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Molecular phylogeny of species in the genera *Amylostereum* and *Echinodontium*

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Analyses of DNA sequences from the internal transcribed spacer (ITS) region of the nuclear rDNA and from a portion of a manganese-dependent peroxidase gene were used to assess the species in *Amylostereum*, including isolates from the mycangia of horntails, decay, and basidiomes. Four species are recognized: *A. areolatum, A. chailletii, A. laevigatum*, and *A. ferreum*. An unidentified *Amylostereum* isolate from the mycangium of *Xoanon matsumurae* had an ITS sequence identical to that of *A. areolatum*. Another unidentified *Amylostereum* isolate from the mycangium of *Sirex areolatus* was near *A. laevigatum*, which appears to be the mycangial symbiont for those horntails attacking cedar-like trees. The other horntail isolates, primarily from Pinaceae, proved to be either *A. areolatum* or *A. chailletii*. The DNA sequences of *Echinodontium tinctorium, E. tsugicola* and *E. japonicum* were similar to those of the *Amylostereum* species, and *Amylostereum* species are now recognized as members of the family Echinodontiaceae rather than the family Stereaceae. *Echinodontium taxodii* was found to be distinct from the Echinodontiaceae and *Stereum*, and *E. taxodii* is recognized as a *Laurilia* species.

Key Words——*Amylostereum*; *Echinodontium*; Echinodontiaceae; internal transcribed spacer; manganese-dependent peroxidase.

Excluding *Dextrinocystidium sacratum* (G. H. Cunningham) S. H. Wu (1995), there are four recognized species of *Amylostereum* (Stereaceae): *A. areolatum* (Fr.: Fr.) Boidin, *A. chailletii* (Pers.: Fr.) Boidin, *A. ferreum* (Berk. & Curt.) Boidin & Lanquetin, and *A. laevigatum* (Fr.: Fr.) Boidin (Boidin and Lanquetin, 1984). All of the *Amylostereum* species occur on coniferous trees. *Amylostereum areolatum, A. chailletii*, and *A. laevigatum* are associated with wood decay of Pinaceae and other conifers in the Northern Hemisphere (Breitenbach and Kranzlin, 1988; Chamuris, 1988; Eriksson and Ryvarden, 1973; Eriksson et al., 1978; Ginns and Lefebvre, 1993), and *A. ferreum* decays wood of *Podocarpus* spp. in Latin America (Boidin and Lanquetin, 1984). Some *Amylostereum* species are also known as symbionts of mycophagus horntails [*Sirex* and *Urocerus* species, (Hymenoptera: Siricinae)], which carry their fungal symbiont in mycangia and inoculate the wood of the plant host with hyphal fragments or arthrospores as they oviposit (Gaut, 1969, 1970; Sano et al., 1995; Tabata and Abe, 1997; Tabata and Abe, 1999; Terashita, 1970).

The genus *Amylostereum* has been traditionally placed in the Stereaceae (Donk, 1964), and species in *Amylostereum* superficially resemble *Stereum*. However, molecular systematics has called for re-evaluation of many of the families of Aphyllophorales. Boidin et al. (1998) placed *Amylostereum* in the monotypic family Amylostereaceae. Analyses of sequences of mitochondrial small subunit rDNA (Hsiau, 1996) suggested an affinity between *A. chailletii* and a group of Aphyllophorales informally recognized as "group 2" by Hibbett and Donoghue (1995). In comparing the sequences of the internal transcribed spacer (ITS) region of the nuclear rDNA, the sequence of *A. chailletii* was found to be very similar to the sequence of *Echinodontium tinctorium* (Ell. & Ev.) Ell. & Ev. (Harrington, unpublished).

Gross (1964) monographed the monotypic family Echinodontiaceae and recognized six *Echinodontium* species with smooth to spinose hymenophores. *Echinodontium tinctorium* and *E. tsugicola* (P. Henn. & Shirai) Imaz. cause white heartrot of living Pinaceae in USA and Japan, respectively (Gilbertson and Ryvarden, 1986). *Echinodontium ballouii* (Banker) Gross was described from *Chamaecyparis thyoides* (L.) B. S. P. in the eastern USA (Gross, 1964). *Echinodontium japonicum* Imaz. is known to occur on *Quercus* spp. in Japan (Imazeki, 1935). Two *Echinodontium* species, *E. taxodii* (Lentz & Mckay) Gross and *E. sulcatum* (Burt) Gross, are often placed in the genus *Laurilia*, which is in the Stereaceae (Parmasto, 1968; Pouzar, 1959) or in the Echinodontiaceae (Jülich, 1981).

We used phylogenetic analyses to re-evaluate the genera *Amylostereum* and *Echinodontium*. We se-

Table 1. *Amylostereum, Echinodontium, Stereum, Perenniporia*, and *Bondarzewia* isolates used for molecular phylogeny.

