Evolution, 55(10), 2001, pp. 2011–2027

THE EVOLUTION OF AGRICULTURE IN BEETLES (CURCULIONIDAE: SCOLYTINAE AND PLATYPODINAE)

BRIAN D. FARRELL,[1,2] ANDREA S. SEQUEIRA,[1] BRIAN C. O'MEARA,[1] BENJAMIN B. NORMARK,[1,3] JEFFREY H. CHUNG,[1,4] AND BJARTE H. JORDAL[1,5]

[1]*Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts 02138*

[2]*E-mail: bfarrell@oeb.harvard.edu*

[3]*Department of Entomology, Fernald Hall, University of Massachusetts, Amherst, Massachuseets 01003*

[4]*Harvard Medical School, 25 Shattuck Street, Boston, Massachusetts 02115*

[5]*Department of Zoology, University of Bergen, Allegaten 41, N-5007 Bergen, Norway*

Abstract.—Beetles in the weevil subfamilies Scolytinae and Platypodinae are unusual in that they burrow as adults inside trees for feeding and oviposition. Some of these beetles are known as ambrosia beetles for their obligate mutualisms with asexual fungi—known as ambrosia fungi—that are derived from plant pathogens in the ascomycete group known as the ophiostomatoid fungi. Other beetles in these subfamilies are known as bark beetles and are associated with free-living, pathogenic ophiostomatoid fungi that facilitate beetle attack of phloem of trees with resin defenses. Using DNA sequences from six genes, including both copies of the nuclear gene encoding enolase, we performed a molecular phylogenetic study of bark and ambrosia beetles across these two subfamilies to establish the rate and direction of changes in life histories and their consequences for diversification. The ambrosia beetle habits have evolved repeatedly and are unreversed. The subfamily Platypodinae is derived from within the Scolytinae, near the tribe Scolytini. Comparison of the molecular branch lengths of ambrosia beetles and ambrosia fungi reveals a strong correlation, which a fungal molecular clock suggests spans 60 to 21 million years. Bark beetles have shifted from ancestral association with conifers to angiosperms and back again several times. Each shift to angiosperms is associated with elevated diversity, whereas the reverse shifts to conifers are associated with lowered diversity. The unusual habit of adult burrowing likely facilitated the diversification of these beetle-fungus associations, enabling them to use the biomass-rich resource that trees represent and set the stage for at least one origin of eusociality.

Key words.—Ambrosia, bark beetles, coevolution, haplodiploidy, insect-plant interactions, Platypodinae, Scolytinae.

Received December 19, 2000. Accepted June 1, 2001.

The interactions between insects and plants include the bulk of terrestrial multicellular species (Ehrlich and Raven 1964) and provide the resource basis for most of the rest. The origins of herbivory and, in particular, feeding on angiosperms have resulted in enhanced rates of insect diversification, producing nearly half of all insects (Mitter et al. 1988; Farrell 1998a), but insects often do not act alone in their depredations on plants. Many insect-plant interactions, perhaps most, also involve microbial associates, including plant pathogenic fungi (Paine et al. 1997). By expanding the capacity of the insects to use plant resources, mutualistic interactions between herbivorous insects and fungi may themselves promote diversification by fungus-associated lineages (Wilding et al. 1989; Holldobler and Wilson 1990; Wingfield et al. 1993; Chapela et al. 1994; Mueller et al. 1998).

In Neotropical forests, the most prodigious consumers of tree leaves are probably the attine ants and the symbiotic basidiomycete fungi they culture in colonies below ground. Because the ant fungi are unusually polyphagous—they are able to utilize 50–77% of the species in a rainforest (Funk 1985; Cherrett et al. 1989)—these leaf-cutting ants feed their fungal gardens with leaves cut from a wide array of plant taxa, an apparent advantage in hyperdiverse forests (Cherrett et al. 1989; Holldobler and Wilson 1990; Mueller et al. 1998). Recent phylogenetic studies of the ants and their fungi has revealed a single origin of gardening, approximately 50 million years ago, and repeated acquisitions of fungi that are apparently propagated as clones and passed between successive generations of ants (Chapela et al. 1994; Mueller et al. 1998; Currie et al. 1999).

Whereas leaf-cutting attine ants strip trees of leaves, fungus-carrying beetles (Fig. 1) in the Neotropics and elsewhere bore into the center of tree trunks (Fig. 2) and branches or carve intricate tunnel systems—often termed galleries—in the phloem (Hubbard 1897; Schedl 1956; Browne 1961; Sturgeon and Mitton 1982; Wood 1986; Paine et al. 1997). The sometimes devastating attacks on forest trees by Scolytinae and Platypodinae (approximately 7500 species altogether) are usually mediated by associated fungi, the arthropod-dispersed ascomycetes classified in the order Ophiostomatales but generally referred to as the ''ophiostomatoid fungi'' (Fig. 3; cf. Wingfield et al. 1993).

Rather than build fungal gardens underground like the ants, these beetles bring the fungi to their host trees, often carrying them via a striking array of glandular, invaginated cuticular structures (mycangia) that serve to maintain fungal spores and mycelia in pure, often yeastlike, cultures for inoculation into the galleries ovipositing beetles dig into wood (Batra 1966; Francke-Grossman 1967; Beaver 1989; Malloch and Blackwell 1993). Once introduced, these fungi form mycelia that help curtail tree defenses and/or serve as beetle food (Beaver 1989). The ophiostomatoid fungi comprise the three sexual, often pathogenic genera *Ophiostoma* (the largest genus, mostly associated with conifers), and *Ceratocystis* and *Ceratocystiopsis* (both mostly associated with various angiosperms), plus several dozen genera of asexual anamorphs. Although most of these are known to be at least loosely associated with beetles, some have become, like the ant fungi,

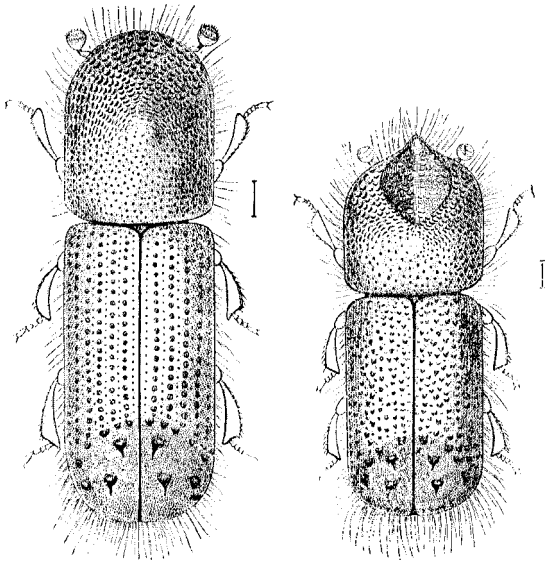

FIG. 1. Dorsal view of adult female (left) and male (right) of *Xyleborus celsus* (from Hubbard 1897). This species ranges in size from 4.0 mm to 4.5 mm.

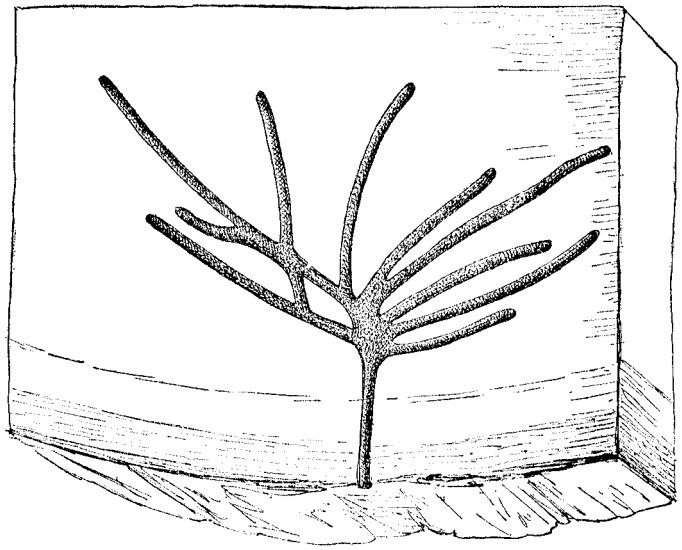

FIG. 2. Gallery of *Xyleborus celsus* in hickory (from Hubbard 1897).

obligately associated, vertically transmitted, polyphagous asexual domesticates and serve as the primary food of their beetle farmers (Malloch and Blackwell 1993). These fungal cultivars are now classified in the genera *Ambrosiella* and *Raffaelea*, but were named ambrosia fungi for their enigmatic identity to early naturalists perplexed over the food of certain tunneling beetles (ambrosia beetles), that seemed not to consume wood (Hubbard 1897).

