

# Phylogenetic Comparison of Ascomycete Mycangial Fungi and *Dendroctonus* Bark Beetles (Coleoptera: Scolytidae)

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**ABSTRACT** The existence of a long-shared evolutionary history among *Dendroctonus* bark beetles and their symbiotic mycangial fungi (Ascomycotina: Ophiostomataceae) was investigated by comparing independently derived phylogenies of the 2 groups of organisms. Two phylogenetic comparisons were made. In the 1st comparison, all mycangium-associated fungi were included in the fungal phylogeny (some beetles possessed 2 mycangial associates). In the 2nd comparison, only the most common mycangial associate of each beetle was included. Statistical tests did not support the existence of widespread cospeciation among the beetles and fungi in the 1st comparison. In the 2nd comparison, a maximum of 4 cospeciation events was statistically supported.

**KEY WORDS** *Dendroctonus*, *Ophiostoma*, bark beetles, mycangia, mycangial fungi, cospeciation

THE EXISTENCE OF a shared evolutionary history between symbiotic organisms can be investigated by comparing independently derived phylogenies of the 2 (or more) associates. Evidence of parallel phylogenesis indicates a high probability that the associations in question are obligate and that the symbionts have faithfully tracked their hosts through evolutionary time (Page 1994). Conversely, if the host and symbiont phylogenies are highly discordant, more casual associations, or the common occurrence of colonization events, are indicated (Page 1994).

The association investigated here occurs among *Dendroctonus* Erichson bark beetles and symbiotic fungi. Several species of beetles in this genus possess mycangia (invaginations of the adult integument specialized for the dissemination of fungi). Mycangia have evolved at least twice in *Dendroctonus*. Two species, *Dendroctonus ponderosae* Hopkins and *D. jeffreyi* Hopkins, possess paired mycangia located on the maxillary cardines and are functional in both sexes (Whitney and Farris 1970; T.D.P., unpublished data). Five species—*Dendroctonus adjunctus* Blandford, *D. approximatus* Hopkins, *D. brevicomis* LeConte, *D. frontalis* Zimmermann, and *D. mexicanus* Hopkins—possess mycangia located beneath a pronotal callus and functional only in females (Francke-Grosmann 1967, Barras and Perry 1971, Happ et al. 1971, Paine and Birch 1983). In these species, an apparently homologous but nonfunctional structure (pseudomycangium) exists in males (Barras and Perry 1971).

Several species of fungi may be associated incidentally with bark beetles; however, usually only 1 or 2 specific fungi are carried in the mycangia of a particular beetle species. The mycangia of *Dendroctonus* are

associated with fungi from 2 major taxa—Ascomycotina and Basidiomycotina. The ascomycetes, which are the focus of this study, are classified in the order Ophiostomatales, family Ophiostomataceae. The ascomycete mycangial fungi include species in *Ophiostoma* H. & P. Sydow, *Leptographium* Lagerb. & Melin (a form genus which includes the anamorphs of several *Ophiostoma* species), and *Ceratocystiopsis* Upadhyay & Kendrick. The mycangia of *D. ponderosae* are consistently associated with *Ophiostoma clavigerum* (Robinson-Jeffrey & Davidson) Harrington and *O. montium* (Rumbold) von Arx (Whitney and Farris 1970), whereas the mycangia of its sibling species, *D. jeffreyi*, is associated only with *O. clavigerum* (Six and Paine 1997). *Dendroctonus adjunctus* carries *Leptographium pyrinum* Davids. (Six and Paine 1996), *D. brevicomis* carries *C. brevicomi* (Hsiau and Harrington 1997), and *D. frontalis* carries *C. ranuculosus* (Barras and Taylor 1973, Harrington and Zambino 1990). The mycangial fungi of *D. approximatus* and *D. mexicanus* are undescribed.

The objectives of this study were to develop a phylogeny of the ascomycete fungi associated with the mycangia of *Dendroctonus* bark beetles, and to compare this phylogeny with an independently derived phylogeny of the host bark beetles to test for evidence of widespread cospeciation, and hence of a long shared evolutionary history, within this assemblage.

## Materials and Methods

**Collections of Beetles.** *Dendroctonus jeffreyi* were collected from *P. jeffreyi* Grev. & Balf. at 10 sites across California. *D. ponderosae* were collected from *P. contorta* Engelm. at 2 sites and from *P. lambertiana* Dougl. at 1 site in California. Adults of both beetle species were collected from under bark just prior to emer-

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Table 1. Isolates of fungi studied

