

Supplementary material

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Table S1. AM fungal taxa for which *de novo* genome sequencing was attempted, organized according to the classification on the INVAM homepage (2020-08-01). The whole genome amplification (WGA) kit used for amplification of the single nuclei is indicated for each isolate. Number (Nr.) of spores indicates how many spores were pooled to extract nuclei for FACS, and number of nuclei shows how many nuclei samples were individually selected for sequencing, resulting in the same number of libraries successfully sequenced.

Family	Genus	Species	Strain	Collection ³	Nuclei sorted ⁴	WGA	Nr. spores	Nr. nuclei
Glomeraceae	Funneliformis	<i>Funneliformis mosseae</i>	87-6 pot B 2015	Kansas		Epicentre	~15	20
		<i>Funneliformis caledonius</i>	UK204	INVAM		Qiagen	~15	24
	Septoglomus	<i>Septoglomus viscosum</i>	MD215	INVAM		Qiagen	~15	20
		<i>Septoglomus constrictum</i>	KS890	INVAM		Qiagen	~15	-
	Glomus	<i>Glomus microaggregatum</i>	UT126B	INVAM		Qiagen	~15	-
		<i>Glomus gold</i>	KS906B	INVAM		Qiagen	~15	-
	Rhizophagus	<i>Rhizophagus irregularis</i>	DAOM197198	Canada		Qiagen	1	24
		<i>Rhizophagus intraradices</i>	FL208A	INVAM		Epicentre	1	24
Acaulosporaceae	Acaulospora	<i>Entrophospora infrequens</i> ¹	110 2015	Kansas	No	na	~15	-

		<i>Entrophospora infrequence</i> ¹	CA203	INVAM		Qiagen	~15	-
		<i>Acaulospora colombiana</i>	CL356	INVAM		Qiagen	~15	15
		<i>Acaulospora morrowiae</i>	CL551	INVAM		Qiagen	~15	19
Diversisporaceae	Diversispora	<i>Diversispora epigaea</i>	AZ150B	INVAM		Qiagen	~15	7
		<i>Diversispora eburnea</i>	AZ414A	INVAM		Qiagen	~15	24
Gigasporaceae	Gigaspora	<i>Gigaspora margarita</i>	120-4 pot B 10/14	Kansas		Qiagen	~15	24
		<i>Gigaspora rosea</i>	FL105	INVAM		Epicentre	~15	20
	Dentiscutata	<i>Dentiscutata heterogama</i>	IL203A	INVAM		Qiagen	~15	7
		<i>Dentiscutata erythropus</i>	MA453B	INVAM		Qiagen	~15	24
	Cetraspora	<i>Cetraspora pellucida</i>	FL966	INVAM		Qiagen	~15	24
		<i>Cetraspora pellucida</i> ²	28 12/20/2015	Kansas		Epicentre	~15	17
	Racocetra	<i>Racocetra persica</i>	MA461A	INVAM		Qiagen	~15	24

	Scutellospora	<i>Racocetra fulgida</i>	IN212	INVAM		Qiagen	~15	22
		<i>Scutellospora calospora</i>	AU212A	INVAM		Epicentre	~15	13
Claroideoglomeraceae	Claroideoglomus	<i>Claroideoglomus candidum</i>	NC172	INVAM		Epicentre	1	24
		<i>Claroideoglomus candidum</i>	(CCK) pot B 6-9	Kansas		Qiagen	7	24
Paraglomeraceae	Paraglomus	<i>Paraglomus occultum</i>	IA702	INVAM		Epicentre	~15	23
		<i>Paraglomus brasilianum</i>	BR232B	INVAM		Epicentre	~15	20
Archaeosporaceae	Archaeospora	<i>Archaeospora trappei</i>	IL203B	INVAM	No	na	~15	-
		<i>Archaeospora schencki</i>	CL383	INVAM		Qiagen	~15	24
Ambisporaceae	Ambispora	<i>Ambispora leptoticha</i>	FL130A	INVAM		Qiagen	~15	22
		<i>Ambispora gerdemannii</i>	MT106	INVAM		Qiagen	~15	24

1. Taxonomic placement of *Entrophspora infrequences* remains unresolved
2. The strain was previously named *Scutellospora pellucida*. It is the same culture as INVAM collection *Cetraspora pellucida* (INVAM IN211)
3. Collections from where the strains were obtained: Kansas: James D. Bever's lab, University of Kansas, USA; INVAM: International culture collection of (vesicular) arbuscular mycorrhizal fungi, West Virginia University, Morgantown, WV, USA; Canada: Agriculture and Agri-food Canada.
4. Shadowed boxes indicate successful nuclei sorting.

Table S2. Published data used in phylogenomic analyses. Annotations of the whole genome assemblies were downloaded from their original source. Final column shows in which figures each isolate was included.

Phyla	Species	Isolate	Publication	Used for Fig.
Glomeromycota	<i>Rhizophagus irregularis</i>	A1	(Chen <i>et al.</i> , 2018)	1-2, S3-S9, S13
	<i>Rhizophagus diaphanus</i>	MUCL43196	(Morin <i>et al.</i> , 2019)	1-2, S3-S9, S13
	<i>Rhizophagus cerebriforme</i>	DAOM227022	(Morin <i>et al.</i> , 2019)	1-2, S3-S9, S13
	<i>Rhizophagus irregularis</i>	DAOM234181	(Beaudet <i>et al.</i> , 2018)	S6
	<i>Funneliformis mosseae</i>	DAOM236685	(Beaudet <i>et al.</i> , 2018)	S6
	<i>Acaulospora morrowiae</i>	CR315B	(Beaudet <i>et al.</i> , 2018)	S6
	<i>Diversispora epigaea</i>	IT104	(Sun <i>et al.</i> , 2019)	1-2, S3-S9, S13
	<i>Diversispora versiforme</i>	W475-40	(Beaudet <i>et al.</i> , 2018)	S6
	<i>Gigaspora rosea</i>	DAOM194757	(Morin <i>et al.</i> , 2019)	1-2, S3-S9, S13
	<i>Racocetra castanea</i>	BEG 1	(Beaudet <i>et al.</i> , 2018)	S6
	<i>Scutellospora calospora</i>	IL209	(Beaudet <i>et al.</i> , 2018)	S6
	<i>Claroideoglomus claroideum</i>	DAOM234280	(Beaudet <i>et al.</i> , 2018)	S6
	<i>Claroideoglomus claroideum</i>	SA101	(Montoliu-Nerin <i>et al.</i> , 2020)	1-2, S3-S14
	<i>Ambispora lepototicha</i>	JA116	(Beaudet <i>et al.</i> , 2018)	S6
	<i>Paraglomus brasilianum</i>	DAOM240472	(Beaudet <i>et al.</i> , 2018)	S6
Mortierellomycota	<i>Mortierella elongata</i>	AG 77	(Uehling <i>et al.</i> , 2017)	1, S3-S6
	<i>Lobosporangium transversale</i>	NRR 3116	(Mondo <i>et al.</i> , 2017a)	1, S3-S6
Mucoromycota	<i>Endogone sp.</i>	FLAS 59071	(Chang <i>et al.</i> , 2019)	1, S3-S6

	<i>Jimgerdemannia lactiflua</i>	OSC 166217	(Chang <i>et al.</i> , 2019)	1, S3-S6
	<i>Jimgerdemannia flammicorona</i>	AD 002	(Chang <i>et al.</i> , 2019)	1, S3-S6
	<i>Syncephalastrum racemosum</i>	NRRL 2496	(Mondo <i>et al.</i> , 2017a)	1, S3-S6
	<i>Lichtheimia corymbifera</i>	JMRC FSU 9682	(Schwartz <i>et al.</i> , 2014)	1, S3-S6
	<i>Hesseltinella vesiculosa</i>	NRRL 3301	(Mondo <i>et al.</i> , 2017a)	1, S3-S6
	<i>Absidia repens</i>	NRRL 1336	(Mondo <i>et al.</i> , 2017a)	1, S3-S6
	<i>Saksenaea vasiformis</i>	B4078	(Chibucos <i>et al.</i> , 2016)	1, S3-S6
	<i>Phycomyces blakesleeanus</i>	NRRL 1555	(Corrochano <i>et al.</i> , 2016)	1, S3-S6
	<i>Rhizopus microsporus</i> var. <i>microsporus</i>	ATCC 52813	(Mondo <i>et al.</i> , 2017b)	1, S3-S6
	<i>Rhizopus microsporus</i> var. <i>chinensis</i>	CCTCCM 201021	(Wang <i>et al.</i> , 2013)	1, S3-S6
	<i>Mucor circinelloides</i>	CBS 277 49	(Corrochano <i>et al.</i> , 2016)	1, S3-S6
	<i>Rhizopus delemar</i>	RA99 880	(Ma <i>et al.</i> , 2009)	1, S3-S6
Basidiomycota	<i>Laccaria bicolor</i>	S238N-H82	(Martin <i>et al.</i> , 2008)	S3-S5
	<i>Ustilago maydis</i>	521, DSMZ14603	(Kämper <i>et al.</i> , 2006)	S3-S5
	<i>Puccinia striiformis</i> f. sp. <i>tritici</i>	104 E137 A-	(Schwessinger <i>et al.</i> , 2018)	S3-S5
Ascomycota	<i>Tuber melanosporum</i>	Mel28	(Martin <i>et al.</i> , 2010)	S3-S5
	<i>Schizosaccharomyces pombe</i>	-	(Wood <i>et al.</i> , 2002)	S3-S5
	<i>Yarrowia lypolitica</i>	FKP355	(Pomraning <i>et al.</i> , 2018)	S3-S5

Table S3. Whole genome assembly statistics for all *de novo* genome assemblies generated in this study. Showing results from the QUAST analysis (Estimated size, Size, #Contigs, N50, Largest contig and GC), estimated completeness analysis (BUSCO), results from gene prediction and repeat annotation pipeline (# Genes and (content), Repeat content). Final column list figures where taxon is included.