Ambrosia beetles total some 3400 described species in 10 tribes. They lay their eggs in fungal gardens they culture in galleries usually dug deep in the interior xylem. Extant ambrosia beetle genera in several tribes are represented in 30-million-year-old amber, suggesting that at least some origins of this association occurred even earlier in the Tertiary, perhaps roughly contemporaneous with the origin of attine ants. Whether the contemporary descendents of ambrosia beetles and fungi are themselves of comparable ages, however, has been an open question. Ant fungal gardening arose once and is hypothesized to have arisen through intermediate stages of fungal consumption and then dispersal or the reverse (Mueller et al. 2001). Beetle fungal gardening differs both in numbers of origins and in the persistence of apparently earlier stages. In contrast to ants, there are several independent instances of ambrosia associations, in different beetle tribes, each of which also contains phloem-feeding beetles associated with *Ophiostoma* or other sexual free-living relatives of the ambrosia fungi (Whitney 1982). The nonambrosia-feeding Scolytinae total approximately 4100 described species and are largely host-specialised phloem-feeders properly known as bark beetles, plus approximately 400 species of typically more polyphagous beetles that feed on the pith of twigs or in seeds (Schedl 1956; Browne 1961; Wood 1982; Kirkendall 1993).

Bark beetles that attack conifers are especially well studied, and their associated *Ophiostoma* fungi have been shown to circumvent the conifers' resinous defenses by fast growth (3–10 mm/day) and thus quick blocking of the resin canals (Paine et al. 1997). These associations with resin-bearing conifers are ancestral in the Scolytinae (Sequeira et al. 2000) and remain principal affiliations of temperate bark beetles. Numerous authors have remarked on a similar association of tropical bark beetles with resin- or latex-bearing angiosperms (e.g., in Mexico: Atkinson and Equihua 1986a,b; Noguera-Martinez and Atkinson 1990; Africa: Schedl 1956; Malaysia: Browne 1958, 1961; also see Wood and Bright 1992), whose sticky defenses they may likewise counter with fungal associates and tunneling strategies (Farrell et al. 1991; Dussourd and Denno 1994). Indeed, these beetles are some of the most ubiquitous insects captured in the fossilized legume and conifer resins that comprise the Dominican and Baltic ambers (Bright and Poinar 1994).

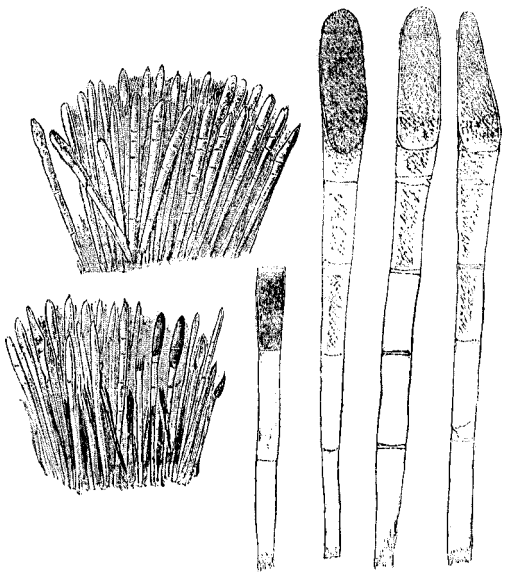

FIG. 3. Ambrosia fungus of *Xyleborus celsus* (from Hubbard 1897).

Unlike the typically specialist bark beetles, individual ambrosia beetle species most often use a wide array of host taxa (Schedl 1956; Browne 1958; Beaver 1979, 1989; Noguera-Martinez and Atkinson 1990), made possible by the beetles' direct feeding on fungi with broad host tolerances (Francke-Grosmann 1967; Beaver 1989). Thus, domesticated fungi seem to enable both leaf-cutting ants and ambrosia beetles to adopt a generalist strategy apparently well suited to hyperdiverse tropical forests (Beaver 1979; Cherrett et al. 1989; Holldobler and Wilson 1990; Mueller et al. 1998). Whereas both adults and larvae of phloem-feeders carve galleries as they feed in isolation, ambrosia beetle larvae do little tunneling; instead they feed gregariously in chambers on asexually produced conidiospores induced in cultures kept pure by the parents (Hubbard 1897; Francke-Grosseman 1967; Beaver 1989; Kirkendall et al. 1997), much as in attine ant gardens (Mueller et al. 1998; Currie et al. 1999). The only other major agricultural insect group, the eusocial macrotermitine termites, are largely detritivores, feeding their basidiomycete fungi (genus *Termitomyces*) with leaf-litter and other plant remains found near the surface of the soil in their African and Indomalayan habitats (Wood and Thomas 1989).

Recent progress in molecular systematics of the ambrosia fungi *Ambrosiella* and *Raffaelea* and other ophiostomatoid fungi (Cassar and Blackwell 1996; Jones and Blackwell 1998) provide opportunities for comparisons with bark and ambrosia beetle phylogenesis. On the fungal side, the phylogeny offers support for the acquisition-clonality hypothesis developed for ants (Chapela et al. 1994), in that these asexual ambrosia fungal genera are each polyphyletic and multiply derived from the sexual genera *Ceratocystis, Ceratocystiopsis,* and *Ophiostoma* (Cassar and Blackwell 1996; Jones and Blackwell 1998). There have not been comparable molecular surveys across the many groups of ambrosia beetles from which these fungi have been studied. We have therefore sampled all of the major lineages of Scolytinae and Platypodinae to identify the number, placement, and apparent ages of origins of ambrosia beetles and to evaluate whether this habit is ever reversed. Identification of ambrosia beetle sister groups will also make it possible to test whether polyphagy tends to promote diversification, by preventing extinction and increasing geographic range, or depress diversification, by increasing gene flow among distant populations (Kelley and Farrell 1998; Dobler and Farrell 1999; Kelley et al. 1999).

The striking variation in resource use in Scolytinae is paralleled by variation in reproductive strategies, and numerous hypotheses have been developed on the interplay between these two dimensions of their life histories (Kirkendall 1993; Kirkendall et al. 1997). For example, most bark beetles are monogynous, presumably because the tunnels facilitate mate-guarding; and gregarious feeding in ambrosia chambers or in pith or seeds has been thought to lead to the many instances of inbreeding (Kirkendall 1993). Moreover, ambrosia beetles show parental care, at least one origin of haplodiploidy (Normark et al. 1999) and eusociality (Kent and Simpson 1992). Thus, it seems that all three agricultural insect groups (ambrosia beetles, attine ants, and macrotermitine termites) show some degree of sociality.

To provide a phylogenetic framework for tests of hypotheses on the evolution of interactions between beetles, fungi, and host plants, we conducted a molecular phylogenetic study of 86 species of Scolytinae and Platypodinae, representing the major tribes and ecological variation worldwide, plus outgroups. We used six genes, including two divergent copies of enolase that have not previously been used for phylogenetic studies in insects.

Materials and Methods

Beetles were collected from colonized hosts (see Appendix for collecting localities) and include genera from 20 of the 25 tribes proposed by Wood and Bright (1992) for the Scolytinae and from two of the five tribes of Platypodinae (both classified as families in Wood and Bright 1992). Previous comparative morphological studies of adults (Kuschel 1966; Thompson 1992) and cladistic analyses of larvae (Marvaldi 1997) have proposed a close relationship between the weevil subfamily Cossoninae and the Scolytinae. We were able to obtain two genera from the Cossoninae belonging to two different tribes (Cossonini and Araucarini) to include as outgroups.

Amplification and Sequencing

Polymerase chain reaction (PCR) and cycle sequencing were used to obtain partial sequences of six genes: EF-1α; COI; two copies of enolase, here termed enolase 1ni (no intron) and enolase 2I (intron-containing; Fig. 4); 18S; and 28S (D2 and D3 expansion segments). DNA was extracted from individual beetles preserved in ethanol following Sunnucks and Hales (1996) with the modifications introduced by Normark (1999).

PCR reactions (50 μl) typically contained 0.2 mM of each primer, 0.8 mM dNTPs, Qiagen (Valencia, CA) PCR buffer with additional $MgCl_2$ to a final concentration of 2 mM (18S and 28S) or 2.5 mM (EF-1α, COI, and enolase), and 1.25 units Qiagen *Taq* DNA polymerase. For 18S and COI, the temperature profile was 40 cycles of 95°C for 30 sec, 47°C for 60 sec, and 72°C for 60 sec. The cycling profile for 28S was the same except that the annealing temperature was 50°C. For EF-1α, a touchdown profile of 42 cycles was used, with annealing temperature decreasing from 58°C to 42°C by 2°C every third cycle and the final 18 cycles at 42°C. After amplification, double-stranded PCR products were purified using the Qiagen PCR purification kit to remove primers and unincorporated dNTPs prior to sequencing. Cycle sequencing reactions were performed with the ABI prism Dye Terminator Cycle Sequencing Kit (Perkin-Elmer, Norwalk, CT) on an ABI 370 automated sequencer. Primer sequences are given for enolase in Table 1, for EF-1α and COI in Normark et al. (1999); and for 18S and 28S in Sequeira et al. (2000).