Species	Isolates	Geographic origin	Association	
<i>Ceratocystiopsis brevicomi</i>	946	James Resv., San Jacinto Mtns, CA	<i>P. coulteri</i> , <i>D. brevicomis</i> mycangium	
	947	James Resv., San Jacinto Mtns, CA	<i>P. coulteri</i> , <i>D. brevicomis</i> mycangium	
	981	James Resv., San Jacinto Mtns, CA	<i>P. coulteri</i> , <i>D. brevicomis</i> mycangium	
	983	James Resv., San Jacinto Mtns, CA	<i>P. coulteri</i> , <i>D. brevicomis</i> mycangium	
	984	James Resv., San Jacinto Mtns, CA	<i>P. coulteri</i> , <i>D. brevicomis</i> mycangium	
	985	James Resv., San Jacinto Mtns, CA	<i>P. coulteri</i> , <i>D. brevicomis</i> mycangium	
	987	James Resv., San Jacinto Mtns, CA	<i>P. coulteri</i> , <i>D. brevicomis</i> mycangium	
	944	Baldy Mtn, San Jacinto Mtns, CA	<i>P. coulteri</i> , <i>D. brevicomis</i> mycangium	
	945	Baldy Mtn, San Jacinto Mtns, CA	<i>P. coulteri</i> , <i>D. brevicomis</i> mycangium	
	957	Baldy Mtn, San Jacinto Mtns, CA	<i>P. coulteri</i> , <i>D. brevicomis</i> mycangium	
	988	Baldy Mtn, San Jacinto Mtns, CA	<i>P. coulteri</i> , <i>D. brevicomis</i> mycangium	
	<i>C. ranaculosus</i>	C210 <sup>a</sup>	Unknown	Associated with <i>D. frontalis</i>
		C237 <sup>a</sup>	Unknown	<i>P. echinata</i> , <i>D. frontalis</i> mycangium
		1020	Kisatchie Nat'l Forest, LA	<i>P. taeda</i> , <i>D. frontalis</i> mycangium
1025		Kisatchie Nat'l Forest, LA	<i>P. taeda</i> , <i>D. frontalis</i> mycangium	
1026		Kisatchie Nat'l Forest, LA	<i>P. taeda</i> , <i>D. frontalis</i> mycangium	
1028		Kisatchie Nat'l Forest, LA	<i>P. taeda</i> , <i>D. frontalis</i> mycangium	
958		Sabine Nat'l Forest, TX	<i>D. frontalis</i> mycangium	
966		Sabine Nat'l Forest, TX	<i>D. frontalis</i> mycangium	
973		Sabine Nat'l Forest, TX	<i>D. frontalis</i> mycangium	
980		Sabine Nat'l Forest, TX	<i>D. frontalis</i> mycangium	
<i>Ceratocystiopsis</i> sp.	948	Gainesville, FL	<i>P. taeda</i> , <i>D. frontalis</i> mycangium	
	949	Gainesville, FL	<i>P. taeda</i> , <i>D. frontalis</i> mycangium	
	953	Gainesville, FL	<i>P. taeda</i> , <i>D. frontalis</i> mycangium	
	954	Gainesville, FL	<i>P. taeda</i> , <i>D. frontalis</i> mycangium	
	956	Gainesville, FL	<i>P. taeda</i> , <i>D. frontalis</i> mycangium	
	961	Gainesville, FL	<i>P. taeda</i> , <i>D. frontalis</i> mycangium	
	962	Gainesville, FL	<i>P. taeda</i> , <i>D. frontalis</i> mycangium	
	964	Gainesville, FL	<i>P. taeda</i> , <i>D. frontalis</i> mycangium	
<i>Leptographium pyrimum</i>	791	Twilight Camp, Pinaleno Mtns, AZ	<i>P. arizonica</i> , <i>D. adjunctus</i> mycangium	
	878	Riggs Flat, Pinaleno Mtns, AZ	<i>P. monticola</i> , <i>D. adjunctus</i> mycangium	
	879	Turkey Flat, Pinaleno Mtns, AZ	<i>P. arizonica</i> , <i>D. adjunctus</i> mycangium	
	882	Turkey Flat, Pinaleno Mtns, AZ	<i>P. arizonica</i> , <i>D. adjunctus</i> mycangium	
	886	Riggs Flat, Pinaleno Mtns, AZ	<i>P. ponderosa</i> , <i>D. adjunctus</i> mycangium	
	1092	Sacramento Mtns., NM	<i>D. adjunctus</i> mycangium	
	1093	Sacramento Mtns., NM	<i>D. adjunctus</i> mycangium	
	1094	Sacramento Mtns., NM	<i>D. adjunctus</i> mycangium	
	L. sp. A	276	Riggs Flat, Pinaleno Mtns, AZ	<i>P. ponderosa</i> , <i>D. approximatus</i> mycangium
		278	Riggs Flat, Pinaleno Mtns, AZ	<i>P. ponderosa</i> , <i>D. approximatus</i> mycangium
L. sp. B	281	Riggs Flat, Pinaleno Mtns, AZ	<i>P. ponderosa</i> , <i>D. approximatus</i> mycangium	
<i>Ophiostoma clavigerum</i> ( <i>D. ponderosae</i> )	1006	Inyo Craters, Inyo Nat'l. For., CA	<i>P. contorta</i> , <i>D. ponderosae</i> mycangium	
	C295 <sup>a</sup>	Invermero, Br. Columbia, Canada	Associated with <i>D. ponderosae</i>	
	C292 <sup>a</sup>	Carbondale, Alberta, Canada	Associated with <i>D. ponderosae</i>	
	C291 <sup>a</sup>	West Castle, Alberta, Canada	<i>P. contorta</i> , associated with <i>D. ponderosae</i>	
	C187 <sup>a</sup>	Yosemite Valley, CA	Associated with <i>D. ponderosae</i>	
	C186 <sup>a</sup>	California	<i>P. ponderosa</i> , associated with <i>D. ponderosae</i>	
	C132 <sup>a</sup>	California	<i>Pinus</i> sp., associated with <i>D. ponderosae</i>	
	1037	Truckee, Sierra Nevada Mtns, CA	<i>P. contorta</i> , <i>D. ponderosae</i> mycangium	
	1039	Truckee, Sierra Nevada Mtns, CA	<i>P. contorta</i> , <i>D. ponderosae</i> mycangium	
	1073	Deerlick, San Bernard Mtns, CA	<i>P. contorta</i> , <i>D. ponderosae</i> mycangium	
	<i>O. clavigerum</i> ( <i>D. jeffreyi</i> )	22	Heartbar, San Bernard Mtns, CA	<i>P. jeffreyi</i> , <i>D. jeffreyi</i> mycangium
		218	Lassen Nat'l Forest, CA	<i>P. jeffreyi</i> , <i>D. jeffreyi</i> mycangium
		422	Indiana Summit, CA	<i>P. jeffreyi</i> , <i>D. jeffreyi</i> mycangium
		570	Deadman Summit, CA	<i>P. jeffreyi</i> , <i>D. jeffreyi</i> mycangium
635		Bell Mdws, Sierra Nevada Mtns, CA	<i>P. jeffreyi</i> , <i>D. jeffreyi</i> mycangium	
671		Monitor Pass, S. Nevada Mtns, CA	<i>P. jeffreyi</i> , <i>D. jeffreyi</i> mycangium	
687		Meyers, Sierra Nevada Mtns, CA	<i>P. jeffreyi</i> , <i>D. jeffreyi</i> mycangium	
755		Monitor Pass, S. Nevada Mtns, CA	<i>P. jeffreyi</i> , <i>D. jeffreyi</i> mycangium	
898		Arrowbear, San Bernard Mtns, CA	<i>P. jeffreyi</i> , <i>D. jeffreyi</i> mycangium	
<i>O. montium</i>		15	Lassen Nat'l Forest, CA	<i>P. ponderosa</i> , <i>D. ponderosae</i> mycangium
	24	Lassen Nat'l Forest, CA	<i>P. ponderosae</i> mycangium	
	933	Sawtooth NRA, UT	<i>D. ponderosae</i> mycangium	
	934	Sawtooth NRA, UT	<i>D. ponderosae</i> mycangium	
	1057	Truckee, Sierra Nevada Mtns, CA	<i>P. contorta</i> , <i>D. ponderosae</i> mycangium	