* Indicate that the strain removed due to unclear identity prior to phylogenomic analysis and only included in Fig. S1.

Species	Estimated size (Mb)	Size (Mb)	# Contigs	N50	Largest contig (Kb)	GC (%)	BUSCO (%)	# Genes and (content, Mb)	Repeat content (Mb)	Used for Fig.
<i>F. mosseae</i>	156.54	145.72	27,134	11,988	97.04	25.22	C: 86, F: 6	16,857 (42.65)	77.14	1-2, S1-S14
<i>F. caledonius</i>	150.38	146.16	31,052	9,933	81.73	26.00	C: 89, F: 4	17,946 (41.00)	75.48	1-2, S1-S14
<i>Se. viscosum</i> *	86.48	69.04	13,413	14,169	159.44	36.25	C: 90, F: 4	14,815 (37.81)	17.27	S1
<i>Rh. irregularis</i> DAOM197198	133.86	116.45	15,939	21,163	222.15	27.28	C: 94, F: 1	23,258 (68.13)	33.80	1-2, S1-S14
<i>Rh. intraradices</i> *	133.86	116.45	15,939	21,163	222.15	27.28	C: 94, F: 1	23,258 (68.13)	33.80	S1
<i>Ac. colombiana</i>	247.44	299.81	7,5131	8,208	86.29	29.39	C: 78, F: 9	14,505 (23.78)	240.52	1-2, S1-S14
<i>Ac. morrowiae</i>	154.35	218.77	64,585	6,189	54.03	27.89	C: 74, F: 14	18,394 (31.29)	159.05	1-2, S1-S9, S11, S13
<i>Di. eburnea</i>	87.85	65.11	9,803	22,612	184.86	25.84	C: 92, F: 3	12,017 (37.55)	25.62	1-2, S1-S14
<i>Di. epigaea</i> *	54.32	64.27	16,527	8,159	138.06	38.11	C: 76, F: 12	15,972 (29.58)	10.12	S1
<i>G. margarita</i>	492.60	574.57	173,647	5,875	76.23	27.40	C: 88, F: 3	46,492 (82.99)	378.98	1-2, S1-S14
<i>G. rosea</i>	200.66	240.31	90,143	4,479	50.41	28.74	C: 46, F: 13	26,343 (36.22)	154.42	1-2, S1-S9, S11, S13
<i>De. heterogama</i>	167.37	181.57	61,528	4,991	40.56	27.81	C: 44, F: 11	16,277 (23.62)	121.82	1-2, S1-S9, S11, S13
<i>De. erythropus</i>	248.76	293.54	73,964	7,545	61.80	28.34	C: 87, F: 4	28,764 (58.16)	190.17	1-2, S1-S14
<i>Ra. persica</i>	176.70	353.07	181,541	2,886	30.66	27.09	C: 47, F: 24	37,045 (36.01)	238.55	1-2, S1-S9, S11, S13
<i>Ra. fulgida</i>	212.27	295.99	105,307	4,839	74.97	28.40	C: 68, F: 14	19,906 (29.68)	205.34	1-2, S1-S14
<i>Ce. pellucida</i> 28 K ¹	214.21	226.81	79,972	4,932	51.05	26.61	C: 43, F: 12	18,058 (26.55)	164.40	1-2, S1-S9, S11, S13
<i>Ce. pellucida</i> FL966	418.72	435.08	90,930	10,017	77.05	25.97	C: 89, F: 5	22,053 (51.29)	325.42	1-2, S1-S14

<i>S. callospora</i>	143.92	147.04	50,548	4,999	47.38	26.36	C: 29, F: 15	11,479 (16.87)	107.94	1-2, S1-S9, S11, S13
<i>Cl. candidum</i> NC172	86.47	68.12	12,232	15,216	101.84	27.83	C: 88, F: 3	15,761 (42.66)	21.83	1-2, S1-S14
<i>Cl. candidum</i> B6-9K	87.88	69.90	12,603	15,877	114.29	27.86	C: 87, F: 4	16,088 (43.88)	22.68	1-2, S1-S14
<i>Ar. schenckii</i> *	86.41	89.56	17,380	12,048	123.55	36.28	C: 86, F: 8	19,726 (47.95)	24.04	S1
<i>Am. gerdemannii</i>	102.08	87.97	19,363	10,266	96.67	28.22	C: 90, F: 3	13,690 (33.64)	42.05	1-2, S1-S14
<i>Am. leptoticha</i>	163.75	197.20	58,506	5,886	99.48	23.62	C: 90, F: 3	14,642 (30.94)	144.29	1-2, S1-S14
<i>P. occultum</i>	49.53	50.06	8,053	16,033	146.03	36.55	C: 76, F: 7	11,385 (30.35)	10.57	1-2, S1-S9, S11, S13
<i>P. brasilianum</i>	61.76	58.47	7,115	21,894	153.20	36.59	C: 90, F: 3	11,842 (36.52)	15.09	1-2, S1-S14

1. The strain was named *Scutellospora pellucida* in Bever lab collection (K for Kansas). It is the same culture as INVAM collection *Cetraspora pellucida* (INVAM IN211)

Table S4. BUSCO statistics for all *de novo* genome assemblies generated using single nuclei sequencing and assembly of combined reads. Number of genes out of a total of 290 conserved single copy genes. Column “15 selected” marks the 15 genome assemblies selected for in-depth analysis of Glomeromycota.

Species	Complete single copy	Complete duplicated	Fragmented	Missing	15 selected
<i>F. mosseae</i>	248	1	18	23	*
<i>F. caledonius</i>	256	2	12	20	*
<i>Se. viscosum</i> ³	257	5	11	17	
<i>Rh. irregularis</i>	271	1	4	14	*
<i>Rh. intraradices</i> ³	249	2	14	25	
<i>Ac. colombiana</i>	188	37	25	40	*
<i>Ac. morrowiae</i>	207	8	42	33	
<i>Di. eburnea</i>	265	3	9	13	*
<i>Di. epigaea</i> ³	181	41	36	32	
<i>G. margarita</i>	252	4	9	25	*
<i>G. rosea</i>	131	1	38	120	
<i>De. heterogama</i>	127	1	31	131	
<i>De. erythropus</i>	246	5	13	26	*
<i>Ra. persica</i>	135	0	70	85	
<i>Ra. fulgida</i>	195	3	40	52	*
<i>Ce. pellucida</i> 28 K ¹	124	1	34	131	
<i>Ce. pellucida</i> FL966	255	4	14	17	*
<i>S. calospora</i>	84	1	42	163	
<i>Cl. claroideum</i>	224	31	13	22	*
<i>Cl. candidum</i>	222	33	10	25	*
<i>Cl. candidum</i> K ²	223	30	12	25	*
<i>Ar. schenckii</i> ³	200	50	23	17	
<i>Am. gerdemannii</i>	256	6	9	19	*
<i>Am. leptoticha</i>	254	5	10	21	*
<i>P. occultum</i>	219	3	40	52	
<i>P. brasilianum</i>	258	4	10	18	*

1. The strain was named *Scutellospora pellucida* in Bever lab collection (K for Kansas). It is the same culture as INVAM collection *Cetraspora pellucida* (INVAM IN211)
2. K is added to indicate that this is the *Claroideoglossum candidum* from Kansas (CCK) pot B 6-9 is from the Bever lab collection.
3. Phylogenetic placement based on rDNA SSU genes did not support the names assigned to these strains and they were thus removed from further analysis.

Table S5. Six datasets with different taxon and SCOs sampling were used for the phylogenomic analyses. For each dataset figure numbers are listed for results from the five types of phylogenomic analysis performed: maximum likelihood (ML), Bayesian, coalescent-based approach (ASTRAL), split networks in IQ_tree (Network) and topology visualization (DensiTree). Supplementary figures are indicated with S before the number.

Dataset	Number of taxa	% of taxa with shared SCOs	Number of SCOs	ML tree	Bayesian tree	Astral tree	Net-work	Densi-Tree
Dikarya + Glomeromycota + Mucoromycota + Mortierellomycota	47	50%	178	S3, S4A		S4B, S5		
Glomeromycota + transcriptome data + sister phyla	53	50%	17	S6				
Glomeromycota + sister phyla	41	50%	371	Fig 1, S7A	S7A	S7B, S8		
Glomeromycota	27	50%	1,737	S9A		Fig. 2A, S9B	S13	
Glomeromycota	27	100%	31	S11A		Fig. 2B, S11B		
Glomeromycota selected	15	100%	799	S10A		Fig. 2C S19B	S14	S12

Table S6. Mapping of single nuclei reads of *Rh. irregularis* DAOM197198 to the reference genome assembly v.2.0 (ref v.2.0) (Chen *et al.*, 2018), and the *de novo* assembly produced in this study (*de novo*). Percentage of mapped reads from each nucleus (1-24) and percentage of the assembly covered by the reads from 24 individually amplified and sequenced nuclei. With average in bold. Last two rows indicate % reads mapped and % of assembly covered when all reads are pooled before mapping, separately for 1x and 50x.