Enolase

Enolase, also known as 2-phospho-D-glycerate hydrolase, is a glycolytic enzyme responsible for converting two PG molecules into PEP through a dehydration reaction during glycolysis. The enolase gene is generally a single-copy (in invertebrates) nuclear gene of approximately 1302 bp length. Although cited as a promising candidate for molecular systematics by Friedlander et al. (1992), it had never been de-

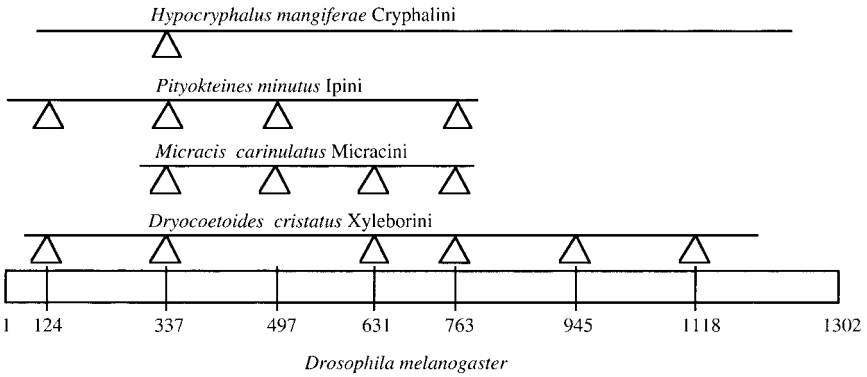

FIG. 4. Diagram of the intron/exon structure of enolase 2I in members of four different tribes included in this study. Numbers refer to the position of the intron with respect to the *Drosophila melanogaster* sequence (Genbank accession no. X17034).

veloped for insect systematics. Initial primers for the single-copy nuclear protein coding gene were developed using GenBank sequences for *Drosophila*, the decapod crustacean *Penaeus monodon* (Boonchuoy et al. 1999), and several vertebrates. A series of PCR, sequencing, and primer modification was performed until sequences could be retrieved from a representative set of taxa. For PCR either a touchdown profile as above or a standard 40-cycle program with 47°C to 42°C annealing temperature were used (depending on the taxon).

Sequence Alignment

All sequences were compiled using Sequencher 3.0 (Genecodes Corp., Ann Arbor, MI). There were no insertions or deletions in COI. Introns were removed from EF-1α and from the intron-bearing copy of enolase prior to phylogenetic analysis, yielding protein-coding sequences with no insertions or deletions. Ribosomal sequences did have insertion-deletion differences, and thus were aligned using Clustal X (Aladdin Systems Inc., Heidelberg, Germany) with the default gap opening:gap extension costs (15:6) and then subjected to eye inspection, where the minimum regions of ambiguous alignment were selected for exclusion, including few gap-bearing regions in the analysis.

TABLE 1. Primers for enolase. Names refer to the gene (en, enolase), the direction (s, sense; a, antisense), and the position of the 3′ end with respect to the coding portion of the *Drosophila melanogaster* sequence (Genbank accession no. X17034). Use refers to amplification (a) and sequencing (s). N, Y, R, S, and X are base ambiguities.

Name	Use	Sequence
ens65c	a,s	GACTCCCGTGGNAACCCCACNGTGGAGGT
ens65b	a,s	GACTCCCGTGGNAACCCCACNGTNGAGGT
ens65a	a,s	GACTCCCGYGGNAAYCCCACNGTNGAGGT
ens287	a,s	GARATYGAYGARTTYATGATYAA
ens312a	a	GACGGCACCGAGAACAAGAGC
ens312b	a	TGGACGGCACCGAGAACAA
ens509	a,s	TGGCSATGCAGGARTTCATGAT
ens552n	a,s	TTYACCGARGCXATGAARATG
ens552i	s	TTYACCGARGCXATGMGXATG
ens791	a,s	TACGATTTGGACTTCAAGA
ena385	s	GCCARATCNGCAATGTGTYTGTAAAGTGG
ena493	s	ATCATRAAYTCYTGCAT
ena474	s	CATGAATTCCTGCATGGCCAGCTTGTTGCC
ena544	a,s	CGTCCATRCCAATYTCAAT
ena776	a,s	TTYGGRTTCTTGAARTCCA
ena764	a,s	TCTTGAAGTCCAAATCGTA
ena886	a,s	CCAGTCRTCYTGRTCRAAXGG
ena1014	a	ATCTGGTTGACCTTCAGSAGSAGGCA
ena1170	a,s	GGAGCACCGGTCTTGATCTGACC
ena1223	a,s	CTGGTTGTACTTGGCCAGACGCTC
ena1228	a,s	CTCCTCCTCAATGCGCARGATCTG

Gene Divergences

The relative sequence divergences by gene were compared by plotting the uncorrected pairwise distance between two taxa for a gene of interest against the HKY + Γ corrected pairwise distance for COI for that pair. The likelihood correction was used to more closely approximate time on the x-axis, although the overall result is similar with uncorrected EF-1α or COI divergence. For ribosomal genes, the distance for all positions was used, but third codon positions were excluded for protein-coding genes because saturation obscures the more slowly evolving first and second positions (Fig. 5a). To visualize the relative divergences per codon position the proportions of overall gene divergence by codon position was graphed and the ratio of total first and second codon position : third position changes calculated (Fig. 5b).

Phylogenetic Analysis

Phylogenetic analysis was performed by maximum-parsimony using PAUP (ver. 4.0b4a, Swofford 2000). Amino acid sequences of COI, the most variable protein coding gene (Fig. 5a), were combined in one matrix with nucleotide sequences for all other genes (EF-1α, 18S, 28S, enolase 1ni, and enolase 2I). All substitutions were weighted equally and the few included gaps were treated as missing data.

Heuristic searches used 100 random-addition-sequence starting trees and starting from random trees with no maxtrees limit, with TBR branch swapping on best trees only. These same parameters were also used with an implementation of the parsimony ratchet procedure (courtesy of P. Lewis and D. Sikes, Univ. Connecticut; based on Nixon 1999), with 1000 replicates and 15% weighting. For bootstrap analyses, 1000 pseudoreplicates were generated with 10 random taxon additions. For incongruence testing among the six datasets the ILD test (Farris et al. 1995) was used as implemented in

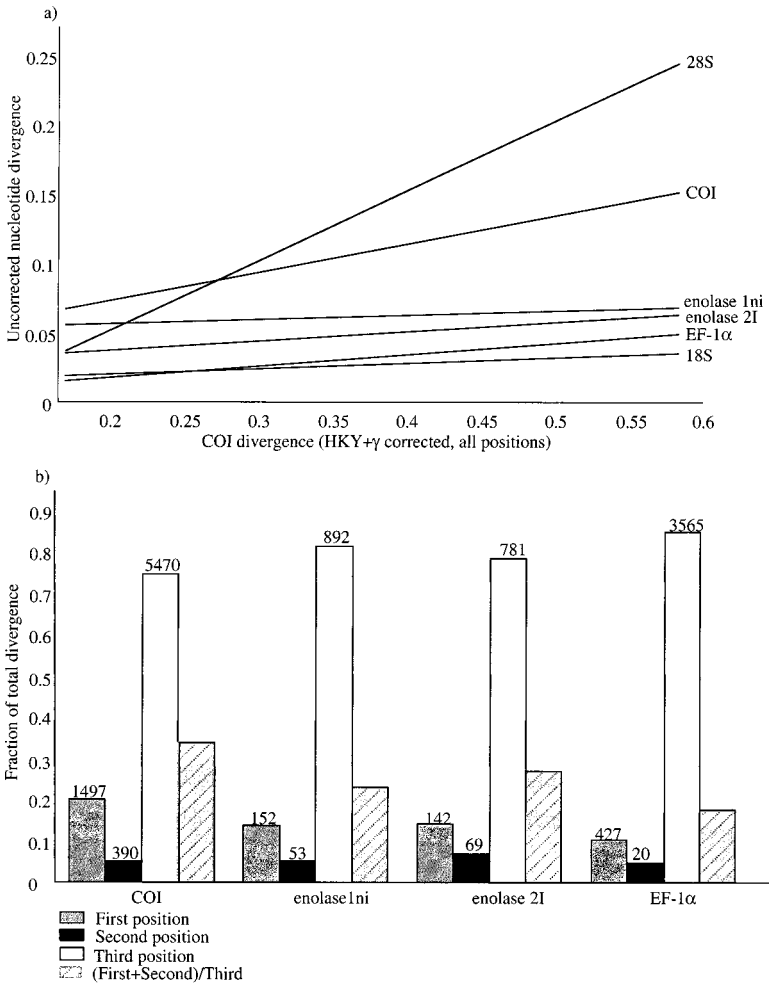

FIG. 5. (a) Divergence by gene. Uncorrected nucleotide divergence (p, uncorrected pairwise divergence) between two taxa for each gene region plotted against the HKY + Γ corrected pairwise distance for COI for that taxon pair. (b) Proportions of overall gene divergence by codon position. Numbers on the y-axis indicate the proportion of a given gene's changes at a particular codon position. Uncorrected total number of changes, summed over all taxa, are reported above the bars. The striped bar in each bar group depicts the relative proportion of changes in first and second codon positions combined with respect to those occurring in third codon positions.

