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Sedecula

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* (Coniophoraceae, Boletales). Stable isotope comparisons with known ectomycorrhizal and saprotrophic fungi together with phylogenetic evidence also suggest that *S. pulvinata* is saprotrophic and that this genus represents a unique morphological transition from a resupinate basidiocarp morphology (in *Coniophora* and relatives) to 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 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, *S. pulvinata*, are from the periphery of the Great Basin, from the Sierra Nevada mountains of eastern California (Hall 1991, Waters et al. 1997) to southeastern Oregon (D. Pankratz, pers. comm.) and southern Idaho (Stanikunaite et al. 2007), and from northern Arizona (States 1984, States and Gaud 1994) through western Colorado (Kotter and Farentinos 1984a). It has also 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 first described by Zeller (1941) who placed it in the family Sclerodermataceae based on its thick leathery peridium, glebal 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, Zeller 1949). Smith (1951) and Guzman (1971) concurred with Zeller's assessment, but Thiers (1971) 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 specifically 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. However, since the ectomycorrhizal nutritional mode is conserved within fungal lineages, phylogenetic relationships have proven useful for distinguishing ectomycorrhizal fungi from non-ectomycorrhizal relatives (Tedersoo & 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) has also 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

70 from the higher $\delta^{13}C$ values in wood and litter cellulose that supply saprotrophic fungi compared
71 to the plant sugars transferred to ectomycorrhizal fungi (Hobbie 2005). In contrast,
72 ectomycorrhizal fungi are usually higher in $\delta^{15}N$ than saprotrophic fungi. Nutritional sources
73 contribute part of this difference, with saprotrophic fungi often assimilating nitrogen from ^{15}N-
74 depleted wood or litter whereas ectomycorrhizal fungi are generally active in deeper soil
75 horizons (Lindahl et al. 2007; Hobbie et al. 2014). In addition, transfer of ^{15}N-depleted nitrogen
76 from ectomycorrhizal fungi to host plants leads to ^{15}N enrichment of the nitrogen remaining in
77 ectomycorrhizal fungi (Hobbie and Högberg 2012).
78 Stable carbon isotope analyses to determine ectomycorrhizal or saprotrophic status rely
79 on the carbon sources (primarily complex carbohydrates in wood or litter for saprotrophic fungi
80 and simple sugars for ectomycorrhizal fungi) for these two life history strategies having different
81 carbon isotope values. However, because altitude, water stress, and other climatic factors can
82 influence the discrimination against $^{13}CO_2$ in primary photosynthesis (Kohn et al. 2010), sample
83 data from herbarium specimens should be normalized to common conditions if it is derived from
84 different locations. In addition, the combustion of fossil fuels of C3 origin to carbon dioxide has
85 changed the $\delta^{13}C$ of atmospheric CO_2 from -6.5‰ in the pre-Industrial era to about -8.2‰ today
86 (the Suess effect; McCarroll & Loader 2004), with a continuing annual decrease of 0.03‰.
87 Accordingly, $\delta^{13}C$ data on samples from different years may also need to be normalized to
88 account for changes in the source CO_2 used in photosynthesis.
89 Neither the trophic mode nor the phylogenetic affiliations of *S. pulvinata* have been
90 studied to date, so the closest relatives and main ecological role of this fungus remains a mystery.
91 Here we analyze the phylogenetic relationships of *Sedecula pulvinata* based on several genes
92 (translation elongation factor 1-a (EF1a), mitochondrial large subunit (mtLSU), the internal