	% mapped reads		% of assembly covered (>= 1X)	
	ref v.2.0 (%)	<i>de novo</i> (%)	ref v.2.0 (%)	<i>de novo</i> (%)
Rh. irregularis - Nucleus 1	99.11	99.61	50.84	53.42
Rh. irregularis - Nucleus 2	99.35	99.58	25.83	26.72
Rh. irregularis - Nucleus 3	99.39	99.63	51.75	54.34
Rh. irregularis - Nucleus 4	99.27	99.62	54.31	57.16
Rh. irregularis - Nucleus 5	99.36	99.67	50.34	52.85
Rh. irregularis - Nucleus 6	99.06	99.52	52.90	55.68
Rh. irregularis - Nucleus 7	99.26	99.67	51.65	54.13
Rh. irregularis - Nucleus 8	93.52	99.65	39.68	41.49
Rh. irregularis - Nucleus 9	99.31	99.62	64.93	68.37
Rh. irregularis - Nucleus 10	99.39	99.69	65.17	68.58
Rh. irregularis - Nucleus 11	99.34	99.65	66.51	70.01
Rh. irregularis - Nucleus 12	99.45	99.68	20.97	22.20
Rh. irregularis - Nucleus 13	99.30	99.67	62.98	66.20
Rh. irregularis - Nucleus 14	92.37	92.79	53.95	56.48
Rh. irregularis - Nucleus 15	99.14	99.53	85.38	90.25
Rh. irregularis - Nucleus 16	98.83	99.71	47.19	49.65
Rh. irregularis - Nucleus 17	98.80	99.58	47.36	49.62
Rh. irregularis - Nucleus 18	99.44	99.63	41.46	43.41
Rh. irregularis - Nucleus 19	99.33	99.65	64.42	67.94
Rh. irregularis - Nucleus 20	99.24	99.57	48.54	50.98
Rh. irregularis - Nucleus 21	99.45	99.65	37.99	39.70
Rh. irregularis - Nucleus 22	99.36	99.68	44.36	46.40
Rh. irregularis - Nucleus 23	99.24	99.60	32.81	34.04
Rh. irregularis - Nucleus 24	99.07	99.42	39.91	41.85
Average across 24 nuclei	98.72	99.34	50.05	52.56
24 nuclei combined 1X	98.67	99.24	94.79	100
24 nuclei combined 50X	98.67	99.24	91.49	97.21

Table S7. OrthoFinder statistics for orthogroups shared between *Rh. irregularis* DAOM197198 reference genome v.2.0 (Chen *et al.*, 2018) and the *de novo* assembly of the same strain produced in this study (Both) followed by numbers for each assembly.

Number of “species” (i.e. genome assemblies)	Both	v.2.0	<i>de novo</i>
Number of genes	49,451	26,183	23,268
Number of genes in orthogroups	43,894	23,429	20,465
Number of unassigned genes	5,557	2,754	2,803
Percentage of genes in orthogroups	88.8	89.5	88.0
Percentage of unassigned genes	11.2	10.5	12.0
Number of orthogroups	13,908	13,528	13,505
Number of species-specific orthogroups	783	403	380
Number of genes in species-specific orthogroups	4,264	2,638	1,626
Percentage of genes in species-specific orthogroups	8.6	10.1	7.0
Mean orthogroup size	3.2		
Median orthogroup size	2.0		
G50 (assigned genes)	3		
G50 (all genes)	2		
O50 (assigned genes)	3,092		
O50 (all genes)	4,324		
Number of orthogroups with all species present	13,125		
Number of single-copy orthogroups	10,138		

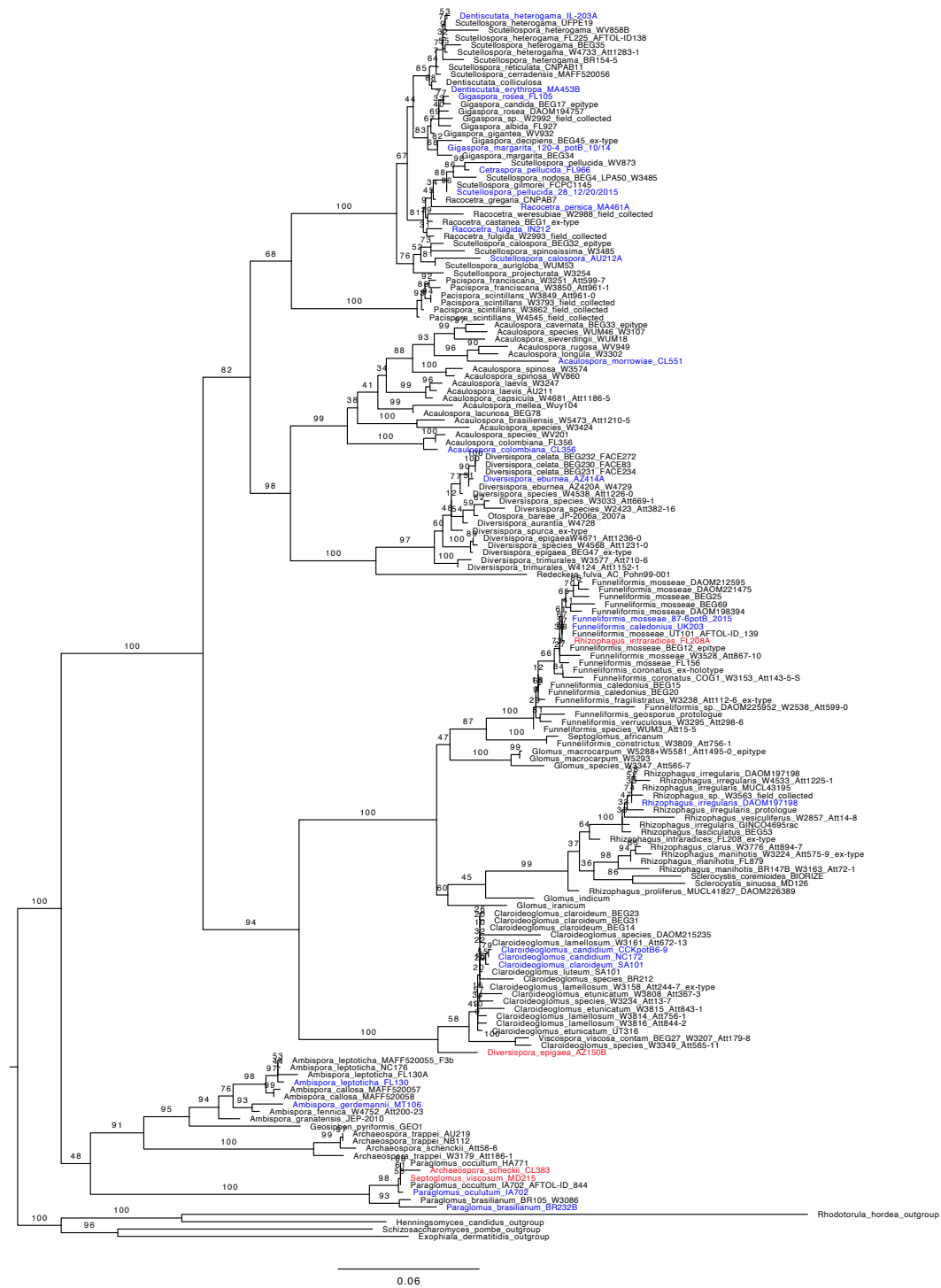


Figure S1. Best maximum likelihood RAxML tree based on a ribosomal small subunit (SSU) alignment from Krüger *et al.*, (2012) including the SSU sequences from the newly generated assemblies (blue and red). Isolates sequenced in the current study are labeled according to initial information from culture collections (Supplementary Table 1). Taxon names in red indicate that the isolate was excluded from further analysis because of suspected contamination or misidentification due to inconsistent placement based on taxon name. Taxa in blue were included in the downstream analyses. Branch labels show bootstrap support (100 replicates).

