American Journal of Botany

RESEARCH ARTICLE

Stable isotope analyses reveal previously unknown trophic mode diversity in the Hymenochaetales

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Manuscript received 24 February 2018; revision accepted 2 August 2018.

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Citation: Korotkin, H. B., R. A. Swenie, O. Miettinen, J. M. Budke, K.-H. Chen, F. Lutzoni, M. E. Smith, and P. B. Matheny. 2018. Stable isotope analyses reveal previously unknown trophic mode diversity in the Hymenochaetales. *American Journal of Botany* 105(11): 1–19.

doi:10.1002/ajb2.1183

PREMISE OF THE STUDY: The Hymenochaetales are dominated by lignicolous saprotrophic fungi involved in wood decay. However, the group also includes bryophilous and terricolous taxa, but their modes of nutrition are not clear. Here, we investigate patterns of carbon and nitrogen utilization in numerous non-lignicolous Hymenochaetales and provide a phylogenetic context in which these non-canonical ecological guilds arose.

METHODS: We combined stable isotope analyses of $\delta^{13}C$ and $\delta^{15}N$ and phylogenetic analyses to explore assignment and evolution of nutritional modes. Clustering procedures and statistical tests were performed to assign trophic modes to Hymenochaetales and test for differences between varying ecologies. Genomes of Hymenochaetales were mined for presence of enzymes involved in plant cell wall and lignin degradation and sucrolytic activity.

KEY RESULTS: Three different trophic clusters were detected – biotrophic, saprotrophic, and a second biotrophic cluster including many bryophilous Hymenochaetales and mosses. Non-lignicolous Hymenochaetales are generally biotrophic. All lignicolous Hymenochaetales clustered as saprotrophic and most terricolous Hymenochaetales clustered as ectomycorrhizal. Overall, at least 15 species of Hymenochaetales are inferred as biotrophic. Bryophilous species of *Rickenella* can degrade plant cell walls and lignin, and cleave sucrose to glucose consistent with a parasitic or endophytic life style.

CONCLUSIONS: Most non-lignicolous Hymenochaetales are biotrophic. Stable isotope values of many bryophilous Hymenochaetales cluster as ectomycorrhizal or in a biotrophic cluster indicative of parasitism or an endophytic life style. Overall, trophic mode diversity in the Hymenochaetales is greater than anticipated, and non-lignicolous ecological traits and biotrophic modes of nutrition are evolutionarily derived features.

KEY WORDS Agaricomycetes; Basidiomycota; ecology; endophytes; functional diversity; mosses; mycorrhizoids; phylogenetics; stable isotopes.

The basidiomycete order Hymenochaetales Oberw. contains some 900 species of mostly polyporoid and corticioid fungi classified in 75 genera worldwide (Hibbett et al., 2014). Most are lignicolous white-rot saprotrophs that decompose wood (Wagner and Fischer, 2002; Binder et al., 2005; Larsson et al., 2006; Tedersoo et al., 2007). The order also includes a few species with diverse mycorrhizal abilities (Nouhra et al., 2013; Tedersoo and Smith, 2013; Kolařík and Vohník, 2018), plant pathogens (Larsson et al., 2006), and several agarics or mushroom-forming fungi that typically occur on or with bryophytes—particularly mosses or liverworts—or that occur on soil (Redhead et al., 2002) (Fig. 1). These non-lignicolous fungi, particularly the bryophilous agarics (Racovitza, 1959; Davey and Currah, 2006), were previously treated as Agaricales Underw. due to similarities in basidiome morphology (Redhead et al., 2002) but were recovered in the Hymenochaetales by molecular phylogenetic analyses (Moncalvo et al., 2000, 2002; Redhead et al., 2002). Later, the group was referred to as the *Rickenella* clade (Larsson et al., 2006) and classified in the family Rickenellaceae Vizzini (Vizzini, 2010; Nakasone and Burdsall, 2012), the name of which, unfortunately, is illegitimate due to inclusion of the type of the earlier described family Repetobasidiaceae Jülich (Jülich, 1981). In addition, the diversity of its constituents is unsettled, the monophyly of the group has been questioned (Larsson, 2007; Miettinen and Larsson, 2010), and their nutritional modes are largely unclear (Felix, 1988; Kost, 1988; Bresinsky and Schötz, 2006).

FIGURE 1. Ecological and trophic diversity of Hymenochaetales. (A) *Hymenochaete cinnamomea* (lignicolous saprotroph). (B) *Trichaptum abietinum* (lignicolous saprotroph). (C) *Contumyces rosellus* (terricolous and possible biotroph). (D) *Coltricia perennis* (terricolous ECM biotroph). (E) *Rickenella swartzii* (bryophilous biotroph). (F) *Rickenella minuta* (terricolous ECM biotroph). (A–E) Photos M. G. Wood. (F) Photo P. B. Matheny. Scale bars = 10 mm.

The phyogenetic placement of the *Rickenella* clade in the Hymenochaetales raises several questions about its ecology and evolution: (1) is the non-lignicolous association derived or ancestral in the Hymenochaetales? (2) What are the trophic modes of bryophilous and terricolous Hymenochaetales? That is, are they saprotrophs that decay dead organic matter for carbon nutrition, or are they biotrophs that acquire carbon nutrition from a live symbiont? Or; (3) can they do both? If biotrophs, do they engage in a nutrient-exchange mutualism with bryophytes, or are they parasites of bryophytes?

Analysis of stable isotope signatures of nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) has been used to infer trophic modes of fungi (Hobbie et al., 2001; Trudell et al., 2004; Mayor et al., 2009; Seitzman et al., 2011; Hobbie and Högberg, 2012; Tedersoo et al., 2012; Birkebak et al., 2013; Trappe et al., 2015; Sánchez-García and Matheny, 2017). However, analysis of stable isotope data has not been used to explore the trophic status of bryophilous and terricolous Hymenochaetales, and very few Hymenochaetales overall have even been evaluated, probably due to the fact that most Hymenochaetales are lignicolous saprotrophs (Wagner and Fischer, 2002). Stable isotope data are useful to discriminate between Hymenochaetales that acquire their nutrition from a live associate or from dead organic matter, thus providing a powerful means to discern the degree of trophic diversity in the order without direct observations of trophic interactions in the field or laboratory.

Analysis of fungal nutritional modes can also be augmented by an array of approaches that include anatomical studies, pure syntheses or *in vitro* experiments, molecular ecology (e.g., barcoding ectomycorrhizal (ECM) root tips), and phylogenetic relatedness (Tedersoo et al., 2010). In addition, the presence or absence of unique suites of genes that encode enzymes involved in nutrient acquisition can also be used to characterize trophic modes in fungi (Parrent et al., 2009; Wolfe et al., 2012). To this end, the U.S. Department of Energy Joint Genome Institute has sequenced the whole genomes of two species of bryophilous Hymenochaetales, *Rickenella fibula* (Bull.) Raithelh. and *R. mellea* (Singer & Clémençon) Lamoure, and five genomes of lignicolous Hymenochaetales as of this writing.

Here we carry out a cluster analysis of stable C and N stable isotope data from nearly 1000 samples of Agaricomycetes Doweld, phylogenetic analyses of a multigene dataset, ancestral state reconstruction analyses, and genome searches for key enzymes used to degrade plant cell walls and lignin and sucrolytic activity, in order to determine whether bryophilous, lignicolous, and terricolous Hymenochaetales are saprotrophic or biotrophic, whether these states are derived or ancestral, and whether these ecological guilds share similar or dissimilar modes of nutrition.

MATERIALS AND METHODS

Stable isotope analyses

A total of 108 specimens, including 92 samples of Hymenochaetales (Table 1), were analyzed at the University of New Hampshire

TABLE 1. Metadata for 108 collections analyzed by stable isotope mass spectrometry, $\delta^{15}N$ and $\delta^{13}C$ stable isotope values, and trophic cluster assigned by *mclust* analysis. liv. = living gametophyte tissue; sen. = senescent gametophyte tissue. Herbarium abbreviations follow Thiers [continuously updated].

Taxon	Herbarium accession no.	Specimen-voucher no.	Location	Long./ Lat.	Ecology	Date of collection	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)
Alloclavaria purpurea	TENN071489	HRL0146	Quebec	48.7081 −67.3889	Terricolous On soil	2008-08-26	4.92	−24.28
Alloclavaria purpurea	H6047394	Korhonenen 10305	Finland	66.5039 25.7294	Terricolous On soil	1991-08-18	3.10	−22.18
Alloclavaria purpurea	H6047434	Korhonen 10411	Finland	65.9646 29.1887	Terricolous On soil	1991-08-26	1.41	−24.86
Alloclavaria purpurea	H6034567	Niskanen 01-053	Finland	62.5000 22.7500	Terricolous On soil with *Picea*, *Betula*	2001-08-20	3.61	−24.46
Alloclavaria purpurea	H6034547	Niskanen 01-044	Finland	64.8675 27.6752	Terricolous On soil	2001-08-17	4.01	−26.51
Alloclavaria purpurea	H6047663	Ahokas s.n.	Finland	60.9367 26.3996	No data	2005-09-04	2.80	−23.26
Amanita multisquamosa	TENN070497	MGW1516	Tennessee	35.7525 −83.27305	Terricolous Soil	2015-07-11	4.11	−27.04
Bjerkandera adusta	TENN059170	MF2	Tennessee	35.70778 −83.3800	Lignicolous On Wood	2001-05-23	2.53	−23.59
Blasiphalia pseudogrisella	H6059323	Hoijer 3034	Finland	63.7530 26.9889	Bryophilous With *Blasia*	2001-08-14	1.07	−23.54
Blasiphalia pseudogrisella	H7031951	Hoijer 4393	Estonia	59.3591 27.4211	Bryophilous With *Blasia*	2006-05-02	3.41	−26.18
Blasiphalia pseudogrisella	H6059312	Hoijer 4118	Finland	62.2833 21.3833	Bryophilous With *Blasia*	2005-09-20	1.68	−23.76
Blasiphalia pseudogrisella	H6019812	Hoijer 4539	Finland	69.0500 20.8000	Bryophilous With *Blasia*	2007-08-21	2.04	−24.66
Cantharellopsis prescotii	TENN071487	HRL2135	Quebec	50.25 −63.6	Terricolous On soil	2015-09-10	0.42	−27.79
Cantharellopsis prescotii	H6035464	Kytovuori 08-0808	Finland	60.2512 24.0675	Terricolous In damp depression, rich mixed forest	2008-09-03	−0.70	−32.82
Cantharellopsis prescotii	H6050719	Kytovuori 93-850	Finland	60.2512 24.0675	Terricolous In grass-herb *Picea* forest	1993-09-09	0.39	−26.66
Cantharellopsis prescotii	H6059300	Ohenoja s.n.	Finland	64.8833 28.9166	Terricolous In moss with *Picea*	1992-08-24	−0.58	−28.85
Cantharellopsis prescotii (as *Gerronema albidum*)	H6050710	Kytovuori 851422	Finland	60.3011 22.3022	Terricolous In rich *Picea* forest	1985-10-05	−1.86	−28.79
Cantharellopsis prescotii (as *Gerronema albidum*)	H6059277	Kytovuori 90-231	Finland	64.8675 27.6752	Terricolous In rich *Picea* forest	1990-08-14	−1.56	−29.12
Coltricia cinnamomea	H6059308	Niemela 6911	Finland	61.0900 25.0000	Terricolous On soil	2000-09-22	6.64	−26.91
Coltricia cinnamomea	H6059331	Niemela 8199	Finland	61.8000 29.0170	Terricolous On soil	2005-09-15	5.65	−26.79
Coltricia montagnei	TENN066402	MR070111-02	Tennessee	35.7388 −83.4222	Terricolous On soil	2011-07-11	8.19	−25.24
Coltricia montagnei	TENN065217	SAT1017611	North Carolina	35.6111 −83.1250	Terricolous On soil	2010-06-25	8.28	−25.21
Coltricia perennis	H6029159	Salo 10282	Finland	61.9126 29.2814	Terricolous On soil	2004-05-01	4.45	−24.94
Coltricia perennis	H6002974	Salo 11024	Finland	60.3932 25.6653	Terricolous On soil	2007-04-05	11.86	−24.62
Coltricia perennis	H6013608	Miettinen 18513	Finland	61.8000 29.0170	Terricolous On soil	2014-09-04	8.49	−25.66
Coltricia perennis	H6059309	Haikonen 22781	Finland	61.9833 26.2666	Terricolous On soil	2003-09-10	8.56	−24.76
Contumyces rosellus	TENN071494	MGW1462	California	38.2918 −122.4580	Terricolous On soil	2015-01-19	4.82	−27.62
Contumyces vesuvianus	TUR203608	—	Italy	40.4350 18.0422	Bryophilous Moss	2015-11-30	−1.75	−27.83
Cortinarius corrugatus	TENN070645	PBM4040	Tennessee	35.6725 −83.4930	Terricolous Soil	2015-07-16	12.04	−26.65
Cotylidia diaphana	TENN071490	HRL1509	Quebec	45.4948 −73.8907	Terricolous On soil	2013-07-27	2.42	−23.20
Cotylidia pannosa	TENN071488	HRL0287	Quebec	48.1841 −68.9843	Terricolous On soil	2009-08-30	8.32	−24.06

