: The holotype of *G. parma* resides at L (L0054078) and the ITS2 was successfully sequenced via Illumina sequencing in this study. Although all collections of *G. japonica* were originally labeled as *G. parma*, it is clear that they represent two different species.

Sequence similarity comparisons

The two regions of ITS were compared to investigate whether only one region (i.e., either ITS1 or ITS2) could be used for molecular identification of these taxa (Table 2). The ITS1 region was extremely variable and difficult to align among taxa. It contained almost seven times the number of parsimony-informative characters compared to the ITS2 region (520 vs. 76). Infraspecific ITS1 sequence variation on average was 0.2% in *G. martinii*, 0.3% in

Table 2 Infraspecific and interspecific sequence variation of the ITS1 and ITS2 regions for specimens in the *G. brunnea* lineage. Mean and range (in parentheses) of percent differences based on uncorrected "p" sequence differences are shown for ITS1 along the upper diagonal and for ITS2 along the lower diagonal

	G. brunnea	*G. caroliniana*	*G. grandis*	*G. japonica*	*G. martinii*	*G. parma*
G. brunnea	0.3 (0–1.4) 0.1 (0–1.0)	33.3 (32.6–34.9)	30.6 (30–33.4)	35.2 (34.6–35.8)	33.3 (32.8–35.5)	8.4 (6.4–11.1)
G. caroliniana	6.2 (5.0–9.0)	1.0 (0–2.1) 0 (0–0)	24.9 (24–26.1)	38.2 (37.9–38.7)	36 (35.2–37.6)	31.9 (28.8–36.5)
G. grandis	4.7 (4.0–6.0)	5.0 (4.0–7.0)	0.4 (0–1.9) 0.2 (0–1.0)	36.4 (35.7–36.9)	11.1 (10.8–11.7)	28.9 (25.8– 33.3)
G. japonica	7.4 (7.0–8.0)	8.9 (8.0–11.0)	7.9 (7.0–9.0)	0.3 (0–0.5) 0 (0–0)	38.6 (38.5–38.7)	33.2 (30.8–36.6)
G. martinii	6.1 (5.0–8.0)	6.6 (6.0–8.0)	5.2 (5.0–7.0)	7.9 (7.0–9.0)	0.2 (0–0.3) 0 (0–0)	31.2 (28.1–34.8)
G. parma	4.4 (4 .0– 6.0)	7.6 (6.0–10.0)	6.8 (6.0–9.0)	7.9 (7.0–10.0)	7.9 (7.0–10.0)	2.0 (0–2.9) 0 (0–0)

G. brunnea and *G. japonica*, 0.4% in *G. grandis*, 1% in *G. caroliniana*, and 2% in *G. parma*. Infraspecific ITS2 sequence variation on average was zero in *G. caroliniana*, *G. japonica*, *G. martinii*, and *G. parma*; 0.1% in *G. brunnea*; and 0.2% in *G. grandis*. Interspecific ITS1 sequence variation on average ranged from 8.4% between *G. brunnea* and *G. parma* to 38.6% between *G. japonica* and *G. martinii*, and most comparisons differed by 30% or more. Interspecific ITS2 sequence variation on average ranged from 4.4% between *G. brunnea* and *G. parma* to 8.9% between *G. caroliniana* and *G. japonica*, and most comparisons differed by 6–8%. Infraspecific variation for all species comparisons averaged less than 0.7% and 0.1% for ITS1 and ITS2, respectively, whereas interspecific variation averaged more than 30% and 6%.

Two single specimens could possibly represent new species based solely on the differences in their ITS sequences. One is the specimen of *G. grandis* (originally *G. slonevskii*, H.561) from Turkey, which possessed 21/1165 (1.8%) infraspecific nucleotide differences. The other is a specimen of *G. japonica* (TNS: F18001) from Hiroshima, Japan, which possessed 76/1360 (5.6%) infraspecific nucleotide differences. This specimen (TNS: F18001) was not used in the sequence similarity comparisons due to its highly variable sequence variation. However, more collections are needed before either of these putative taxa are described as new (Aime et al. 2021).

The LSU and *TEF-1* were compared to investigate the infraspecific and interspecific variability of these two common molecular markers. Infraspecific LSU sequence variation on average was 0.04% in *G. parma*, 0.1% in *G. brunnea*, *G. caroliniana*, *G. grandis*, and *G. japonica*, and 0.2% in *G. martinii* (Table 3). Infraspecific *TEF-1* sequence variation on average was 0.3% in *G. grandis*, 0.5% in *G. caroliniana*, *G. japonica*, and *G. parma*, 0.6% in *G. brunnea*, and 0.7% in *G. martinii*. Interspecific LSU sequence variation on average ranged from 0.3% between *G. brunnea* and *G. parma* as well as *G. martinii* and *G. parma* to 1.8% between *G. caroliniana* and *G. japonica*. Interspecific *TEF-1* sequence variation on average ranged from 2.3% between *G. brunnea* and *G. parma* to 7.7% between *G. caroliniana* and *G. japonica*. Infraspecific variation for all species comparisons averaged less than 0.1% and 0.5% for LSU and *TEF-1*, respectively, whereas interspecific variation averaged less than 1% and 5%.

