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Citation	Trappe, M. J., Smith, M. E., & Hobbie, E. A. (2015). Exploring the phylogenetic affiliations and the trophic mode of Sedecula pulvinata (Sedeculaceae). Mycologia, 107(4), 688-696. doi:10.3852/14-110
DOI	10.3852/14-110
Publisher	Mycological Society of America
Version	Version of Record
Terms of Use	http://cdss.library.oregonstate.edu/sa-termsofuse

Mycologia, 107(4), 2015, pp. 688–696. DOI: 10.3852/14-110

Exploring the phylogenetic affiliations and the trophic mode of *Sedecula pulvinata* (Sedeculaceae)

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Abstract: *Sedecula* is a monotypic genus of hypogeous fungi that is rare and endemic to dry conifer forests of the western United States. The only known species, *Sedecula pulvinata*, was described in 1941 and its taxonomic placement and trophic status have remained uncertain ever since. Here we employ isotopic and molecular phylogenetic analyses to determine its nutritional mode and placement on the fungal tree of life. Phylogenetic analysis indicates that *S. pulvinata* is closely related to the genus *Coniophora*, in Coniophoraceae (Boletales). Stable isotope comparisons with known ectomycorrhizal and saprotrophic fungi together with phylogenetic evidence also suggest that *S. pulvinata* is saprotrophic. We conclude that *Sedecula* likely represents a unique morphological transition between a resupinate basidiocarp morphology (in *Coniophora* and relatives) and a hypogeous, sequestrate basidiocarp morphology (in *Sedecula*). Spore dimensions are amended from the original description.

Key words: Boletales, Coniophoraceae, Great Basin, isotopes, mycorrhizal, saprotrophic

Introduction

Sedecula Zeller is a monotypic genus of hypogeous or erumpent fungi endemic to upper elevation xeric conifer forests of the western United States. Most collections of the only described species, *Sedecula pulvinata* Zeller, are from the periphery of the Great Basin, from the Sierra Nevada of eastern California (Hall 1991, Waters et al. 1997) to southwestern and south central Oregon (D. Pankratz pers. comm.) and southern Idaho (Stanikunaite et al. 2007), and from northern Arizona (States 1984, States and Gaud 1997) through Utah (herbarium collections MICH 26608, 71424, 71425) and western Colorado (Kotter and Farentinos 1984a). It also has been reported from the eastern Cascades of Washington (Lehmkuhl et al. 2004). *Sedecula pulvinata* is considered rare and is on the Interagency Special Status/Sensitive Species Program (ISSSSP) list of organisms requiring protection of known sites (Castellano et al. 1999).

The genus was described by Zeller (1941) who placed it in the family Sclerodermataceae based on its thick, leathery peridium, gleba chambers and the dark spore mass that becomes powdery at maturity. Based on subsequent studies of spore morphology and the apparent centripetal development of the gleba, Zeller recognized that *Sedecula* was distinct from any members of the Sclerodermataceae. Accordingly he established the new family Sedeculaceae to accommodate the genus (Zeller 1948, 1949). Smith (1951) and Guzmán (1971) concurred with Zeller's assessment, but Thiers (1984) speculated that *Sedecula* might be related to *Agaricus* because its large, smooth spores are morphologically similar to members of that genus. Evidence from hyphal morphology (Agerer 1999) and molecular phylogenetic data (Binder and Bresinsky 2002, Binder and Hibbett 2006) have since shown that the family Sclerodermataceae is nested within the order Boletales. However, none of the recent phylogenetic or morphological studies of Sclerodermataceae or Boletales have addressed the evolutionary origins of *Sedecula* or Sedeculaceae, leaving the taxonomic status of this group in limbo. For example, both Mycobank (www.mycobank.org/) and Index Fungorum (www.indexfungorum.org/) list *Sedecula* and Sedeculaceae as incertae sedis within Agaricales.

Most hypogeous fungi in North America are ectomycorrhizal (Trappe et al. 2007), and because *Sedecula pulvinata* is found in western coniferous forests it has been assumed that this fungus also forms ectomycorrhizas with conifers (Kotter and Farantinos 1984b, Molina et al. 1992, Barroetaveña et al. 2007). Colonization of root tips and development of a fungal mantle and Hartig net are anatomical hallmarks of ectomycorrhizal associations, but there are no morphological or molecular data thus far indicating that *S. pulvinata* forms these associations. Because the ectomycorrhizal nutritional mode is conserved within fungal lineages, phylogenetic relationships have

Submitted 1 May 2014; accepted for publication 2 Feb 2015.
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TABLE I. GenBank accession numbers of sample sequences

MICH	Locality	mtLSU	ITS	28S	*TEF*1
26608	Washington, Utah	KJ882296			
26633	Boulder, Colorado	KJ882294			
67760	San Miguel, New Mexico	KJ882295	KJ882287	KJ882287	KP033141

proven useful for distinguishing ectomycorrhizal fungi from non-ectomycorrhizal relatives (Tedersoo and Smith 2013).

Analysis of ^{13}C:^{12}C and ^{15}N:^{14}N ratios in sporocarps (expressed as $\delta^{15}N$ and $\delta^{13}C$ signatures) also has been established as a fairly reliable method of ascertaining trophism within fungi (Hobbie et al. 2001, Taylor et al. 2003). Mycorrhizal taxa tend to have higher $\delta^{15}N$ and lower $\delta^{13}C$ than saprotrophic fungi (Mayor et al. 2009). Such differences in $\delta^{13}C$ appear to arise from the higher $\delta^{13}C$ values in wood and litter cellulose that supply saprotrophic fungi compared to the plant sugars transferred to ectomycorrhizal fungi (Hobbie 2005). In contrast, ectomycorrhizal fungi are usually higher in $\delta^{15}N$ than saprotrophic fungi. Nutritional sources contribute part of this difference, with saprotrophic fungi often assimilating nitrogen from ^{15}N-depleted wood or litter whereas ectomycorrhizal fungi are generally active in deeper soil horizons (Lindahl et al. 2007, Hobbie et al. 2014). In addition, transfer of ^{15}N-depleted nitrogen from ectomycorrhizal fungi to host plants leads to ^{15}N enrichment of the nitrogen remaining in ectomycorrhizal fungi (Hobbie and Högberg 2012).