(continues)

TABLE 1. Continued

Taxon	Herbarium accession no.	Specimen-voucher no.	Location	Long./Lat.	Ecology	Date of collection	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)
Cotylidia pannosa	H6059288	Haikonen 25824	Finland	61.1721 25.5471	Terricolous On bank with mosses	2007-10-10	−1.87	−24.04
Cotylidia undulata	TENN071491	HRL0625	Quebec	46.5220 −72.3213	Terricolous Moss site	2010-10-02	−4.41	−24.29
Cotylidia undulata	H6059276	Haikonen 26475	Finland	60.7178 24.4417	Terricolous In *Funaria* old fire site	2008-09-08	−3.97	−24.34
Cotylidia undulata	H6059299	Saarenoksa 21494	Finland	60.2236 25.0678	Terricolous Among mosses and ECM veg.	1994-09-20	−2.04	−23.29
Dicranum scoparium liv.	TENN-B-071478	HBK013	Tennessee	35.9606 −83.9207	Terricolous On soil	2014-09-11	−2.07	−32.00
Dicranum scoparium sen.	TENN-B-071478	HBK013	Tennessee	35.9606 -83.9207	Terricolous On soil	2014-09-11	−1.57	−31.18
Dicranum scoparium liv.	TENN-B-0102885	DUKE bf20-3	North Carolina	35.9940 −78.8986	Terricolous On soil	2015-04-15	−4.67	−30.59
Dicranum scoparium sen.	TENN-B-0102885	DUKE bf20-3	North Carolina	35.9940 −78.8986	Terricolous On soil	2015-04-15	−3.81	−30.80
Dicranum scoparium liv.	TENN-B-071477	RAS051	Tennessee	36.2423 −83.9305	Terricolous On soil	2015-11-09	−4.39	−31.00
Dicranum scoparium sen.	TENN-B-071477	RAS051	Tennessee	36.2423 −83.9305	Terricolous On soil	2015-11-09	−3.89	−30.93
Dicranum scoparium liv.	TENN-B-071473	HBK016	Tennessee	35.6016 −83.9246	Terricolous On soil	2015-11-14	−1.20	−28.82
Dicranum scoparium sen.	TENN-B-071473	HBK016	Tennessee	35.6016 −83.9246	Terricolous On soil	2015-11-14	−3.96	−27.90
Dicranum scoparium	TENN-B-071474	HBK014	Tennessee	35.6016 −83.9246	Terricolous On soil	2015-11-14	−3.90	−32.42
Dicranum scoparium sen.	TENN-B-071474	HBK014	Tennessee	35.6016 −83.9246	Terricolous On soil	2015-11-14	−3.10	−32.18
Galerina marginata	TENN071079	FPD26	Tennessee	35.9338 −83.8774	Lignicolous On wood	2016-02-13	0.42	−23.33
Inocybe subochracea	TENN062488	PBM2659	Tennessee	35.6019 −83.8136	Terricolous On soil	2013-09-13	−3.06	−24.84
Loreleia marchantiae	TUR83652	—	Finland	No data	Bryophilous Liverwort	No data	1.51	−26.07
Loreleia marchantiae	TUR203090	Lahti 24/14	Finland	61.3230 25.7322	Bryophilous Liverwort	2014-07-20	1.89	−25.58
Loreleia postii	H6055478	Harmaja s.n.	Finland	60.9300 27.6000	Bryophilous Soil, old fire place	1976-08-29	−0.66	−23.41
Loreleia postii	H6059335	Saarenoksa 48384	Finland	60.2236 25.0678	Bryophilous In moss-covered old fire place	1984-10-21	−2.56	−25.71
Muscinupta laevis	H6059292	Haikonen 19745	Finland	60.9827 25.6612	Bryophylous *Polytrichum*	1999-10-10	−0.90	−25.81
Muscinupta laevis	H6059303	Saarenoksa 43385	Finland	60.2129 25.0779	Bryophylous *Polytrichum*	1985-09-14	−3.55	−27.24
Muscinupta laevis	H6003362	Salo 9493	Finland	60.2371 25.1371	Bryophylous *Polytrichum*	2003-05-14	1.15	−25.93
Odonticium romellii	H6059330	Murdoch 11	Finland	61.6987 23.7896	Lignicolous On wood	2006-09-30	−2.90	−22.32
Onnia tomentosa	H6048516	Niemela 9079	Finland	61.7695 23.0658	Lignicolous Ground	2013-08-30	−1.43	−21.37
Onnia tomentosa	H6048491	Niemela 9081	Finland	61.7695 23.0658	Lignicolous Ground	2013-08-30	−4.28	−22.66
Phellinus nigricans	H6012648	Miettinen 14031	Finland	64.6791 28.4840	Lignicolous On wood	2010-04-12	0.44	−23.44
Phellinus nigricans	H6048524	Niemela 9065	Finland	61.5496 23.5961	Lignicolous On wood	2013-07-22	−4.48	−23.56
Rickenella fibula	TENN066160	SAT1117302	Tennessee	35.7055 −83.3833	BryophilousOn moss	2011-06-22	−3.25	−26.04
Rickenella fibula	TENN060941	TFB13109	Tennessee	35.6111 −83.8166	BryophilousOn moss	2006-05-24	−3.79	−28.44
Rickenella fibula	TENN071481	JMB101914-06	Washington	47.6080 −122.3351	BryophilousOn moss	2014-10-19	1.87	−28.23

(continues)

TABLE 1. Continued

Taxon	Herbarium accession no.	Specimen-voucher no.	Location	Long./ Lat.	Ecology	Date of collection	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)
Rickenella fibula	TENN071478	HBK013	Tennessee	35.6016 –83.9246	Bryophilous *Dicranum*	2014-09-11	2.08	–25.42
Rickenella fibula	TENN071480	BPL872	Tennessee	36.1028 –84.7200	Bryophilous *Dicranum*	2015-10-03	–4.96	–28.71
Rickenella fibula	TENN071477	RAS051	Tennessee	36.2423 –83.9305	Bryophilous *Dicranum*	2015-09-15	–4.52	–27.37
Rickenella "fibula" (*R. mellea*)	TENN071476	HBK012	Washington	47.6080 –122.3351	Bryophilous *Dicranum*	2014-10-10	0.73	–28.54
Rickenella fibula	TENN071482	HBK015	Washington	47.6080 –122.3351	Bryophilous *Dicranum*	2014-10-11	–2.92	–23.40
Rickenella fibula	TENN071474	HBK014	Tennessee	35.6016 –83.9246	Bryophilous *Dicranum*	2015-11-14	–3.25	–29.67
Rickenella fibula	TENN071473	HBK016	Tennessee	35.6016 –83.9246	Bryophilous *Dicranum*	2015-11-14	–2.70	–25.54
Rickenella fibula	TENN071479	JMB101914-07	Washington	47.6080 –122.3351	Bryophilous On moss	2014-10-19	–2.09	–29.22
Rickenella fibula	TENN060305	TFB12057	Tennessee	35.7075 –83.3825	Bryophilous On moss	2006-09-04	–2.09	–28.67
Rickenella fibula	TENN066877	PBM2506	Mass.	42.2627 –71.8019	Bryophilous On moss	2003-10-31	–0.16	–24.87
Rickenella fibula	TENN066876	PBM2503	Mass.	42.2627 –71.8019	Bryophilous On moss	2003-10-25	0.32	–25.95
Rickenella fibula	TENN066245	MGW992	Tennessee	35.7583 –83.2125	Bryophilous *Dicranum*	2011-06-25	–4.87	–28.63
Rickenella fibula	H6059291	Ohenoja s.n.	Finland	62.1332 22.5581	Bryophilous On moss	2006-09-22	–2.49	–25.81
Rickenella fibula	H6034921	Kytovuori 93-040	Finland	62.2409 23.7702	Bryophilous On moss	1993-08-04	–2.82	–28.36
Rickenella fibula	H6034922	Kytovuori 94-482	Finland	60.2251 25.0201	Bryophilous On moss	1994-04-14	–0.25	–25.26
Rickenella fibula	H6019327	Salo 1882	Finland	67.6507 24.9158	Bryophilous On moss	1995-10-15	–1.59	–24.89
Rickenella fibula	H6059302	Kytovuori 96-071	Finland	60.4748 25.0971	Bryophilous On moss	1996-07-12	–0.13	–25.09
Rickenella fibula	CORD	MES950	Chile	–40.1738 –73.4735	Bryophilous On moss	2015-05-01	–1.21	–22.95
Rickenella fibula	TENN061243	TFB13157	North Carolina	35.5016 –83.4008	Bryophilous On moss	2006-07-19	–2.93	–30.03
Rickenella fibula	TENN061272	TFB13163	Quebec	52.9527 –73.5488	Bryophilous On moss	2006-07-29	–5.04	–28.89
Rickenella minuta	TENN055098	TFB8528	Argentina	–42.7244 -71.7530	Terricolous On soil	1996-05-12	6.45	–25.43
Rickenella minuta	TENN055094	TFB8524	Argentina	–42.7244 –71.7530	Terricolous On soil	1996-05-06	2.37	–25.28
Rickenella minuta	CORD	MES1535	Chile	–40.6785 –72.1120	Terricolous On soil	2016-05-03	–3.77	–25.98
Rickenella minuta	TENN071469	MES1781	Chile	–40.6549 –72.1121	Terricolous On soil	2016-05-07	0.72	–25.36
Rickenella minuta	TENN071467	MES1950	Argentina	–41.0000 –71.500	Terricolous On soil	2016-05-13	0.78	–24.83
Rickenella minuta	CORD	MES2168	Argentina	–41.1499 –71.2999	Terricolous On soil	2016-05-18	2.36	–24.46
Rickenella minuta	TENN071472	MES1558	Chile	–40.5785 –73.1335	Terricolous On soil	2016-05-03	–1.65	–26.44
Rickinella minuta	TENN071468	MES1892	Argentina	–41.1499 –71.2999	Terricolous On soil	2016-05-13	5.74	–26.45
Rickinella minuta	CORD	MES1891	Argentina	–41.1499 –71.2999	Terricolous On soil	2016-05-13	8.14	–25.30
Rickinella minuta	TENN071471	MES2110	Argentina	–41.0000 –71.5000	Terricolous On soil	2016-05-16	3.28	–25.12
Ricknella minuta	CORD	MES1965	Argentina	–41.1499 –71.2999	Terricolous On soil	2016-05-13	2.96	–26.35

(continues)