93 transcribed spacer region (ITS) and the ribosomal large subunit (LSU)) to establish its taxonomic
94 placement and employ isotopic analysis to gain insights to its trophic status.
95 MATERIALS AND METHODS
96 Sporocarp tissue (TABLE I) was ground with a micropestle and DNA was extracted with a
97 modified CTAB method (Gardes and Bruns 1993). We performed PCR using published methods
98 for the following loci: ITS with primers ITS1F and ITS4 (Gardes and Bruns 1993), LSU with
99 primers LROR and LR5 (Vilgalys and Hester, 1990), mtLSU with primers ML5 and ML6 (Bruns
100 et al., 1998), EF1a with primers EF983F and EF1567R (Rehner and Buckley, 2005). PCR
101 products were visualized on 1.5% agarose gels with SYBR Green I (Molecular Probes, Eugene,
102 Oregon, USA) and amplicons were cleaned for sequencing with EXO and SAP enzymes (Glenn
103 and Schable 2005). DNA was sequenced with the same primers as above at the University of
104 Florida Interdisciplinary Center for Biotechnology Research (ICBR). Sequences were manually
105 examined and edited with Sequencher v.4.1 (Gene Codes, Ann Arbor, Michigan, USA).
106 Sequences were then compiled into nucleotide alignments for each gene (ITS, mtLSU, EF1a, and
107 LSU) using sequence data from GenBank and from several published phylogenies (Binder and
108 Hibbett 2006, Skrede et al. 2011). Each nucleotide alignment was subjected to Maximum
109 Parsimony (MP) analysis with the PAUP* software package (Swofford 2002) and Maximum
110 Likelihood (ML) analysis using the GTR+I+G model using the GARLI software package
111 (Zwickl 2006). Consistency of relationships was then evaluated based on 500 bootstraps with
112 both ML and MP methods. Analyses of the LSU rDNA and the EF1a loci contained mostly the
113 same taxa and the phylogenies for these two individual genes had no supported incongruence, so
114 they were concatenated and analyzed together in a single matrix (1465 characters, 380 parsimony
115 informative characters). Unfortunately, the ITS (470 characters, 140 parsimony informative

characters) and mtLSU (377 characters, 132 parsimony informative characters) datasets contained mostly different species so they had to be analyzed separately.

We analyzed $\delta^{13}C$ and $\delta^{15}N$ signatures in tissue of *Sedecula* collections from California, Colorado, and Utah that were archived in the 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.

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 $\delta^{13}C$) = $(R_{sample}/R_{standard}-1) \bullet 1000‰$, 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 et al. 2010), these factors were also 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). Statistical analysis used JMP (SAS Institute, Cary, North Carolina).

RESULTS

Phylogenetic analyses based on all four DNA loci place *Sedecula pulvinata* in the family Coniophoraceae and order Boletales with strong MP and ML bootstrap support (FIG. 1). Data from all four loci confirm that *Sedecula pulvinata* is distantly related to all members of Agaricales and also to *Scleroderma* and other genera of gasteroid fungi in Sclerodermataceae (*Pisolithus, Calostoma*). Although our three different phylogenies all show *S. pulvinata* nested within Coniophoraceae, *Sedecula* is placed on a long branch in both the ITS and the EF1a + LSU phylogenies and none of the phylogenetic analyses could resolve the placement of *Sedecula* within Coniophoraceae. Although only ML phylogenies are depicted in FIG. 1, MP analyses produced trees with similar overall topologies and also resolved *Sedecula* in the Coniophoraceae.

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 ($p = 0.205$), as given in 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 and V).

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

DISCUSSION

Our DNA analysis indicates 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* and more closely related to *C. arida* or *C. puteana* (ITS rDNA, EF1a + LSU). Binder and Hibbett (2006) noted that gasteromycetation occurs in most lineages of Boletales except Tapinellineae, Coniophorineae and Hygrophoropsidaceae, which are basal to Boletales and dominated by resupinate sporocarps. This work indicates that Coniophorineae does indeed include a gasteromycete member, and *Sedecula* may in fact represent one of the earlier non-resupinate taxa in the evolution of Boletales.

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‰ (TABLES II and III). 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. There is evidence that the mycorrhizal/saprotrophic divide may not be absolute, with some mycorrhizal fungi demonstrating the ability to decompose organic soil carbon (Talbot et al. 2008) and some saprotrophic fungi forming 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 and ecological differences between *Sedecula* and *Coniophora*. *Sedecula* is almost certainly saprotrophic based on its phylogenetic position and its isotopic similarity to known saprotrophic fungi. *Sedecula* is also distantly related to other sequestrate fungi and apparently represents an independent evolutionary transition to a gasteroid fruiting body (FIG. 3). Because of this unique phylogenetic position within a lineage representing mostly resupinate saprotrophs 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.