Stable carbon isotope analyses to determine ectomycorrhizal or saprotrophic status rely on the carbon sources (primarily complex carbohydrates in wood or litter for saprotrophic fungi and simple sugars for ectomycorrhizal fungi) for these two life history strategies having different carbon isotope values. However, because altitude, water stress and other climatic factors can influence the discrimination against $^{13}CO_2$ in primary photosynthesis (Kohn 2010) sample data from herbarium specimens should be normalized to common conditions if these fungi are obtained from different locations. In addition, the combustion of fossil fuels of C3 origin to carbon dioxide has changed the $\delta^{13}C$ of atmospheric CO_2 from −6.5‰ in the pre-industrial era to about −8.2‰ today (the Suess effect; McCarroll and Loader 2004), with a continuing annual decrease of 0.03‰. Accordingly $\delta^{13}C$ data on samples from different years also may need to be normalized to account for changes in the source CO_2 used in photosynthesis.

Neither the trophic mode nor the phylogenetic affiliations of *S. pulvinata* have been studied to date, so the closest relatives and main ecological role of this fungus remain a mystery.

Here we analyze the phylogenetic relationships of *Sedecula pulvinata* based on several loci—the translation elongation factor 1-α gene (*TEF*1), the ML5-ML6 region of the mitochondrial large subunit rRNA gene (mtLSU), the rDNA internal transcribed spacer regions (ITS1–5.8S–ITS2, referred to as ITS) and partial 28S region to establish its taxonomic placement—and we employ isotopic analysis to gain insights to its trophic status.

MATERIALS AND METHODS

Sporocarp tissue (TABLE I) was ground with a micropestle and DNA was extracted with a modified CTAB method (Gardes and Bruns 1993). We performed PCR using published methods for the following loci: ITS rDNA with primers ITS1F and ITS4 (Gardes and Bruns 1993), the D1 and D2 regions of the 28S rDNA with primers LR0R and LR5 (Vilgalys and Hester 1990), mtLSU with primers ML5 and ML6 (Bruns et al. 1998), *TEF*1 with primers EF983F and EF1567R (Rehner and Buckley 2005). PCR products were visualized on 1.5% agarose gels with SYBR Green I (Molecular Probes, Eugene, Oregon), and amplicons were cleaned for sequencing with EXO and SAP enzymes (Glenn and Schable 2005). DNA was sequenced with the same primers as above at the University of Florida Interdisciplinary Center for Biotechnology Research (ICBR). Based on preliminary BLAST results we determined that *S. pulvinata* was a member of Boletales. Sequences were compiled into nucleotide alignments for each gene (ITS rDNA, mtLSU, *TEF*1, and 28S rDNA) using sequence data from GenBank and from several published Boletales phylogenies (Binder and Hibbett 2006, Skrede et al. 2011). Preliminary sequence alignments were performed with MUSCLE (Edgar 2004) followed by manual adjustments in Mesquite 1.1 (Maddison and Maddison 2006). The data for each individual nucleotide alignment was as follows: 28S (972 characters analyzed, 216 parsimony informative characters), *TEF*1 (440 characters analyzed, 148 parsimony informative characters), ITS rDNA (470 characters analyzed, 140 parsimony-informative characters) and 28S (324 characters analyzed, 108 parsimony informative characters).

Each nucleotide alignment first was subjected to maximum parsimony (MP) analysis using the default parameters followed by bootstrapping with 1000 replicates with the ''fast, stepwise addition'' approach with the PAUP* software package (Swofford 2002). Maximum likelihood (ML) analysis was performed separately on each of the four loci using the GTR+I+G model followed by bootstrapping with 500 replicates using the GARLI software package (Zwickl 2006). To ensure a robust analysis of *TEF*1, this dataset was