Species	Isolate No.*	Other isolate No.	Host	Insect	Location	GenBank Accession Nos. (ITS)	GenBank Accession Nos. (Peroxidase)
A. areolatum	B1352	CBS305.82	*Picea abies*		Germany		
A. areolatum	B1353	CBS655.93	*Picea abies*		Denmark		
A. areolatum	B1350	FD-241	*Pinus densiflora*	*S. nitobei* mycangium	Japan	AF218389	AF218404
A. areolatum	B1351	FD-242	*Pinus densiflora*	*S. nitobei* mycangium	Japan		
A. areolatum	B1395	FD-299	*Pinus densiflora*	*S. nitobei* mycangium	Japan		
A. areolatum	B1385	CCFC010138		*S. juvencus* mycangium	Germany		
A. areolatum	B1386	CCFC010474		*S. noctilio* mycangium	Australia		
A. areolatum	B1394	CCFC007961	*Pinus radiata*	*S. noctilio* oviposition bores	New Zealand		
A. chailletii	B1356	CBS631.84	*Abies alba*		Sweden		
A. chailletii	B1358	CBS482.83	*Picea abies*		United Kingdom		
A. chailletii	B1354	CBS483.83		*U. gigas* mycangium	United Kingdom	AF218391	AF218406
A. chailletii	B1387	CCFC010139		*U. gigas* mycangium	Germany	AF218392	
A. chailletii	B1355	ATCC44179	*Abies balsamea*		Canada	AF218393	
A. chailletii	B1390	CCFC009979	*Abies balsamea*		Quebec, Canada		
A. chailletii	B105		*Abies balsamea*		N. Hampshire, USA		
A. chailletii	B1391	CCFC002263	*Abies lasiocarpa*		B. Columbia, Canada		
A. chailletii	B29	FP-105519-SP	*Larix occidentalis*		Oregon, USA		
A. chailletii	B1389	CCFC007757	*Pseudotsuga taxifolia*		B. Columbia, Canada		
A. chailletii	B1392	CCFC007540	*Thuja plicata*		B. Columbia, Canada		
A. chailletii	B1388	CCFC007758	*Tsuga heterophylla*		B. Columbia, Canada		
A. ferreum	B1359	CBS634.84	*Podocarpus lambertii*		Brazil	AF218390	AF218405
A. ferreum	B1360	CBS635.84	*Podocarpus lambertii*		Brazil		
A. laevigatum	B1367	FD-4	*Cryptomeria japonica*		Japan		
A. laevigatum	B1368	FD-120	*Cryptomeria japonica*		Japan		
A. laevigatum	B1369	FD-124	*Chamaecyparis obtusa*		Japan		
A. laevigatum	B1370	FD-311	*Chamaecyparis pisifera*		Japan		
A. laevigatum	B1371	CBS624.84	*Juniperus nana*		France	AF218396	AF218409
A. laevigatum	B1372	CBS625.84	*Juniperus nana*		France		
A. laevigatum	B1397	CBS419.50	*Juniperus nana*		France		
A. laevigatum	B1364	FD-166	*Cryptomeria japonica*	*U. antennatus* mycangium	Japan		
A. laevigatum	B1365	FD-309	*Cryptomeria japonica*	*U. antennatus* mycangium	Japan		
A. laevigatum	B1366	FD-310	*Chamaecyparis obtusa*	*U. antennatus* mycangium	Japan		
A. laevigatum	B1361	FD-3	*Cryptomeria japonica*	*U. japonicus* mycangium	Japan	AF218395	AF218408
A. laevigatum	B1362	FD-9	*Cryptomeria japonica*	*U. japonicus* mycangium	Japan		
A. laevigatum	B1363	FD-112	*Chamaecyparis obtusa*	*U. japonicus* mycangium	Japan		
A. sp.	B1373	FD-308	*Larix kaempferi*	*Xo. matsumurae* mycangium	Japan		
A. sp.	B1393	CCFC010375		*S. areolatus* mycangium	California, USA	AF218394	AF218407
E. japonicum	B1374	IFO30308	*Quercus gilva*		Japan		
E. japonicum	B1375	IFO30309	*Quercus gilva*		Japan	AF218399	AF218412
E. taxodii	B1376	WD-1448	*Chamaecyparis formosensis*		Japan	AF218402	
E. tinctorium	B13	PA-1	Unknown		Oregon, USA		
E. tinctorium	B1122		*Tsuga* sp.		Alaska, USA	AF218397	AF218410
E. tsugicola	B1377	WD-1215	*Tsuga diversifolia*		Japan	AF218398	AF218411
E. tsugicola	B1378	WD-1218	*Tsuga diversifolia*		Japan		
P. subacida	B37	C3A-2	*Abies balsamea*		N. Hampshire, USA	AF218403	
S. annosum		FPL8562			Wisconsin, USA	AF218401	
S. hirsutum		FPL8805			Wisconsin, USA	AF218400	
B. montana		DAOM415			Ontario, Canada		AF218413, AF218414

* Isolate numbers of the Iowa State University Collection. Insect: *S., Sirex*; *U., Urocerus*; *Xo., Xoanon*.

quenced the ITS regions and a portion of a peroxidase gene from isolates obtained from the Institute for Fermentation, the Centraalbureau voor Schimmelcultures, the Canadian Collection of Fungal Cultures, and from isolates we obtained from horntails and woody substrata in Japan. We also examined the morphology of basidiomes of *A. laevigatum* collected from Japan and Sweden.

Materials and Methods

Isolates The source and substrate of isolates are given in Table 1. Extracted DNA of *Stereum annosum* Berk. & Br., *S. hirsutum* (Willd.: Fr.) S. F. Gray, and *Bondarzewia montana* (Fr.) Sing. was kindly provided by David Hibbett.