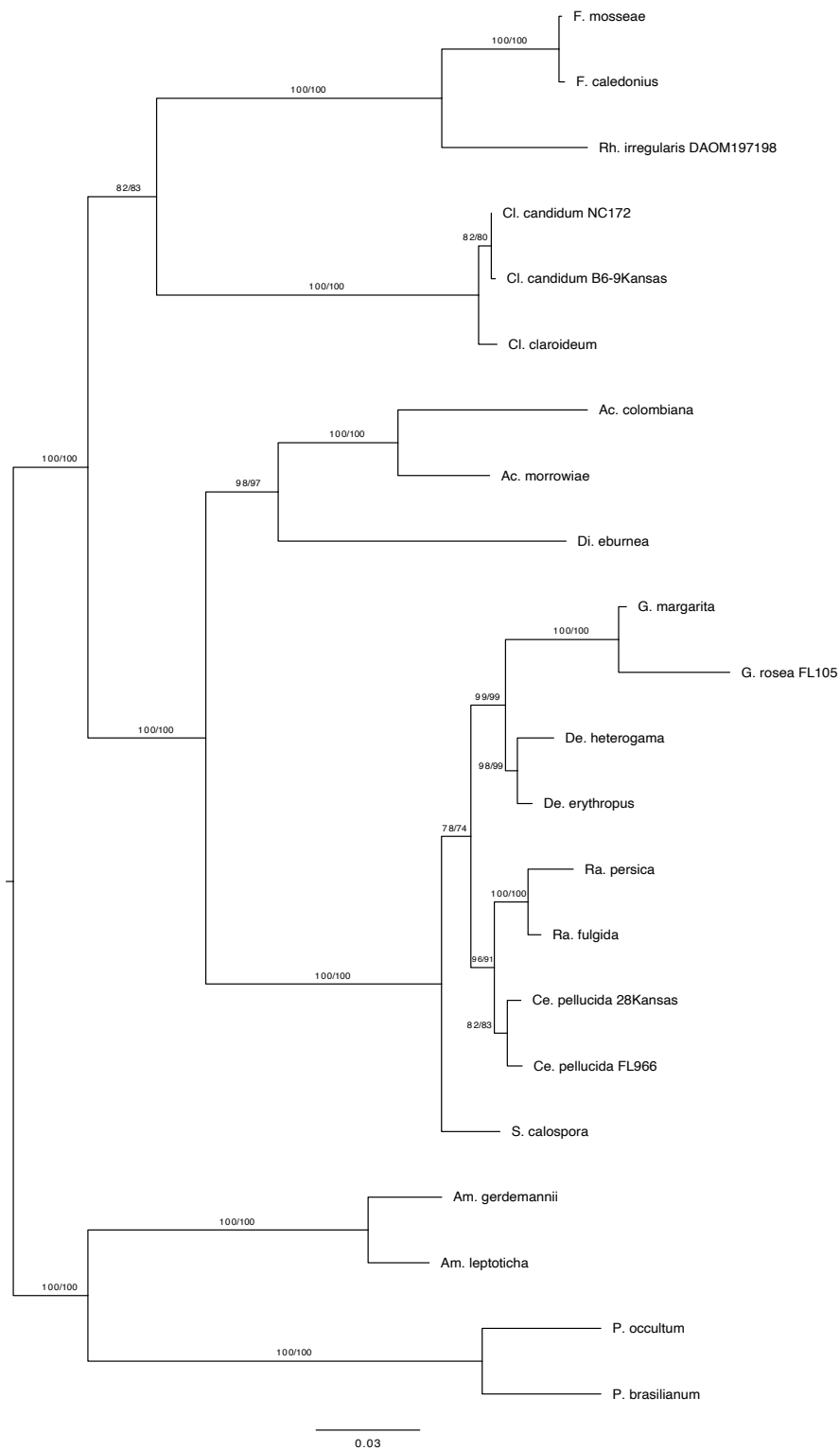


Figure S2. Best maximum likelihood RAXML tree from an alignment of the rDNA sequences (SSU+5.8s+LSU) of the newly assembled genomes. The same topology was obtained from an IQ-TREE analysis. Bootstrap values (1000 replicates) are shown above or next to the branches (RAXML/IQ-TREE). Full species names are presented in Table S1. Strain identifiers are indicated when two or more strains of the same species are included. (Supplementary Table 1, 2).

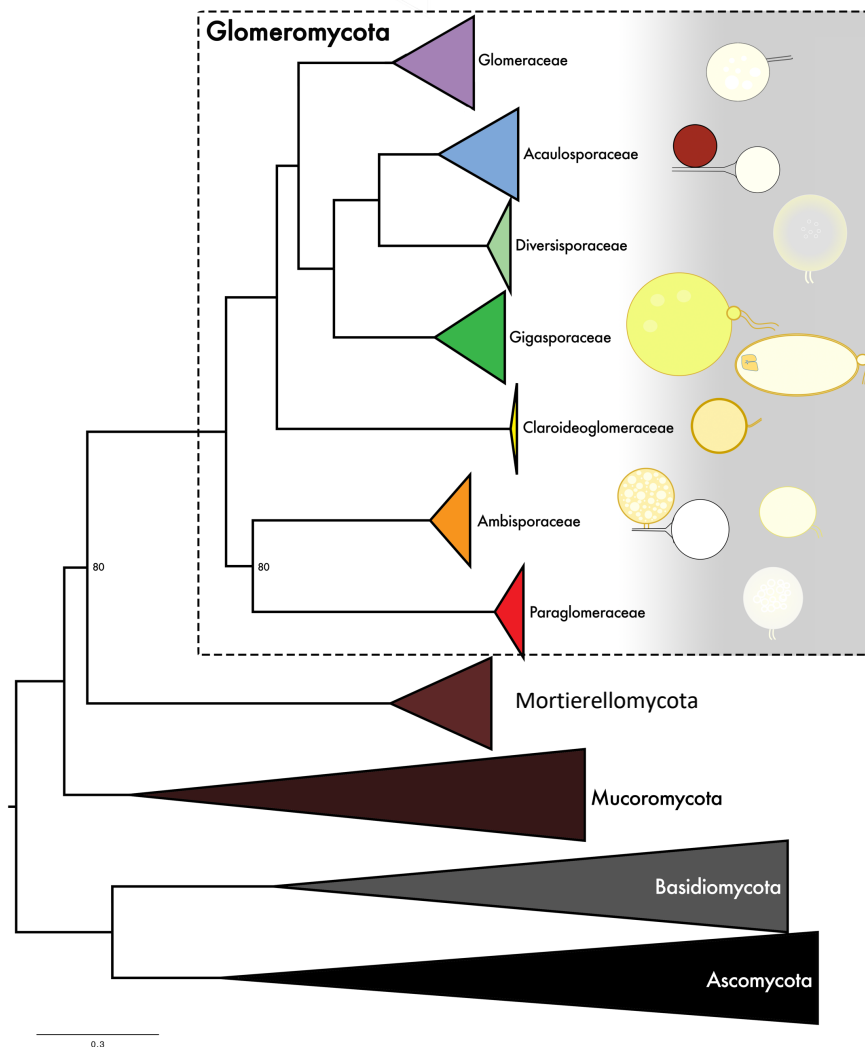


Figure S3. Best maximum likelihood RAxML tree from a concatenated alignment of 178 single copy orthologs shared among > 50% of the taxa. All branches have bootstrap support of 100 unless indicated. Basidiomycota and Ascomycota (Dikarya) were used as outgroup. All phyla are collapsed with the exception of Glomeromycota, where seven families are visualized in the dashed box. Typical spore morphologies are schematically illustrated to the right of each family and not drawn to scale. For expanded tree see Supplementary Figure 4A.