TABLE 1. Continued

Taxon	Herbarium accession no.	Specimen-voucher no.	Location	Long./ Lat.	Ecology	Date of collection	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)
Rickenella minuta	CORD	MES1656	Chile	−40.6785 −72.1120	Terricolous On soil	2016-05-04	0.43	−26.14
Rickenella minuta	TENN071466	MES1054	Chile	−40.6785 −72.1119	Terricolous On soil	2015-05-05	−2.13	−25.11
Rickenella minuta	TENN071470	MES1259	Argentina	−41.0006 −71.4999	Terricolous On soil	2015-05-14	1.41	−26.64
Rickenella setipes	H6059279	Kytovuori 82-234	Finland	60.1900 25.0400	Bryophilous Soil, garden meadow	1982-09-01	−0.60	−27.22
Rickenella setipes	H6059290	Saarenoksa 06978	Finland	60.2091 24.9647	Bryophilous On moss	1978-07-18	−1.84	−26.38
Rickenella swartzii	TENN071475	HBK017	Washington	46.9474 −122.3091	Bryophilous On moss	2014-10-11	−3.22	−29.59
Rickenella swartzii	TENN071493	MGW1075	California	39.2683 −123.7871	Bryophilous On moss	2011-11-18	0.73	−24.23
Rickenella swartzii	TENN071492	MGW1341	California	39.2682 −123.7871	Bryophilous On moss	2013-11-16	−0.18	−26.68
Rickenella swartzii	TENN071484	HRL1399	California	39.3649 −123.8144	Bryophilous On moss	2012-12-17	0.49	−26.30
Rigidoporus populinus	H6059304	Airaksinen s.n.	Finland	60.3768 25.2690	Lignicolous On wood	1993-04-11	−3.64	−23.39
Rigidoporus populinus	H6052662	Kotiranta 27027	Finland	60.0459 24.0046	Lignicolous On wood	2004-04-23	−0.09	−22.11
Sphagnomphalia brevibasidita (=*Gerronema cinctum*)	H6059278	Askola 1633	Finland	60.5256 24.7621	Bryophilous In moist spring site	1985-06-27	−1.51	−23.48
Trametes ochracea	TENN060148	TFB12211	Russia	53.3655 49.8927	Lignicolous On wood	2004-08-21	−3.50	−22.42
Trichaptum fuscoviolaceum	H6059286	Miettinen 7555,3	Finland	61.3509 25.2781	Lignicolous On wood	2003-09-04	−4.15	−22.12
Trichaptum fuscoviolaceum	H6059317	Haikonen 25849	Finland	61.2102 26.0458	Lignicolous On wood	2007-10-16	−2.14	−23.11

Isotope Lab (www. isotope.unh.edu) on an Elementar Americas Pyrocube (Elementar Americas Inc., Mt. Laurel, New Jersey, USA) elemental analyzer combined with a GeoVision isotope ratio mass spectrometer (GeoVision Inc., Taipei, Taiwan). Samples were prepared by grinding 2–3 mg of dried basidiome tissue. Several known biotrophic ECM (i.e., *Amanita* Pers., *Cortinarius* (Pers.) Gray, *Inocybe* (Fr.) Fr.), saprotrophic (i.e., *Galerina marginata* (Batsch: Fr.) Kühner, *Trametes* Fr., *Trichaptum* Murrill), plant pathogen (i.e., *Rigidoporus populinus* (Schumach.: Fr.) Pouzar), and autotrophic controls (i.e., *Dicranum scoparium* Hedw.) were also used. The analyses produced stable isotope signatures of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) and elemental percent (%C, %N) (only $\delta^{13}C$ and $\delta^{15}N$ are shown in Table 1). Abundance values of ^{13}C were measured relative to the Vienna Pee Dee Belemnite (Mayor et al., 2009) and ^{15}N abundance values relative to atmospheric N.

In order to assign trophic states to Hymenochaetales samples, we assembled a global data set of 957 taxa (Appendix S1; see Supplemental Data with this article). $\delta^{15}N$ and $\delta^{13}C$ data were available for 849 of these taxa from Mayor et al. (2009), Birkebak et al. (2013), and Sánchez-García and Matheny (2017). Stable isotope data for the remaining 108 taxa were produced during this study. Previously available data contained only five Hymenochaetales samples (i.e., two from *Inonotus tomentosus* (Fr.) Teng and one each from *Onnia vallata* (Berk.) Y.C. Dai & Niemelä, *Oxyporus cuneatus* (Murrill) Aoshima, and *Trichaptum biforme* (Fr.) Ryvarden). Of the $\delta^{15}N$ and $\delta^{13}C$ measurements from our 108 samples, 92 originated from the Hymenochaetales (thus 97 total in the global dataset), 49 of which were scored as bryophilous following Redhead et al. (2002), 14 as lignicolous, and 34 as terricolous based on specimen-voucher metadata or the literature (Hansen and Knudsen, 1997; Bernicchia and Gorjón, 2010; Ryvarden, 2010; Table 1). Of the 849 taxa in the global dataset, these were assigned known trophic modes following Mayor et al. (2009), Seitzman et al. (2011), Birkebak et al. (2013), and Sánchez-García and Matheny (2017) – saprotrophic (SAP), ectomycorrhizal (ECM), neither saprotrophic nor ectomycorrhizal (NS-NE), autotrophic (AUTO), or, in the case of most our Hymenochaetales samples, as unknown. Of our samples, trophic states in the Hymenochaetales were scored for *Coltricia* Gray (ECM; Danielson, 1984; Tedersoo et al., 2007), the five samples from Mayor et al. (2009) (SAP), and nine lignicolous samples in the genera *Onnia* P. Karst., *Phellinus* Quél., *Rigidoporus* Murrill, and *Trichaptum* (SAP; Larsson et al., 2006; Wu et al., 2017).

We used the *mclust* package in R version 3.3.1 to formulate discrete clusters of functional trophic groups (R Development Core Team, 2014; Scrucca et al., 2016). This package models the stable isotope values as a Gaussian finite mixture while permitting differences in covariance structures, sample sizes of clusters, and number of clusters (components). Different models are compared using Bayesian information criterion (BIC) values. The package has an advantage over other clustering procedures, viz., k-means clustering (Steinley, 2006), where the number of clusters (k) need not be pre-assigned.

After assignment to clusters, the distribution of bryophilous, lignicolous, and terricolous Hymenochaetales was tabulated

according to *mclust* results from the best-fit model. Tests for equal distribution of ecological guild by trophic cluster were performed using Fisher's exact test (Fisher, 1922). Analyses of variance (ANOVA) and Tukey-Kramer post-hoc tests (Tukey, 1949) were performed to test null hypotheses of no differences between stable N and C isotope values of trophic modes (SAP, ECM, NS-NE, AUTO) of taxa in the same trophic cluster. The clustering procedures and statistical tests were conducted in R. Because corrections or normalizations for the Suess effect (Tans et al., 1979; McCarroll and Loader, 2004) do not significantly improve analysis of the stable isotope data (Mayor et al., 2009; Trappe et al., 2015), no adjustments were made to the results presented here.

Collections and taxon sampling for phylogenetic analyses

Samples of Hymenochaetales were selected for phylogenetic analyses from the University of Tennessee (TENN), the University of Helsinki (H), University of Turku (TUR), Universidad Nacional de Córdoba (CORD), the University of Florida (FLAS), and independent collectors (Appendix S2; herbarium abbreviations follow Thiers [continuously updated; http://sweetgum.nybg.org/science/ih/]. For phylogenetic analyses of the order, we downloaded available sequences of nuclear 28S rRNA (28S), 18S rRNA (18S), and RNA polymerase II second largest subunit (*rpb2*) from GenBank.

Fungal DNA extractions, PCR, and sequencing

Genomic DNA was extracted from 10–30 mg of dried basidiome tissue from fungal samples. These were ground with liquid nitrogen and a pinch of sterile sand using a porcelain mortar and pestle. DNA was then extracted using an E.Z.N.A. HP Fungal DNA kit (Omega Bio-Tek, Norcross, Georgia, USA). Elutions of genomic DNA were then diluted with sterile water into two 1:10 serial dilutions. PCR, PCR purification, and sequencing were performed following protocols outlined in Judge et al. (2010). PCR products were viewed on 1% agarose gels prepared with SYBR safe (Thermo Fisher Scientific, Rockwood, Tennessee, USA) and a UV trans-illuminator. The following primer pairs were used for PCR and sequencing: (1) LR0R-LR7 or LR0R-LR5 (28S; Vilgalys and Hester, 1990); (2) PNS1-NS41 (18S; Hibbett, 1996); and (3) *rpb2*-b6F-*rpb2*-b7.1R (*rpb2*; Matheny, 2005). In addition, we amplified and sequenced nuc rDNA ITS1-5.8S-ITS (ITS) to aid in species confirmation using primers ITS1F-ITS4 (White et al., 1990; Gardes and Bruns, 1993). Raw sequences were edited and assembled using Sequencher 5.0.1 (Gene Codes Corp., Ann Arbor, Michigan, USA). All new DNA sequences produced during this study were deposited at GenBank (Appendix S2).

Phylogenetic analyses

Nucleotide sequences of 18S, 28S, and *rpb2* were assembled and aligned in ClustalX (Larkin et al., 2007), manually adjusted in AliView (Larsson, 2014) or MacClade (Maddison and Maddison, 2005), and concatenated into a supermatrix in SeaView version 4.5.2 (Gouy et al., 2010) after inspection for strongly supported interlocus conflict (ML bootstraps >70% for conflicting clades). The supermatrix included taxa across the Hymenochaetales and represents a three-locus alignment of concatenated 28S, 18S, and *rpb2* gene regions. For members of the *Rickenella* clade sensu Larsson et al. (2006), samples were included in the Hymenochaetales dataset if represented by at least one locus, usually 28S. DNA sequences were not available for *Kurtia argillacea* (Bres.) Karasiński, an ericoid mycorrhizal member of the *Rickenella* clade (Kolařík and Vohník, 2018). Gene regions absent for taxa were coded as missing data. To this data set we added sequences of outgroups from the Polyporales Gäum – *Phlebia brevispora* Nakasone, *Bjerkandera adusta* (Willd.: Fr.) P. Karst., *Polyporus brumalis* (Pers.: Fr.) Fr., and *Punctularia strigosozonata* (Schwein.) P.H.B. Talbot; Phallomycetidae Hosaka et al. – *Ramaria rubella* (Schaeff.) R.H. Petersen; and Auriculariales J. Schröt. – *Auricularia subglabra* Looney et al. Outgroup choice was based on Hibbett et al. (2014). *Auricularia subglabra* was used to root resulting phylogenetic trees. Ambiguously aligned sites were excluded prior to phylogenetic analyses.

The supermatrix was analyzed using maximum likelihood (ML) and Bayesian inference (BI) phylogenetic methods. Single and linked locus data sets were analyzed with ML only. RAxML version 8 (Stamatakis, 2014) was used to conduct phylogenetic analyses under the ML criterion with 1000 bootstrap replicates, and MrBayes version 3.2.6 (Ronquist et al., 2012) was used to estimate posterior probabilities (PP) from a sample of trees drawn from a posterior distribution. In the RAxML analyses, a GTRGAMMA model of evolution was assigned to gene partitions as recommended by the user manual. Data were partitioned using the best partitioning scheme from PartitionFinder version 2.1.1 (Lanfear et al., 2017) with the 28S+18S loci modeled in combination but separate from the three *rpb2* codon positions resulting in four partitions total. A GTR model with gamma distributed rate heterogeneity was also used in BI analyses. The Hymenochaetales dataset was run for 15 million generations, and trees were sampled every 1000 generations. Convergence diagnostics and run length were determined based on recommendations in the MrBayes user manual. The first 25% of the trees in the posterior distribution were removed prior to sump and sumt commands and PP calculations.

Ancestral state reconstructions (ASR) were conducted in Mesquite version 2.74 (Maddison and Maddison, 2010) using the maximum parsimony criterion on the best ML tree. The strength of ASR assignments was assessed by examination of a given internode and its frequency in 1000 posterior trees sampled from the BI analysis. Trophic states were scored for tips in the supermatrix dataset based on the *mclust* analysis of the best-fit model (Appendix S1). Datasets and resulting tree files were submitted to TreeBASE (submission 21259; www.treebase.org) and are available from the corresponding author (PBM).