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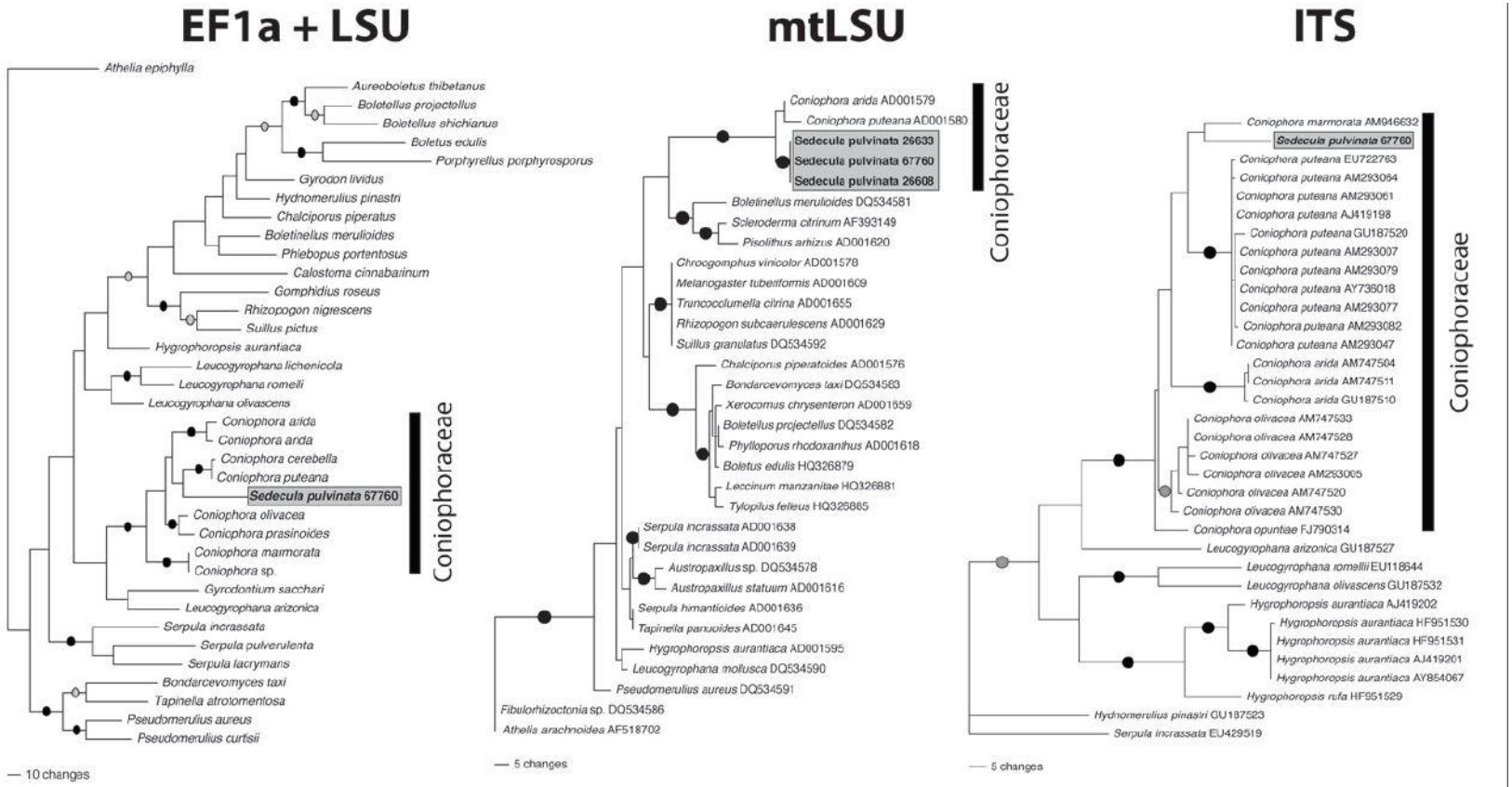
345 FIGURES AND TABLES

346 FIGURE 1. Three Maximum Likelihood phylogenies depict the phylogenetic placement of *Sedecula pulvinata* within the family
347 Coniophoraceae based on combined analysis of elongation factor 1 alpha and ribosomal large subunit (likelihood score = -lnL
348 12773.52, left), mitochondrial large subunit (likelihood score = -lnL 2270.938, middle), and internal transcribed spacer region
349 (likelihood score = -lnL 2867.627, right).