<sup>a</sup> From the culture collection of T. C. Harrington.

gence. *D. ponderosae* were also collected from bark of *P. contorta* and using pheromone traps baited with trans-verbenol, exo-brevicommin, and myrcene in Idaho (B. J. Bentz, U.S. Forest Service, Logan, UT).

*Dendroctonus brevicomis* were collected from 2 sites in the San Jacinto Mountains, CA, using pheromone traps baited with exo-brevicommin, frontalin, and myrcene and placed near infested *P. coulteri* D. Don. *D.*

**Table 2. Enzymes, buffers, and staining procedures used in starch gel electrophoresis of *Ceratocystopsis*, *Leptographium*, and *Ophiostoma* associated with *Dendroctonus* bark beetles**

Enzyme (EC number) <sup>a</sup>	Abbreviation <sup>b</sup>	Buffer systems <sup>c</sup>	Staining references
Aconitase (4.2.1.3)	ACO	A	Marty et al. (1984)
Aspartate aminotransferase (2.6.1.1)	AAT	B	Marty et al. (1984)
Fumerase (4.2.1.2)	FUM	A	Marty et al. (1984)
Glucose-6-phosphate dehydrogenase (1.1.1.49)	G-6PD	A	Marty et al. (1984)
Isocitrate dehydrogenase (1.1.1.42)	IDH	C	Marty et al. (1984)
Malic enzyme (1.1.1.40)	ME1,2	C	Micales et al. (1986)
Malate dehydrogenase (1.1.1.37)	MDH1,2	C	Marty et al. (1984)
Menadiione reductase (1.6.99.2)	MNR	A	Conkle et al. (1982)
Peptidase (3.4.13.1)	PEP	A	Vallejos (1983) <sup>d</sup>

<sup>a</sup> Code of the Nomenclature Committee of the International Union of Biochemistry (1984).

<sup>b</sup> Multiple forms of enzymes are designated in order of decreasing anodal migration.

<sup>c</sup> A, discontinuous TRIS citrate/lithium borate system, pH 8.5/8.1; B, discontinuous TRIS citrate/sodium borate system, pH 8.8/8.0; C, continuous morpholine citrate system, pH 8.1 (Conkle et al. [1982]).

<sup>d</sup> Filter paper overlay method.

*adjunctus* were collected from newly attacked *P. arizonica* Engelm., *P. ponderosae* Laws., and *P. monticola* Dougl. at several sites in the Pinaleno Mountains, AZ; and also in the Sacramento Mountains in New Mexico (M. E. Schultz, U.S. Forest Service, Juneau, AK). *D. approximatus* were collected from a single *P. ponderosa* co-infested with *D. adjunctus* at 1 site in the Pinaleno Mountains, AZ. *D. frontalis* were collected from bark of *P. taeda* in Gainesville, FL (J. R. Meeker, Division of Forestry, Florida Department of Agriculture and Consumer Services, Gainesville), Kisatchie National Forest, LA (J. D. Reeve, U.S. Forest Service, Pineville, LA), and Sabine National Forest, TX (F. M. Stephen, Department of Entomology, University of Arkansas, Fayetteville).