Presence/absence of genes involved in plant tissue degradation

We used enzyme names as key words to search against genome annotations based on InterPro (Jones et al., 2014) and Pfam (Finn et al., 2016) to assess the presence of enzymes involved in plant tissue degradation across seven available genomes of Hymenochaetales at MycoCosm (http://jgi.doe.gov/fungi) on 17 June 2017. The seven genomes searched were: (1) *Rickenella fibula* (JGI 333301 v1.0; (2) *R. mellea* (JGI 334780 v1.0); (3) *Fomitiporia mediterranea* M. Fisch. (JGI 56107 v1.0); (4) *Onnia scaura* (Lloyd) Imazeki (JGI 245618 v1.0); (5) *Porodaedalea niemelaei* M. Fisch. (JGI 333975 v1.0); (6) *Schizopora paradoxa* (Schrad.: Fr.) Donk (JGI 239088 v1.0); and (7) *Trichaptum abietinum* (Pers.: Fr.) Ryvarden (JGI 210203 v1.0) (Grigoriev et al., 2011, 2014). The functional annotations were conducted according to the DOE-JGI Microbial Genomic Annotation Pipeline (Huntemann et al., 2015). The protein sequences were

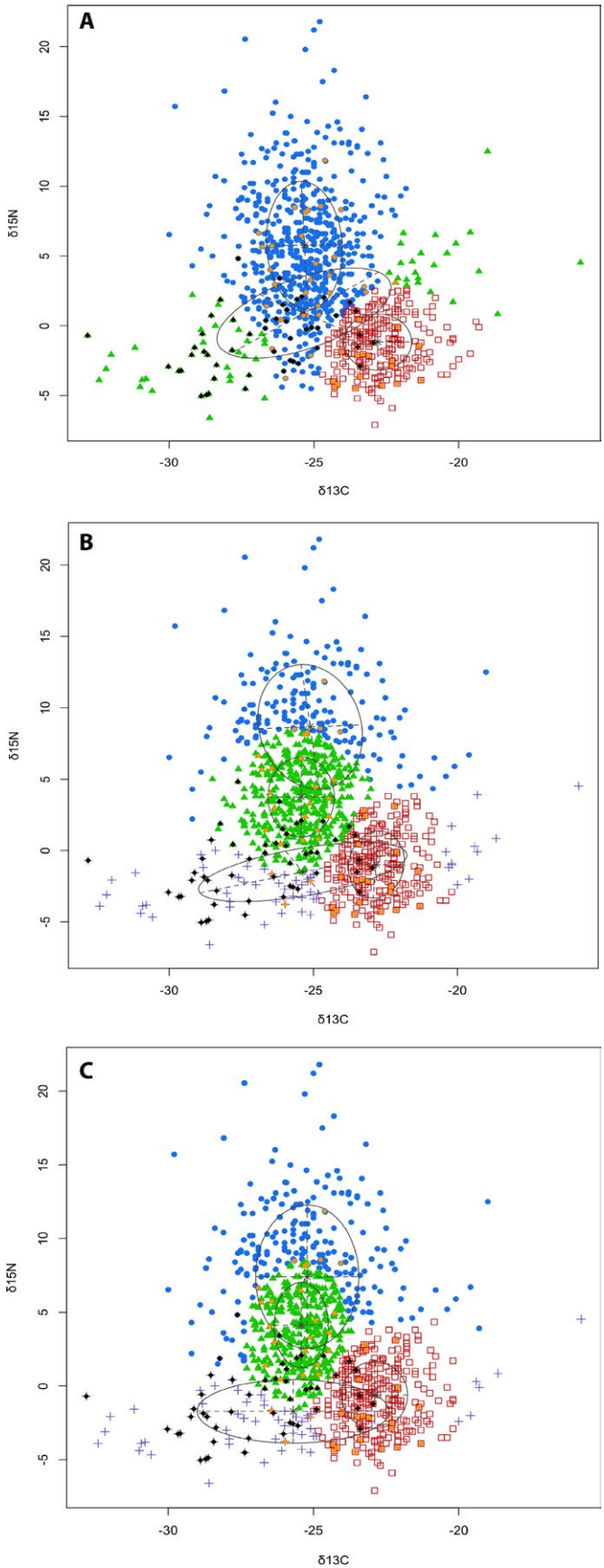

FIGURE 2. *Mclust* analysis of 957 stable isotope samples from Appendix S1. (A) *Mclust* classification according to the best-fit model (VVV, 3 components). Circles = trophic cluster 1; squares = trophic cluster 2; triangles = cluster 3. Spheres indicate covariance for each cluster. 78% of cluster 1 (circles) is composed of known ECM fungi and all NS-NE fungi. 88% of cluster 2 (squares) includes known SAP fungi; cluster 3 (triangles) includes known autotrophs and a small number of ECM and SAP fungi and nearly half of bryophilous Hymenochaetales. The latter are represented by black-filed circles, squares, and triangles, and appear in all three clusters. Cluster 3 (triangles) contains no lignicolous samples and only one terricolous sample. Terricolous Hymenochaetales are indicated by orange-filled circles (cluster 1) and one orange-filled triangle (cluster 3). Lignicolous Hymenochaetales are indicated by orange-filled squares. (B) *Mclust* classification according to the second best-fit model (VEV, 4 components). Circles = trophic cluster 1 (mainly NS-NE and ECM taxa); squares = trophic cluster 2 (SAP); triangles = trophic cluster 3 (other ECM); crosses = trophic cluster 4 (biotrophic other). Spheres indicate covariance for each cluster. (C) *Mclust* classification according to the third best-fit model (VIV, 4 components). Circles = trophic cluster 1 (mainly NS-NE and ECM taxa); squares = trophic cluster 2 (SAP); triangles = trophic cluster 3 (other ECM); crosses = trophic cluster 4 (biotrophic other). Spheres indicate covariance for each cluster.

searched against the Pfam database using HMMER version 3.0 (Eddy, 2011). The gathering threshold (–cut_ga) was chosen when using the pfam_scan.pl script. InterProScan was conducted with default settings.

Search words included the following: arabinosidase, cellulase, cellobiohydrolase, chitinase, galactosidase, glucanase, invertase, mannosidase, polygalacturonase, and xylanase, which are mainly enzymes of various glycoside hydrolase families involved in degradation of plant cell walls common in biotrophic parasites and saprotrophic fungi (Talbot et al., 2008; Zhao et al., 2013; Kohler et al., 2015). In addition we searched for the enzymes laccase, ligninase, and peroxidase involved in lignin degradation (Read et al., 2004; Talbot et al., 2008; Zhao et al., 2013; Kohler et al., 2015) under the assumption that the *Rickenella* genomes should lack such enzymes because bryophytes do not produce wood. For enzymes involved in lignin degradation and sucrolytic activity in the two *Rickenella* genomes, we searched the Conserved Domain Database (CDD: https://www.ncbi.nlm.nih.gov/Structure/cdd/cdd.shtml) with amino acid sequences of each enzyme to identify active sites across these enzymes to ensure protein homology.

RESULTS

Stable isotope data and cluster analyses

Ratios of stable isotopes are recorded as $\delta^{15}N$ and $\delta^{13}C$ values in Table 1, including 108 new samples, 92 of which represent Hymenochaetales. The remaining new samples are from control saprotrophic, ectomycorrhizal, and autotrophic taxa (the latter from the moss *Dicranum scoparium*, an associate of *Rickenella fibula*). In summary, 97 Hymenochaetales samples are included in Table 1.

The program *mclust* produced BIC values for three models: (1) *mclust* VVV,3 (ellipsoidal, varying volume, shape, and orientation, model with three components; BIC −9631.316 – the best-fit model); (2) *mclust* VEV,4 (ellipsoidal, equal shape, model with

TABLE 2. Three trophic clusters recovered by *mclust* analysis of stable isotope values provided in Appendix S1. The total number of taxa by cluster is indicated along with the number of Hymenochaetales samples that are bryophilous, lignicolous, and terricolous. For each cluster the percentage (%) and total number of taxa that are considered saprotrophic (SAP), ectomycorrhizal (ECM), neither saprotrophic nor ectomycorrhizal (NS-NE), autotrophic (AUTO), and unknown (UKN) are also shown.

Cluster	Trophic status	Total taxa	Bryophilous Hymeno-chaetales	Lignicolous Hymeno-chaetales	Terricolous Hymeno-chaetales	% / Total Trophic States from Appendix S1				
						SAP	ECM	NS-NE	AUTO	UKN
1	Biotrophic	637	23	0	29	9%	78%	7%	0%	6%
						58	495	45	0	39
2	Saprotrophic	248	5	14	4	88%	7%	0%	0%	3%
						218	17	0	0	13
3	Biotrophic	72	21	0	1	36%	18%	0%	14%	32%
						26	13	0	10	23

four components; BIC −9631.394 – second best-fit model); and (3) *mclust* VVI (diagonal, varying volume and shape; model with four components; BIC −9633.893 – third best-fit model).

According to the best-fit model, the 957 stable isotope samples cluster into three discrete groups or components in the *mclust* analyses (Fig. 2A). A biotrophic cluster of 637 samples (cluster 1) is dominated by ECM taxa (78%) and indicated by circles (Table 2). A saprotrophic cluster of 248 samples (cluster 2) is dominated by SAP taxa (88%) and indicated by squares. A third cluster was formed from 72 samples, including 10 *Dicranum* autotrophic samples and 62 samples of Agaricomycetes of varying trophic states (SAP, ECM, unknown) and is indicated by triangles. No NS-NE trophic samples grouped in clusters 2 and 3. Nearly half of the unknown samples of bryophilous Hymenochaetales grouped in cluster 3. This cluster contained none of our lignicolous samples and only one terricolous sample. Bryophilous Hymenochaetales are represented by black-filled circles, squares, and triangles, thus appearing in all three clusters, whereas non-bryophilous Hymenochaetales are indicated by orange-filled circles or squares (and one triangle) and appear almost exclusively in clusters 1 and 2.

The second and third best-fit models produced a fourth component into which taxa typically with high $\delta^{15}N$ ratios (*Camarophyllopsis* Herink, *Clavaria* Vaill. ex L., *Clavulinopsis* Overeen, *Ramariopsis* (Donk) Corner – all NS-NE taxa of Seitzman et al. (2011) and Birkebak et al. (2013)) clustered with mainly known ECM taxa also with high $\delta^{15}N$ ratios (Appendix S1; Fig. 2B–C). The only Hymenochaetales samples to group into this cluster were four samples of the known ECM genus *Coltricia*.

Hypothesis testing

Table 2 shows the number of samples of bryophilous, lignicolous, and terricolous Hymenochaetales distributed across the three different trophic clusters (clusters 1, 2, and 3) inferred by the best-fit model (Fig. 2A). This table also includes the number of samples and their percentages by known and unknown trophic assignment from Appendix 1 for each cluster according to the best-fit model. ANOVA results and subsequent Tukey-Kramer post-hoc tests strongly support N and C stable isotope mean differences between each of the three clusters (Table 3). If bryophilous Hymenochaetales have more or less equal ratios across clusters (the null hypothesis), then we can conclude that bryophilous Hymenochaetales are more functionally diverse than expected in that bryophilous Hymenochaetales exhibit different trophic modes. Indeed, this is what is observed from Fisher's exact test where one-third of the samples are expected to group in the biotrophic cluster, and the remaining two-thirds in the other two clusters (saprotrophic and unknown cluster 3) ($P = 0.22$) in support of the null hypothesis. That is, bryophilous Hymenochaetales do not particularly favor one trophic cluster over another.

TABLE 3. Comparison of trophic cluster assignments by *mclust* to test differences in sample means using ANOVA and Tukey-Kramer post-hoc tests. Stable isotope data from Mayor et al. (2009), Birkebak et al. (2013), Sánchez-García and Matheny (2017), and this study. An asterisk (*) indicates a statistically significant difference at the 0.05 level.