PAUP, using 100 replicates and 50 random addition sequences in each replicate (TBR, maxtrees = 2000, excluding uninformative characters).

To create the constraint trees for the nodes from the combined maximum-parsimony tree and to calculate decay indexes (Bremer 1994), we used Autodecay 4.0 (Eriksson 1998), again with 100 random additions for the heuristic PAUP runs (TBR limited to 10^6 rearrangements per addition sequence replicate).

To test whether the distribution of each of three life-history traits (host used: conifers vs. angiosperms; feeding substrate used: phoem, xylem, ambrosia, pith, or seeds; and inbreeding vs. outcrossing; Wood 1982; Kirkendall 1993) on the topology differs significantly from a randomly distributed character, we compared the observed number of changes separately for each of these three characters on the tree with a randomized distribution of the respective states of each character produced using the PTP utility in PAUP and holding their relative frequencies and the beetle topology constant (Kelley and Farrell 1998).

For comparisons of ambrosia-fungus phylogeny with beetle phylogeny, we obtained the 18S sequences for the ophiostomatoid fungi from TreeBase (matrix accession number M712; Berbee and Taylor 2001) and additional sequences for *Ophiostoma* and all available *Ceratocystis* and *Raffaelea* sequences from Genbank (*Ceratocystis virescens* U32419, *C. fimbriata* U43777, *Ambrosiella brunnea* U40023, *A. ferruginea* U40016, *A. gnathotrichi* U40015, *A. hartigii* U40017, *A. ips* U40018, *A. macrospora* U40019, *A. sulcati* U40020, *A. sulfure* U40021, *A. xylebori* U40022, *Leucostoma personii* M83259, *Ophiostoma bicolor* AB007666, *O. europhioides* AB007667, *O. penicillatum* AB007668, *O. piceae* AB007663, *O. piliferum* U20377, *Raffaelea albimanens* U44474, *R. arxii* U44475, *R. canadensis* U44480, *R. santoroi* U44477, *R. sulcati* U44481, *R. tritirachium* U44478). The very similar 18S sequences for 28 fungal taxa were aligned using Sequencher 3.1 and yield a matrix of 1960 characters (174 informative) that was used in maximum-parsimony analyses with heuristic search parameters and bootstrap analyses as above. With Modeltest 3.0 (Posada 1998) the following parameters were se-

TABLE 2. Sister group comparison of diversity of angiosperm feeding and conifer feeding. Comparisons 1–4 are from the present study, 5–10 are published in Farrell (1998a). Comparisons 1, 5–10 represent shifts from conifers to angiosperms, and 2–4 represent shifts from angiosperms to conifers.

Angiosperm feeding		Gymnosperm feeding	
1. Scolytinae (− Ipini)	5200	Hylastini + Tomicini	180
2. *Acanthotomicus* +	95	*Orthotomicus*	11
Premnobius	24		
3. Xyleborini/Dryocoetini	1500	Ipini (− *Acanthotomicus* + *Premnobius*)	195
4. Corthylina	458	*Pityophthorus*	200
5. *Apion*	1500	Antliarhininae	12
6. Belinae	150	Allocoryninae + Oxycorinae	30
7. Higher Curculionidae	44,002	Nemonychidae	85
or Anthribidae	3000		
8. Higher Cerambycidae	25,000	Aseminae + Spondylinae	78
9. Megalopodinae	400	Palophaginae	3
10. Higher Chrysomelidae	33,400	Aulacoscelinae + Orsodacninae	26

lected for the 18S fungal dataset (TrN + I + G, general time reversible model, submodel abaaea, estimating the proportion of invariable sites and the shape of the Γ parameter, with the empirical base frequencies, and estimating the ts/tv ratio). This model was used to choose between the maximum-parsimony trees obtained from the 100 random adition sequences search. Maximum-likelihood branch-length optimizations were performed on the topology of the most likely tree using the Describe Tree feature in PAUP. These were then used with the Berbee and Taylor (1995, 2001) calibration for fungal 18S. Ages for nodes in Figure 7 were inferred using the divergence time between *Neurospora* and *Hypomyces* in Berbee and Taylor (2001) and the maximum-likelihood-optimized branch lengths under a molecular clock. Standard errors correspond to branch length/age ranges given by the maximum-likelihood optimization on the maximum-parsimony fungal topologies.

Diversity Tests

Each of the life-history traits of interest were mapped on the most parsimonious tree, and sister groups identified for diversity contrasts (Tables 2, 3). Tallying of relative diversities of the tribes and genera requires assumptions of monophyly of taxa not included in our analysis. Inasmuch as this is true of both sides of each sister group comparison, this approach should not bias the results.

Relative Age of Ambrosia Associations

To evaluate whether there is a correspondence between the ages of origin of ambrosia fungi and the origin of ambrosia associated beetles, the following analysis was carried out: Modeltest 3.0 was used on the 18S beetle dataset to choose the most likely model (maximum-likelihood parameters), and then the selected model was used to select among the maximum-parsimony trees (see Results) obtained from the combined analyses. Branch lengths were optimized on the selected tree using the corresponding parameters. Linear correlation was performed for the 18S maximum-likelihood branchlengths optimized on the fungal 18S topology and the 18S beetle branchlengths optimized on the combined beetle topology from the root to each origin of ambrosia beetles and beetle associated fungi.

TABLE 3. The diversity of ambrosia-feeding groups is contrasted with the diversity of their sister groups of other habits.

Ambrosia beetle group	Diversity	Sister group	Diversity
Xyleborini	1300	*Ozopemon*	25
Corthylina	458	*Pityophthorus*	385
Platypodini	1500	Scolytini	192
Premnobius	24	*Acanthotomicus*	95

RESULTS

Our matrix of 159 previously published sequences and 106 new sequences, across six genes, contains 6104 characters with 1613 parsimony-informative sites (see Appendix for Genbank Accession nos.). Partition homogeneity tests indicate no significant incongruence among the six datasets ($P = 0.0698$). From the 1936 bp of the 18S alignment, 272 bases were excluded because they could not be unambiguously aligned, producing a final matrix of 1664 characters. The 28S alignment produced a matrix of 993 bp, which displays two 400-bp regions of great apparent homology plus one much more variable region with ambiguous alignment. The variable region was excluded from the analysis, leaving 892 positions included in the analysis with a few gaps analyzed as missing data. For EF-1α, evidence of two loci that differ in intron/exon structure was found in some members of the subfamily Scolytinae (Normark et al. 1999). For this study we used only the copy having one intron between coding positions 753 and 754. Two copies of enolase were discovered (enolase 1ni and enolase 2I) and were analyzed separately (see Fig. 4 for intron structure diagrams). Although considerable variation was found in intron lengths, the alignment of the coding sequences was unequivocal. All introns were removed prior to phylogenetic analysis.

Compared Gene Divergences

The uncorrected pairwise distance against the HKY + Γ corrected pairwise distance for COI for each pair plotted in Figure 5a indicates a trend in decreasing rate of evolution from 28S, COI, the enolases, EF-1α, and 18S. When comparing the proportions of overall gene divergence by codon

position in the four protein-coding regions (Fig. 5b), COI displays the highest ratio of first and second codon position substitutions to third codon position substitutions (0.339 vs. 0.178 for EF-1α), suggesting that a greater proportion of changes could be obscured due to multiple hits at third codon positions. To reduce the effect of saturation we used the COI aminoacid sequences in the combined dataset.

Combined Phylogenetic Analyses

The parsimony searches (100 random addition sequences) starting with random trees resulted in 33 maximum-parsimony trees of length 11,319 steps (the parsimony ratchet found trees four steps longer). Tree support varied among nodes at different levels of relationship, with strongest support for nodes at some of the deepest and most shallow groupings (Fig. 6).