ITS sequences Mycelia were grown at room temperature (20–25°C) for 10–12 days in 15–20 ml of MY liquid medium (2% malt extract, 1% yeast extract). A fresh mycelial mat was collected by vacuum filtration, ground to a fine powder in liquid nitrogen with a mortar and pestle, and DNA was extracted following the protocol of DeScenzo and Harrington (1994). Template DNA of most of the isolates was extracted from liquid cultures, but mycelia of three *Amylostereum* isolate (B29, B1358, B1360) were scraped lightly with a pipette tip for template DNA (Harrington and Wingfield, 1995).

A fragment of nuclear rDNA about 700 bp long from the 3′ end of the 18S (small subunit gene) to the 5′ end of the 28S (large subunit gene) was amplified and sequenced using the primers ITS1-F (Gardes and Bruns, 1993) and ITS4 (White et al., 1990). The polymerase chain reaction (PCR) products were purified using QIAquick PCR Purification Kit (QIAGEN Inc., USA) and sequenced with the ABI PRISM 377 DNA sequencer (Perkin-Elmer) in the DNA Sequencing Facility at Iowa State University. Both the coding and template strands were sequenced, with complete overlap of the complementary sequences. Sequences were visually aligned and analyzed using heuristic searches in PAUP 4.0, with stepwise additions (simple) and tree-bisection-reconnection (Swofford, 1998). Uninformative characters were ignored. Gaps were coded as a newstate (fifth character) because the gaps were consistently found within species and, except for outgroup taxa, most gaps were only one or two bases in length. Bootstrapping (100 bootstrap replicates) was used to determine confidence in the branches.

Manganese-dependent peroxidase In an earlier study (Maijala et al., 1998), a portion of a manganese (Mn)—dependent peroxidase gene was identified in an isolate of *E. tinctorium*, and a homologous gene was found in each of the *Echinodontium* and *Amylostereum* species studied except *E. taxodii*. A PCR fragment of about 570 bp was amplified using degenerative primers and the reaction conditions outlined by Maijala et al. (1998). Amplified fragments were cloned into the pGEM-easy vector (Promega, Madison, Wisconsin, USA) and sequenced at the DNA Sequencing Facility with the vector primers T7 and SP6. Phylogenetic analyses were as outlined for the ITS sequences, except that 1000 bootstrap replications were made.

Results

ITS sequences The ITS sequences of each of the *Amylostereum* species aligned well with each other. In order to find potential outgroup taxa and identify genera that may be closely related to *Amylostereum*, we compared these ITS sequences with those generated in our studies (Harrington et al., 1998) of *Heterobasidion*. *Heterobasidion* and other genera were placed in "group 2" based on mitochondrial rDNA sequences (Hibbett and Donoghue, 1995; Hsiau, 1996). Extracted DNA from representatives of group 2 genera was provided by Dr. D. Hibbett, and we generated ITS sequences for representatives of *Bondarzewia, Lentinellus, Auriscalpium, Hericium, Echinodontium*, and *Russula*. Of these genera, the ITS sequence of *E. tinctorium* matched closely to ITS sequences of *Amylostereum*. We subsequently generated ITS sequences for isolates of *E. tinctorium, E. tsugicola*, and *E. japonicum*, and these sequences were easily aligned with those of *Amylostereum* species. However, the ITS sequence of *E. taxodii* did not match closely to those of the other *Echinodontium* species. Because *Amylostereum* has been placed in the Stereaceae, we also included ITS sequences of two *Stereum* species in the analyses, and the ITS sequence of *Perenniporia subacida* (Pk.) Donk was also compared as a potential outgroup taxon.

In the complete data set of ITS sequences of *Amylostereum, Echinodontium, Stereum*, and *P. subacida*, there were 614 aligned characters with the insertions of gaps. However, 261 of these characters were excluded from the initial analysis because of ambiguous alignment. Of the included characters, 259 were constant, 39 were parsimony uninformative, and 55 characters were informative. Ten most parsimonious trees of 150 steps were found, with a consistency index (CI) of 0.8200, a retention index (RI) of 0.8767, and a rescaled consistency index (RC) of 0.7189 (Fig. 1). *Perenniporia subacida*, the only polypored species studied, was selected as an outgroup taxon, rooting the tree at an internal node with basal polytomy.

The ITS sequence of *E. taxodii* was quite distinct from those of the other ingroup taxa. Strong bootstrap support was found for the inferred clade that included two *Stereum* species, the *Amylostereum* species, and the *Echinodontium* species, exclusive of *E. taxodii* (Fig. 1). The inferred clade containing the *Amylostereum* species, *E. tinctorium, E. tsugicola*, and *E. japonicum* was well supported, as was the clade of *E. tinctorium* and *E. tsugicola*. The two isolates of *E. japonicum* formed a well-supported clade, as did the nine tested isolates of *A. areolatum*. The inferred *Amylostereum clade* was only weakly supported, but there were few phylogenetically informative characters among the *Amylostereum* species after deletion of the ambiguously aligned characters. Each of the branches with bootstrap support (Fig. 1) was found in each of the 10 most parsimonious trees. Fur-