Test	Trophic comparison	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)
ANOVA	All clusters	$F_{2,954} = 301.1$, $P < 0.00001$*	$F_{2,954} = 301.1$, $P < 0.00001$*
Tukey-Kramer post-hoc	cluster 1 vs. cluster 3	$P < 0.00001$*	$P < 0.001$*
	cluster 1 vs. cluster 2	$P < 0.00001$*	$P < 0.00001$*
	cluster 2 vs. cluster 3	$P = 0.03$*	$P < 0.00001$*

At this stage, we then tested whether bryophilous taxa in cluster 1 (ECM) have stable isotope values similar to those of known saprotrophic and NS-NE taxa, and whether bryophilous taxa in cluster 3 (biotrophic other) share similar isotope values with known ECM fungi, saprotrophs, and autotrophs (*Dicranum scoparium)* (Table 4). Bryophilous samples in cluster 1 share similar C ratio signatures as known ECM and NS-NE taxa in the global dataset. Their N ratio signatures, however, are significantly different compared to ECM and NS-NE taxa but similar to saprotrophs. These results support the hypothesis that bryophilous Hymenochaetales in cluster 1 receive their C source from photosynthetic partners, as do ECM and NS-NE taxa, but do not engage in nutrient exchange of N with their bryophyte associates similar to saprotrophic fungi.

Bryophilous Hymenochaetales in cluster 3, however, have significantly different C and N values than those of ECM and saprotrophic fungi (Table 4). Indeed, their N values overall are no different from N values of the autotrophs in this cluster. These results support the hypothesis that the bryophilous fungi in cluster 3 are not ECM (and thus not N exchange mutualists), NS-SE (none are in this cluster), or saprotrophic. Instead, these fungi are deriving their N similar to that of autotrophs. Given their overall very low $\delta^{13}C$ ratios (Appendix S1) it seems reasonable to assume their source of C is from live autotrophs. We thus conclude that the bryophilous unknown samples in cluster 3 are best interpreted as parasites or endophytes.

We also tested whether lignicolous Hymenochaetales are distributed equally across trophic clusters under the expectation that one-third of these should group in the saprotrophic cluster, because they produce basidiomes on wood, and two-thirds in clusters 1 and 3. All 14 lignicolous samples grouped in the saprotrophic cluster

TABLE 4. Comparison of stable isotope results from Appendix S1 to test differences in sample means between trophic clusters and trophic states using ANOVA and Tukey-Kramer post-hoc tests. Stable isotope data from Mayor et al. (2009), Birkebak et al. (2013), Sánchez-García and Matheny (2017), and this study. ECM = ectomycorrhizal; NS-NE = neither saprotrophic nor ectomycorrhizal (both ECM and NS-NE are biotrophic); SAP = saprotrophic; Moss samples are from *Dicranum scoparium*. An asterisk (*) refers to a statistically significant difference at the 0.05 level.

Test	Trophic comparison[a]	δ ^{15}N (‰)	δ ^{13}C (‰)
ANOVA	Bryophilous Hymenochaetales in cluster 1; ECM non-Hymenochaetales; SAP non-Hymenochaetales; NS-NE	$F_{3,863} = 278.2$, $P < 0.00001$*	$F_{3,863} = 194.6$, $P < 0.00001$*
Tukey-Kramer post-hoc	Bryophilous Hymenochaetales in cluster 1 vs. ECM non-Hymenochaetales	$P < 0.00001$*	$P = 0.95$
	Bryophilous Hymenochaetales in cluster 1 vs. SAP non-Hymenochaetales	$P = 0.89$	$P < 0.00001$*
	Bryophilous Hymenochaetales in cluster 1 vs. NS-NE non-Hymenochaetales	$P < 0.00001$*	$P = 1$
ANOVA	Bryophilous Hymenochaetales in cluster 3; ECM non-Hymenochaetales; SAP non-Hymenochaetales; moss	$F_{3,833} = 180.1$, $P < 0.00001$*	$F_{3,833} = 311.6$, $P < 0.00001$*
Tukey-Kramer post-hoc	Bryophilous Hymenochaetales in cluster 3 vs. ECM non-Hymenochaetales	$P < 0.00001$*	$P < 0.00001$*
	Bryophilous Hymenochaetales in cluster 3 vs. SAP non-Hymenochaetales	$P = 0.047$*	$P < 0.00001$*
	Bryophilous Hymenochaetales in cluster 3 vs. moss	$P = 0.88$	$P = 0.001$*
ANOVA	Terricolous Hymenochaetales in cluster 1; ECM non-Hymenochaetales; SAP non-Hymenochaetales; NS-NE	$F_{3,874} = 268.7$, $P < 0.00001$*	$F_{3,874} = 194.7$, $P < 0.00001$*
Tukey-Kramer post-hoc	Terricolous Hymenochaetales in cluster 1 vs. ECM non-Hymenochaetales	$P = 0.22$	$P = 0.95$
	Terricolous Hymenochaetales in cluster 1 vs SAP non-Hymenochaetales	$P < 0.00001$*	$P < 0.00001$*
	Terricolous Hymenochaetales in cluster 1 vs NS-NE non-Hymenochaetales	$P < 0.00001$*	$P = 0.90$
ANOVA	Lignicolous Hymenochaetales in cluster 2; ECM non-Hymenochaetales; SAP non-Hymenochaetales	$F_{2,816} = 243.9$, $P < 0.00001$*	$F_{2,816} = 292.3$, $P < 0.00001$*
Tukey-Kramer post-hoc	Lignicolous Hymenochaetales in cluster 2 vs. ECM non-Hymenochaetales	$P < 0.00001$*	$P < 0.00001$*
	Lignicolous Hymenochaetales in cluster 2 vs. SAP non-Hymenochaetales	$P = 0.03$*	$P = 0.76$

Note: [a]Trophic states were not tested in Tukey-Kramer tests if absent from a given cluster. For example, mosses (autotrophs) are absent from clusters 1 and 2, and thus not tested for comparison.

2 (Fig. 2; Table 2). Doing Fisher's exact test, we can reject the null hypothesis ($P < 0.001$). Therefore, lignicolous Hymenochaetales are saprotrophic significantly more so than expected by chance. Do lignicolous Hymenochaetales in cluster 2 share similar stable isotope values to taxa of other known trophic assignments (ECM, saprotrophic)? This hypothesis is partially supported in that lignicolous Hymenochaetales share similar C values with known saprotrophs (both acquire C from dead organic material) but have deviating N values (Table 4; see also Fig. 2, orange-filled squares). This raises further questions whether these differences in N ratios might be due to small sample size, geographic location, substrate, or other factors.

Finally, we tested whether terricolous Hymenochaetales are distributed equally across the three trophic clusters. If so, then one-third should group in the biotrophic cluster and two-thirds in clusters 2 and 3. However, as Table 2 shows, 29 of 34 terricolous Hymenochaetales group in the biotrophic cluster dominated by known ECM samples. Results from Fisher's exact test strongly reject a null distribution ($P < 0.0001$). Therefore, terricolous Hymenochaetales are biotrophic, and likely mycorrhizal, more often than expected by chance. Do terricolous Hymenochaetales in cluster 1 (biotrophic) have similar stable isotope values to those of known saprotrophic and NS-NE taxa? Tukey-Kramer post-hoc tests (Table 4) significantly reject stable isotope similarities of terricolous Hymenochaetales to C and N values of known saprotrophic taxa and N values of NS-NE taxa. Thus, the stable isotope values of members of this guild are most similar to known ECM taxa.

Phylogenetic analyses of the Hymenochaetales and the Rickenella clade

Two hundred twelve new DNA sequences were produced during this study from herbarium specimens (66 for 28S, 54 for 18S, 33 for *rpb2*, and 59 for ITS; Appendix S2). No strongly supported conflict was observed between individual gene trees (rRNA and *rpb2*; data not shown but trees available at TreeBASE or from the corresponding author). The Hymenochaetales supermatrix of 18S, 28S, and *rpb2* included 157 taxa and 3880 sites. 22,502 trees were sampled from the posterior distribution after the burn-in and used to calculate PP values in the BI analysis.

Members of the *Rickenella* clade sensu Larsson et al. (2006) do not form a monophyletic group (Fig. 3; Appendix S3). Rather, we recover a poorly supported clade of lineages (Fig. 3A) belonging to the genera *Alloclavaria* Dentinger & McLaughlin, *Atheloderma* Parmasto, *Blasiphalia* Redhead, *Cantharellopsis* Kuyper, *Contumyces* Redhead et al., *Cotylidia* P. Karst., *Globulicium* Hjortstam, *Leifia* Ginns, *Loreleia* Redhead et al., *Muscinupta* Redhead et al., *Odonticium* Parmasto, *Peniophorella* P. Karst., *Rickenella* Raithelh., and *Sphagnomphalia* Redhead et al. (Fig. 3A). All of these are bryophilous or terricolous with the exception of *Globulicium*, *Leifia*, *Odonticium*, and *Peniophorella*, which are lignicolous. Note that *Sidera* Miettinen & K.H. Larss. (lignicolous and presumed saprotroph) and *Kurtia* Karasiński (lignicolous, but ericoid biotroph) were not included in the study.

Examination of the *Rickenella* clade (Fig. 3A) also reveals the paraphyly of *Contumyces* with respect to *Loreleia*, and polyphyly of the genus *Cotylidia* and the species *Odonticium romellii*. *Loreleia postii* (Fr.) Redhead et al., a species implicated as a parasite of the liverwort *Marchantia* (Kost, 1988), was excluded because blast results from the single sample supported an alliance with the Agaricales, namely, *Omphalina* Quél., outside the fungal order of focus for this study. *Rickenella minuta* comprises two strongly supported sister clades (Fig. 3A) suggesting this single morphological species is composed of two phylogenetic species.

The paraphyletic group from which the *Rickenella* clade is shown as derived (Fig. 3B) is dominated by lignicolous saprotrophs with the exception of the ECM lineage *Coltricia*. Taxa mostly considered by Larsson et al. (2006) as members of the *Rickenella* clade can be found in this portion of the tree and include *Repetobasidium* J. Erikss.,

Hyphoderma capitatum J. Erikss. & Å. Strid, *Tsugacorticium* Nakasone & Burds., *Resinicium* Parmasta, *Mycoacia* Donk, *Skvortzovia* Bononi & Hjortstam, and *Phlebia georgica* Parmasto. The Hymenochaetaceae is recovered as a paraphyletic group (85% ML support / PP > 0.95) including members of the Schizoporaceae Jülich – *Basidioradulum* Nobles, *Hyphodontia* J. Erikss., *Schizopora* Velen., and *Xylodon* (Pers.) Gray (per Index fungorum) – and taxa of uncertain position in the order – *Fibricium* J. Erikss., *Oxyporus* (Bourdot & Galzin) Donk (recently regarded as *Rigidoporus* Murrill), and *Trichaptum* Murrill.

MP ancestral state reconstruction (ASR) analyses support several switches to an ECM state from the ancestral saprotrophic state of the Hymenochaetales (Fig. 3). These are inferred to have occurred in *Coltricia* and in some taxa of the *Rickenella* clade. The exact number of transitions in the *Rickenella* clade is not entirely clear as it is not possible to reconstruct ancestral states with confidence in this portion of the tree. The evolution of ECM *Coltricia* from lignicolous, white-rot, saprotrophic ancestors is strongly supported. In the *Rickenella* clade several other lineages are inferred as ECM or ECM-like as well: *Alloclavaria purpurea* (O.F. Müll: Fr.) Dentinger & D.J. McLaughlin*, *Cotylidia diphana* (Appendix S1, not shown in Fig. 3), *Blasiphalia* Redhead, *Muscinupta* Redhead, Lücking & Lawrey, *Loreleia marchantiae* (Singer & Clémençon) Redhead et al., *Contumyces rosellus* (M.M. Moser) Redhead et al., *Rickenella minuta* (Singer & Digilio) Raithelh, and *R. swartzii* (Fr.) Kuyper. Putative ECM lineages that also include trophic samples that cluster as parasites (cluster 3) include *Cantharellopsis prescotii* (Weinm.) Kuyper, *Muscinupta laevis* (Fr.) Redhead, and *Rickenella fibula* (Appendix S1). The latter is primarily indicated as biotrophic but stable isotope samples of this species were found in all three trophic clusters. Thus, *R. fibula* appears capable of multiple trophic modes (see below).