Species concepts

Previous authors had confusing species concepts for some of these taxa. For example, McKnight (1971) used the name *G. fastigiata* to refer to North American specimens that are most likely *G. brunnea* or *G. caroliniana*; one specimen (Rhoads 24.VI.1945 from Utah, BPI 566998) examined by McKnight (1971) was molecularly annotated to be *G. caroliniana* in this study. Later collections identified by

Table 3 Infraspecific and interspecific sequence variation of the LSU and *TEF-1* regions for specimens in the *G. brunnea* lineage. Mean and range (in parentheses) of percent differences based on uncorrected "p" sequence differences are shown for LSU along the upper diagonal and for *TEF-1* along the lower diagonal

	G. brunnea	*G. caroliniana*	*G. grandis*	*G. japonica*	*G. martinii*	*G. parma*
G. brunnea	0.1 (0–0.5) 0.6 (0–1.6)	1.2 (1.1–2.1)	0.5 (0.3–0.9)	1.3 (1.1–1.7)	0.5 (0.3–1.4)	0.3 (0.2–0.6)
G. caroliniana	5.0 (3.4–6.1)	0.1 (0–0.7) 0.5 (0–1.4)	1.0 (0.8–1.5)	1.8 (1.6–2.0)	1.6 (1.4–2.7)	1.4 (1.3–2.0)
G. grandis	4.7 (3.0–6.8)	3.2 (1.8–4.7)	0.1 (0–0.3) 0.3 (0–1.1)	0.9 (0.6–1.2)	0.9 (0.6–1.6)	0.6 (0.5– 0.8)
G. japonica	7.0 (5.3–7.7)	7.7 (4.9–8.7)	7.2 (6.0–9.8)	0.1 (0–0.3) 0.5 (0–1.1)	1.4 (1.1–2.0)	1.1 (0.9–1.3)
G. martinii	4.8 (3.8–5.5)	4.4 (2.8–5.6)	3.5 (2.0–5.1)	5.7 (4.7–6.3)	0.2 (0–0.8) 0.7 (0–1.4)	0.3 (0.2–0.9)
G. parma	2.3 (1.4– 3.0)	4.3 (2.8–5.5)	4.0 (2.4–6.9)	6.2 (4.7–6.9)	3.8 (3.2–4.7)	0.04 (0–0.2) 0.5 (0–1.4)

McKnight as *G. fastigiata* have been molecularly annotated in this study to be *G. korfii*. Following Svrček and Moravec (1972), Harmaja (1973), and Weber and Weber (1988), Abbott and Currah (1997) also used the name *G. fastigiata* to refer to eastern and southern North American specimens of *G. brunnea*. In addition, the two Turkish specimens claimed to be *G. brunnea* based on morphology and LSU sequence data (Acar et al. 2018) are most likely *G. fastigiata*. Since the LSU is a poor marker for species delimitation in *Gyromitra* (Miller et al. 2020), attempts were made, but ignored, to obtain samples of these two specimens for ITS sequencing. Numerous specimens occur in the Mycology Collections Portal (MyCoPortal 2024) that are identified as *G. fastigiata* from North America, but these could be one of several different *Gyromitra* species. Twelve specimens labeled as *G. fastigiata* from Illinois, Kansas, Maryland, New York, Ohio, Utah, Virginia, and Wisconsin were sequenced for the ITS (Table 1). None were identified as *G. fastigiata*. These were molecularly annotated as *G. americanigigas*, *G. brunnea*, *G. caroliniana*, *G. esculenta*, and *G. korfii*. Thus, we have chosen to not discuss historical species concepts, but rather focus on specimens for which ITS2 sequence data are available.

Species within the *G. brunnea* lineage are characterized by stipitate or sessile ascomata and ellipsoid, coarsely reticulate ascospores that possess multiple apiculi. Taxa included in *Gyromitra* subgenus *Caroliniana* section *Caroliniana* (*G. brunnea*, *G. caroliniana*, and *G. grandis*) feature stipitate ascomata that are irregularly lobed or cerebriform. *Gyromitra brunnea*, known only from Canada and the USA, is characterized by brown ascomata that are irregularly lobed to plicate with a fluted, white stipe. The ascospores of *G. brunnea* are usually under 30 µm in length (25–30×11–14 µm in our study). *Gyromitra caroliniana*, known only from the USA, is characterized by brown, rounded to cerebriform ascomata that are deeply and irregularly wrinkled and folded with a furrowed, white stipe. In addition to the differences in the morphology of the ascomata, the ascospores of *G. caroliniana* are longer and wider (30–36×12–16 µm) than those in *G. brunnea.* Although *G. grandis* is morphologically similar to *G. brunnea*, *G. grandis* is known only from Europe and Japan and is characterized by brown, often trilobed, folded, wavy ascomata with a white stipe that is widened toward the base and rarely furrowed. The ascospores of *G. grandis* are slightly longer and wider (27–32×12–15 µm) than those of *G. brunnea.*