TABLE II. Collections analyzed by isotope ratio mass spectrometry

Taxon	Location	Herbarium	Trophic group	$\delta^{15}N$ (‰)	%N	$\delta^{13}C$ (‰)	%C	C/N
Elaphomyces granulatus	Boulder, CO	OSC 44460	M	10.05	3.38	−23.8	33.64	9.95
Gautieria crispa	Larimer, CO	OSC 61399	M	9.41	2.01	−21.4	44.26	21.97
Gautieria crispa	Taos, NM	OSC 61395	M	17.05	4.79	−24.9	47.32	9.88
Gautieria monticola	Boulder, CO	OSC 44445	M	12.39	4.35	−23.4	45.91	10.54
Gautieria monticola	Yuba Pass, CA	OSC 44487	M	17.34	3.9	−24.0	45.16	11.57
Gautieria monticola	Donner Pass, CA	OSC 60056	M	10.70	2.38	−25.4	43.45	18.24
Gautieria monticola	Taos, NM	OSC 61398	M	13.32	3.97	−23.2	47.26	11.89
Geopora clausa	Montrose, CO	OSC 41486	M	3.84	3.03	−22.2	38.76	12.79
Rhizopogon hysterangioides	Grand, CO	OSC 44372	M	10.91	2.05	−24.3	48.69	23.77
Rhizopogon ochraceorubens	Clear Creek, CO	OSC 40838	M	10.36	1.81	−25.0	42.2	23.37
Rhizopogon subcaerulescens	Donner Pass, CA	OSC 63445	M	2.14	1.68	−25.7	45.61	27.12
Rhizopogon vulgaris	Donner Pass, CA	OSC 63424	M	3.12	2.64	−24.8	46.01	17.41
Armillaria viscidipes	Medicine Bow, WY	OSC 5796	P	12.40	2.32	−22.1	41.82	18.04
Agrocybe praecox	Yuba Pass, CA	OSC 50297	S	−0.91	2.68	−23.7	41.84	15.64
Fomitopsis cajanderi	Larimer, CO	OSC 35268	S	−2.03	1.25	−20.6	48.26	38.46
Fomitopsis cajanderi	Graham, AZ	OSC 35269	S	0.12	0.98	−17.8	45.67	46.76
Fomitopsis cajanderi	Pima, AZ	OSC 35270	S	−1.10	1.81	−18.4	44.29	24.45
Nivatogastrium nubigenum	Yuba Pass, CA	OSC 69802	S	−1.77	1.58	−19.9	42.33	26.76
Nivatogastrium nubigenum	Yuba Pass, CA	OSC 69803	S	−1.45	2.05	−20.4	44.28	21.63
Phellinus chrysoloma	Medicine Bow, WY	OSC 31677	S	−3.25	1.99	−18.9	48.36	24.28
Phellinus pini	Donner Pass, CA	OSC 34283	S	0.92	2.17	−17.5	46.14	21.26
Sedecula pulvinata	Boulder, CO	MICH 26629		4.28	3.11	−20.9	44.22	14.24
Sedecula pulvinata	Boulder, CO	MICH 26630		3.81	3.72	−21.1	45.0	12.11
Sedecula pulvinata	Boulder, CO	MICH 00340		4.01	3.57	−20.3	45.94	12.88
Sedecula pulvinata	Garfield, Utah	MICH 00329		−1.48	1.99	−19.7	30.37	15.27
Sedecula pulvinata	Yuba Pass, CA	MICH 00324		2.38	3.5	−21.1	42.72	12.19
Sedecula pulvinata	Yuba Pass, CA	OSC 39125		1.89	3.46	−19.9	39.03	11.27
Sedecula pulvinata	Yuba Pass, CA	MICH 00326		2.79	3.46	−22.1	43.76	12.65

Abbreviations: CO = Colorado. CA = California. NM = New Mexico. WY = Wyoming. AZ = Arizona. MAP = mean annual precipitation. MAT = mean annual temperature. Lat = latitude. Long = longitude. ATM = ospheric. Coll. = collection. Species names are as they appear on the voucher.

subjected to a separate ML analysis using RAxML on the CIPRES Science Gateway (www.phylo.org, Stamatkis 2006, Stamatakis et al. 2008). For this analysis the codon positions were partitioned and evaluated separately and the GTRGAMMA setting was used to determine the best ML tree and for rapid bootstrapping with 500 replicates.

We analyzed $\delta^{13}C$ and $\delta^{15}N$ signatures in tissue of *Sedecula* collections from California, Colorado and Utah that were archived at Oregon State University and University of Michigan herbaria. Reference samples of known mycorrhizal and saprotrophic taxa were similarly analyzed (TABLE II). Different ecotypes or regions can have different isotopic background profiles (Taylor et al. 2003), so reference samples collected from nearby or similar regions were employed. Although regional terricolous reference samples were not always available, Hobbie et al. (2012) found the differences in isotopic signatures between terricolous and lignicolous saprotrophs to be so small as not to influence results.

Samples were analyzed for $\delta^{15}N$, $\delta^{13}C$, %N, and %C on a ThermoFisher Delta-Plus isotope ratio mass spectrometer linked to a Carlo Erba NC2500 elemental analyzer (ThermoFisher GmbH, Bremen, Germany) at the University of New Hampshire Stable Isotope Lab. The internal standards for isotopic and concentration measurements were tuna, pine needles (NIST 1575a), orchard leaves (NIST 1515) and a ground mushroom standard. We report stable isotope abundances as $\delta^{15}N$ (or $\delta^{13}C$) = $(R_{sample}/R_{standard}\text{-}1) \cdot 1000‰$, where $R = {}^{15}N/{}^{14}N$ or ${}^{13}C/{}^{12}C$ of either the sample or the reference standard (atmospheric N_2 for nitrogen, PeeDee belemnite for carbon). The average precision of isotopic measurements of the standards was 0.17‰ for $\delta^{15}N$ and 0.13‰ for $\delta^{13}C$. When comparing between samples, samples with more of the heavy isotope are referred to as heavier, or enriched; samples with more of the light isotope are lighter, or depleted.

We tested a mixed linear regression model to assess what factors influenced $\delta^{13}C$. Because of known correlations between plant carbon isotope data and site altitude, precipitation, and latitude (Kohn 2010), these factors were included in regression models for their potential covariance with fungal $\delta^{13}C$. An additional correction for the Suess effect used 2000 as the reference year and yearly values of the $\delta^{13}C$ of atmospheric carbon dioxide from McCarroll and Loader (2004). Data were analyzed statistically in JMP (SAS Institute, Cary, North Carolina).