Inferred trophic mode of species of Hymenochaetales

Table 5 summarizes 26 species of Hymenochaetales, including taxonomic synonyms, sampled in the global data set and Table 1, their ecology, trophic cluster, inferred tropic mode, and phylogenetic placement by clade or family in the Hymenochaetales. Generally, ecology is a useful predictor of trophic mode for terricolous and lignicolous species in the order. Almost all terricolous Hymenochaetales are ECM including *Alloclavaria purpurea*, species of *Coltricia*, *Cotylidia diaphana* (Schwein.) Lentz, and *Rickenella minuta*. An exception to this is *Cotylidia undulata* (Fr.) P. Karst., which is inferred as saprotrophic. All lignicolous Hymenochaetales sampled are saprotrophic. These include *Odonticium romellii* (S. Lundell) Parmasto, *Onnia tomentosa* (Fr.) P. Karst., *Onnia vallata*, and species of *Oxyporus* (recently revised as *Rigidoporus*; Wu et al., 2017), and *Trichaptum*.

The trophic mode of bryophilous Hymenochaetales varies depending on the species. Two species that produce basidiomes on liverworts such as *Blasia* and *Marchantia* are supported as mycorrhizal-like sharing similar biotrophic signatures with ECM Agaricomycetes. *Contumyces rosellus*, most samples of *Rickenella swartzii*, and nearly half of our *R. fibula* samples are also inferred as having a mycorrhizal-like trophic status but produce basidiomes on mosses. Only one bryophilous species is inferred as saprotrophic – *Sphagnomphalia brevibasidiata* (Singer) Redhead et al. Other bryophilous Hymenochaetales are neither ECM, NS-NE, or saprotrophic and are likely candidates as endophytes or parasites. These include *Cantharellopsis prescotii*, *Contumyces vesuvianus* (V. Brig.) Redhead et al., and half of our samples of *Rickenella fibula*.

A few species are ambiguous with respect to their trophic state, due to assignment to multiple trophic clusters and low sampling, or exhibit dual trophic signatures. These include *Cotylidia pannosa*, *Loreleia postii* (one sample of which is confirmed as Agaricales), *Muscinupta laevis*, Lücking & Lawrey, *Rickenella fibula*, and *R. swartzii* (=*R. setipes* (Fr.) Raithelh. (Appendix S1).

Trophic modes do not appear to be phylogenetically conserved. Biotrophic ECM and saprotrophic taxa are distributed in both the distantly related *Rickenella* clade and Hymenochaetaceae supporting the contention that biotrophy evolved on multiple occasions in the Hymenochaetales. However, most species sampled in the *Rickenella* clade are inferred as biotrophic, either as ECM or "other"; none of these feature trophic signatures similar to biotrophic (NS-NE) Hygrophoraceae and Clavariaceae (Appendix S1; Fig. 2B–C).

Decomposition enzyme repertoire

The genomes of *Rickenella fibula* and *R. mellea* are very similar overall to those of white-rot saprotrophic Hymenochaetales in terms of presence of enzymes or suites of enzymes used in plant cell wall and lignin degradation (Appendix S4). Some exceptions include the presence of invertase, involved in sucrolytic activity and indicative of plant parasitism and endophytism, in both *Rickenella* genomes but absent from all other saprotrophic Hymenochaetales genomes. Xylanase, involved in the breakdown of hemicelluose, was found only in the genomes of *R. fibula* and *Porodaedalea niemelaei*.

When searching CDD with the invertase amino acid sequences of *Rickenella fibula* and *R. mellea*, three active sites were identified consistent with those reported in Parrent et al. (2009). Both appear to be extracellular invertase based on blastp searches at the National Center for Biotechnology Information (NCBI). The laccase enzyme of *R. fibula* and *R. mellea* possesses multiple active sites of cupredoxin domains of laccases similar to Tv-LCC from *Trametes versicolor* (L.) Lloyd (Polyporales). The ligninase enzymes (class II peroxidases) share multiple heme, substrate, Mn, and Ca binding sites similar to Mn peroxidases of *Sistotrematsrum niveocremeum* (Höhn. & Litsch.) J. Erikss. (Trechisporales) and other Agaricomycetes. The peroxidase enzymes are similar to cytochrome C peroxidase in *Schizopora paradoxa* (Schrad.) Donk (Hymenochaetales) and share the possession of numerous heme, substrate, and K^+ binding sites with ascorbate and cytochrome C peroxidases. No active sites were identified by CDD in the xylanase of *R. fibula*; however, the enzyme was identified as a member of glycosyl hydrolase family 10 and is similar to other GH10 family proteins of other Agaricomycetes.

DISCUSSION

Revealing unknown trophic diversity in the Hymenochaetales

This is the first study to use stable C and N isotope data to predict or affirm the trophic status of numerous fungi with various ecologies in

*The citation of *Clavaria purpurea* Fr. (Syst. mycol. (Lunndae) 1: 480. 1821) by Dentinger and McLaughlin (2006) as the basionym for *Alloclavaria purpurea*, type species of *Alloclavaria*, is an indirect reference to *Clavaria purpurea* O.F. Müll (1780), cited by Fries, as permitted by ICN Art. 40.3 (Turland et al., 2018). Direct citation of the basionym is *Clavaria purpurea* O.F. Müll: Fr. Thus, *Alloclavaria purpurea* should be cited as *A. purpurea* (O.F. Müll: Fr.) Dentinger & D.J. McLaughlin. The name, because it is sanctioned in Fries' 1821 work, is conserved against the earlier *Clavaria purpurea* Schaef. (1774).

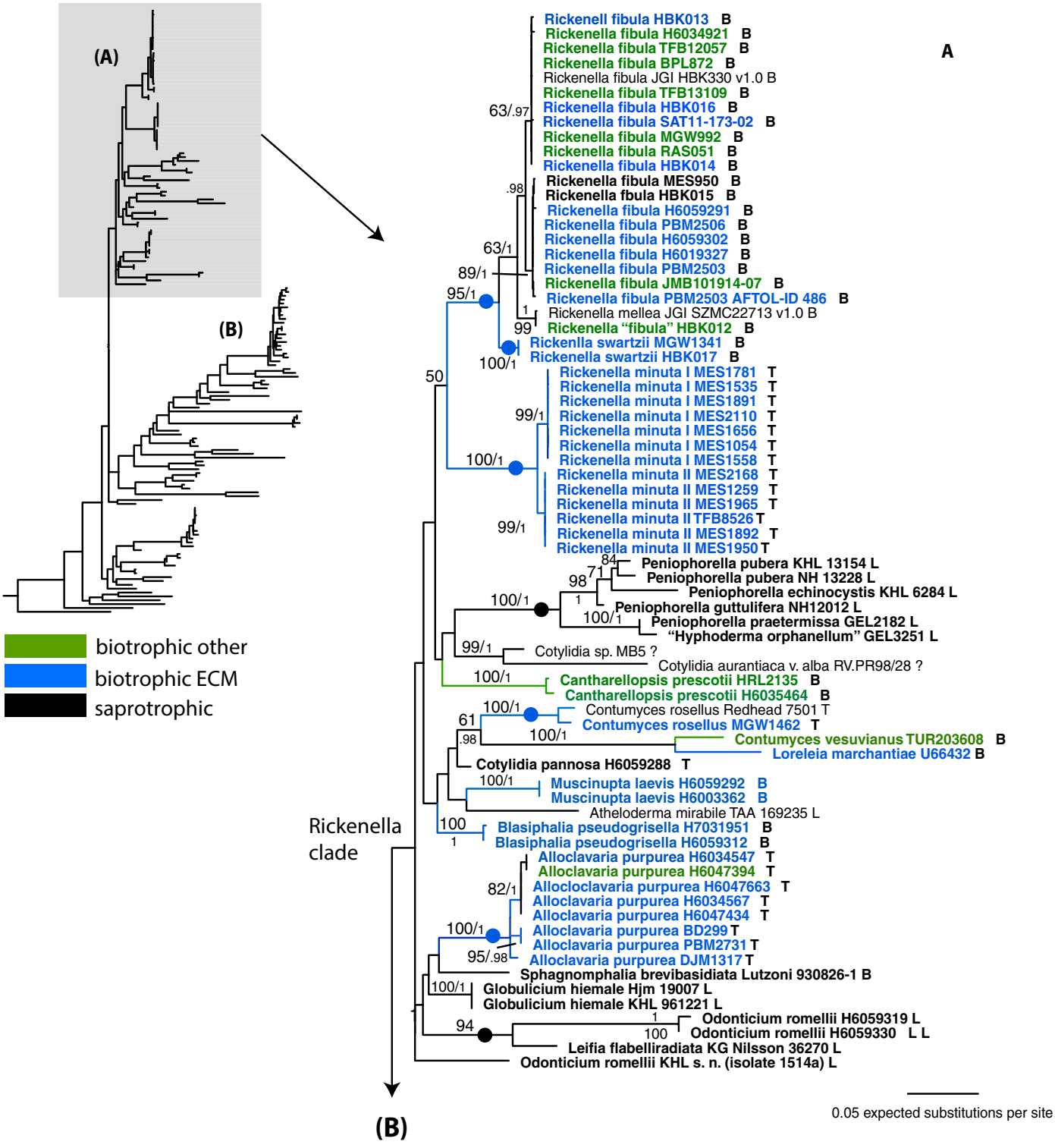

FIGURE 3. Maximum likelihood (ML) phylogeny of the order Hymenochaetales based on analyses of a 28S, 18S, and *rpb2* supermatrix. (A) *Rickenella* clade. (B) Remainder of the Hymenochaetales and outgroups. Taxon names in quotes are mislabeled. An asterisk (*) indicates lineages recovered in the *Rickenella* clade sensu Larsson et al. (2006). Values >50% above or below branches represent proportions from 500 bootstrap replicates. Posterior probabilities >0.95 are also indicated at internodes. Blue bold taxon labels group in trophic cluster 1 (ECM). Black bold taxon labels group in trophic cluster 2 (saprotrophic). Green bold taxon labels group in cluster 3 (biotrophic other). Tips labeled black but not in bold (excluding outgroups) lack stable isotope data. A lower case delta symbol (δ) next to a tip indicates that stable isotope data were produced from the sequenced specimen (Table 2). B, L, and T next to labeled tips indicate bryophilous (B), lignicolous (L), and terricolous (T) substrates. Ancestral states with >0.9 posterior probability are indicated with filled circles at or along internodes leading to more than one species. Black circles indicate saprotrophic states and blue circles ECM states.