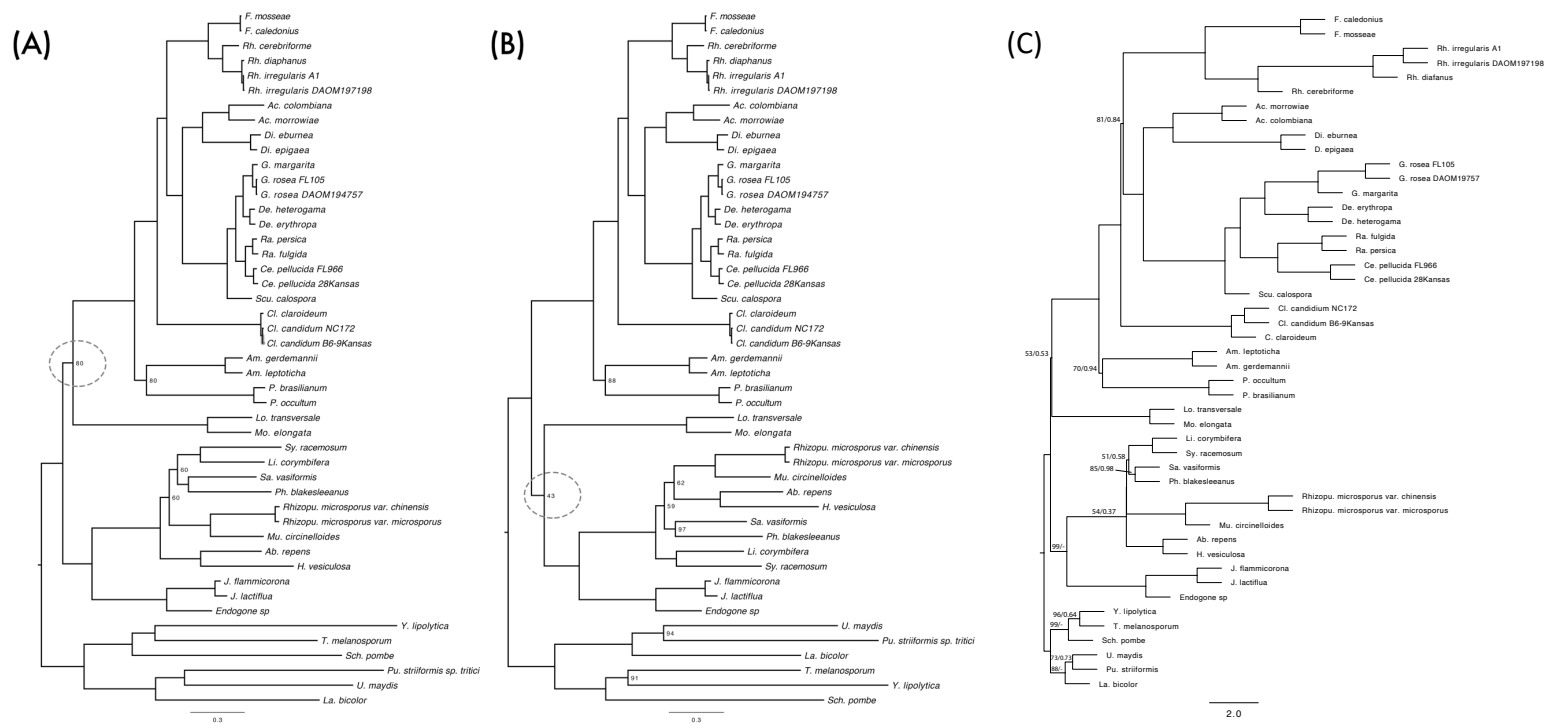


Figure S4. Phylogeny of Glomeromycota, Mucoromycota and Mortierellomycota including members of Dikarya as outgroup. Best maximum likelihood **(A)** RAxML and **(B)** IQ-TREE tree from a concatenated alignment of 178 single copy orthologs shared among >50% of the taxa. All branches have bootstrap support of 100 unless indicated in the tree. Circled nodes indicate conflict between the two maximum likelihood topologies, in which Mortierellomycota is recovered as a sister group of Glomeromycota (in A) or of Mucoromycota (in B). **(C)** ASTRAL phylogeny based on 178 individual gene trees inferred with IQ-TREE. Multi-locus bootstrapping and local posterior probabilities are indicated at the nodes when below 100 or 1.0 (MLBS/LPP). Full species names are presented in Supplementary Table 1, 2. Strain identifiers are indicated when two or more strains of the same species are included.

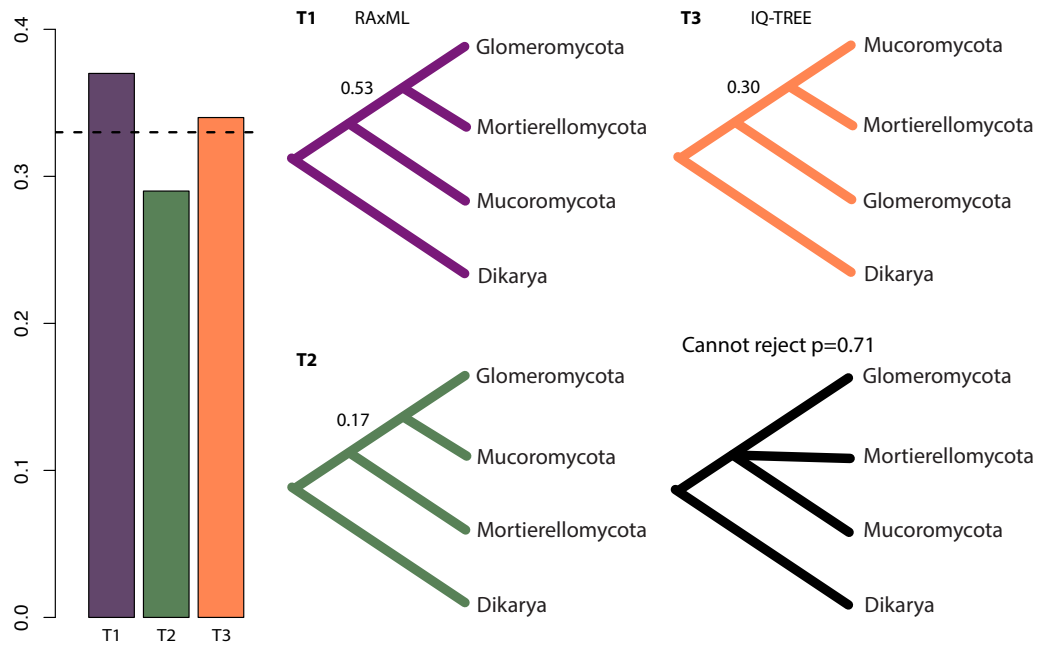


Figure S5. Evaluation of support among individual gene trees for alternative hypotheses of the relationships among Glomeromycota, Mucoromycota and Mortierellomycota. Bar graphs represent the gene tree quartet frequencies for three possible branching orders. T1 corresponds to the ASTRAL topology, T2 and T3 correspond to alternative topologies in ASTRAL. The topologies recovered by RAxML and IQ-TREE are indicated. Dashed horizontal lines marked the expectation of a hard polytomy. Local posterior probabilities are indicated only when below 1.0.

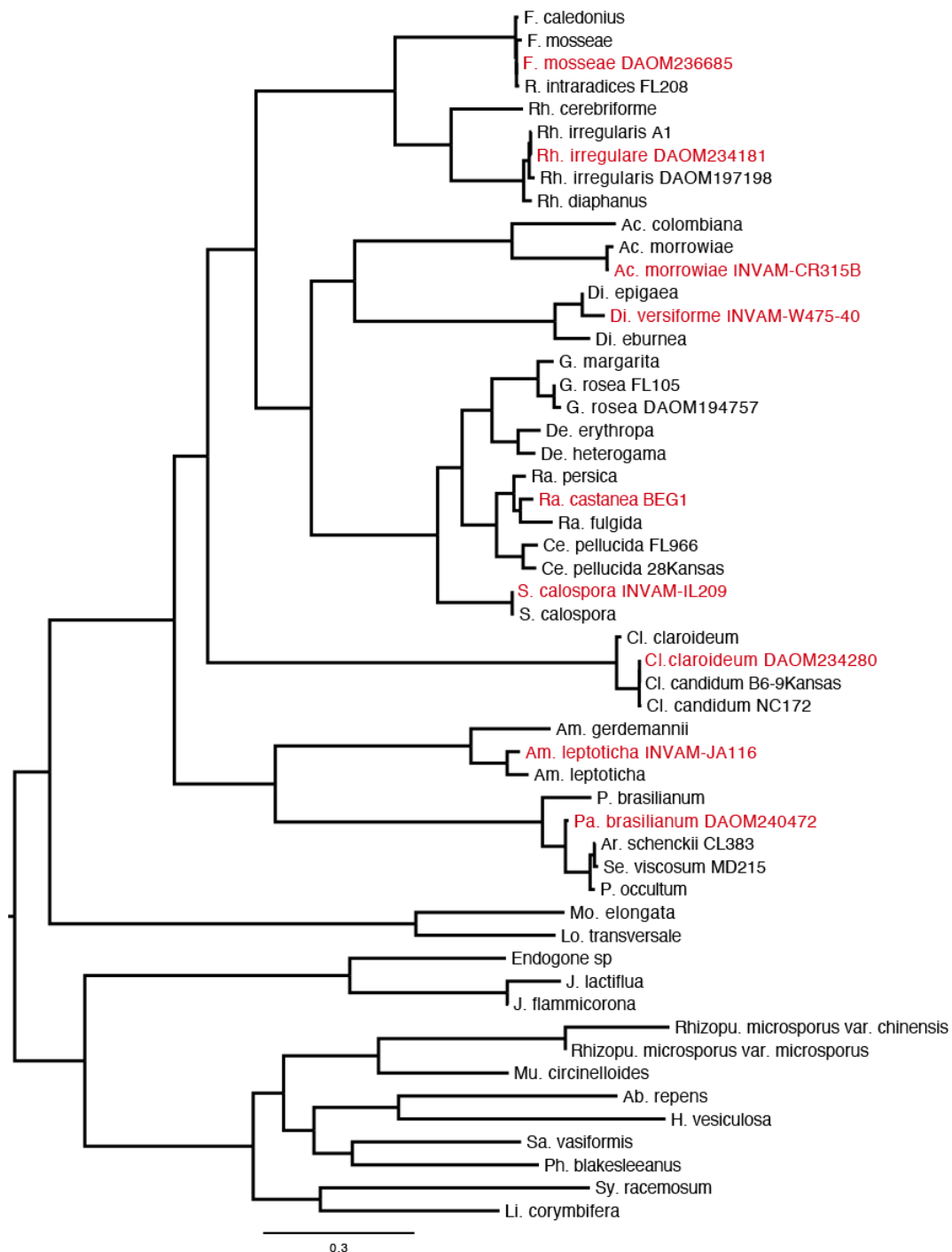


Figure S6. Best maximum likelihood RAXML tree from a concatenated alignment of 17 single copy orthologs shared among >50% of the taxa. All branches have bootstrap support of 100. Taxa in red show transcriptomic data from Beaudet *et al.*, 2018 and include species name and strain identifier. The transcriptomic data from Beaudet *et al.*, 2018 was not included in downstream analysis. Strain identifiers are indicated when two or more strains of the same species are included (Supplementary Table 1, 2).