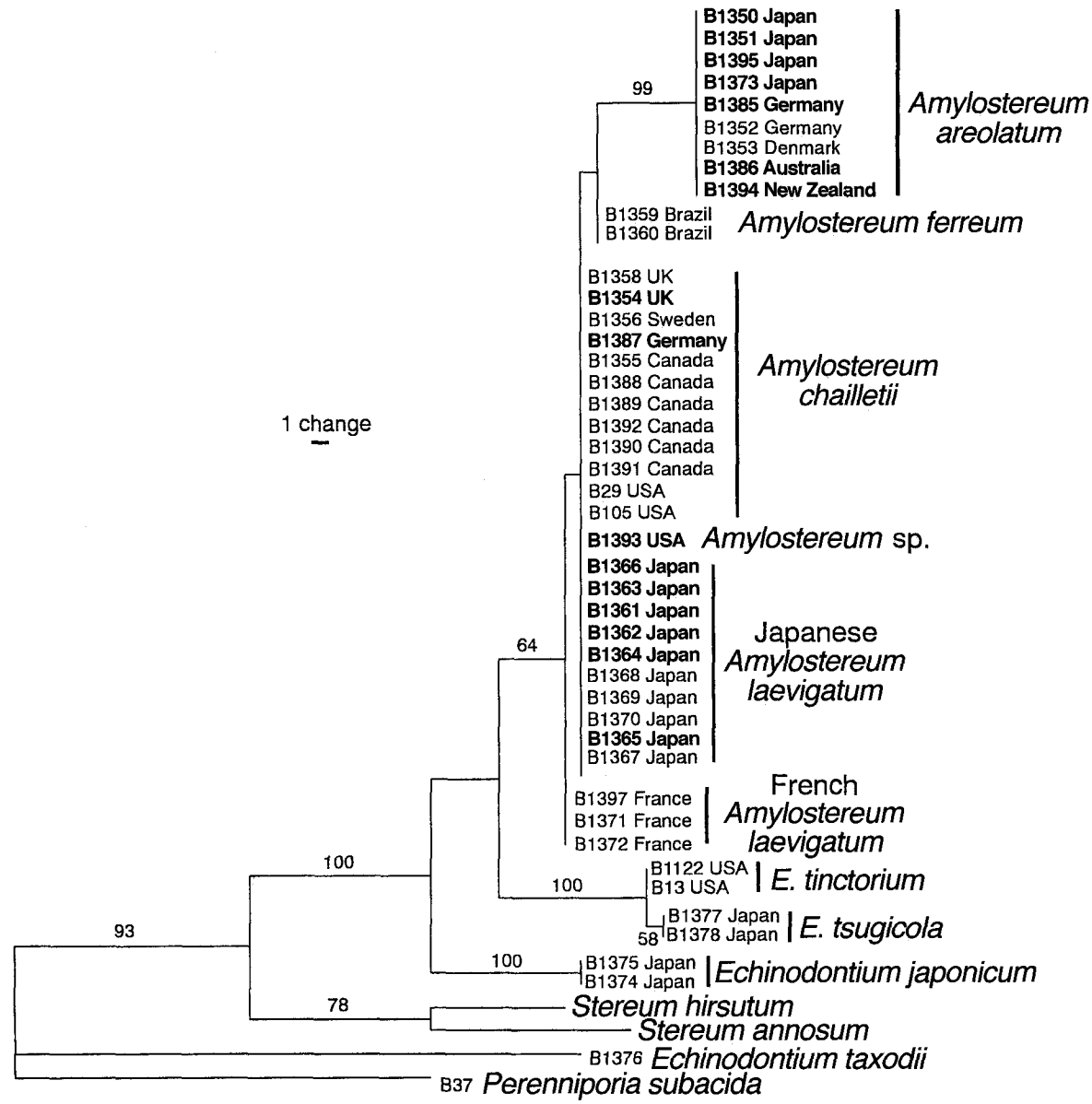

Fig. 1. One of 10 most parsimonious trees based on 355 alignable characters of the ITS-1, 5.8S, and ITS-2 regions of the nuclear rDNA of *Amylostereum, Echinodontium*, and *Stereum* species. The tree is rooted to *Perenniporia subacida*. Bootstrap values (100 replicates) greater than 50% are indicated above the branches. Isolate numbers in bold are isolates from horntails.

ther, neighbor-joining analysis of the same data resulted in a tree with the same topology as that in the most parsimonious tree (Fig. 1), except for minor changes in the relationships among *A. chailletii, A. laevigatum* and *A. ferreum*.

A second ITS data set of fewer taxa was used to look more closely at the relationships among the *Echinodontium* and *Amylostereum* species. The sequences of *S. hirsutum, E. taxodii*, and *P. subacida* were eliminated. Of the aligned 564 characters, including gaps, 364 characters were constant, and 77 variable characters were parsimony-uninformative, leaving 123 parsimony-informative characters. Using *S. annosum* as an outgroup taxon, 152 most parsimonious trees of 308 steps were found, with CI=0.8279, RI=0.9161, and RC=0.7585. The inferred clade containing the *Amylostereum* species was well supported, as was the clade containing *E. tinctorium* and *E. tsugicola* (Fig. 2). With rooting the tree at an internal node with basal polytomy, *E. japonicum* did not clearly group with the other *Echinodontium* species or with *Amylostereum*.