**Fungal Isolations.** *Beetles Possessing Maxillary Mycangia.* In the laboratory, each beetle was decapitated. The head was dissected to remove the maxillary cardines that contain the fungus-bearing mycangia. The cardines were surface-sterilized for 2 min in modified White's solution (Barras 1972) and submerged into malt agar and/or *Ophiostoma* selective agar (malt agar with 100 ppm cycloheximide and 10 ppm streptomycin) (Harrington 1981). Once fungi began to grow from the mycangia, a plug of agar containing mycelia was removed from the growing edge and placed onto

2% water agar to facilitate hyphal tipping. After a few days of growth on water agar, hyphal tips were taken from each subculture and placed on malt agar. All isolates are listed in Table 1. Isolates 276, 281, 791, 878, 879, 946, 948, and 1020 were deposited in the American Type Culture Collection, Rockville, MD.

*Beetles Possessing Pronotal Mycangia.* Female beetles were rinsed in sterile water and decapitated. The pronotal callus containing the mycangium was clipped from the edge of the orbital occipitum, and the inner contents of the thorax were scraped away. The portion of the thorax containing the mycangium was then surface-sterilized and clipped into several pieces using sterile scissors. Isolations then proceeded as described above for maxillary mycangia.

**Culture Acquisitions of Fungi.** Several strains of *Dendroctonus*-associated fungi were acquired from the culture collection of Thomas C. Harrington (Department of Plant Pathology, Iowa State University, Ames) (Table 1).

**Isozyme Electrophoresis.** Mycelia for enzyme extraction were obtained by placing agar plugs taken from growing cultures of each isolate into 30 ml of liquid medium (20 mg malt extract, 1 mg yeast extract/ml) in 125-ml Erlenmeyer flasks (Zambino and Harrington 1992). The cultures were held for 14 d at 21°C

**Table 3. Isozyme electromorphs of species and isolates of *Ceratocystopsis*, *Leptographium*, and *Ophiostoma* associated with *Dendroctonus* bark beetles**

Species	n	Enzymes <sup>a</sup>										
		AAT	ACO	FUM	G6PD	IDH	MDH1	MDH2	ME1	ME2	MNR	PEP
<i>C. brevicornis</i>	11	AC <sup>c</sup>	E	ACD	C	AB	BCD	A	BCD	A	C	B
<i>C. ranaculosus</i>	10	ABC	C	BCD	C	AB	ACD	AB	ABCD	AB	C	B
<i>Ceratocystopsis</i> sp.	8	B	E	BCD	D	B	ABC	AB	AC	AB	C	BC
<i>L. pyrinum</i>	8	B	BC	C	A	A	C	A	B	A	B	B
<i>Leptographium</i> sp. A	2	B	E	A	AB	B	B	E	B	C	A	CD
<i>Leptographium</i> sp. B	1	A	B	B	F	A	C	F	C	D	A	A
<i>O. clavigerum</i> (DP) <sup>b</sup>	9	B	B	C	A	B	D	AB	B	A	B	ABC
<i>O. clavigerum</i> (DJ) <sup>c</sup>	10	B	B	CD	A	B	BCD	A	B	AB	B	ABC
<i>O. montium</i>	5	BCD	AB	C	A	CD	C	AD	CDE	AE	B	ABC

<sup>a</sup> Electromorphs are designated alphabetically in order of decreasing anodal migration.

<sup>b</sup> Associated with *D. ponderosae*.

<sup>c</sup> Associated with *D. jeffreyi*.

Table 4. Cord distance of Cavalli-Sforza and Edwards for mycangial fungi of 6 species of *Dendroctonus* bark beetles

	<i>L. pyrinum</i>	<i>L. sp. A</i>	<i>L. sp. B</i>	<i>O. clavigerum</i> DJ	<i>O. clavigerum</i> DP	<i>O. montium</i>	<i>Ceratocystiopsis</i> sp.	<i>C. brevicomi</i>
<i>L. pyrinum</i>								
<i>L. sp. A</i>	0.8091	—	—	—	—	—	—	—
<i>L. sp. B</i>	0.7861	0.9737	—	—	—	—	—	—
<i>O. clavigerum</i> DJ	0.2803	0.6908	0.8863	—	—	—	—	—
<i>O. clavigerum</i> DP	0.1859	0.6370	0.8512	0.0663	—	—	—	—
<i>O. montium</i>	0.3999	0.8785	0.8321	0.4690	0.3916	—	—	—
<i>Ceratocystiopsis</i> sp.	0.6716	0.7204	0.9250	0.6388	0.5589	0.7693	—	—
<i>C. brevicomi</i>	0.5670	0.6809	0.9451	0.5848	0.4876	0.7169	0.4516	—
<i>C. ranaculosus</i>	0.4923	0.8714	0.7802	0.5599	0.4846	0.7118	0.3507	0.2750

in natural light. Enzymes were extracted by vacuum drying the fungal mats, grinding the mats in liquid nitrogen to a fine powder, then grinding the powder in extraction buffer (Zambino and Harrington 1989). The resulting liquid extract was absorbed onto 4-mm #1 Whatman filter paper wicks (Whatman, Kent, England) and loaded onto 10% horizontal Sigma starch gels (Sigma, St. Louis, MO) with 3 standards. Standards were made from homogenates of an isolate of *O. clavigerum* associated with *D. jeffreyi* and an isolate of *C. ranaculosus*. Following electrophoresis, gels were sliced horizontally and stained for enzyme activity.