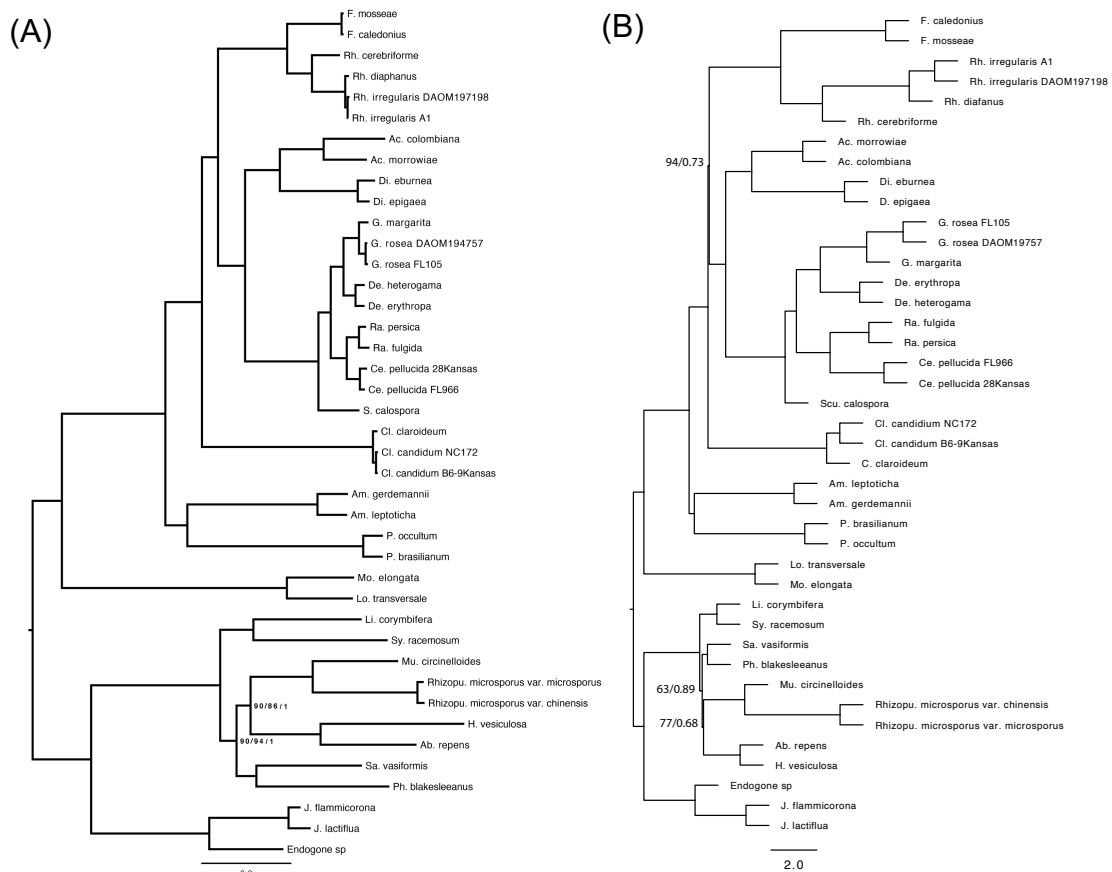


Figure S7. Phylogeny of Glomeromycota including Mortierellomycota and Mucoromycota as outgroup **(A)** Expanded tree corresponding to Figure 1. Best maximum likelihood tree inferred with RAxML from a concatenated alignment of 371 single copy orthologs shared by >50% of the taxa. The same topology was recovered using IQ-TREE and Bayesian inference. Support values are indicated at the nodes when below 100 or 1.0 (RAxML/IQ-TREE/Bayesian). **(B)** ASTRAL phylogeny based on 371 individual gene trees inferred with IQ-TREE. Support values are indicated at the nodes when below 100 or 1.0 (MLBS/LPP). Strain identifiers are included when two or more strains of the same species are included (Supplementary Table 1, 2).

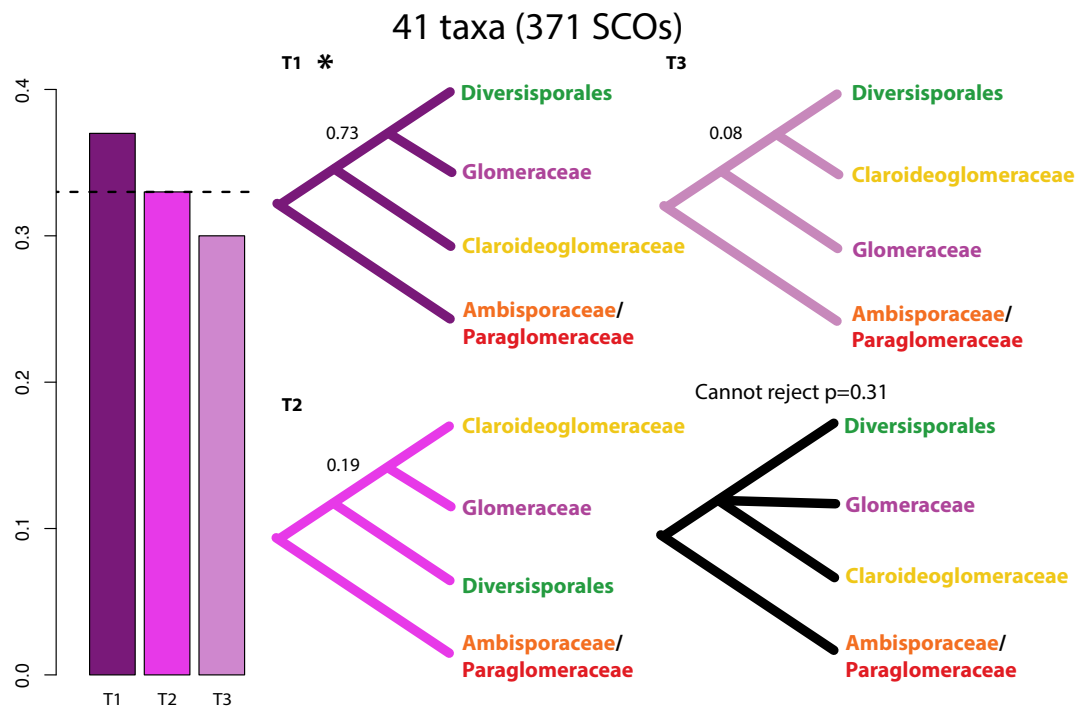


Figure S8. Evaluation of support among individual gene trees for alternative hypotheses of the relationships within Glomeromycota based on the dataset of Glomeromycota plus its sister phyla. Bar graphs represent the gene tree quartet frequencies for three possible branching orders. T1 corresponds to the ASTRAL topology, T2 and T3 correspond to alternative topologies in ASTRAL. Dashed horizontal lines marked the expectation of a hard polytomy. Local posterior probabilities are indicated only when below 1.0.