The phylogeny estimate is in broad agreement with the outline provided by Wood (1982) and Nobuchi (1969), with some notable exceptions. Of the tribes recognized by Wood and represented by multiple genera in our sample, several are monophyletic in our results (Hylastini, Hypoborini, Corthylini, Crypturgini, Xyloterini, Platypodini); a few are paraphyletic with respect to a few close relatives (Phloeosinini, Ipini, Dryocoetini), and one is paraphyletic with respect to all other bark beetles (Tomicini). Three tribes are strikingly polyphyletic in our results: Xyleborini, Cryphalini, and Hylesinini. Xyleborini is polyphyletic due to the distant placement of *Premnobius*, whose membership in Xyleborini had long been controversial (Normark et al. 1999). Polyphyly of Cryphalini and Hylesinini is less well supported, the hypothesis of monophyly for the Hylesinini has been rejected in previous studies where topologies constraining the monophyly of the group were found to be significantly longer than the maximum-parsimony tree ($P = 0.001$, Sequeira et al. 2000). This can be due to the inclusion of several members of the extremely species-rich and morphologically diverse *Hylesinopsis* (R. Beaver, pers. comm.). Of the subfamilies recognized by Wood, only Platypodinae is monophyletic; the tribes of Hylesininae and Scolytinae are interdigitated to some extent. Striking similarities to Wood's topology include the basal position of Tomicini, Hylastini, and most Hylesinini, and the close relationship of Platypodinae to Scolytini, also suggested in Kuschel et al. (2000).

Primary associations with conifers versus angiosperms show significant deviation from a random distribution across the phylogeny (PTP test, $P = 0.001$; Kelley and Farrell 1998). Conifer associations are basal in the group, and followed by several shifts between angiosperms and conifers (Fig. 6).

Feeding substrate (phloem, xylem, pith, seeds, and ambrosia) also shows significant deviation from a random distribution with respect to the phylogeny (PTP test, $P < 0.001$). Phloem feeding is basal, followed by seven separate origins of ambrosia feeding, all unreversed (Fig. 6).

Distribution of breeding systems (inbreeding vs. outcrossing) also departs significantly from a random distribution with respect to the phylogeny (PTP test, $P < 0.001$).

Sister Group Diversity Tests

The three sister group contrasts between angiosperm-feeding and conifer-feeding lineages show the angiosperm feeders to be consistently more diverse (Table 2, $P < 0.001$, sign test), consistent with the general pattern in beetles (Farrell 1998a). The seven origins of ambrosia feeding are less clear (Table 3). Compared to their respective sister groups, Xyleborini, Platypodini, and Corthylina are more diverse, but *Premnobius* is less diverse. The three remaining origins of ambrosia beetles represented in our samples are much less confidently placed. Of these the Xyloterini is more diverse than the species of *Hylesinus*, Scolytoplatypodini is either more or less diverse than these respective genera of Micracini, depending on which represents the actual sister group, and *Sueus* is less diverse than the Ipini + Xyleborini.

Fungal Phylogeny and Age of Ambrosia Associations

We found 18 maximum-parsimony trees in our reanalysis of 18S sequences for *Ambrosiella, Raffaelea* plus the other ophiostomatoid genera, and outgroups, and these do not conflict with the previously published separate analyses of these genera (Cassar and Blackwell 1996; Jones and Blackwell 1998). As reported by M. Blackwell and associates, we find three separate origins of *Ambrosiella* cultivars, with near relatives from *Ophiostoma* and *Ceratocystis* (Fig. 7). The *Ambrosiella* associated with Corthylini shares an ancestor with *Raffaelea* associates of Platypodinae. The best fitting maximum-likelihood model is the general time reversible (GTR) model estimating the proportion of invariable sites and estimating the shape of the Γ parameter, with the empirical base frequencies, and estimating the ts/tv ratio. Using this model to calculate branchlengths for the best fitting maximum-parsimony tree, and the Berbee and Taylor (2001) calibration, the age estimates for ambrosia fungi are 60 (±7.9) million years for the *Ambrosiella* and *Raffaelea* associates of Corthylini and Platypodinae, 35 (±4.3) million years for the *Ambrosiella* cultivars associated with Xyleborini, and 21 (±2.7) million years for the presumably facultative *Ambrosiella* associates of *Ips.* Significant correlation was found of the maximum-likelihood-optimized 18S branch lengths of ambrosia beetles with those of their associated ambrosia fungi ($R^2 = 0.8872$, $P = 0.019$; Fig. 8).

Discussion

Our estimate of Scolytinae phylogeny illuminates some of the consequences of shifts in resource use and mating systems. As in other groups of beetles and other insects, much of the diversification in life-history traits and lineages seems occasioned by use of angiosperm hosts (Farrell 1998a).

Ambrosia

Fungus gardening has evolved at least seven times in these beetles (Fig. 6). It is not yet clear whether adoption of the fungus-gardening habit generally enhances diversification rate (Table 3), but these obligate associations with fungi span a range of ages that underscores their apparent stability. The three largest radiations, Platypodinae, Xyleborini, and Corthylini, are primarily tropical and comprise 98% of the 3400 described ambrosia beetle species, whereas the three small groups, Xyloterini, Scolytoplatypodini, and Hyorhynchini, have a higher proportion of temperate species. The temperate

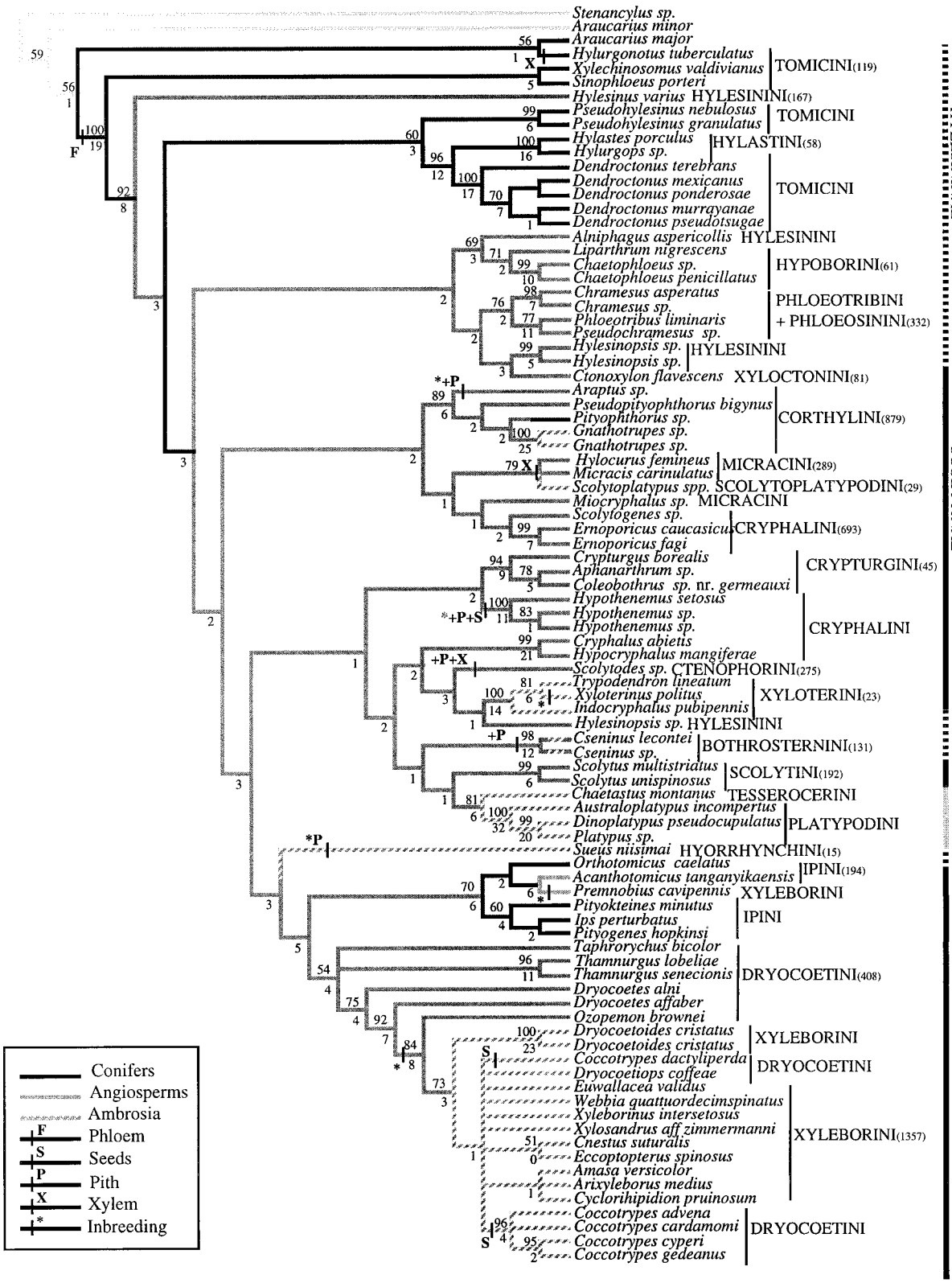

FIG. 6. Strict consensus of 33 most parsimonious trees from the combined analysis of COI amino acids, 18S and 28S (hypervariable regions excluded), EF-1α (intron excluded), and both copies of enolase (enolase 1ni and enolase 2I; introns excluded). Length = 11,319 steps; CI (informative characters only) = 0.2995; RI = 0.4573. Above each internal branch are the bootstrap values, and the decay indices are below, both for the combined analysis. Branch color for the nonambrosia-feeding Scolytinae and Platypodinae indicates the host plant group for the respective genera (black, conifers; gray, angiosperms). Ambrosia feeding is indicated by striped branches, whereas direct feeding on other host tissues is indicated by hatch marks with letters (F, phloem; X, xylem; P, pith; S, seeds; letters are marked