The ITS sequence of the *A. ferreum* isolates was near that of isolates of *A. chailletii* and *A. laevigatum*. Three of the isolates of *A. chailletii* from Europe had slightly different ITS sequences from the North American and a single German isolate of *A. chailletii*, but there was no bootstrap support (<50%) for the branch connecting these two *A. chailletii* groups. Similarly, *A. laevigatum*

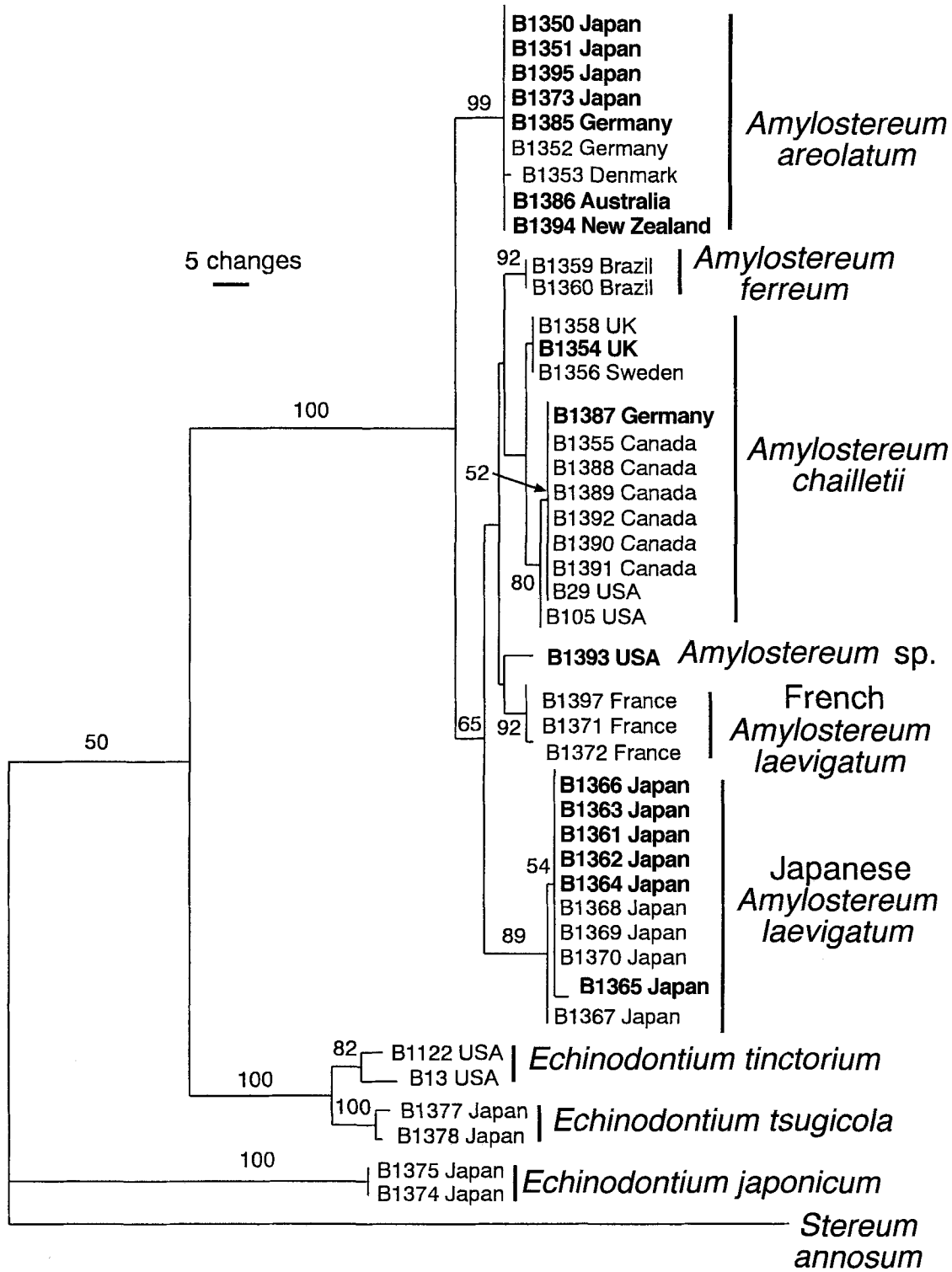

Fig. 2. One of 152 most parsimonious trees based on 564 alignable characters of the ITS-1, 5.8S, and ITS-2 regions of the nuclear rDNA of *Amylostereum* and *Echinodontium* species. The tree is rooted to *Stereum annosum*. Bootstrap values (100 replicates) greater than 50% are indicated above the branches. Isolate numbers in bold are isolates from horntails.

isolates from Japan and France were found in two well-supported branches. An unidentified *Amylostereum* isolate (B1393) from *S. areolatus* Cr. in California, USA had a distinct ITS sequence. The ITS sequence of an unidentified *Amylostereum* isolate (B1373) from *Xo. matsumurae* Rohwer was identical to that of *A. areolatum* (Fig. 2).