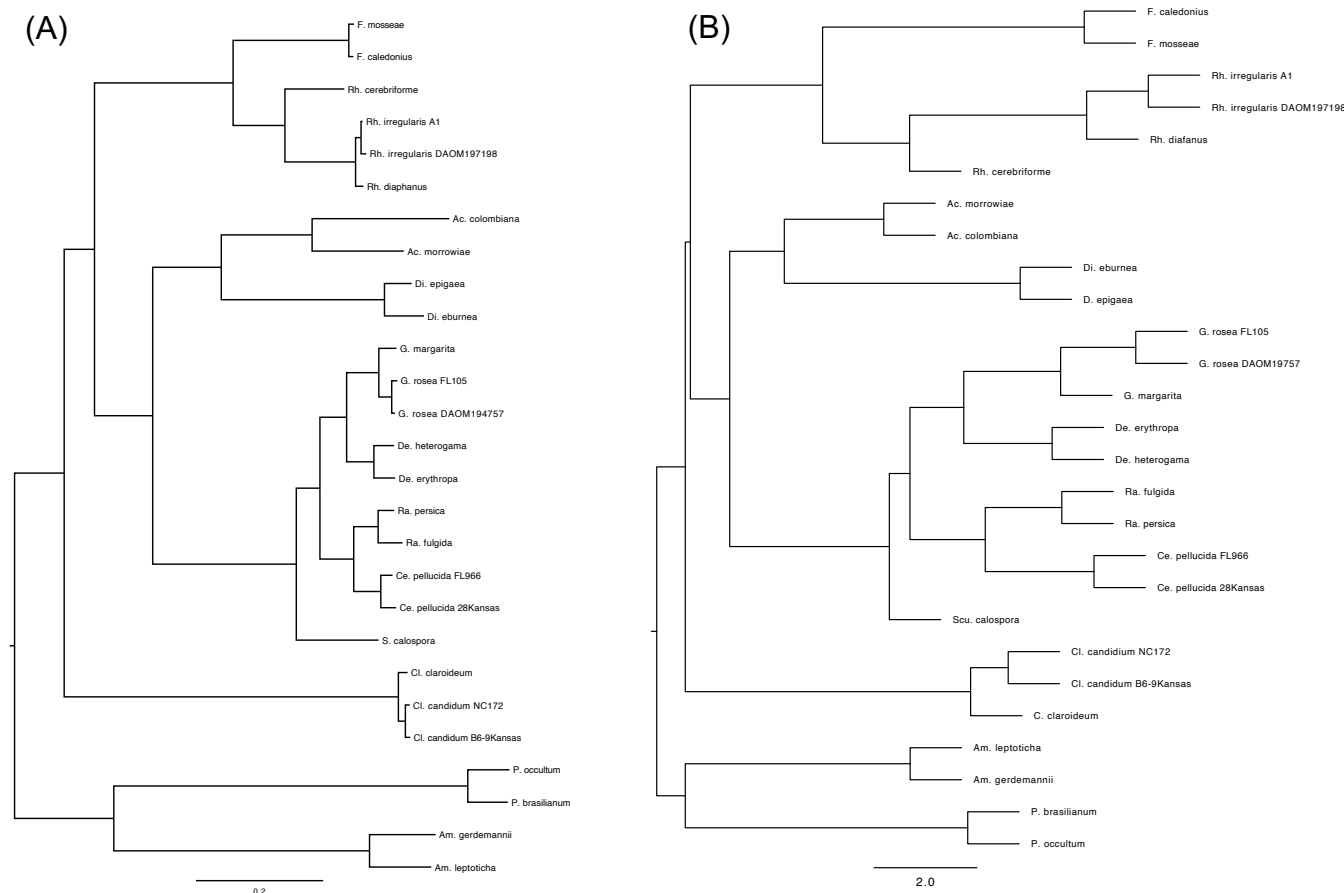


Figure S9. Phylogeny of Glomeromycota based on 1,737 single copy orthologs shared among 27 taxa. **(A)** Best maximum likelihood tree inferred with RAXML from a concatenated alignment of 1,737 single copy orthologs shared among >50% of the taxa. The same topology was recovered using IQ-TREE. All nodes have bootstrap value support of 100. **(B)** ASTRAL phylogeny based on 1,737 individual gene trees inferred with IQ-TREE. All nodes have multi-locus bootstrap support of 100 and local posterior probabilities of 1.0. Strain identifiers are included when two or more strains of the same species are included (Supplementary Table 1).

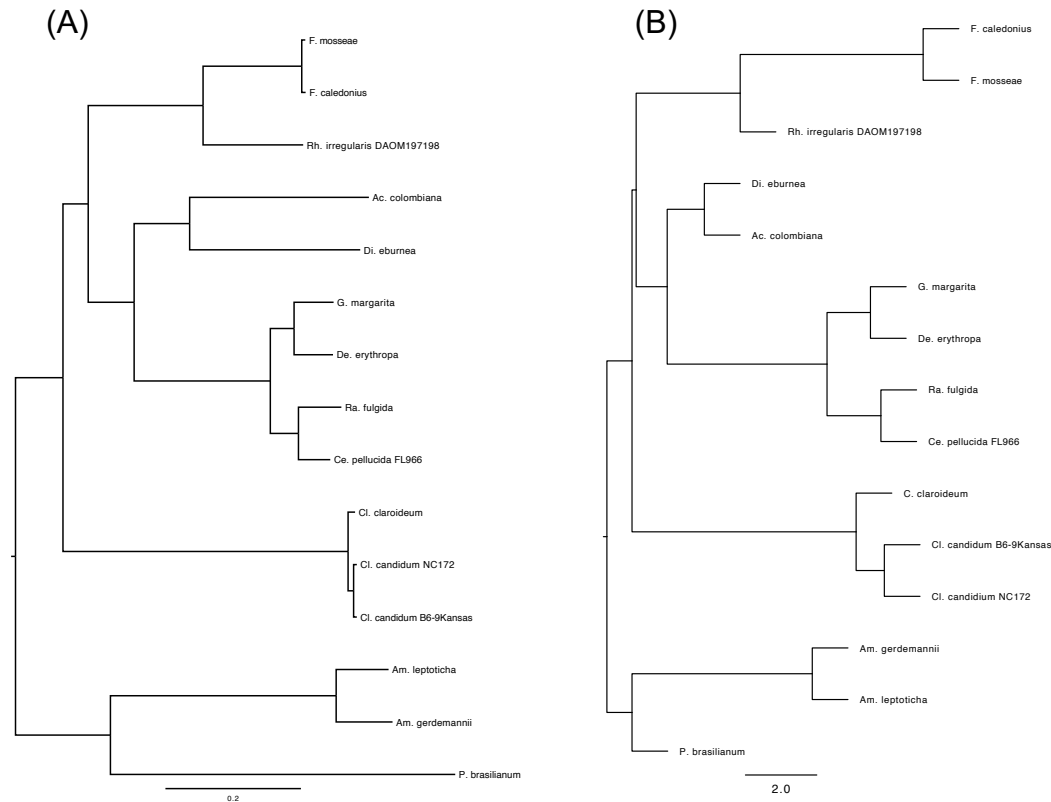


Figure S10. Phylogeny of Glomeromycota based on 799 single copy orthologs shared among 15 selected taxa (Supplementary Table 4). **(A)** Best maximum likelihood tree inferred with RAXML from a concatenated alignment of 799 single copy genes shared among all taxa. Same topology was recovered using IQ-TREE. All nodes have bootstrap value support of 100. **(B)** ASTRAL phylogeny based on 799 individual gene trees inferred with IQ-TREE. All nodes have multi-locus bootstrap support of 100 and local posterior probabilities of 1.0. Strain identifiers are included when two or more strains of the same species are included (Supplementary Table 1).

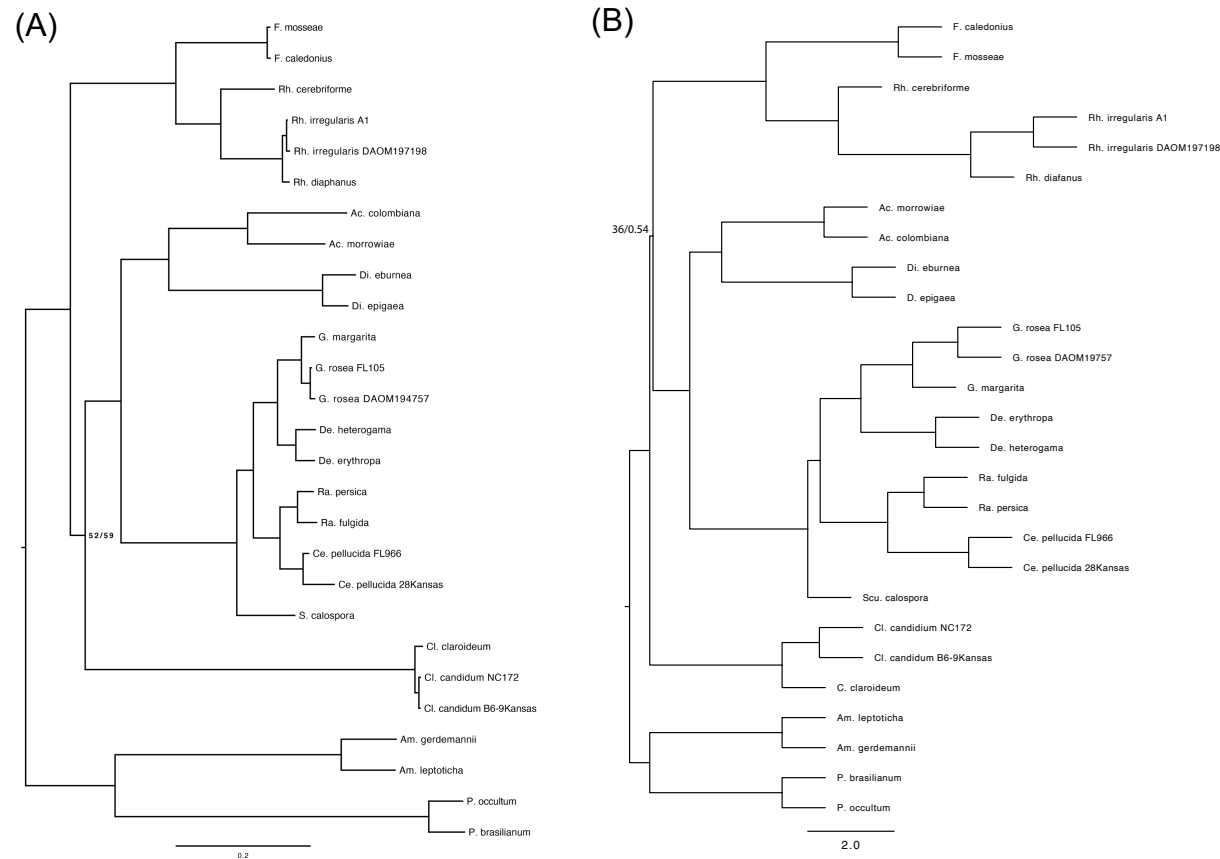


Figure S11. Phylogeny of Glomeromycota based on 31 single copy orthologs shared all 27 taxa. **(A)** Best maximum likelihood tree inferred with RAxML from a concatenated alignment of 31 single copy genes shared among all taxa. Same topology was recovered using IQ-TREE. Support values are indicated at the nodes when below 100 (RAxML/IQ-TREE). **(B)** ASTRAL phylogeny based on 31 individual gene trees inferred with IQ-TREE. Support values are indicated at the nodes when below 100 or 1.0 (MLBS/LPP). Strain identifiers are included when two or more strains of the same species are included (Supplementary Table 1).