TABLE II. Extended

Lat	Long	Elev.	Coll. date	MAP (mm)	MAT (C)	ATM CO_2 $\delta^{13}C$	Suess Effect	$\delta^{13}C$ (‰) adjusted for Suess
40.07	−105.59	3200	8 Aug 1984	1103	−0.4	−7.54	0.46	−24.25
40.74	−105.61	2896	3 Sep 1978	631	2.7	−7.37	0.63	−21.98
36.13	−105.53	2835	10 Aug 1992	466	4.6	−7.77	0.23	−25.11
40.07	−105.59	3200	8 Aug 1984	1103	−0.4	−7.54	0.46	−23.83
39.58	−120.61	1646	12 Jun 1984	1170	8.9	−7.54	0.46	−24.46
39.34	−120.17	1800	1 Jun 1997	926	7.8	−7.91	0.09	−25.50
36.69	−105.40	2743	15 Aug 1992	468	3.1	−7.77	0.23	−23.39
38.59	−107.71	2286	5 Jul 1983	671	5.1	−7.51	0.49	−22.66
40.42	−105.81	3170	7 Aug 1984	1060	−0.9	−7.54	0.46	−24.78
39.68	−105.51	3200	19 Sep 1982	745	0.4	−7.48	0.52	−25.51
39.34	−120.17	2134	28 Jun 1996	1986	7.7	−7.88	0.12	−25.84
39.34	−120.17	2134	28 Jun 1996	1986	7.7	−7.88	0.12	−24.92
41.30	−106.18	2865	23 Aug 1923	848	2.1	−6.74	1.26	−23.31
39.32	−120.60	1743	8 Jun 1989	1488	8.3	−7.68	0.32	−24.06
40.65	−105.53	2365	25 Sep 1963	560	−2.3	−6.95	0.05	−21.64
32.70	−109.91	2896	20 Feb 1964	770	6.2	−6.98	1.02	−18.79
32.42	−110.74	2469	13 Jul 1963	1078	9.5	−6.95	1.05	−19.48
39.65	−120.60	2030	9 Jun 1999	1332	5.9	−7.96	0.04	−19.91
9.65	−120.60	2030	9 Jun 1999	1332	5.9	−7.96	0.04	−20.45
41.06	−106.15	2774	2 Oct 1914	706	2.2	−6.70	1.30	−20.22
39.25	−120.99	975	1 May 1928	1840	12.7	−6.76	1.24	−18.72
40.00	−105.30	1920	19 Aug 1979	449	8.3	−7.40	0.60	−21.53
40.00	−105.29	1920	31 Jul 1979	449	8.3	−7.40	0.60	−21.66
40.00	−105.29	1920	14 Aug 1978	449	8.3	−7.37	0.63	−20.96
37.82	−111.90	2679	7 Jul 1992	448	5.1	−7.77	0.23	−19.90
39.26	−120.38	1829	18 Aug 1982	2192	7.0	−7.48	0.52	−21.60
39.32	−120.60	1743	2 Sep 1969	2202	8.1	−7.12	0.88	−20.78
39.26	−120.38	1829	6 Oct 1982	2192	7.0	−7.48	0.52	22.60

RESULTS

Phylogenetic analyses based on all four DNA loci suggested that *Sedecula pulvinata* has affinities with the family Coniophoraceae and order Boletales (FIG. 1). Data from all four loci confirmed that *Sedecula pulvinata* is distantly related to members of Agaricales and also to *Scleroderma* and other genera of gasteroid fungi in Sclerodermataceae (*Pisolithus*, *Calostoma*). Phylogenies based on ML analysis of four loci (FIG. 1) all showed *S. pulvinata* nested within Coniophoraceae, although the placement of the species varied based on different genes. The ITS and 28S analyses with both the ML and MP methods resolved *Sedecula* within the genus *Coniophora*, but neither locus provided bootstrap support for a particular placement within *Coniophora*. The mtLSU dataset based on ML and MP resolved *Sedecula* as the sister group to the two species of *Coniophora* for which DNA sequences were available (*C. arida*, *C. puteana*). The ML analysis of *TEF*1 resolved *Sedecula* within the genus *Coniophora* with weak bootstrap support, but the two most parsimonious trees resolved *Sedecula* outside *Coniophoraceae* with weak bootstrap support. In the *TEF*1 analyses, bootstrap values were low for most nodes in both MP and ML analyses. Although ML trees generated using similar models in GARLI and RAxML had slightly different topologies and bootstrap support values, both ML trees produced a monophyletic *Coniophora* with *Sedecula* resolved inside *Coniophora* and as sister to the *C. olivacea* clade with weak bootstrap support (FIG. 1).

In our regression models the model with the highest adjusted r^2 included trophic status ($p = 0.002$), a correction for the Suess effect ($P = 0.025$), latitude ($P = 0.106$), and an interactive term including the Suess effect and trophic status ($P = 0.205$) (TABLE III). *Sedecula* samples did not significantly differ from saprotrophic samples in $\delta^{13}C$ or $\delta^{15}N$ but did differ from mycorrhizal samples (TABLES IV, V).

Zeller (1941) listed spore dimensions from the sole collection of *Sedecula pulvinata* as 23–26 × 13–16.2 μm. With more specimens now available, we observed spore sizes of 18–12 μm × 27–20 μm and thus amend the spore dimensions to (18–)23–26(–27) × (12–)13–16(–20) μm.