Taxa included in *Gyromitra* subgenus *Caroliniana* section *Parmae* feature cupulate ascomata that are sessile to substipitate. *Gyromitra japonica*, currently known only from temperate forests in Japan, is characterized by dark brown ascomata that are 3–6 cm in diameter. The ascospores of *G. japonica* are ellipsoid to fusoid, measure 27.5–33.75×12.5–15 µm with apiculi up to 3 µm in length, and are ornamented with a complete reticulum. *Gyromitra martinii*, known only from rotten beech wood

in Germany, France, Slovenia, and Spain, is characterized by purple, wrinkled ascomata that are 10–15 cm in diameter. The ascospores of *G. martinii* are narrowly ellipsoid, measure 29–35 × 12.5–15 µm with apiculi up to 2.5 µm in length, and are ornamented with an incomplete reticulum that is lower in height than the ascospore reticulum of *G. japonica* and *G. parma*. *Gyromitra parma*, known from Europe on the soil in association with beech, is characterized by yellow–brown ascomata that are 7–10 cm in diameter. The ascospores of *G. parma* are fusoid, measure 27.5–35 × 11.25–13.75 µm with apiculi up to 4.5 µm in length, and are ornamented with a complete reticulum.

Several studies of *Gyromitra* (McKnight 1973; Donadini and Astier 1974; McKnight and Batra 1974; Donadini 1986; Abbott and Currah 1997) included scanning electron micrographs (SEMs) of the ascospores of five of the taxa in *Gyromitra* subgenus *Caroliniana*. Four of the SEMs of the ascospores illustrate the reticulate ornamentation and multiple terminal apiculi which characterize *Gyromitra* subgenus *Caroliniana*: *Gyromitra brunnea* (McKnight 1973; McKnight and Batra 1974), *G. caroliniana* (McKnight and Batra 1974), *G. martinii* (Donadini and Astier 1974; Donadini 1986), and *G. parma* (McKnight and Batra 1974; Donadini 1986; Abbott and Currah 1997). While Baute (2006) suggested that *G. martinii* may be the same as *G. parma*, the differences in the height and development of the reticulate ascospore ornamentation observed in the SEMs as well as the ITS2 phylogram in this study confirm that these are separate taxa. The SEMs of the ascospores of *G. fastigiata* (McKnight and Batra 1974) also feature reticulate ornamentation but have knob-like apiculi. In our opinion, these SEMs suggest that this collection (McKnight 12880 (BPI)) may be representative of *G. korfii* and not *G. fastigiata*, although we have not examined the specimen.

Key to species of *Gyromitra* subgenus *Caroliniana*

1. Apothecia stipitate..... 2
1. Apothecia sessile to substipitate..... 4
2. Apothecia cerebriform; eastern North America ..... *G. caroliniana*
2. Apothecia lobed..... 3
3. Ascospores often less than 30 µm in length; eastern North America..... *G. brunnea*
3. Ascospores often more than 30 µm in length; Europe, Asia..... *G. grandis*
4. Ascospore ornamentation verrucose to subreticulate; Europe..... *G. martinii*
4. Ascospore ornamentation reticulate..... 5
5. Apothecia 3–6 cm diam., dark brown; Japan..... ..... *G. japonica*
5. Apothecia 7–10 cm diam., yellow–brown; Europe..... ..... *G. parma*

Discussion

Two well-supported clades of sister taxa occur in the *Gyromitra brunnea* lineage: *G. brunnea*–*G. parma* and *G. caroliniana*–*G. grandis*. For several decades, mycologists have questioned whether *G. brunnea*, *G. caroliniana*, and *G. grandis* (as *G. fastigiata*) are separate taxa and which taxonomic name to apply to North American specimens that resemble *G. fastigiata* (McKnight 1973; Abbott and Currah 1997; Methven et al. 2013; Miller et al. 2015). A search in the MyCoPortal (MyCoPortal 2024) reveals that the name *Gyromitra fastigiata* has been applied to 99 specimens occurring in Canada and USA, whereas 667 specimens are labeled as *G. brunnea* and 243 specimens as *G. caroliniana*. Based on our multi-gene phylogeny of taxa widely sampled throughout North America and Europe, three distinct species exist with *G. brunnea* and *G. caroliniana* occurring primarily in Midwestern and Eastern North America, whereas *G. grandis* is found throughout Europe with single occurrences in Russia and Japan. The 99 mislabeled specimens of *G. fastigiata* occurring in 15 fungaria would need to be morphologically examined or molecularly annotated using the ITS2 region to determine whether these represent *G. brunnea* or *G. caroliniana* or possibly another species.