From an initial screening of 18 enzyme systems, 9 were selected for use (Table 2). These exhibited 11 putative gene loci that resolved well in all fungi. Different electromorphs were assumed to result from different alleles (Table 3). Buffer systems and staining procedure references are listed in Table 2.

**Phylogeny Construction and Comparison.** Phylogenetic relationships among the beetle-associated ascomycetes were estimated using a matrix of allele frequencies developed for each species (or taxonomic unit). During electrophoresis it became apparent that fungi isolated from mycangia of the Florida population of *D. frontalis* were distinct from *C. ranaculosus* carried by the Texas and Louisiana populations of *D. frontalis*. Therefore, isolates from Florida *D. frontalis* were treated as a distinct taxonomic unit (*Ceratocystiopsis* sp.) in all further analyses. Although both *D. jeffreyi* and *D. ponderosae* are associated with *O. clavigerum*, we treated isolates associated with the 2 beetles independently.

The phylogeny of the host beetles was developed using allele frequencies taken from prior isozyme studies on the systematics of *Dendroctonus* by Bentz and Stock (1986) and Higby and Stock (1982). Bentz and Stock (1986) assessed phylogenetic relationships among 10 *Dendroctonus* species including 5 mycangium-bearing species—*D. adjunctus*, *D. approximatus*,

*D. brevicomis*, *D. frontalis*, and *D. ponderosae*. Higby and Stock (1982) assessed the relationship between the sibling species *D. ponderosae* and *D. jeffreyi*. Using 11 loci in common in the 2 studies where alleles could be determined as equivalent, we developed an allele frequency matrix that included all 6 beetles whose mycangial fungi we consider in this study.

The GENDIST program in the PHYLIP package of computer software, version 3.4 (Felsenstein 1993) was used to develop matrices of the cord distances of Cavalli-Sforza and Edwards (1967) for both the fungi and beetles from allele frequencies.

To determine whether the host beetles or symbiotic fungi possessed similar or dissimilar rates of molecular evolution, bivariate plots were used in correlation analysis. Correlation analyses were done using StatView statistical software (Abacus Concepts 1991).

Phylogenies of the host beetles and symbiotic mycangial fungi were constructed using the CONTML program in PHYLIP (Felsenstein 1993). The CONTML program estimates phylogeny using the restricted maximum likelihood method. Using this program, each locus is assumed to evolve independently by genetic drift, and an evolutionary clock is not assumed.

Comparisons and statistical tests of probabilities of congruence between the bark beetle and fungal phylogenies were done using TreeMap computer software, version 1.0b (Page 1995). The TreeMap program compares dendrograms of hosts and symbionts in a way that the number of possible cospeciation events among the host and symbiont dendrogram pair is maximized. In these comparisons, host cladogenesis is considered to be the primary cause of symbiont cladogenesis (the host phylogeny is treated as the independent variable and the symbiont phylogeny is treated as the dependent variable) (Page 1994).

Table 5. Cord distance of Cavalli-Sforza and Edwards for 6 mycangia-bearing *Dendroctonus* bark beetles

	<i>D. adjunctus</i>	<i>D. approximatus</i>	<i>D. brevicomis</i>	<i>D. frontalis</i>	<i>D. jeffreyi</i>
<i>D. adjunctus</i>	—	—	—	—	—
<i>D. approximatus</i>	0.0332	—	—	—	—
<i>D. brevicomis</i>	0.2764	0.2888	—	—	—
<i>D. frontalis</i>	0.3598	0.3505	0.3762	—	—
<i>D. jeffreyi</i>	0.3049	0.3380	0.3841	0.5488	—
<i>D. ponderosae</i>	0.3086	0.3455	0.5472	0.5761	0.2766