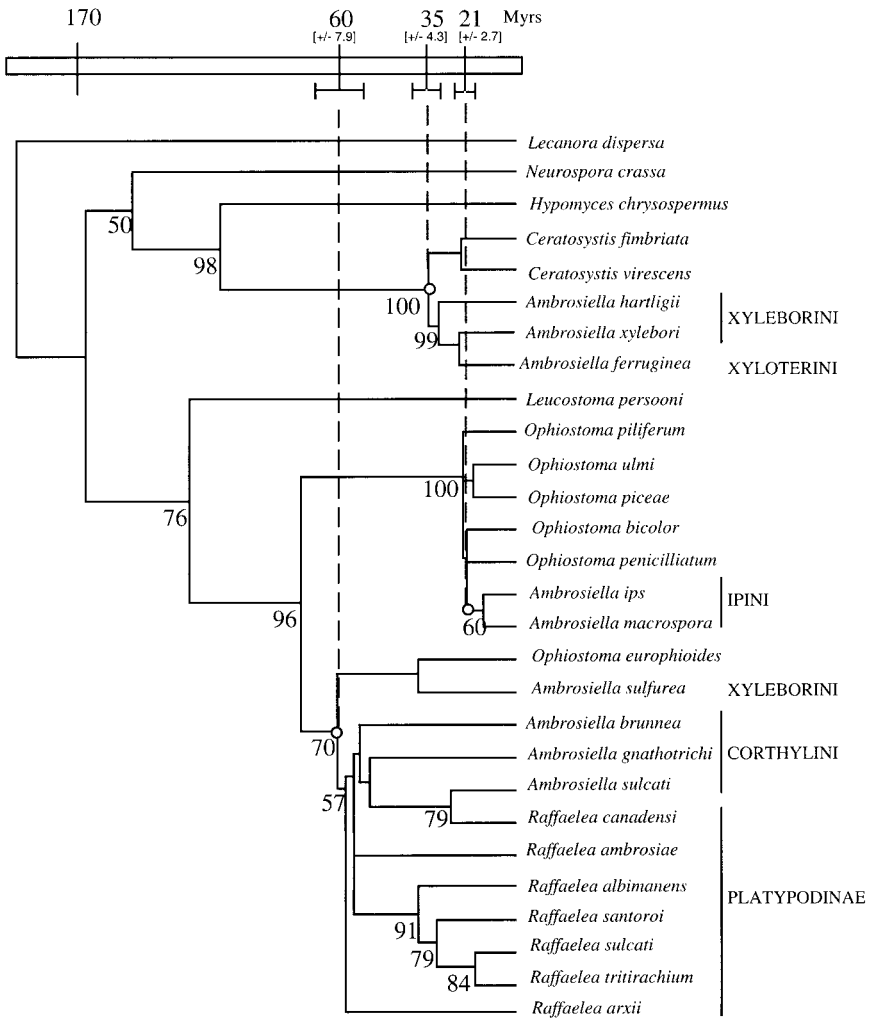

FIG. 7. One of the 18 most parsimonious trees from the analysis of the 18S dataset for ophiostomatoid fungi and allied genera (Length = 623 steps) displaying the most likely score under the model chosen for this dataset with Modeltest 3.0 (Posada 1998). Branch lengths correspond to the maximum-likelihood optimization of the 18S data over the chosen topology enforcing a molecular clock. Numbers on the time scale correspond to the ages of bark beetle associated clades using the calibration for fungal 18S introduced by Berbee and Taylor (2001) and standard errors correspond to branch length/age ranges given by the maximum-likelihood optimization on the 18 maximum-parsimony fungal topologies.

zone is generally drier than the tropics and less conducive to fungal growth, which may explain the greater abundance and diversity of ambrosia beetles in the tropics (Beaver 1989). However, further investigation of the relationships of temperate and tropical ambrosia beetles would be required to determine whether diversification rates are actually higher in the tropics.

The two genera of ambrosia fungi, *Raffaelea* and *Ambrosiella,* are both polyphyletic, and each arose at least five times (Cassar and Blackwell 1996; Jones and Blackwell 1998) from within the ophiostomatoid clade that includes *Ophiostoma* and *Ceratocystis* (Berbee and Taylor 1995, 2001). The ophiostomatoids apparently arose some 200 million years ago, soon after the onset of diversification of conifers, the principal hosts of *Ophiostoma* (Harrington 1993; Kile 1993). Thus, these pathogens predate the Scolytinae and were probably vectored by other insects, perhaps including the weevil antecedents of Scolytinae. The oldest origin of ambrosia fungi is the early Tertiary, some 60 million years ago, in the platypodine-associated *Raffaelea* and the related corthyline-associated *Ambrosiella* (Cassar and Blackwell 1996; Jones and Blackwell 1998; Fig. 7). The genus *Ambrosiella,* associated with the scolytine ambrosia beetle tribes Xyloterini, Xyleborini, and Corthylini (and apparently, Scolytoplatypodini: Kinuura and Hijii 1991), comprises two primary clades of which one is associated with Xyleborini and Xy-

←

with a + if the group is known to contain species that feed on different tissues). The presence of inbreeding in a group is indicated by an asterisk. Bars beside taxon names indicate tribe, subfamily, and family classification after Wood (1986) and Wood and Bright (1993).

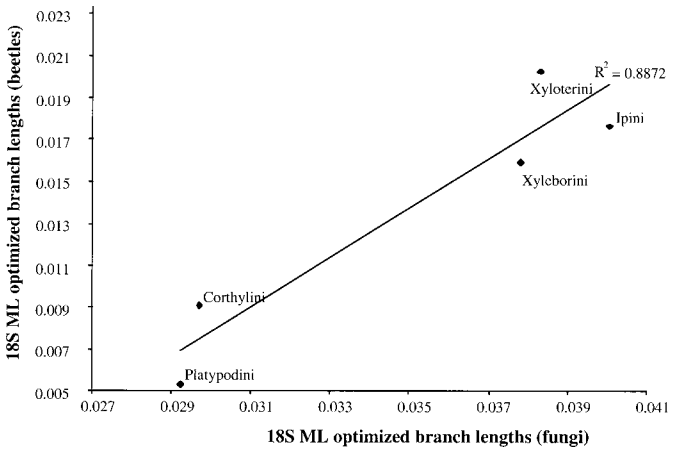

FIG. 8. Maximum-likelihood (ML) optimized branch lengths calculated on the MP selected fungal 18S topology and the optimized branchlengths for 18S on the beetle topology for each of the beetle-ambrosia associations show significant linear correlation (R^2 = 0.8872, P = 0.019).

loterini and the other is associated with Corthylini. Because Xyleborini and Xyloterini represent independent origins of the ambrosia beetle habit, lateral transmission or independent acquistion of ambrosia fungi has apparently occurred, as has been also been demonstrated for the fungi associated with attine ants (Mueller et al. 1998).

The most recent origin of *Ambrosiella* comprises fungi that are associated with the largely conifer phloem-feeding *Ips* and *Hylurgops*, also associated with the largely conifer-attacking *Ophiostoma*, and may thus represent the initial stages of obligate fungal mutualisms (Lutzoni and Pagel 1997). Although strictly asexual lineages are susceptible to accumulation of deleterious mutations (Moran 1996) and are thought to go extinct rapidly in most cases (Barton and Charlesworth 1998), ambrosia fungi appear to have persisted since the early to mid Tertiary, like a few other asexual clades (Judson and Normark 1996; Mark Welch and Meselson 2000).

The correlated branchlengths (Fig. 8) and inferred ages of ambrosia beetles and fungi (Fig. 7) apparently reflects their co-descent from less tightly associated ancestors and corroborates our view that 30 million year old Dominican amber is too ancient to have captured the Xyleborini (Jordal et al. 2000). Thus, Xyleborini, Corthylini, and Platypodinae are all abundant ambrosia beetles on Hispaniola today, and all attack resinous legumes (as well as many other plants) related to the source of Dominican amber (Wood and Bright 1992; Bright and Poinar 1994). Although the corthyline and platypodine fossils in Dominican amber are common, Xyleborini is conspicuously absent from these amber deposits. The origin of 1300 xyleborine species in 20 million years is more than twice the rate for the Platypodinae (1500 species in 60 million years) and may reflect the combination of polyphagy enabled by ambrosia-feeding together with inbreeding and haplodiploidy in this group (Jordal et al. 2000). Moreover, the colonizing ability of xyleborines afforded by the combined inbreeding and haplodiploidy may also explain their disproportionate representation on islands (Jordal et al. 2001). Indeed, both *Xyleborus* and the outcrossing *Platypus* are famously species-rich and morphologically homogeneous genera, which are consistent with rapid radiation (Schedl 1956; Browne 1961; Nobuchi 1969; Beaver 1989; Wood and Bright 1992).