350

FIGURE 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 lower case m (=mycorrhizal), p (=parasitic), or s (=saprotrophic) prefix; *Sedecula pulvinata* has no prefix. The first letters are given of the genus (in upper case) and species names as listed in TABLE II with the exception of Ge for *Geopora clausa*. For the three groups, mean±SE is also plotted with error bars.

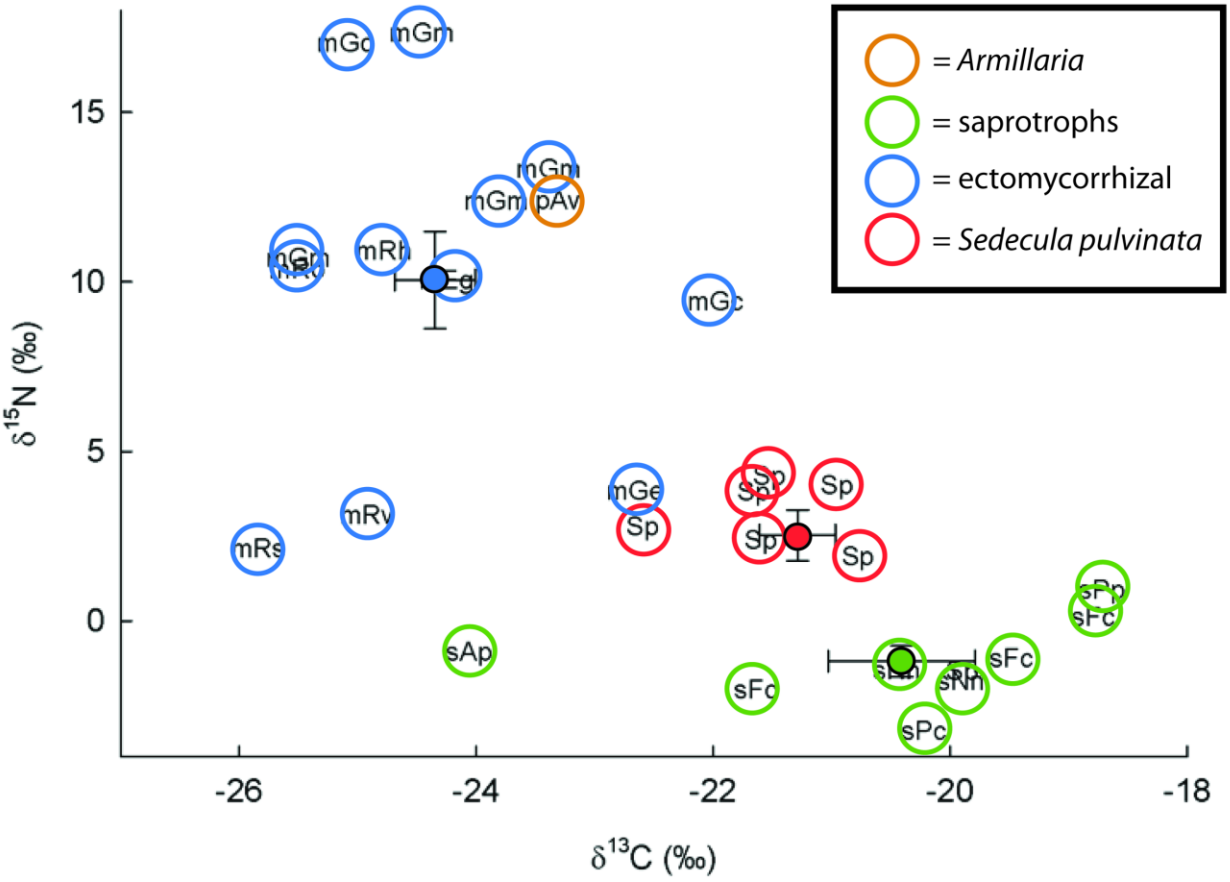


FIGURE 3. *Sedecula pulvinata* basidiocarp. Michael Wood photo.