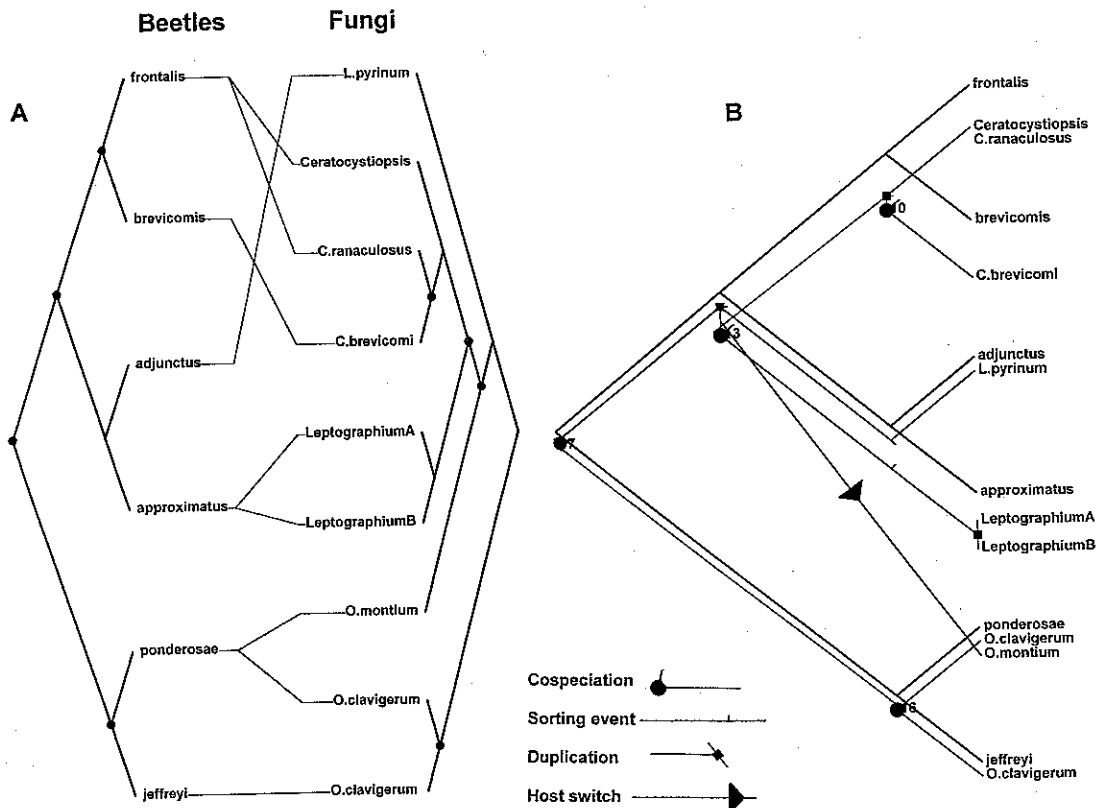


Fig. 1. (A) Comparisons of host (mycangium-bearing *Dendroctonus* bark beetles) and (B) symbiont (mycangium-associated ascomycete fungi) phylogenies. All mycangial associates were included in the analysis.

An exact search was made for optimal (best fit) reconstructions of the host-symbiont dendrogram pair. Reconstructions were developed by superimposing the symbiont (fungal) phylogeny onto the host (beetle) phylogeny so that each node in the symbiont phylogeny was adjacent to the node of its host in the host phylogeny (Page 1994). Where branches are congruent between the 2 phylogenies, the symbiont is assumed to have diverged whenever the host diverged. In the case of incongruent branches, 3 factors were used to explain the discordance. Host-switching was used to reconcile trees when a symbiont was associated with a host different than predicted under the maximal cospeciation criterion. A duplication event was assigned if the direct ancestor of a symbiont diverged independently of its host. A sorting event was assigned when a symbiont was expected to occur with a particular host but did not. Both duplication and host switching involve the independent divergence of the symbiont. Duplication events are the consequence of descendants remaining on the ancestral host, whereas host switching involves at least 1 descendant colonizing a new host (Page 1994).

Two comparisons of the host and symbiont phylogenies were made, one using all fungal associates (some beetle species possessed 2 mycangial associates), and another using only the most common mycangial as-

sociate of each beetle (less common fungal associates were dropped from analysis).

One thousand randomizations of the symbiont (fungus) tree in each beetle-fungus phylogenetic comparison were generated to test the probability that the number of cospeciations predicted in the reconstructions were consistent with chance expectations.

### Results

**Genetic Distance.** Cavalli-Sforza and Edwards' cord distances for all fungi assayed are shown in Table 4. Relatively great distance was exhibited among many of the fungi, especially those that were the less common associates of beetles that possessed >1 associate. However, *L. pyrinum*, *O. clavigerum* associated with *D. jeffreyi*, *O. clavigerum* associated with *D. ponderosae*, and *O. montium* exhibited low distance values among one another. As would be expected for conspecifics, the distance between *O. clavigerum* associated with *D. jeffreyi* and *O. clavigerum* associated with *D. ponderosae* was very low. The 3 *Ceratocystiopsis* species also exhibited relatively low distances among each other. Genetic distances for the host beetles are shown in Table 5.