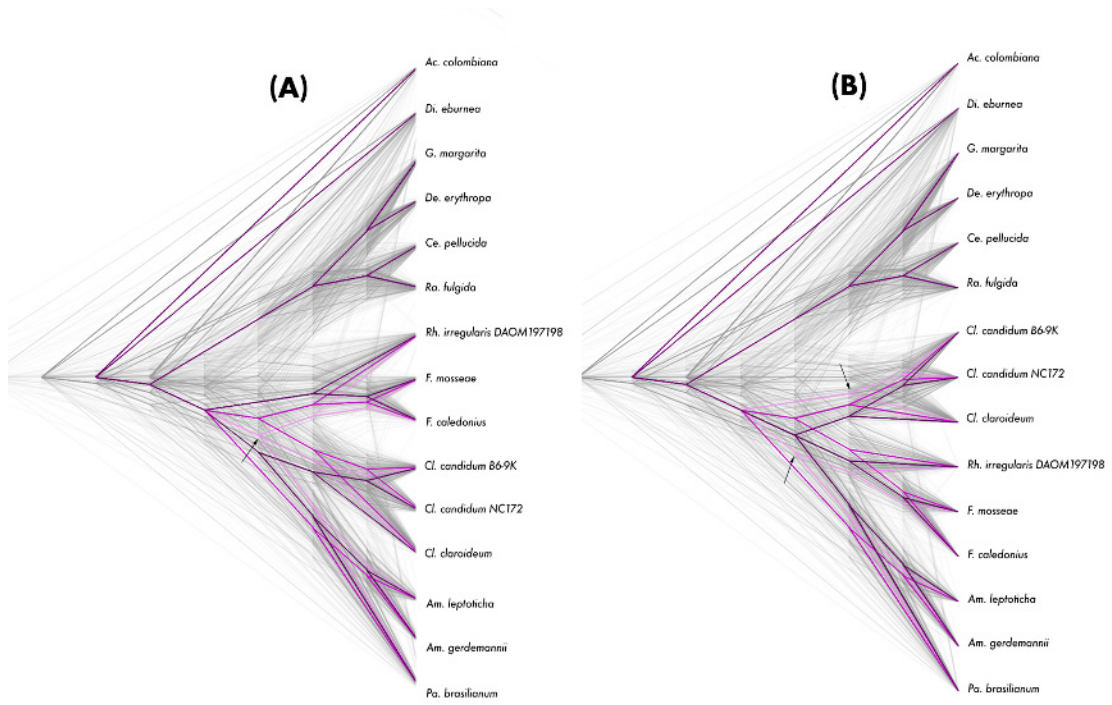


Figure S12. DensiTree based on 799 individual gene trees inferred with IQ-TREE for 15 selected taxa with black arrows indicating topology 3. The order of taxa is rearranged from (A) to (B) to better visualize topology 3. Full species names are presented in Supplementary Table 1 and strain identifiers are included when two or more strains of the same species are included.

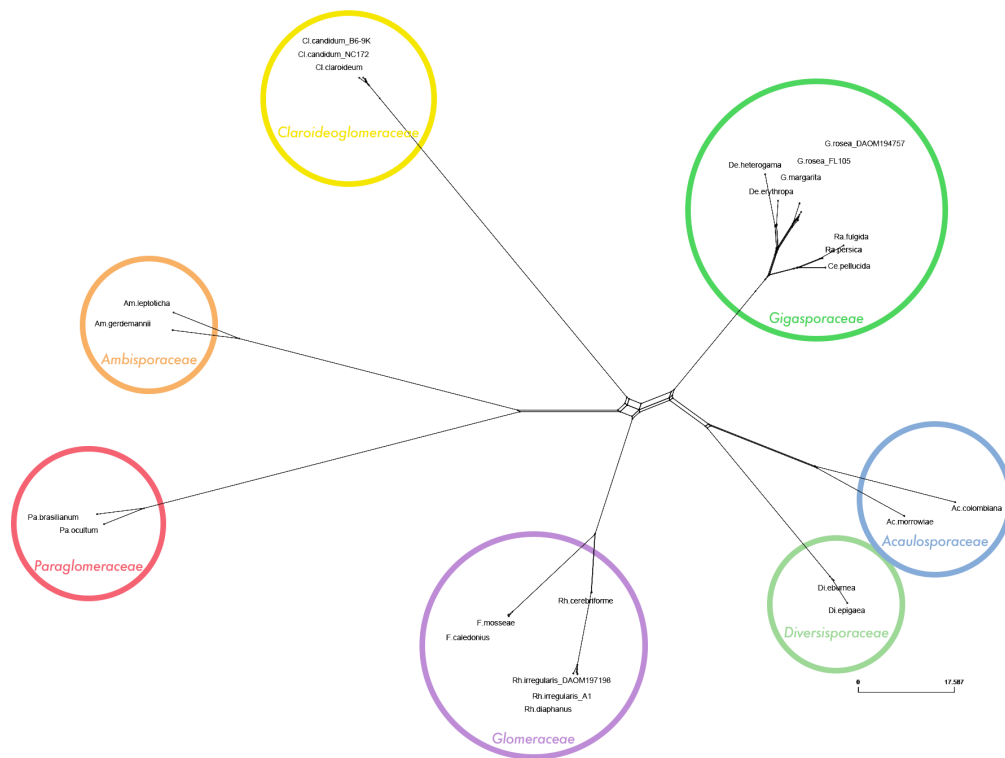


Figure S13. Split network produced with IQ-TREE network analysis and visualized in SplitsTree5 with maximum dimension splits filter of 2, using the dataset containing 27 Glomeromycota taxa, and 1,737 SCOs shared among >50% of the taxa. Branch lengths between families (Circled and color coded according to Figure 1 and Supplementary Table 1) are visualize compared to Figure 3 that provides a zoom in of the reticulation at the base. Strain identifiers are included when two or more strains of the same species are included (Supplementary Table 1).

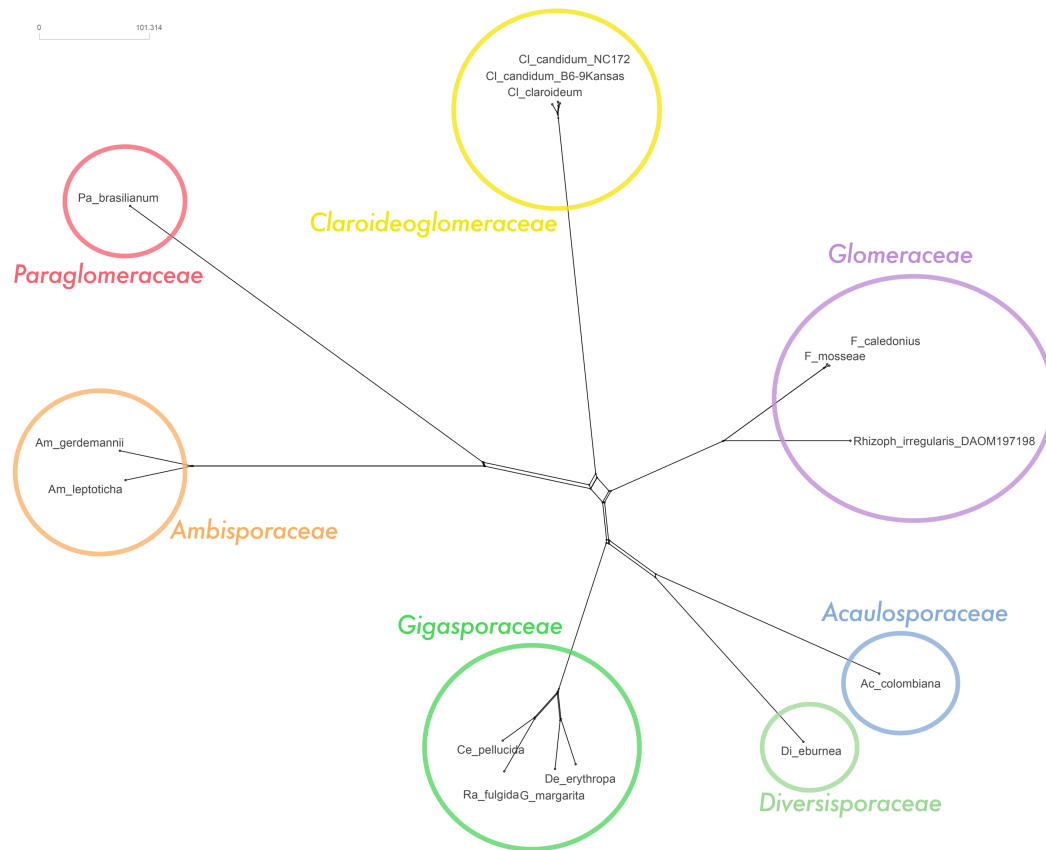


Figure S14. Network from IQ-TREE network analysis, using 799 single gene trees shared among 15 selected taxa (Supplementary Table 4), visualized in SplitsTree5, with a maximum dimension filter of 2. Families are circled and color coded according to Figure 1 and Supplementary Table 1 where full species names are presented.

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