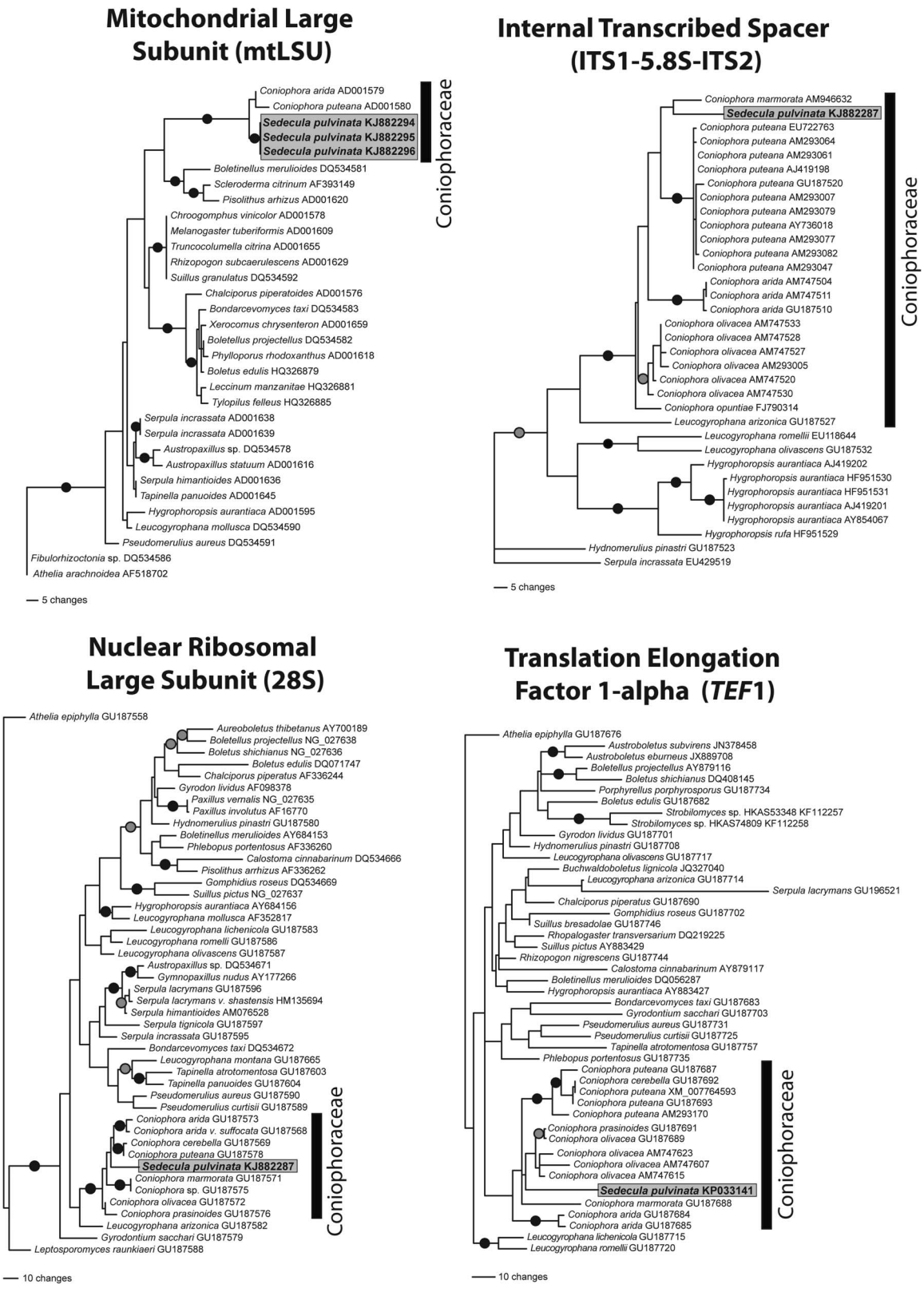

FIG. 1. Maximum likelihood (ML) phylogenies based on four loci (*TEF*1, mtLSU, 28S and ITS) depict the phylogenetic placement of *Sedecula pulvinata* within the family Coniophoraceae. Filled black circles denote nodes supported ≥ 70% bootstrap values for both ML and maximum parsimony (MP) analyses, whereas filled gray circles were supported by only one of the two methods. TreeBASE submission 16950.

TABLE III. Regression model of $\delta^{13}C$ values for sporocarps

Term	Estimate±se	Prob>|t|	(%)	Prob> F
Intercept	−14.85±4.62	0.0044		
Group	—	—	60	**0.0016**
Mycorrhizal	−1.68±0.39	**0.0004**		
Saprotrophic	1.03±0.39	**0.0159**		
Sedecula	[1]0.65			
Suess effect	2.69±1.11	**0.0253**	19.3	**0.0253**
Suess effect • Group	—	—	11.3	0.2048
Suess effect • Mycorrhizal	2.91±1.58	0.0808		
Suess effect • Saprotrophic	−0.72±1.26	0.5717		
Suess effect • *Sedecula*	[1]−2.19			
Latitude	−0.20±0.12	0.1061	9.4	0.1061

Adjusted $r^2 = 0.761$, n = 27, $P < 0.0001$. se = standard error.
[1] Calculated from the coefficients as *Sedecula* = Mycorrhizal, Saprotrophic. Values significant at $\alpha < 0.05$ shown in boldface.

DISCUSSION

Our DNA analysis suggests that *Sedecula* falls within the Coniophoraceae and is phylogenetically distant from other ectomycorrhizal and gasteroid fungi in Boletales as well as members of the Agaricales, where this taxon is currently placed. Although the exact phylogenetic position within the family Coniophoraceae could not be determined based on our analyses (FIG. 1), *Sedecula* could be sister to the entire genus *Coniophora* (mtLSU) or might be nested within *Coniophora* (ITS, partial 28S, *TEF*1). Although our MP analysis of *TEF*1 was incongruent with the other analyses and placed *Sedecula* outside Coniophoraceae, there was no bootstrap support for this placement. Furthermore, the *TEF*1 analysis generally did not resolve well-established relationships detected in Boletales based on other loci and analyses (Binder and Hibbett 2006, Skrede et al. 2011). The overwhelming evidence supports *Sedecula* as a member of Coniophoraceae, but more analyses based on additional and more informative loci are needed to determine the exact placement of this species.