The strict consensus tree included branches for *A.*

areolatum, A. ferreum, A. laevigatum from Europe, *A. laevigatum* from Japan, *A. chailletii, E. tinctorium, E. tsugicola*, and *E. japonicum*. The branch grouping *E. tinctorium* and *E. tsugicola* was found in each of the most parsimonious trees, and the branch grouping all of the *Amylostereum* species was also found in the strict consensus tree. However, the relationships among *Amylostereum, E. tinctorium/E. tsugicola*, and *E. japonicum* were not resolved in the consensus tree. The neighbor-joining analysis gave a tree with the same topology as the most parsimonious tree shown in figure 2, except in the branches relating *A. areolatum, A. ferreum, A. laevigatum* from Europe, *A. laevigatum* from Japan, and *A. chailletii*, and these branches also had no bootstrap support in parsimony analysis (Fig. 2).

Mn-dependent peroxidase Portions of four apparently nonorthologous genes of Mn-dependent peroxidase, designated A, C, D and E, were identified among amplification products of *Echinodontium* and *Amylostereum* species using the degenerative primers (Maijala et al., 1998). These nonorthologous genes were distinguished by differences in their putative amino acid sequences and unique sequences of three included introns (Maijala et al., 1998). The peroxidase A gene was the most commonly encountered among the hundreds of cloned fragments that were sequenced, and we were able to generate per-

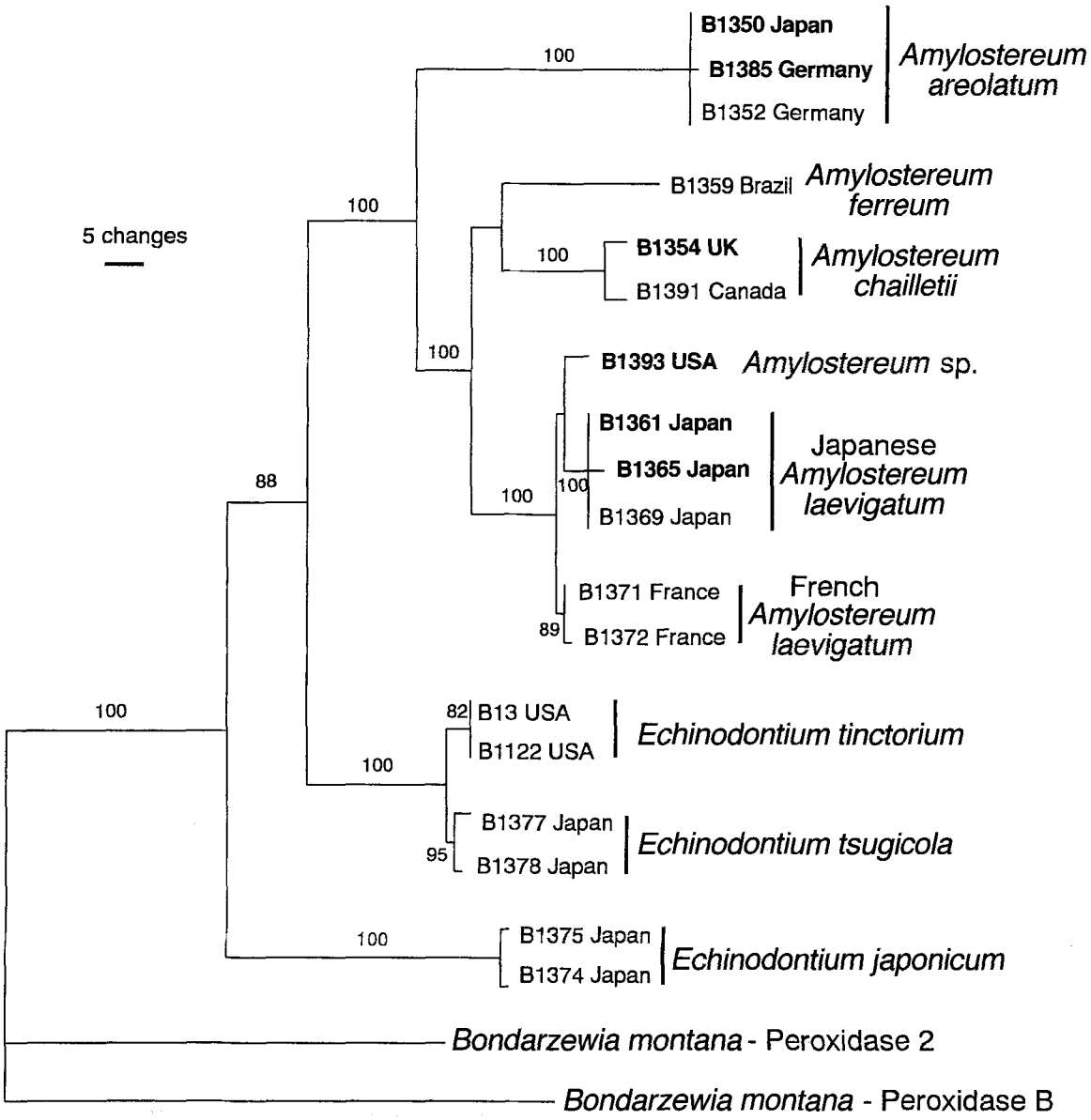

Fig. 3. One of two most parsimonious trees based on 410 characters of exons of a partial sequence of manganese-dependent peroxidase A of *Amylostereum* and *Echinodontium* species. The tree is rooted to *Bondarzewia montana* peroxidase 2 and peroxidase B sequences. Bootstrap values (1000 replicates) greater than 50% are indicated above the branches. Isolate numbers in bold are isolates from horntails.