**Comparisons of Beetle-Fungus Phylogeny.** The comparison in which all mycangial fungi were in-

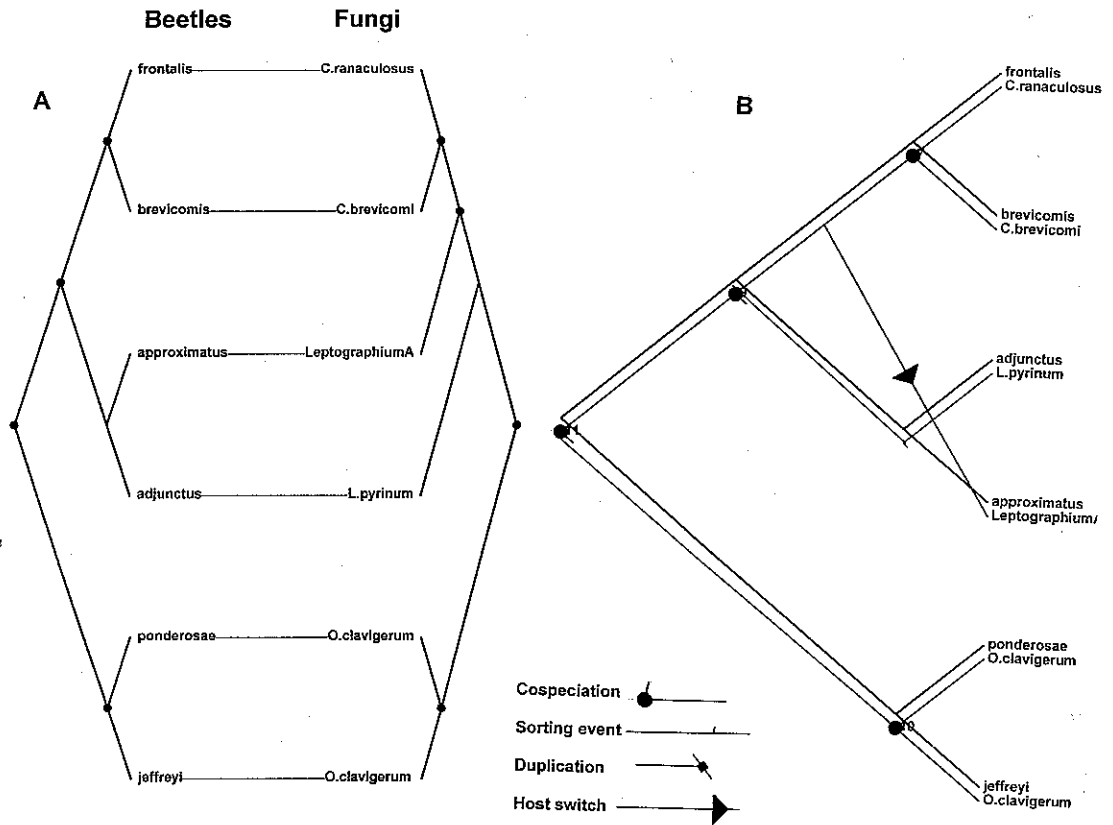


Fig. 2. (A) Comparisons of host (mycangium-bearing *Dendroctonus* bark beetles) and (B) symbiont (mycangium-associated ascomycete fungi) phylogenies. Only the most common mycangial associate of each beetle was included in the analysis.

cluded is shown in Fig. 1A. The best-fit reconstruction comparing the host beetle phylogeny with the phylogeny containing all mycangial fungi is presented in Fig. 1B. This reconstruction indicates that a maximum of 4 cospeciation events were possible. Three dupli-

cations, 4 sorting events, and 1 host switch were required to reconcile the phylogenies. One thousand randomizations of the fungal phylogeny indicated that the probability of the 4 cospeciation events predicted in the best-fit reconstruction could have resulted from chance alone ( $P = 0.32$ ).

The 2nd comparison where only 1 fungal associate per beetle was used is shown in Fig. 2A. In the best-fit reconstruction for this phylogeny pair (Fig. 2B), a maximum of 4 cospeciation events also was indicated; however, only 1 host switch and 1 sorting event were required to reconcile the phylogenies. Randomizations of this fungal phylogeny indicated that the occurrence of the 4 maximum cospeciation events predicted is unlikely to be caused by chance alone ( $P = 0.031$ ).

**Relative Rates of Evolution.** In correlation analysis of the genetic distances of 2 organisms, a correlation coefficient of 1 indicates that both organisms have evolved, on average, at the same rate. In such analyses, only those associations showing evidence of cospeciation can be included; therefore, in our analysis, we used only the fungal associates and their host beetles that exhibited cospeciation in the phylogenetic comparisons. The rates of molecular evolution of the bee-

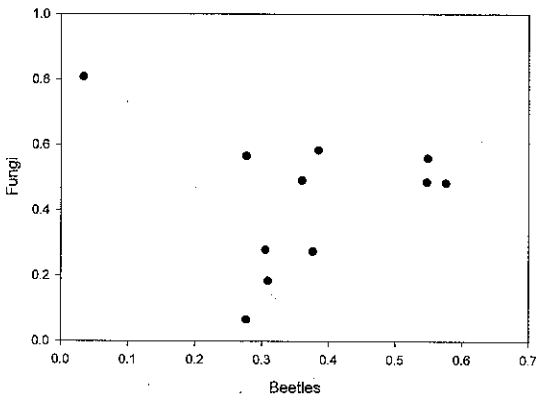


Fig. 3. Bivariate plot comparing cord distances of Cavalli-Sforza and Edwards of *Dendroctonus* bark beetles and their ascomycete mycangial fungi associates.

ties and fungi were not statistically correlated ( $r = -0.109$ ,  $P = 0.751$ ). In a bivariate plot of the 2 organisms, a slightly greater number of points lay closer to the beetle axis, indicating that the beetles are evolving at a slightly more rapid rate than are the fungi (Fig. 3).

### Discussion

Evidence for widespread cospeciation in *Dendroctonus*-mycangial fungus associations was found only when the most common fungal associates were used in the analysis. Therefore, it appears that these most common mycangial fungi have been involved in associations with their host beetles for relatively greater periods of time than the less common associates whose presence is likely to be the result of more recent colonization events.

The roles of the various fungal associates of *Dendroctonus* are not well understood. While some may be mutualists (Barras 1973, Bridges 1983, Goldhammer et al. 1990), others may be "weedy" species (Harrington 1993) or even parasitic upon established mutualisms (Six and Paine 1998). Determining the exact roles each of these fungi have in bark beetle biology and the mode of specificity of mycangia may aid our understanding of how these associations form, are maintained, and how prone they are to colonization events or parasitism.

Future research should include further isolations from *D. adjunctus* and *D. approximatus* to ensure an accurate description of their full complement of fungal associates. Some incongruence between the beetle and fungal phylogenies in this study may be the result of sampling error. Many, although not all, isolations from *D. adjunctus* and *D. approximatus* were made on *Ophiostoma*-selective agar. *Ceratocystiopsis* species grow slowly on *Ophiostoma*-selective agar and, therefore, may have been overlooked when isolations from mycangia were made. Further, *D. approximatus* was difficult to acquire, and only 3 isolates from this beetle were available for use in this study.