Binder and Hibbett (2006) noted that gasteromycetation occurs in most lineages of Boletales except Tapinellineae, Coniophoraceae and Hygrophoropsidaceae, which are basal to Boletales and dominated by resupinate sporocarps. Most authors place four genera within Coniophoraceae: *Coniophora*, *Gyrodontium*, *Coniophoropsis* and *Chrysoconia* (Indexfungorum.org). *Coniophora* is a diverse and widespread genus. Species in this group form resupinate fruiting bodies on wood. *Coniophoropsis obscura* is a monotypic genus of resupinate fungi that are thought to belong to Coniophoraceae, but this group has not been included in molecular analyses (Larsson 2007). *Gyrodontium sacchari* is a widespread polypore that forms pileate to resupinate fruiting bodies with a hydnoid hymenophore (Robledo et al. 2014). The fungus *Chrysoconia orthospora* also was described in a monotypic genus in Coniophoraceae (McCabe and Escobar 1979). This fungus forms small (0.1–0.2 mm diam) hemispherical to dendritic basidiocarps with exposed, ballistosporic basidia. This species grows on wood and has smooth, brown spores with a cyanophilous wall layer (McCabe and Escobar 1979). However, the phylogenetic position of this fungus has not been evaluated and this species might fall outside Boletales. In addition, the genus *Leucogyrophana* is highly polyphyletic but at least one species, *L. arizonica*, is allied with Coniophoraceae (Skrede et al. 2011). Our phylogenetic analyses indicate that Coniophoraceae does indeed include a gasteromycete member and *Sedecula* might in fact represent one of the earlier non-resupinate taxa in the evolution of Boletales. Although we cannot be certain, it seems likely that the gasteroid *Sedecula* evolved from resupinate or polyporoid ancestors in the Coniophoraceae. We know of only one other case of a truffle-like fungus that likely was derived from resupinate ancestors. That taxon, *Stephanospora*, putatively evolved from resupinate

TABLE IV. Carbon, nitrogen and isotopic measurement means with standard deviations

Group (n)	$\delta^{15}N$	N%	$\delta^{13}C$	C%	C/N
Mycorrhizal (12)	9.9 (4.4)^A	3.5 (1.5)^A	−24.3 (1.3)^A	44.2 (3.7)^A	14.8 (6.6)^A
Saprotrophic (8)	−1.2 (1.3)^B	1.8 (0.5)^B	−19.7 (2.0)^B	45.0 (2.4)^A	27.4 (10.2)^B
Sedecula (7)	2.5 (2.0)^B	3.3 (0.6)^A	−20.7 (0.8)^B	41.6 (5.4)^A	12.9 (1.4)^A

Statistical differences of $\alpha < 0.05$ among the three groups are indicated by superscript letters following values.

TABLE V. Tukey post-hoc test for differences in means between trophic groups

Comparison	$\delta^{15}N$	%N	$\delta^{13}C$	%C	C:N
Mycorrhizal vs. Saprotrophic	<0.0001	0.0124	<0.0001	0.8242	0.0062
Mycorrhizal vs. *Sedecula*	0.0005	0.7908	0.0003	0.4407	0.5319
Saprotrophic vs. *Sedecula*	0.1307	0.0071	0.3512	0.2386	0.0014

ancestors allied with *Lindtneria* in the Stephanosporaceae (Lebel et al. 2015).

Most mycorrhizal reference samples in our analysis were high in $\delta^{15}N$ (to > 9‰) and low in $\delta^{13}C$, from −27‰ to −22‰ (TABLE II). In contrast, all saprotrophic reference samples had $\delta^{15}N$ values of −5‰ to 5‰, and $\delta^{13}C$ values of −17‰ to −22‰. Samples of *Sedecula pulvinata* fell within the range occupied by saprotrophic reference samples, with $\delta^{15}N$ values of −5 to 5‰, and $\delta^{13}C$ values of −19‰ to −22‰ (FIG. 2). Conversely, while *Sedecula* grouped more closely with saprotrophic fungi than ectomycorrhizal fungi in $\delta^{15}N$, it was less depleted in ^{15}N than most of the saprotrophic samples. Some overlap of isotopic signatures between mycorrhizal and saprotrophic fungi might be possible, based on overlapping functionalities. For example, some mycorrhizal fungi can decompose organic soil carbon (Talbot et al. 2008) and some saprotrophic fungi form mantles on root tips (Vasiliauskas et al. 2007). Taylor et al. (2003) reported that the $\delta^{15}N$ values of terricolous saprotrophs were closer to those of mycorrhizal fungi than other saprotrophs, however, their $\delta^{13}C$ signature clearly associated them with other saprotrophic fungi. Although the Suess effect significantly affected $\delta^{13}C$, it did not alter the relative ordering in $\delta^{13}C$ of saprotrophic fungi, mycorrhizal fungi and *Sedecula*.

The argument could be made that *Sedecula pulvinata* should be considered a member of the genus *Coniophora*. However, we refrain from proposing nomenclatural changes here due to the unresolved position of *Coniophora* in our phylogenies and the significant morphological differences between *Sedecula* and *Coniophora*. *Sedecula* is almost certainly saprotrophic based on its phylogenetic position and its isotopic similarity to known saprotrophic fungi. Because of this unique phylogenetic position within a lineage representing mostly resupinate saprotrophs forming brown rot, we suggest that *Sedecula pulvinata* should be cultured on axenic media, have its genome sequenced and be studied in the laboratory to understand more about its evolution and development.

ACKNOWLEDGMENTS

The authors thank Greg Bonito, Jonathan Frank and Darlene Southworth for preliminary sequencing. Michael Wood generously provided a color photo of *Sedecula*

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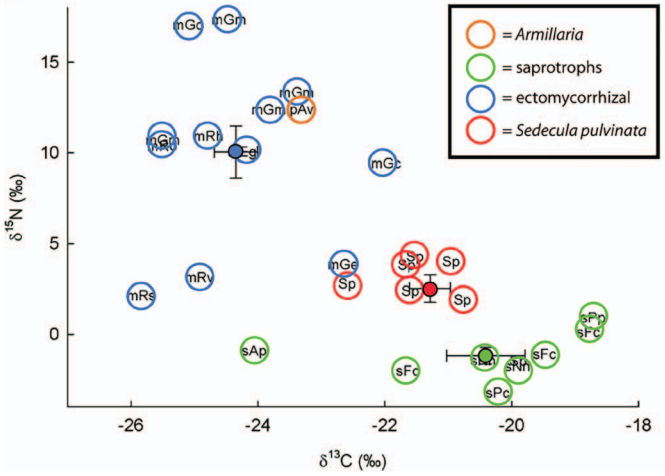

3

FIG. 2. $\delta^{13}C$ and $\delta^{15}N$ values of *Sedecula* and reference samples, adjusted for the Suess effect to a common year of 2000. Trophic group is indicated by the first lowercase m (= mycorrhizal), p (= parasitic), or s (= saprotrophic) prefix; *Sedecula pulvinata* has no prefix. The first letters are of the genus (in uppercase) and species names (TABLE II) with the exception of Ge for *Geopora clausa*. For the three groups, mean ± SE is also plotted with error bars.