oxidase A sequences for each of the *Amylostereum* and *Echinodontium* species, except for *E. taxodii*. In comparing the putative amino acid sequence of peroxidase A with that of other Mn-dependent peroxidase genes, the peroxidase B and peroxidase 2 genes of *B. montana* (Maijala et al., 1998) were most similar, and these DNA sequences were used as outgroups in the parsimony analysis of the putative exons of peroxidase A (Fig. 3). The peroxidase A introns of the *Echinodontium* and *Amylostereum* species could not be aligned without ambiguity.

Of the included 410 characters from the four exons, 213 were constant and 57 were parsimony-uninformative, leaving 140 parsimony-informative characters. Two most parsimonious trees of 348 steps and identical topology were found, with CI=0.7356, RI=0.8221, and RC=0.6047. The inferred relationships among the ingroup taxa (Fig. 3) were very similar to those inferred from the ITS analysis. *Echinodontium japonicum* was basal to the other ingroup taxa, *E. tinctorium* and *E. tsugicola* formed a strongly supported group, and the genus *Amylostereum* was well supported by a 100% bootstrap value. As in the ITS analysis, *A. areolatum* was distinct from the other *Amylostereum* species (Fig. 3). The Japanese and French isolates of *A. laevigatum* formed two distinct, sister clades, and these two clades grouped strongly with the unidentified *Amylostereum* isolate (B1393) from California. Neighbor-joining of the exon DNA sequences gave a tree identical to that from parsimony analysis (Fig. 3), except that *E. japonicum* grouped with the other two *Echinodontium* species in the neighbor-joining analysis. The putative amino acid sequences for peroxidase A were included in a much larger data set for another study (Maijala, Harrington, and Raudaskoski, unpublished), and parsimony analysis of these amino acid sequences gave trees with identical topology to the parsimony analysis of the exon sequences with respect to the *Amylostereum* and *Echinodontium* species.

Discussion

The four recognized species of *Amylostereum* appear to form a monophyletic group based on both ITS and peroxidase A sequence analyses. Further, *A. ferreum, A. chailletii*, and *A. laevigatum* form a separate group, sister to *A. areolatum*. Similarly, Slippers et al. (1998) found by the sequences of small-subunit mt-rDNA and the first intergenic spacer (IGS-1) region of the nuclear rDNA that *A. ferreum* and *A. laevigatum* are more closely related to *A. chailletii* than to *A. areolatum*, and Vasiliauskas et al. (1999) also found that *A. chailletii* and *A. laevigatum* from Europe have similar ITS sequences. Boidin and Lanquetin (1984) showed that *A. ferreum* was partially interfertile with *A. laevigatum* but was completely intersterile with *A. areolatum*.

Isolates of *A. areolatum, A. chailletii*, and *A. laevigatum* from horntails had similar or identical ITS and peroxidase sequences to the respective isolates from basidiomes or decayed wood. Our DNA sequence analyses confirm that *A. areolatum* is the associate of *S. nitobei, S. juvencus* and *S. noctilio* from Japan and Europe. *Sirex noctilio* and its symbiont *A. areolatum* have also been introduced to South America, South Africa, Australia, and New Zealand (Gilbert and Miller, 1952; Gilmoure, 1965; Slippers et al., 1998; Vibrans, 1991).

An unidentified *Amylostereum* isolate (B1373) from the mycangium of *Xo. matsumurae* in Japan had an ITS sequence identical to that of other *A. areolatum* isolates. Isolate B1373 from *Xo. matsumurae* and two isolates (FD-241 and FD-242) from *S. nitobei* were grown on potato-dextrose agar in Petri plates at 25°C, and the cultural characteristics were noted. These three isolates formed mycelia that were white at first, later becoming brown, cottony, and with a sweet odor. They had pale brown, thick-walled, clavate cystidia with an acute to somewhat rounded edge, encrusted with granular material. They produced arthrospores that were 5–21 × 1–4 μm. From the ITS sequences and morphological comparisons, we conclude that the fungal symbiont of *Xo. matsumurae* is *A. areolatum*. This is the first report of a mycangial fungus from any *Xoanon* species, but only one isolation was made from a single horntail.

Amylostereum chailletii is a common decay fungus in both North America and Europe, and it also has been recorded as a symbiont of *S. areolatus, S. cyaneus* Fab., *Urocerus augur augur* Klug, *U. augur sah* Mocsàry, *U. californicus* Nort., and *U. gigas* L. (Gaut, 1970). Isolates from *U. gigas* in Europe had ITS and peroxidase A sequences that were similar to those from decayed wood or basidiomes.