Although there has been confusion about the identity of *G. caroliniana* and *G. costata*, the ITS2 sequences of the specimens of *G. costata* were nearly identical to the sequences of *G. caroliniana*. Complete ITS for the isoepitypes of *G. grandis* and *G. fastigiata* were nearly identical and *G. grandis* is the accepted name (Van Vooren and Carbone 2019). Since no specimens of *G. krombholzii* were retained by Bezděk and the name is invalid, *G. krombholzii* appears to be a synonym of *G. fastigiata* (= *G. grandis*) based on the illustration of Luňáček (1908). While the lectotype of *G. pratensis* was preserved in formaldehyde and a sequence could not be generated, Svrček (1978) examined the specimen and placed it in synonymy with *G. fastigiata* (= *G. grandis*). Even though the holotype of *G. slovneskii* was destroyed by insects, ITS sequences from authentic material presented by Carbone et al. (2018) suggest it is a synonym of *G. fastigata* (= *G. grandis*).

Gyromitra martinii and *G. parma* are morphologically similar and both are associated with beech wood, although *G. martinii* may occasionally occur on other types of trees (e.g., *Populus*, *Corylus*). Their synonymy has been suggested (Baute 2006). However, these two species are phylogenetically distinct and can be distinguished based on the larger ascomata and slightly wider ascospores of *G. martinii*. The apiculi in *G. parma* are longer and the ascospores possess a complete reticulum compared to the incomplete reticulum seen in *G. martinii*. *Gyromitra japonica* represents an example of species discovery in fungaria, as has been seen with plants in herbaria (Bebber et al. 2010; Davis

2022). *Gyromitra japonica* and *G. parma* are morphologically very similar, which explains why all collections of *G. japonica* at TNS were identified as *G. parma*. However, *G. japonica* has smaller ascomata and is only known from Japan, whereas *G. parma* is found throughout Europe. While the macro- and micromorphology of specimens of *G. japonica*, *G. martinii*, and *G. parma* overlap to varying degrees, the ITS2 sequences clearly delineate these three taxa as separate species.

The North American *G. brunnea* and the European *G. parma* occur as moderately supported sister species, as do the North American *G. caroliniana* and Asian/European *G. grandis*. Since we find stipitate and cerebriform/lobed species and sessile/substipitate and cupulate species in the same clades, the sections erected by Van Vooren and Moreau (2009) (sect. *Caroliniana* and sect. *Parmae*) are unsupported.

Both the ITS1 and ITS2 displayed a significant barcode gap (Table 2) and either or both regions could be used to easily molecularly identify species of *Gyromitra* as has been shown in previous studies (Miller et al. 2020, 2022a). The ITS2 region is shorter (ca. 400 vs. 800–1000 bp) and successfully amplified much better than the entire ITS or the ITS1 region alone (104 sequences vs 54), especially for older specimens (Table 1). The LSU varied little among taxa and exhibited almost no barcode gap (Table 3). It should not be used as a molecular barcode marker for *Gyromitra* species. The *TEF-1* was difficult to amplify for most taxa with only 34 sequences included in this study. However, it displayed a significant barcode gap comparable to ITS2 and could be used as a molecular marker for identifying taxa in this lineage.

An interesting discovery was the infraspecific variation in the ITS1 and ITS2 regions of *G. japonica*, which were all collected between 2006 and 2024. Although sequences of *G. japonica* from the Tokyo area displayed almost zero nucleotide differences, the single specimen from Hiroshima (TNS-F18001) exhibited 5.6% infraspecific variation in the ITS. Whether this is a result of extreme genetic change caused by atomic fallout in 1945, some other genetic anomaly specific to the ITS region, or if this specimen merely represents a new species requires further investigation. Neither the LSU or *TEF-1* displayed this infraspecific variation.

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Author contribution Andrew N. Miller was responsible for the study conception and design. Andrew N. Miller and Alden Dirks generated molecular sequence data. Andrew N. Miller, Alden Dirks, and Nicolas Van Vooren provided voucher specimens. Molecular analyses were performed by Andrew N. Miller and Alden Dirks. Morphological analyses were performed by Andrew S. Methven. The first draft of the manuscript was written by Andrew N. Miller, Andrew S. Methven edited the manuscript, and all authors commented on subsequent versions of the manuscript. All authors read and approved the final manuscript.

Data availability All data and materials have been deposited in publicly accessible holdings.

Declarations

Conflict of interest The authors declare no competing interests.

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