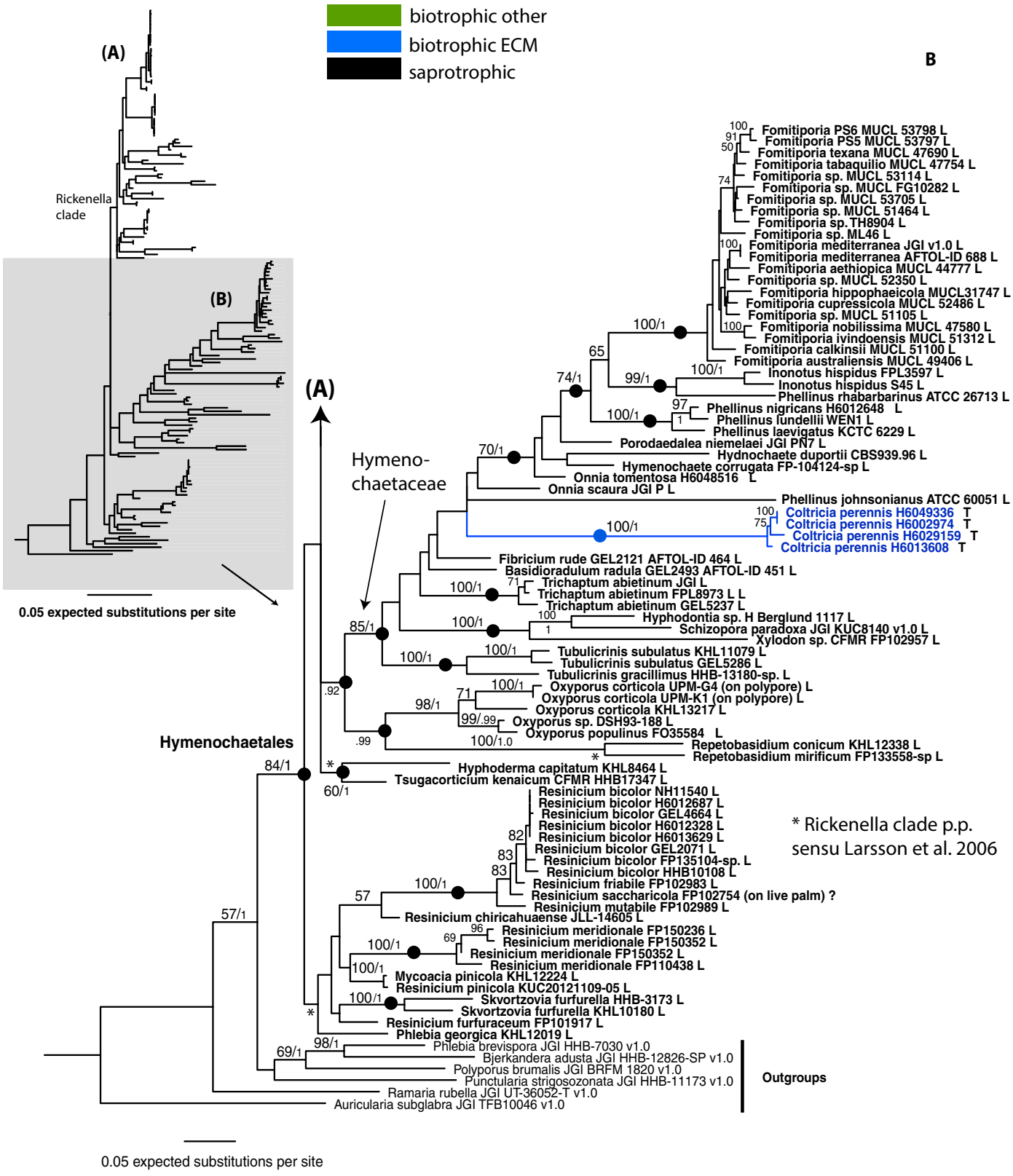

FIGURE 3. Continued

TABLE 5. Overview of species of Hymenochaetales with stable isotope data, their ecology, trophic cluster, inferred trophic mode, and phylogenetic placement.

Species	Ecology	Trophic cluster (No. samples)	Inferred trophic mode	Phylogenetic placement in Hymenochaetales
Alloclavaria pupurea	Terricolous	1 (5), 3 (1)	Biotrophic – ECM	Rickenella clade
Blasiphalia pseudogrisella	Bryophilous	1 (3), 2 (1)	Biotrophic – ECM with liverworts (*Blasia*)	Rickenella clade
Canthrellopsis prescotii (=*Gerronema albidum*)	Bryophilous	3 (5), 1 (1)	Biotrophic – other	Rickenella clade
Coltricia cinnamomea	Terricolous	1 (2)	Biotrophic – ECM	Hymenochaetaceae[a]
Coltricia montagnei	Terricolous	1 (2)	Biotrophic – ECM	Hymenochaetaceae[a]
Coltricia perennis	Terricolous	1 (4)	Biotrophic – ECM	Hymenochaetaceae
Contumyces rosellus	Terricolous	1 (1)	Biotrophic – ECM	Rickenella clade
Contumyces vesuvianus	Bryophilous	3 (1)	Biotrophic – other	Rickenella clade
Cotylidia diaphana	Terricolous	1 (1)	Biotrophic – ECM	Rickenella clade
Cotylidia pannosa	Terricolous	1 (1), 2 (1)	Biotrophic – ECM or saprotrophic	Rickenella clade
Cotylidia undulata	Terricolous	2 (3)	Saprotrophic	Rickenella clade[b]
Loreleia marchantiae	Bryophilous	1 (2)	Biotrophic – ECM with liverworts (*Marchantia*, *Conocephalum*, *Lunularia*)	Rickenella clade
Loreleia postii	Bryophilous	1 (1), 2 (1)	Biotrophic – ECM or saprotrophic	Agaricales
Muscinupta laevis	Bryophilous	1 (2), 3 (1)	Biotrophic – ECM or other	Rickenella clade
Odonticium romellii	Lignicolous	2 (1)	Saprotrophic	Rickenella clade
Onnia tometnosa (=*Inonotus tomentosus*)	Lignicolous	2 (4)	Saprotrophic	Hymenochaetaceae
Onnia vallata	Lignicolous	2 (1)	Saprotrophic	Hymenochaetaceae (lacking DNA verification)
Oxyporus cuneatus (=*Rigidiporus cuneatus*)	Lignicolous	2 (1)	Saprotrophic	Oxyporus clade[c]
Oxyporus populinus (=*Rigidiporus populinus*)	Lignicolous	2 (2)	Saprotrophic	Oxyporus clade
Phellinus nigricans	Lignicolous	2 (2)	Saprotrophic	Hymenochaetaceae
Rickenella fibula	Bryophilous	1 (9), 2 (2), 3 (12)	Biotrophic – ECM or other, infrequently saprotrophic	Rickenella clade
Rickenella minuta	Terricolous	1 (14)	Biotrophic – ECM	Rickenella clade
Rickenella swartzii (=*R. setipes*)	Bryophilous	1 (4), 3 (2)	Biotrophic – ECM or other	Rickenella clade
Sphagnomphalia brevibasidiata (=*Gerronema cinctum*)	Bryophilous	2 (1)	Saprotrophic	Rickenella clade
Trichaptum biforme	Lignicolous	2 (1)	Saprotrophic	Incertae sedis[d]
Trichaptum fuscoviolaceum	Lignicolous	2 (2)	Saprotrophic	Incertae sedis[d]

Notes: [a]per Larsson et al., 2006; [b]per Sjökvist et al., 2012; [c]per Wu et al., 2017; [d]per NCBI taxonomy.

the Hymenochaetales, an order otherwise dominated by lignicolous saprotrophs. We used stable isotope evidence (Table 5) to infer at least 15 biotrophic non-lignicolous lineages of Hymenochaetales (12 of which are shown in Fig. 3), all of which are ECM or exhibit other modes of biotrophy that depart from previous trophic characterizations (Hobbie et al., 2001; Mayor et al., 2009; Seitzman et al., 2011; Birkebak et al., 2013; Sánchez-García and Matheny, 2017). Moreover, a few taxa such as moss-inhabiting species of *Rickenella* are characterized by multiple trophic modes, expressing ECM-like and/or possibly parasitic or endophytic signatures, or, to a lesser extent, saprotrophic modes of nutrition (Bresinsky and Schötz, 2006; Chen et al., 2018). Data were grouped in three different components or clusters according to the best-fit model in *mclust*. The results discussed below are not sensitive to model choice with respect to Hymenochaetales samples as somewhat less fit models cleaved the ECM and ECM-like cluster into two components reflecting high $\delta^{13}N$ ratios among samples in the fourth cluster. Only samples of the known ECM *Coltricia* lineage clustered into this fourth component.

Aside from *Coltricia* and its close ally *Coltriciella* Murrill (Tedersoo et al., 2007), most biotrophic Hymenochaetales are concentrated in the *Rickenella* clade (Fig. 3A; Table 5). However, it is not clear how many shifts to biotrophy (clusters 1 and 3) occurred in the *Rickenella* clade (Fig. 3A) due to uncertainty about phylogenetic relationships in this portion of the phylogeny. Future studies will need to sample additional gene regions to ascertain the extent that evolutionary shifts to biotrophy may be phylogenetically conserved. Nonetheless, these results reinforce a general trend observed elsewhere in the Agaricomycetes that support the evolution of biotrophic lineages from saprotrophic ancestors (Martin et al., 2016), as the most recent common ancestor of the Hymenochaetales is reconstructed as saprotrophic with robust support (Fig. 3B).

Five non-lignicolous species in the *Rickenella* clade, viz. *Alloclavaria purpurea*, *Contumyces rosellus*, *Cotylidia diaphana*, *Loreleia marchantiae*, and *Rickenella minuta*, possess stable isotope signatures similar to those of ECM Agaricomycetes. Studies on the morphology of roots or rhizoids colonized by these fungi are needed to confirm, or have confirmed in some cases (Redhead, 1981), the presence of anatomical features consistent with an ECM habit. Nevertheless, *Alloclavaria purpurea* is most likely an ECM lineage with Pinaceae. In general the literature suggests *A. purpurea* is a moss associate (Dentinger and McLaughlin, 2006). However, three collections from North America were collected on sandy soil under pines, on ground among moss near fir, and on a moss-covered bank under ericaceous shrubs, bayberry, and pines. Corner (1950) noted that *A. purpurea* fruits exclusively near coniferous trees. Walker et al. (2012) recovered ITS matches of *A. purpurea* from ECM root tips of Douglas fir, but interpretation of stable isotope data produced in that study was ambiguous depending on the method of analysis used. This led Tedersoo and Smith (2013) to regard *A. purpurea* as a likely endophyte or saprobe. Our results do not support the suggestion that this species is endophytic or saprotrophic because five of six samples of *A. purpurea* from North America and Europe consistently produced a stable isotope ECM signature. We thus conclude *A. purpurea* is a novel ECM lineage in the Hymenochaetales.

ITS sequences of *Rickenella minuta* from basidiomes match those sampled from ECM root tips of Nothofagaceae in Argentina (Nouhra et al., 2013; data not shown). In addition, basidiomes of *R. minuta* are produced directly on soil in ectotrophic forests, although at times they can also be found among mosses (P.B. Matheny and G.R. Smith, personal observation). Thus, at least two lines of evidence support the ECM status of *R. minuta* – root tip molecular data and stable isotope signatures. This confirms the third known ECM lineage in the Hymenochaetales.

Contumyces rosellus (as *Omphalina rosella* (M.M. Moser) M.M. Moser) was documented by Redhead et al. (1995) from variable habitats, but it most commonly occurs on soil, generally in lawns among grasses or bryophytes. Stable isotope data from one sample of this species suggests an ECM status as well; however, additional collections from different populations need to be assayed to confirm this mode of nutrition.

The ecology of the genus *Cotylidia* is poorly understood, and prior evidence regarding its trophic status was lacking (Kout and Zíbarova, 2013). Species in the genus have been variably described as terrestrial and producing basidiomes on mineral soils often among mosses or as possibly bryophilous (Redhead et al., 2002). However, bryophilous associations are not constant among the few species described in the genus (Kout and Zíbarova, 2013). Moreau and Audet (2008) suggested *C. carpatica* (Pilát) Huijsman is a parasite of mosses, but *C. pannosa* (Sowerby) D.A. Reid has not been reported in association with mosses. We produced stable isotope data from three different species of *Cotylidia*. One of these, *C. diaphana*, is terricolous and bears an ECM signature (Table 5). However, *C. undulata* (Fr.) P.Karst., which is also terricolous, is inferred as saprotrophic. Two samples of *C. pannosa* were inconsistent regarding trophic assignment (one ECM, one SAP). Additional samples, including from different species, are required to determine how consistent and well supported such inferences are. Note that *Cotylidia* is not monophyletic in our phylogenetic tree (Fig. 3A). Accordingly, variation in trophic modes among *Cotylidia* species is not unexpected.

Loreleia marchantiae also groups in the predominantly ECM cluster 1. This is consistent with observations that the fungus penetrates rhizoids of the thalloid liverwort *Marchantia* L. (Bresinsky and Schötz, 2006), of which it was considered a parasite (Kost, 1988). However, Bresinsky and Schötz (2006) suggested the fungus enables N exchange from cyanobacteria to its liverwort associate. If *L. marchantiae* were acquiring N from a live autotroph, we would expect N isotope samples of this fungus to group it in trophic cluster 3. This, however, is not the case, as its N signatures are more like those of saprotrophs (Table 4). *Loreleia marchantiae* is found producing basidiomes on liverworts in the genera *Marchantia*, *Conocephalum* Hill, and *Lunularia* Adans., in particular on live *M. polymorpha* L. (Kost, 1988; Knudsen and Vesterholt, 2012). *Marchantia* is also associated with arbuscular-mycorrhizal (AM) fungi in the genus *Glomus* (Kottke and Nebel, 2005; Russell and Bulman, 2005), and mycorrhizal-like exchange of nutrients (P, N) has been reported in *Marchantia palaeacea* Bertol (Humphreys et al., 2010). However, at this time there is no evidence to support a N-exchange mutualism between *L. marchantiae* and its liverwort associate.