TABLE I. GenBank accession numbers of sample sequences.

MICH#	Locality	EF1a+LSU	mtLSU	ITS
26608	Washington, UT	XXXXXX	XXXXXX	XXXXXX
26633	Boulder, CO	XXXXXX	XXXXXX	XXXXXX
67760	San Miguel, NM	XXXXXX	XXXXXX	XXXXXX

371 TABLE II. Collections analyzed by isotope ratio mass spectrometry. Abbreviations: CO, Colorado; CA, California; NM, New Mexico; WY,

372 Wyoming; AZ, Arizona; MAP, Mean Annual Precipitation; MAT, Mean Annual Temperature.

Taxon	Location	Herbarium #	Trophic Group	$\delta^{15}N$ (‰)	N%	$\delta^{13}C$ (‰)	C%	C/N	Lat	Long	Elev (m)	Coll. Date	MAP (mm)	MAT (C°)	ATM CO_2 $\delta^{13}C$	Suess Effect	$\delta^{13}C$ (‰) adjusted for Suess
Elaphomyces granulatus	Boulder, CO	OSC 44460	M	10.1	3.38	-23.8	33.64	9.95	40.07	-105.59	3200	8 Aug 1984	1103.0	-0.4	-7.54	0.46	-24.25
Gautieria crispa	Larimer, CO	OSC 61399	M	9.4	2.01	-21.4	44.26	21.97	40.74	-105.61	2896	3 Sep 1978	630.7	2.7	-7.37	0.63	-21.98
Gautieria monticola	Boulder, CO	OSC 44445	M	12.4	4.35	-23.4	45.91	10.54	40.07	-105.59	3200	8 Aug 1984	1103.0	-0.4	-7.54	0.46	-23.83
Geopora clausa	Montrose, CO	OSC 41486	M	3.8	3.03	-22.2	38.76	12.79	38.59	-107.71	2286	5 Jul 1983	671.2	5.1	-7.51	0.49	-22.66
Rhizopogon hysterangioides	Grand, CO	OSC 44372	M	10.9	2.05	-24.3	48.69	23.77	40.42	-105.81	3170	7 Aug 1984	1060.1	-0.9	-7.54	0.46	-24.78
Gautieria monticola	Yuba Pass, CA	OSC 44487	M	17.3	3.9	-24.0	45.16	11.57	39.58	-120.61	1646	12 Jun 1984	1169.8	8.9	-7.54	0.46	-24.46
Gautieria monticola	Donner Pass, CA	OSC 60056	M	10.7	2.38	-25.4	43.45	18.24	39.34	-120.17	1800	1 Jun 1997	926.5	7.8	-7.91	0.09	-25.50
Rhizopogon subcaerulescens	Donner Pass, CA	OSC 63445	M	2.1	1.68	-25.7	45.61	27.12	39.34	-120.17	2134	28 Jun 1996	1985.5	7.7	-7.88	0.12	-25.84
Rhizopogon vulgaris	Donner Pass, CA	OSC 63424	M	3.1	2.64	-24.8	46.01	17.41	39.34	-120.17	2134	28 Jun 1996	1985.5	7.7	-7.88	0.12	-24.92
Gautieria crispa	Taos, NM	OSC 61395	M	17.1	4.79	-24.9	47.32	9.88	36.13	-105.53	2835	10 Aug 1992	465.7	4.6	-7.77	0.23	-25.11
Gautieria monticola	Taos, NM	OSC 61398	M	13.3	3.97	-23.2	47.26	11.89	36.69	-105.40	2743	15 Aug 1992	467.7	3.1	-7.77	0.23	-23.39
Rhizopogon ochraceorubens	Clear Creek, CO	OSC 40838	M	10.4	1.81	-25.0	42.2	23.37	39.68	-105.51	3200	19 Sep 1982	745.0	0.4	-7.48	0.52	-25.51
Armillaria viscidipes	Medicine Bow, WY	OSC 5796	P	12.4	2.32	-22.1	41.82	18.04	41.30	-106.18	2865	23 Aug 1923	847.9	2.1	-6.74	1.26	-23.31
Agrocybe praecox	Yuba Pass, CA	OSC 50297	S	-0.9	2.68	-23.7	41.84	15.64	39.32	-120.60	1743	8 Jun 1989	1487.9	8.3	-7.68	0.32	-24.06
Fomitopsis cajanderi	Larimer, CO	OSC 35268	S	-2.0	1.25	-20.6	48.26	38.46	40.65	-105.53	2365	25 Sep 1963	559.9	-2.3	-6.95	1.05	-21.64
Phellinus chrysoloma	Medicine Bow, WY	OSC 31677	S	-3.3	1.99	-18.9	48.36	24.28	41.06	-106.15	2774	2 Oct 1914	706.1	2.2	-6.7	1.3	-20.22
Nivatogastreum nubigenum	Yuba Pass , CA	OSC 69802	S	-1.8	1.58	-19.9	42.33	26.76	39.65	-120.60	2030	9 Jun 1999	1332.3	5.9	-7.96	0.04	-19.91
Nivatogastreum nubigenum	Yuba Pass, CA	OSC 69803	S	-1.5	2.05	-20.4	44.28	21.6	39.65	-120.60	2030	9 Jun 1999	1332.3	5.9	-7.96	0.04	-20.45
Phellinus pini	Donner Pass, CA	OSC 34283	S	0.9	2.17	-17.5	46.14	21.26	39.25	-120.99	975	1 May 1928	1839.5	12.7	-6.76	1.24	-18.72
Fomitopsis cajanderi	Graham, AZ	OSC 35269	S	0.1	0.98	-17.8	45.67	46.76	32.70	-109.91	2896	20 Feb 1964	770.1	6.2	-6.98	1.02	-18.79
Fomitopsis cajanderi	Pima, AZ	OSC 35270	S	-1.1	1.81	-18.4	44.29	24.45	32.42	-110.74	2469	13 Jul 1963	1078.4	9.5	-6.95	1.05	-19.48
Sedecula pulvinata	Boulder, CO	MICH 26629		4.3	3.11	-20.9	44.22	14.24	40.00	-105.30	1920	19 Aug 1979	449.4	8.3	-7.4	0.6	-21.53
Sedecula pulvinata	Boulder, CO	MICH 26630		3.8	3.72	-21.1	45	12.11	40.00	-105.29	1920	31 Jul 1979	449.4	8.3	-7.4	0.6	-21.66