Whereas the species of ambrosia beetles are extremely uniform, the diversity of their mycangia is striking (Francke-Grossman 1967; Beaver 1989). Within the Platypodinae and Xyleborini, the mycangia of very close relatives (i.e., sharing an ambrosia-associated ancestor) often occur in different body regions (e.g., the mouthparts, basal leg segments, thorax, or elytra; Francke-Grossman 1967; Beaver 1989) or differ between the sexes. These mycangia have thus evolved very rapidly. Tests of the hypothesis that these fungi and their herbivores are undergoing rapid turnover could draw on patterns in molecular evolution and analyses of dispersion on the phylogeny (Moran 1996; Kelley and Farrell 1998). There are a few species that apparently lack mycangia (Beaver 1989), but most species have not been subjected to the detailed histological studies required to detect and demonstrate mycangial presence. The widespread distribution of mycangia (across Xyleborini, Ipini, Drycoetini, Xyloterini, Corthylini, Tomicini, Scolytoplatypodini, Cryphalini, Bothrosternini, Hylesinini, and the Platypodinae) suggests the presence of mycangia close to the ancestor of the Scolytinae, which was evidently feeding on phloem of conifers in association with ophiostomatoid fungi. However, the origins of ambrosia feeding all followed shifts to angiosperms (although some temperate ambrosia beetle species are able to use conifer hosts; Wood 1982).

Shifts between Conifers and Angiosperms

The estimate of Scolytinae phylogeny supports the hypothesis that shifts from conifer feeding to angiosperm feeding have tended to enhance rates of species diversification in beetles (Fig. 5, Table 2) and other herbivores (Farrell 1998a, 1999). While the shifts from conifers to angiosperms in the hylesinine tribes and within Ipini further support the hypothesis that use of angiosperms fosters insect diversity, our results for scolytines strengthen this pattern by providing evidence that shifts back to conifers following angiosperm colonization are associated with lowered diversity (Table 2). Both early Mesozoic (~200 million years ago; Farrell 1998) and late Tertiary (10–20 million years ago; this study) colonizations of conifers are associated with lower diversity, suggesting that the number of conifer host species is the limiting quality of this plant group, rather than the particular time period in which shifts occur.

Nevertheless, the three independent conifer-associations (Hylastini + Tomicini, Ipini, and *Pityophthorus*) each comprise approximately 200 species, and thus are among the most species-rich conifer associations known. Many other small groups of conifer-associated beetles occur in otherwise angiosperm-affiliated clades in the Scolytinae and other groups. These bark beetles collectively use nearly all of the 300 species of conifers (e.g., some *Dendroctonus* use up to 30 species of *Pinus*; Kelley and Farrell 1998), and most conifers have multiple beetle associates (Sturgeon and Mitton 1982; Bright and Stock 1982; Wood and Bright 1992). The relatively great diversity of scolytines associated with conifers may reflect their very small body size range (1–11 mm). Thus, different

scolytine species are dispersed among different tree parts (e.g., phloem, xylem, cones, twigs, roots, as well as distal vs. proximal regions of these parts; Wood 1982), different stages of declining tree health, or different parts of the species' range, and an individual tree may support reproduction of up to 10 species of bark beetles (Paine et al. 1997). Thus, relatively tiny-bodied scolytines appear to partition host resources more finely than their relatively large bodied, wood-boring competitors in the beetle families Cerambycidae and Buprestidae (Strong et al. 1984; Denno et al. 1995), consistent with the implications of body size for resource use (Morse et al. 1985).

For example, the unusually widespread *Pinus ponderosae* is host to 75 scolytine species (of which half are the tiny 1–2-mm species of twig-mining *Pityophthorus*; Wood and Bright 1992), but only 35 cerambycid species (Linsley and Chemsak 1997) and five or six buprestids. In contrast, the greater overall diversity of cerambycid and buprestid wood borers (approximately 20,000 and 12,000 species, respectively) largely reflects their colonization of nonwoody tissues (Bright 1987; Linsley and Chemsak 1997), substrates probably not generally amenable to gallery construction by adult beetles.

The pronounced conservatism of scolytine associations with conifer and angiosperm wood is comparable to that of insect associations with other plant parts (e.g., the leaves and flowers of herbaceous plants: Farrell and Mitter 1994; Farrell 1998a,b; A. Marvaldi, A. S. Sequeira, C. W. O'Brien, and B. D. Farrell, unpubl. ms.). This appears to reflect the persistence, even after the death of woody tissues, of plant qualities that determine herbivore specificity. From the perspective of plant fitness, this may simply be a nonadaptive consequence of reliance on relatively stable chemicals for defense. A large old tree is often a mosaic of healthy, unhealthy, and dead tissues, however, and such a tree may benefit strongly from having wood that is resistant to insect attack long after its death.

Unlike other beetle, wasp, and moth herbivore groups, all of the conifer associations in these weevils postdate the origin of angiosperms (Farrell 1998a,b). The original conifer association in these beetles apparently occurred with their shift to *Araucaria* in the late Cretaceous, when angiosperms were still in the initial stages of diversification and *Araucaria* were still widespread on most continents (Sequeira et al. 2000; Sequeira and Farrell 2001). Relationships within the scolytine sister group Cossoninae remain unstudied, but the ostensibly basal tribe Araucariini are associated with *Araucaria* and many genera are associated with other conifers. Moreover, adults of these weevils also bore tunnels in host trees, an unusual habit (Kuschel 1966). It thus seems probable that the common ancestor of Cossoninae and Scolytinae shifted to conifers, although we cannot rule out independent shifts to conifers within each group. The sister group to these taxa is not firmly established, but our current best estimate is the monocot-associated Derolominae, which is placed well within the angiosperm-feeding weevils (Marvaldi et al., unpubl. ms.). The much later shifts to conifers by Ipini, *Pityophthorus*, and other scolytine lineages occurred in the late Tertiary, when conifers were again becoming more widespread with the Oligocene cooling of climate and expansion of the temperate zone (Graham 1999).

Although the clearest evidence is that the diversity of scolytine beetles reflects their use of angiosperms, their highly unusual abilities to bore as adults into wood permitted use of plants with secretory defenses (and also the origins of ambrosia fungus cultivation). Thus, adult boring permits the dual strategy of carrying fungi and the tunneling activities that block the defensive secretory canals that characterize most of their host groups. The repeated radiations of plant groups with latex and resin canals (Farrell et al. 1991) may have provided opportunities for bark beetles and other insects able to circumvent this defense (Dussourd and Denno 1994), but no comparative phylogenetic studies of the relative diversity of canal-adapted herbivores have been undertaken. It is nevertheless tempting to speculate that present-day bark beetle diversity is linked to use of a resource that seems underutilized by their frequent competitors Cerambycidae and Buprestidae, which either avoid resin or latex plants altogether or avoid the principal resin canals by tunneling deep into xylem (Tavakilian et al. 1997). These and other wood borers were probably among the selective forces initially favoring the resin and latex defense (Farrell et al. 1991). Because the different groups of bark beetle conifer-specialists all sequester host defensive terpenes as precursors in pheromones (Paine et al. 1997), they are consistent with an advanced stage in Ehrlich and Raven's (1964) original scenario of stepwise coevolution in which host defenses become herbivore cues in resource use. Although use of these plants may spur bark beetle diversification, detailed study of additional instances of this habit and the others thought to enhance diversity in the beetles, such as ambrosia feeding and haplodiploid inbreeding, is required to discern their respective impact on scolytine evolution.

Inbreeding and Diversification

There have been at least eight independent origins of inbreeding in the Scolytinae (Fig. 6), totaling some 1500 species. The vast majority of these are ambrosia feeders (Xyleborini, *Premnobius*, *Xyloterinus*) and most of the rest are pith or seed feeders (within Bothrosternini, Hyorhynchini, *Araptus*, *Coccotrypes*, and *Dryocoetiops*)—habits that keep siblings in close quarters throughout their larval development (Kirkendall 1993). There are only a few phloem-feeding inbreeders (in *Ozopemon*, *Coccotrypes*, and *Dendroctonus*; Kelley and Farrell 1998; Jordal et al. 2000, 2001). However, because most inbreeding scolytines are the ambrosia-associated Xyleborini, it is not clear which of these traits, if either, may enhance diversification rates. It may be that the unique combination of ambrosia feeding and inbreeding haplodiploidy has spurred xyleborine diversification, but close study of speciation seems required to test diversification hypotheses concerning unique events.