FIG. 3. Basidiocarp of *Sedecula pulvinata* from mixed conifer forest near Yuba Pass, Sierra County, California (Oct 1998). Photo and collection by Michael Wood.

pulvinata for use in this paper. Grant NSF-0843366 to E. Hobbie supported a portion of this work. Participation of M. E. Smith was financially supported in part by the University of Florida's Institute for Food and Agricultural Sciences (IFAS). MICH and OSC herbaria generously provided sample specimens for analysis. Two reviewers provided helpful suggestions to improve the manuscript.

LITERATURE CITED

Agerer R. 1999. Never change a functionally successful principle: the evolution of Boletales s.l. (Hymenomycetes, Basidiomycota) as seen from below-ground features. Sendtnera 6:5–91.

Barroetaveña C, Cázares E, Rajchenberg M. 2007. Ectomycorrhizal fungi associated with ponderosa pine and Douglas-fir: a comparison of species richness in native western North American forests and Patagonian plantations from Argentina. Mycorrhiza 17:355–373, doi:10.1007/s00572-007-0121-x. 10 p.

Binder M, Bresinsky A. 2002. Derivation of a polymorphic lineage of Gasteromycetes from boletoid ancestors. Mycologia 94:85–98, doi:10.2307/3761848

———, Hibbett DS. 2006. Molecular systematics and biological diversification of Boletales. Mycologia 98: 971–981, doi:10.3852/mycologia.98.6.917

Bruns T, Szaro T, Gardes M, Cullings K, Pan J, Taylor DL, Horton TR, Kretzer A, Garbelotto M, Li Y. 1998. A sequence database for the identification of ectomycorrhizal basidiomycetes by phylogenetic analysis. Mol Ecol 7:257–272.

Castellano MA, Smith JE, O'Dell TE, Cázares E, Nugent S. 1999. Handbook to strategy 1 fungal species in the Northwest Forest Plan. Gen. Tech. Rep. PNW-GTR-476. Portland, Oregon: Department of Agriculture, Forest Service, Pacific Northwest Research Station. 195 p.

Edgar Robert C. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res 32.5:1792–1797, doi:10.1093/nar/gkh340

Gardes M, Bruns TD. 1993. ITS primers with enhanced specificity for basidiomycetes—application to the identification of mycorrhizae and rusts. Mol Ecol 2:113–118, doi:10.1111/j.1365-294X.1993.tb00005.x

Glenn T, Schable N. 2005. Isolating microsatelline DNA loci. In: Zimmer EA, Roalson EH, eds. Molecular evolution: producing the biochemical data, part B. San Diego, California: Academic Press. p 202–222.

Guzmán G. 1971. A new delimitation of probable phylogeny of the family *Sclerodermataceae*. Trans Br Mycol Soc 56: 195–199, doi:10.1016/S0007-1536(71)80030-X

Hall DS. 1991. Diet of the northern flying squirrel at Sagehen Creek, California. J Mamm 72:615–617, doi:10.2307/1382146

Hobbie EA. 2005. Using isotopic tracers to follow carbon and nitrogen cycling of fungi. In: Dighton J, Oudemans P, White J, eds. The fungal community: its organization and role in the ecosystem. 3rd ed. New York: Taylor & Francis. p 361–381.

———, Högberg P. 2012. Nitrogen isotopes link mycorrhizal fungi and plants to nitrogen dynamics. New Phytol 196:367–382, doi:10.1111/j.1469-8137.2012.04300.x

———, Sánchez FS, Rygiewicz PT. 2012. Controls of isotopic patterns in saprotrophic and ectomycorrhizal fungi. Soil Biol Biochem 48:60–68, doi:10.1016/j.soilbio.2012.01.014

———, van Diepen LTA, Lilleskov EA, Ouimette AP, Finzi AC, Hofmockel KS. 2014. Fungal functioning in a pine forest: evidence from a ^{15}N-labeled global change experiment. New Phytol 201:1431–1439, doi:10.1111/nph.12578

———, Weber NS, Trappe JM. 2001. Mycorrhizal vs saprotrophic status of fungi: the isotopic evidence. New Phytol 150:601–610, doi:10.1046/j.1469-8137.2001.00134.x

Kohn MJ. 2010. Carbon isotope compositions of terrestrial C3 plants as indicators of (paleo) ecology and (paleo) climate. Proc Natl Acad Sci 107:19691–19695, doi:10.1073/pnas.1004933107

Kotter MM, Farentinos RC. 1984a. Formation of ponderosa pine ectomycorrhizae after inoculation with feces of tassel-eared squirrels. Mycologia 76:758–760, doi:10.2307/3793237

———, ———. 1984b. Tassel-eared squirrels as spore dispersal agents of hypogeous mycorrhizal fungi. J Mamm 65:684–687, doi:10.2307/1380853

Larsson K-H. 2007. Rethinking the classification of corticioid fungi. Mycol Res 111:1040–1063, doi:10.1016/j.mycres.2007.08.001

Lebel T, Beever RE, Castellano MA. 2015. Cryptic diversity in the sequestrate genus *Stephanospora* (Stephanosporaceae: Agaricales) in Australasia. Fungal Divers 119:201–228, doi:10.1016/j.funbio.2014.12.007 (in press).