Sedecula pulvinata	Boulder, CO	MICH 00340	4.0	3.57	-20.3	45.94	12.88	40.00	-105.29	1920	14 Aug 1978	449.4	8.3	-7.37	0.63	-20.96
Sedecula pulvinata	Garfield, Utah	MICH 00329	-1.5	1.99	-19.7	30.37	15.27	37.82	-111.90	2679	7 Jul 1992	447.8	5.1	-7.77	0.23	-19.90
Sedecula pulvinata	Yuba Pass, CA	MICH 00324	2.4	3.5	-21.1	42.72	12.19	39.26	-120.38	1829	18 Aug 1982	2192.0	7.0	-7.48	0.52	-21.60
Sedecula pulvinata	Yuba Pass, CA	OSC 39125	1.9	3.46	-19.9	39.03	11.27	39.32	-120.60	1743	2 Sep 1969	2201.6	8.1	-7.12	0.88	-20.78
Sedecula pulvinata	Yuba Pass, CA	MICH 00326	2.8	3.46	-22.1	43.76	12.65	39.26	-120.38	1829	6 Oct 1982	2192.0	7.0	-7.48	0.52	-22.60

TABLE III. Regression model of $\delta^{13}C$ values for sporocarps. Adjusted $r^2 = 0.761$, n = 27, $p < 0.0001$. [1]Calculated by difference.

Term	Estimate±se	Prob>|t|	Variance (%)	Effect Prob > F
Intercept	-14.85±4.62	0.0044		
Group	--	--	60.0	**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

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}

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**