Gaut (1970) found by anastomosis, dikaryotization, and interfertility tests that the fungus associated with *S. areolatus* was *A. chailletii*. An *Amylostereum* isolate (B1393=CCFC010375) from the mycangium of *S. areolatus* was isolated by Stillwell and was presumed to be *A. chailletii*. However, this isolate has unique ITS and peroxidase A sequences that were similar to those of *A. laevigatum* and distinct from those of *A. chailletii*. *Sirex areolatus* commonly attacks cedar-like trees such as *Libocedrus, Cupressus, Juniperus*, and *Sequoia* (Furniss and Carolin, 1977), similar to the hosts of *U. japonicus* and *U. antennatus*, which have *A. laevigatum* as its symbiont. More isolates need to be examined to clarify the species of *Amylostereum* associated with *S. areolatus*, but it appears that *A. laevigatum* is the primary symbiont of *Sirex* and *Urocerus* horntails attacking cedar-like tree species, while *A. chailletii* and *A. areolatum* are the primary symbionts of the horntails attacking the Pinaceae.

Although the ITS sequences of *A. laevigatum* isolates from Japan and France were distinct, isolates from these two countries had similar peroxidase A sequences. It has been speculated that *A. laevigatum* in Europe is two distinct taxa, one occurring on *Juniperus* (with basidiospores 7–9 μm long) and another on *Taxus* (basidiospores 9–12 μm long) (Eriksson and Ryvarden, 1973). However, Boidin and Lanquetin (1984) did not find the same distinction between *Taxus* and *Juniperus* isolates from Sweden and France. Specimens of *A. laevigatum* from Japan (Forestry and Forest Products

Research Institute, SFM1, SFM2, and SFM3) and those from *Juniperus* in Sweden (Botanical Museum of Uppsala University, UPS Nos. 142326, 142340, and 142359) were examined, and each had the shorter basidiospores typical of the *Juniperus* type. Thus, the peroxidase A sequences and morphological data support the earlier identification of the symbiotic fungus associated with *U. antennatus* and *U. japonicus* as *A. laevigatum* (Tabata and Abe, 1997; Tabata and Abe, 1999). Further studies of more specimens of *A. laevigatum* from Europe are needed, however.

The results of ITS sequence and peroxidase A analyses show that some of the *Echinodontium* species are quite closely related to *Amylostereum*. *Echinodontium tsugicola* is morphologically very similar to *E. tinctorium* (Gilbertson and Ryvarden, 1986; Imazeki, 1935), and these species are sister to the genus *Amylostereum*, though these *Echinodontium* species have prominently spinose hymenophores while those of *Amylostereum* are smooth. The hymenophore of *E. japonicum*, which occurs on *Quercus* rather than conifers, has fine teeth and is related to the other *Echinodontium* species and *Amylostereum* based on ITS and peroxidase sequences. However, it does not clearly fall into the *Amylostereum* or *Echinodontium* group. The phylogenetic analyses suggest that *Echinodontium* is paraphyletic (contains another genus, i.e., *Amylostereum*) if *Echinodontium* is comprised of *E. tinctorium, E. tsugicola*, and *E. japonicum*. Unfortunately, cultures of *E. ballouii* were not available for study.

The hymenophore of *E. taxodii* is smooth, and it has been considered a species of *Laurilia* (Stereaceae) by Parmasto (1968). The ITS sequence of our isolate of *E. taxodii* was very distinct from the other *Echinodontium* species and from *Stereum*, and we were unable to obtain a peroxidase A gene fragment from this isolate. This supports the exclusion of *E. taxodii* from *Echinodontium* and *Stereum*, and the name *L. taxodii* (Lentz & McKay) Parm. is regarded as appropriate. The related *E. sulcatum* was not studied here, but its similarity to *L. taxodii* in biology and morphology (Davidson et al., 1960) suggests that it, too, may belong outside of *Echinodontium*, most likely in *Laurilia* (Pouzar, 1959).

Amylostereum, Echinodontium, and *Laurilia* have resupinate to effused-reflexed basidiocarps and amyloid basidiospores and cause white rots. However, these genera are distinguished by the following features. *Amylostereum* has an even hymenial surface, monomitic or dimitic hyphal system, smooth basidiospores, and, in three of four species, symbiotic relationships with horntails. *Echinodontium* has a hydnaceous hymenial surface, dimitic hyphal system, and echinulate basidiospores. *Laurilia* has an even to tuberculate hymenial surface, dimitic or trimitic hyphal system, and echinulate basidiospores. Boidin et al. (1998) erected a new monotypic family for *Amylostereum* (Amylostereaceae), but the close phylogenetic relationship shown in our study supports placement of *Amylostereum* in the Echinodontiaceae. Based on morphological characteristics and sequence analyses, we propose that *Amylostereum* be moved from the Stereaceae and placed in the Echinodontiaceae.

The Echinodontiaceae was proposed by Donk (1961) and emended by Gross (1964), recognizing only the genus *Echinodontium*, which included species with smooth hymenophores as well as hydnoid hymenophores. Jülich (1981) adopted Gross's interpretation and described the family in detail, but he added another genus, *Laurilia*, including *E. taxodii* and *E. sulcatum*. The disposition of *Laurilia* is unclear, but affinities of *L. taxodii* with *Echinodontium* and *Stereum* are questioned by our DNA sequence analyses. Ryvarden (1991) suggested that the genus *Haploporus* be placed in the Echinodontiaceae, but more data are needed to determine if *Haploporus* is related to *Echinodontium* and *Amylostereum*.

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