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### References Cited

- Abacus Concepts. 1991. StatView statistical software. Abacus Concepts, Berkeley, CA.
- Barras, S. J. 1972. Improved White's solution for surface sterilization of pupae of *Dendroctonus frontalis*. J. Econ. Entomol. 65: 1504.
1973. Reduction of progeny and development in the southern pine beetle following removal of symbiotic fungi. Can. Entomol. 105: 1295-1299.
- Barras, S. J., and T. Perry. 1971. Gland cells and fungi associated with prothoracic mycangium of *Dendroctonus adjunctus* (Coleoptera: Scolytidae). Ann. Entomol. Soc. Am. 64: 123-126.
- Barras, S. J., and J. J. Taylor. 1973. Varietal *Ceratocystis minor* identified from mycangium of *Dendroctonus frontalis*. Mycopathol. Mycol. Applic. 50: 293-305.
- Bentz, B. J., and M. W. Stock. 1986. Genetic relationships among ten species of *Dendroctonus* bark beetles (Coleoptera: Scolytidae). Ann. Entomol. Soc. Am. 79: 527-534.
- Bridges, J. R. 1983. Mycangial fungi of *Dendroctonus frontalis* (Coleoptera: Scolytidae) and their relationship to beetle population trends. Environ. Entomol. 12: 858-861.
- Cavalli-Sforza, L. L., and A.W.F. Edwards. 1967. Phylogenetic analysis: models and estimation procedures. Evolution 32: 550-570.
- Felsenstein, J. 1993. PHYLIP 3.4. Software and user manual. University of Washington, Seattle.
- Francke-Grosmann, H. 1967. Ectosymbiosis in wood-inhabiting insects, pp. 141-205. In S. M. Henry [ed.], Symbiosis, vol. 2. Academic, New York.
- Goldhammer, D. S., F. M. Stephen, and T. D. Paine. 1990. The effect of the fungi *Ceratocystis minor* (Hedgcock) Hunt var. *barrasii* Taylor, and SJB 122 on reproduction of the southern pine beetle, *Dendroctonus frontalis* Zimmermann (Coleoptera: Scolytidae). Can. Entomol. 122: 407-418.
- Happ, G. M., C. M. Happ, and S. J. Barras. 1971. Fine structure of the prothoracic mycangium, a chamber for the culture of symbiotic fungi, in the southern pine beetle, *Dendroctonus frontalis*. Tissue Cell 3: 295-308.
- Harrington, T. C. 1981. Cycloheximide sensitivity as a taxonomic character in *Ceratocystis*. Mycologia 73: 1123-1129.
1993. Diseases of conifers caused by species of *Ophiostoma* and *Leptographium*, pp 161-172. In M. J. Wingfield, K. A. Seifert, and J. F. Webber [eds.], *Ceratocystis* and *Ophiostoma*: taxonomy, ecology, and pathogenicity. APS, St. Paul, MN.
- Harrington, T. C., and P. J. Zambino. 1990. *Ceratocystiopsis ranaculosus*, not *Ceratocystis minor* var. *barrasii*, is the mycangial fungus of the southern pine beetle. Mycotaxon 38: 103-115.
- Higby, P. K., and M. W. Stock. 1982. Genetic relationships between two sibling species of bark beetle (Coleoptera: Scolytidae), Jeffrey pine beetle and mountain pine beetle, in northern California. Ann. Entomol. Soc. Am. 75: 668-674.
- Hsiau, P.T.W., and T. C. Harrington. 1997. *Ceratocystiopsis brevicomi* sp. nov., a mycangial fungus from *Dendroctonus brevicomis* (Coleoptera: Scolytidae). Mycologia 89: 661-669.
- Page, R.D.M. 1994. Parallel phylogenies: reconstructing the history of host-parasite assemblages. Cladistics 10: 155-173.
1995. TreeMap 1.0b computer software. Department of Zoology, University of Oxford, UK.

- Paine, T. D., and M. C. Birch. 1983. Acquisition and maintenance of mycangial fungi by *Dendroctonus brevicomis* LeConte (Coleoptera: Scolytidae). *Environ. Entomol.* 12: 1384-1386.
- Six, D. L., and T. D. Paine. 1996. *Leptographium pyrinum* is a mycangial fungus of *Dendroctonus adjunctus*. *Mycologia* 88: 739-744.
1997. *Ophiostoma clavigerum* is the mycangial fungus of the Jeffrey pine beetle, *Dendroctonus jeffreyi* (Coleoptera: Scolytidae). *Mycologia* 89: 858-866.
1998. Effects of mycangial fungi and host tree species on progeny survival and emergence of *Dendroctonus jeffreyi* and *D. ponderosae*. *Environ. Entomol.* 27: 1393-1401.
- Whitney, H. S., and S. H. Farris. 1970. Maxillary mycangium in the mountain pine beetle. *Science* (Wash. D.C.) 167: 54-55.
- Zambino, P. J., and T. C. Harrington. 1989. Isozyme variation within and among host-specialized varieties of *Leptographium wageneri*. *Mycologia* 81: 122-133.
1992. Correspondence of isozyme characterization with morphology in the asexual genus *Leptographium* and taxonomic implications. *Mycologia* 84: 12-25.

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