Most isotope samples of the bryophilous *Blasiphalia pseudogrisella* (A.H. Sm.) Redhead, *Muscinputa laevis*, and *Rickenella swartzii* also feature ECM trophic signatures. This would suggest these fungi receive photosynthates as C input and exchange N in return in a nutrient exchange mutualism as is typical for the ECM symbiosis (Smith and Read, 2008). Indeed, Kowal et al. (2018) confirm such a mutualism between liverworts and Ascomycota. However, bryophilous Hymenochaetales that group in the predominantly ECM cluster 1 (Table 4) share similar N signatures with saprotrophic fungi. This pattern suggests that bryophilous Hymenochaetales in this cluster may not be engaging in N exchange. As such, hypotheses that these fungi are commensals cannot be dismissed; unless it can be shown that they convey other fitness benefits to their bryophyte associates (Davey and Currah, 2006). Parasitism (Redhead et al., 2002; Larsson et al., 2006) does not seem likely if the fungi are passively receiving C in the form of glucose from their bryophyte associates (Parrent et al., 2009). Redhead (1981) did observe appressoria produced by *Blasiphalia pseudogrisella*, anatomical structures typical of plant pathogens, which penetrate the rhizoids of the liverwort *Blasia pusilla* L. However, Kost (1988) suggests this structure is analogous to those produced by ECM fungi, so-called "palmetto-structures" and refers to infected caulonemata and rhizoids of bryophytes as "mycorrhizoids". If *B. pseudogrisella* were found to lack invertase involved in active breakdown of sucrose to glucose, then this would support our conclusion of an ECM or ECM-like signature for this fungus.

Muscinupta laevis occurs on live mosses, especially *Polytrichum* Hedw. (Redhead et al., 2002). Ryvarden (2010) and Vizzini (2010) considered the species to be a moss parasite. Stable isotope data we analyzed (Table 2) confirm a biotrophic mode of nutrition in this species, either as ECM-like (cluster 1) or as a parasite or endophyte (cluster 3) (Table 5). More sampling from diverse locations is needed to confirm these results. *Rickenella swartzii* is a third biotrophic bryophilous species that fits in this biotrophic ECM-like functional group. It has been found on a wide range of bryophytes (Bresinsky and Schötz, 2006) and considered a parasite invading chloronemata or caulonemata of mosses forming "lignituber-like" structures similar to ericoid mycorrhizas (Kost, 1988). The former authors suggested this species is most likely saprotrophic or forms endomycorrhizas (biotrophic). Stable isotope data affirm a biotrophic signature with four of six samples consistent with an ECM-like state (Fig. 3A) and two in cluster 3 indicative of parasitism or endophytism (Appendix S1; Table 5).

Kost (1988) and Bresinsky and Schötz (2006) predicted contrasting modes of nutrition for *Rickenella fibula*, which is of particular interest since numerous samples of this species support multiple trophic modes. Kost (1988) suggested *R. fibula* is a parasite (biotroph) forming "lignitubers" on moss rhizoids as in *R. swartzii*, whereas Bresinsky and Schötz (2006) considered *R. fibula* as a saprotroph but could not dismiss an "endomycorrhizal" ecology. Similar to *R. swartzii* above, but with denser stable isotope sampling, we found evidence that *R. fibula* is a biotroph—either ECM-like (cluster 1) or parasitic or endophytic (cluster 3). Only two of 23 stable isotope samples support a saprotrophic state for *R. fibula*. Both biotrophic states do not appear to co-vary with phylogeny (Fig. 3A) and are consistent with a report that *R. fibula* can be found throughout living and senescent moss gametophytes exhibiting dual trophic modes (Chen et al., 2018). However, *R. fibula* does not appear to be saprotrophic to a major extent in contrast to the suggestion made by Chen et al. (2018). Redhead (1981) observed peg-like haustoria produced by *R. fibula* on the rhizoids of a moss indicative of parasitism, behavior not inconsistent with our stable isotope results.

Some authors have suggested that the saprotrophic *Phellinus igniarius* (L.) Quél. can colonize young roots of Norway spruce producing hyphal structures similar to a Hartig net and thus are capable of "facultative biotrophy" switching between saprotrophic and biotrophic nutritional modes (Smith et al., 2017). We produced

stable C and N isotope data from two samples of *Phellinus nigricans* (Fr.) P. Karst., a member of the *P. igniarius* complex, but found no evidence of a biotrophic signature in this species (Appendix S1; Table 5). Indeed, stable isotope data confirm a saprotrophic mode of nutrition in the lignicolous species of *Phellinus*, *Odontcium*, *Onnia*, *Oxyporus* (=*Rigidoporus*), and *Trichaptum*. Among non-lignicolous Hymenochaetales, saprotrophic signatures are suggested for the bryophilous *Sphagnomphalia brevibasidiata* (Singer) Redhead, which occurs on live *Sphagnum* (Redhead et al., 2002), and the terricolous *Cotylidia undulata*, discussed above. Additional stable isotope results are needed from *Sphagnomphalia brevibasidiata* that confirm or reject the saprotrophic signature from our single sample.

Phylogenetic relationships in the Hymenochaetales and the Rickenella clade

Future strategies that increase both gene and taxon sampling are required to resolve relationships of major groups within the Hymenochaetales. Increasing taxon sampling (compared to that shown in Fig. 3) reduced phylogenetic resolution overall in the Hymenochaetales (590 tips represented by at least one gene region), probably due to the large amount of missing data. Inferences about phylogenetic relationships drawn from whole genome data at MycoCosm (Grigoriev et al., 2014) suggest *Rickenella* is sister to the rest of the Hymenochaetales, consistent with the topology shown here (Fig. 3). However, the taxa forming a grade relative to *Rickenella* and other Hymenochaetales (*Rickenella* clade p.p.; Fig. 3B) are lacking whole genome data. If the ML phylogenetic hypothesis is correct, then biotrophic lineages of Hymenochaetales are evolutionarily derived.

Future research efforts should target sequencing whole genomes from ecologically diverse Hymenochaetales such as *Alloclavaria purpurea* (terricolous ECM), *Loreleia marchantiae* (bryophilous ECM-like), *Rickenella minuta* (terricolous ECM), *Coltricia* or *Coltriciella* (terricolous ECM), *Cantharellopsis prescotii* (bryophilous parasite), and *Blasiphalia pseudogrisella* (bryophilous ECM-like). *Phellinus igniarius* could be targeted as well (Smith et al., 2017). At present, only two genomes are available from non-lignicolous Hymenochaetales: *Rickenella fibula* and *R. mellea*, species that appear capable of multiple trophic modes (ECM-like and parasitic or endophytic) discussed below. Furthermore, the phylogenetic affinities of *Loreleia postii* need to be evaluated based on additional taxon sampling as our single result suggests an affiliation of this species with *Omphalina* in the Agaricales.

Genomic traits of bryophilous Hymenochaetales

Different fungal trophic modes are also characterized by different genomic traits, such as presence or number of enzymes involved in cellulose and lignin degradation (Read et al., 2004; Talbot et al., 2008, 2015; Kohler et al., 2015). Biotrophic ECM fungi produce plant cell wall degradative enzymes such as cellobiohydralase, cellulase, chitinase, polygalacturonase, and xylanase, in addition to lignin degradation enzymes such as laccase and peroxidases. Biotrophic ericoid mycorrhizal fungi often produce plant cell wall degradative enzymes arabinosidase, galactosidase, and mannosidase, in addition to those found in ECM fungi. Ericoid mycorrhizal fungi also feature lignin degradative enzymes laccase, lignase, and class II peroxidases. However, mycorrhizal fungi in general contain a much lower number of these enzymes compared to saprotrophs, and ECM fungi lack the plant cell wall degradative enzyme glucanase (Zhao et al., 2013; Kohler et al., 2015). Biotrophic parasitic fungi vary in their functional repertoire depending on whether the fungus is a plant or animal pathogen (Zhao et al., 2013). Invertases, for example, enzymes with sucrolytic activity, are found in plant parasitic fungi such as *Heterobasidion irregulare* Garbelotto & Otrosina (Olson et al., 2012) and endophytes in general but have been lost in animal parasites and are absent in most mycorrhizal lineages (Parrent et al., 2009; Martin et al., 2016; Strullu-Derrien et al., 2018). Since the study by Parrent et al. (2009), genomic studies generally support a negative correlation between invertase presence and most ECM fungi. *Tuber*, an ECM lineage in the Ascomycota (Martin et al., 2010), and *Sebacina incrustans* (Pers.) Tul. & C. Tul., an ECM lineage (Parrent et al., 2009; Weiß et al., 2016) are two exceptions.

Given that bryophytes do not produce wood, it would stand to reason there would be a lack of selection to maintain lignin degradative enzymes in biotrophic Hymenochaetales under the assumption they are derived from white-rot ancestors. Contrary to our expectations, the genomes of *Rickenella* are characterized by the presence of a suite of enzymes used to degrade lignin (Lundell et al., 2010), cellulose, and hemicelluose (xylanase in *R. fibula*). Bryophytes are known to contain lignin-like polymers but not lignin itself (Ligrone et al., 2008), which could explain the maintenance of lignin degradation in bryophilous Hymenochaetales. The presence of invertase in the genomes of *R. fibula* and *R. mellea*, and its absence in white-rot Hymenochaetales genomes, is more consistent with a non-mycorrhizal biotrophic lifestyle, such as that of a parasite or endophyte (Parrent et al., 2009), as exemplified by the assignment of these taxa in cluster 3 (Fig. 2; Table 4). It remains to be explained why some bryophilous *Rickenella* samples cluster more closely with known ECM fungi (Table 4) based on similar C utilization patterns, other than these *Rickenella* species are capable of multiple trophic modes (Olson et al., 2012; Smith et al., 2017; Chen et al., 2018).

ACKNOWLEDGEMENTS

The authors thank the Department of Ecology and Evolutionary Biology at the University of Tennessee, in particular Ken McFarland, and the Missouri Mycological Society and the University of Tennessee Botany Excellence Fund for financial support. The U.S. Department of Energy Joint Genome Institute produced the whole genome and transcriptome for *Rickenella fibula* HBK330-10. We acknowledge Igor Grigoriev, Joseph Spatafora, and Kerry Barry for their support as participants in the 1000 Fungal Genomes Program. The following herbaria provided specimen loans: CMMF, CORD, FLAS, H, TENN, and TUR. We also thank Renée Lebeuf, Steve Trudell, and Mike Wood for sharing specimen-vouchers and Meg Staton for comments on an earlier version of this manuscript. Mike Wood is particularly acknowledged for the photos shown in Fig. 1. We thank the University of Tennessee Genomics Core for sequencing assistance, Jacob Edwards for laboratory assistance, and staff at the University of New Hampshire Stable Isotope Lab for sample preparation. Marisol Sánchez-García, Brian Looney, and Brian O'Meara provided assistance with phylogenetic and related methods. We thank Joshua Birkebak for pointing out and correcting nomenclatural concerns. Portions of this study were supported by funds from NSF DEB-1354802 awarded to MES and PBM. The basis of this work was originally published as a Master's thesis,

 We thank the associate editor and reviewers for their helpful comments and suggestions to earlier versions of this manuscript.

DATA ACCESSIBLITY

DNA sequence data, alignments, and phylogenetic trees are deposited at GenBank and TreeBASE (21259; www.treebase.org) and are also available at http://mathenylab.utk.edu/Site/Alignments_%26_Data_Sets.html.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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