Lehmkuhl JF, Gould LE, Cázares E, Hosford DR. 2004. Truffle abundance and mycophagy by northern flying squirrels in eastern Washington forests. For Ecol Manage 200:49–65, doi:10.1016/j.foreco.2004.06.006

Lindahl BD, Ihrmark K, Boberg J, Trumbore SE, Högberg P, Stenlid J, Finlay RD. 2007. Spatial separation of litter decomposition and mycorrhizal nitrogen uptake in a boreal forest. New Phytol 173:611–620, doi:10.1111/j.1469-8137.2006.01936.x

Maddison WP, Maddison DR. 2006. Mesquite 1.12: a modular system for evolutionary analysis. http://mesquiteproject.org.

Mayor JR, Schuur EAG, Henkel TW. 2009. Elucidating the nutritional dynamics of fungi using stable isotopes. Ecol Lett 12:171–183, doi:10.1111/j.1461-0248.2008.01265.x

McCabe DE, Escobar GA. 1979. *Chyrosoconia*, a gasteroid member of the Coniophoraceae. Mycotaxon 9:239–242.

McCarroll D, Loader NJ. 2004. Stable isotopes in tree rings. Quat Sci Rev 23:771–801, doi:10.1016/j.quascirev.2003.06.017

Molina R, Massicotte H, Trappe JM. 1992. Specificity phenomena in mycorrhizal symbioses: community-ecological consequences and practical implications. In: Allen MF, ed. Mycorrhizal functioning: an integrative plant-fungal process. New York: Chapman & Hall. p 357–423.

Rehner SA, Buckley A. 2005. *Beauveria* phylogeny inferred from nuclear ITS and EF1-α sequences: evidence for cryptic diversification and links to *Cordyceps* teleomorphs. Mycologia, 97:84–98, doi:10.3852/mycologia.97.1.84.

Robledo GL, Giorgio EM, Franco CRP, Popoff O, Decock C. 2014. *Gyrodontium sacchari* (Spreng.: Fr.) Hjortstam (Boletales, Basidiomycota) in America: New records and its geographic distribution. Check List 10:1514–1519.

Skrede I, Engh IB, Binder M, Carlsen T, Kauserud H, Bendiksby M. 2011. Evolutionary history of Serpulaceae (Basidiomycota): molecular phylogeny, historical biogeography and evidence for a single transition of nutritional mode. BMC Evol Biol 11:230, doi:10.1186/1471-2148-11-230

Smith AH. 1951. Puffballs and their allies in Michigan. Ann Arbor: Univ. Michigan Press. 104 p.

Stamatakis A. 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22:2688–2690, doi:10.1093/bioinformatics/btl446

———, Hoover P, Rougemont J. 2008. A rapid bootstrap algorithm for the RAxML web-servers. Syst Biol 75:758–771.

Stanikunaite R, Trappe JM, Khan SI, Ross SA. 2007. Evaluation of therapeutic activity of hypogeous ascomycetes and basidiomycetes from North America. Int J Med Mush 9:7–14, doi:10.1615/IntJMedMushr.v9.i1.20

States JS. 1984. New records of false truffles in pine forest of Arizona. Mycotaxon 19:351–367.

———, Gaud WS. 1997. Ecology of hypogeous fungi associated with ponderosa pine I. Patterns of distribution and sporocarp production in some Arizona forests. Mycologia 89:712–721.

Swofford DL. 2002. PAUP* 4.0: phylogenetic analysis using parsimony (*and other methods) Sunderland: Massachusetts: Sinauer Associates.

Talbot JM, Allison SD, Treseder KK. 2008. Decomposers in disguise: mycorrhizal fungi as regulators of soil C dynamics in ecosystems under global change. Funct Ecol 22:955–963, doi:10.1111/j.1365-2435.2008.01402.x

Taylor AFS, Fransson PM, Högberg P, Högberg MN, Plamboeck AH. 2003. Species level patterns in ^{13}C and ^{15}N abundance of ectomycorrhizal and saprotrophic fungal sporocarps. New Phytol 159:757–774, doi:10.1046/j.1469-8137.2003.00838.x.

Tedersoo L, Smith ME. 2013. Lineages of ectomycorrhizal fungi revisited: Foraging strategies and novel lineages revealed by sequences from belowground. Fungal Biol Rev 27:83–99, doi:10.1016/j.fbr.2013.09.001

Thiers HD. 1984. The secotioid syndrome. Mycologia 76:1–8, doi:10.2307/3792830

Trappe MJ, Evans F, Trappe JM. 2007. Field guide to North American truffles: hunting, identifying, and enjoying the world's most prized fungi. Berkeley, California: Ten Speed Press. 144 p.

Vasiliauskas R, Menkis A, Finlay RD, Stenlid J. 2007. Wood-decay fungi in fine living roots of conifer seedlings. New Phytol 174:441–446, doi:10.1111/j.1469-8137.2007.02014.x

Vilgalys R, Hester M. 1990. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. J Bacteriol 172:4239–4246.

Waters JR, McKelvey KS, Luoma DL, Zabel CJ. 1997. Truffle production in old-growth and mature fir stands in northeastern California. For Ecol Manage 96:155–166, doi:10.1016/S0378-1127(97)00016-9

Zeller SM. 1941. Further notes on fungi. Mycologia 33:196–214, doi:10.2307/3754932

———. 1948. Notes on certain Gasteromycetes, including two new orders. Mycologia 40:639–668, doi:10.2307/3755316

———. 1949. Keys to the orders, families and genera of the Gasteromycetes. Mycologia 41:36–58, doi:10.2307/3755271

Zwickl DJ. 2006. Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion [doctoral dissertation]. Austin: Univ. Texas. 115 p.