There is a famous association between eusociality and haplodiploidy. Although the fact of the association had long been known in the case of the social Hymenoptera, a theoretical explanation for it was first put forward by Hamilton (1964), and an additional example of the association was discovered, in gall forming thrips, by Crespi (1992). Ambrosia-feeding

Scolytinae are remarkable in that they exhibit examples of both haplodiploidy and eusociality but in *separate* lineages, indicating that in this case (and possibly others) haplodiploidy and eusociality are separate adaptations to a similar environment—perhaps to the habit of cavity dwelling. Although there is a large radiation of haplodiploid ambrosia beetles (Fig. 6; Jordal et al. 2000), eusociality in ambrosia beetles is much rarer. So far it is known only in *Australoplatypus incompertus* (Kent and Simpson 1992), which specializes on *Eucalyptus* species and is one of the several unusual ambrosia beetles that are specialists on living trees. Because the trees do not decline in quality, colonies have been reported to persist for up to 37 years (Kent and Simpson 1992). This suggests the hypothesis that, whereas haplodiploidy may be adaptive even in very ephemeral cavities (as ephemeral as a parasitized caterpillar or physogastric mother mite), eusociality requires more persistent cavities. We predict that eusociality should be sought in other ambrosia beetles that attack living trees: the Malaysian genus *Dendroplatypus* that is specialized on the dipterocarp *Shorea* and the Mexican species of *Platypus* that attack living *Quercus.*

Conclusions

The diversification of wood-boring beetles in the subfamily Scolytinae has obviously been shaped by their different forms of mutualism with ophiostomatoid fungi. The ophiostomatoids apparently predate the late Cretaceous origin of the Scolytinae, but both sexual and asexual forms have become largely dependent on these (and a few other) beetles for dispersal. The sexual ophiostomatoids facilitate beetle use of trees with resin or latex defenses by blocking the secretory canals. These facultative mutualisms are widespread across the tribes of this beetle group and characterize the basal lineages. However, the beetles have also repeatedly domesticated asexual, polyphagous forms of these fungi, which become food for both adults and young, creating associations that have persisted through much of the Tertiary. Adult wood boring, by facilitating transport of fungi deep within trees, and fungal gardening permitted the elaboration of these associations between beetles and fungi, enhancing their collective ability to use the biomass-rich resource of trees.

Acknowledgments

We thank L. Kirkendall, D. Bright, and S. Wood for early encouragement and inspiration, as well as for innumerable specimens and identifications. In particular, the monumental tomes and syntheses of biological information therein by S. Wood are resources unavailable for most groups of organisms. Invaluable specimens and information were also supplied by T. Atkinson, B. Ayers, C. T. Fletchman, H. Goto, H. Kajimura, S. Kelley, S. Kühnholz, M. Lombardero, S. P. Quek, R. Rabaglia, G. Morse, A. Schumate, and A. Ueda. M. Blackwell, M. Berbee, and J. Taylor kindly shared preprints on ascomycete fungal phylogeny. We thank S. Pitnick, U. Mueller, and an anonymous reviewer for helpful comments on the manuscript. ASS was supported by postdoctoral grants from Fundacion Antorchas and Consejo Nacional de Ciencia y Tecnica (CONICET), Argentina. This research was supported by USDA National Research Initiative Grant 97-35302-4226 to BDF and by the Putnam Expedition Fund of the Museum of Comparative Zoology.

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Corresponding Editor: S. Pitnick

Appendix

Accession numbers and localities for the taxa included in the study. Italic numbers beside the accessions correspond to Kelley and Farrell 1998 (1), Farrell 1998a (2), Normark et al. 1999 (3), Jordal et al. 2000 (4), and Sequeira et al. 2000 (5).

Taxa	CO1	18S	eno2I	ef1	eno1ni	28S	Locality
Cossoninae (outgroup)							
Araucarius minor	AF375307	AF308304 *5*	—	AF308396 *5*	—	AF308351 *5*	
Araucarius major	—	—	—	—	AF375335	AF308350 *5*	
Stenancylus sp.	—	AF375246	—	AF375264	AF375334	AF375301	
Bothrosternini							
Cnesinus lecontei	—	AF308305 *5*	—	AF308397 *5*	—	AF308352 *5*	Costa Rica
Cnesinus sp.	—	AF308306 *5*	—	AF308398 *5*	—	AF308353 *5*	Argentina: Salta
Hylastini							
Hylastes porculus	AF375321	AF308339 *5*	—	AF308430 *5*	—	AF308387 *5*	USA: MA
Hylurgops sp.	AF375323	AF308317 *5*	—	AF308408 *5*	—	AF308364 *5*	
Hylesinini							
Alniphagus aspericollis	—	AF308320 *5*	—	AF308411 *5*	—	AF308367 *5*	Canada: BC
Hylesinopsis sp.	—	AF308309 *5*	—	AF308401 *5*	—	AF308356 *5*	Uganda
Hylesinopsis sp.	—	AF308321 *5*	—	AF308412 *5*	—	AF308368 *5*	Uganda
Hylesinopsis sp.	—	AF375253	—	—	—	—	Uganda
Hylesinus varius	—	AF308318 *5*	—	AF308409 *5*	—	AF308365 *5*	Norway
Hyorrhinchini							
Sueus niisimai	—	AF308307 *5*	—	AF308399 *5*	—	AF308354 *5*	Singapore
Hypoborini							
Chaetophloeus sp.	—	AF308322 *5*	—	AF308413 *5*	—	AF308369 *5*	
Chaetophloeus penicillatus	—	Af308324 *5*	—	AF308415 *5*	—	—	USA: AZ
Liparthrum nigrescens	—	AF308323 *5*	—	AF308414 *5*	—	AF308370 *5*	
Phloeosinini							
Chramesus asperatus	—	AF308315 *5*	—	AF308406 *5*	—	AF308362 *5*	USA: AZ
Chramesus sp.	—	AF308312 *5*	—	AF308403 *5*	—	AF308359 *5*	Costa Rica
Pseudochramesus sp.	AF375328	AF308313 *5*	—	AF308404 *5*	—	AF308360 *5*	Argentina: Salta
Phoeotribini							
Phloeotribus liminaris	AF375325	AF308326 *5*	—	AF308417 *5*	—	AF308373 *5*	USA: MD
Tomicini							
Dendroctonus mexicanus	AF067988 *1*	AF308335 *5*	—	AF308426 *5*	—	AF308383 *5*	
Dendroctonus murrayanae	AF067989 *1*	AF308336 *5*	—	AF308427 *5*	—	AF308384 *5*	
Dendroctonus ponderosae	AF067987 *1*	AF308337 *5*	—	AF308428 *5*	—	AF308385 *5*	
Dendroctonus pseudotsugae	AF375318	AF308327 *5*	—	AF308418 *5*	AF375341	AF308374 *5*	
Dendroctonus terebrans	AF375315	AF308338 *5*	—	AF308429 *5*	AF375338	AF308386 *5*	USA: GA
Hylurgonotus tuberculatus	AF375313	AF308328 *5*	—	AF308419 *5*	AF375334	AF308375 *5*	Argentina: Neuquen
Pseudohylesinus granulatus	—	AF308330 *5*	—	AF308421 *5*	—	—	USA: WA
Pseudohylesinus nebulosus	AF375316	AF308331 *5*	—	AF308422 *5*	AF375339	AF308379 *5*	USA: WA
Sinophloeus porteri	AF375314	AF308329 *5*	—	AF308420 *5*	—	AF308377 *5*	Argentina: Neuquen
Xylechinosomus valdivianus	AF375312	AF308319 *5*	—	AF308410 *5*	AF375336	AF308366 *5*	Argentina: Neuquen
Corthylini							
Araptus sp.	AF187123 *3*	AF375242	AF375284	AF186671 *3*	—	AF375297	Argentina: Salta
Gnathotrupes sp.	—	AF375252	—	AF375273	—	—	Argentina: Neuquen
Gnathotrupes sp.	—	AF375251	—	AF375274	—	—	Argentina: Neuquen
Pityophthorus sp.	AF375326	—	AF375285	AF375272	AF375345	—	Uganda
Pseudopityophthorus sp.	—	—	—	AF375271	—	AF375305	USA: AZ
Cryphalini							
Cryphalus abietis	AF187109 *3*	AF375247	AF375279	AF186657 *3*	—	—	Norway: Bergen
Ernoporicus caucasicus	—	AF375249	AF375280	—	—	—	Norway: Rosendal
Ernoporicus fagi	—	AF375250	AF375281	—	—	—	Czech Republic
Hypocryphalus mangiferae	—	AF375254	AF375282	AF375269	—	—	Singapore
Hypothenemus setous	—	AF308341 *5*	—	AF308431 *5*	—	AF308389 *5*	Uganda

APPENDIX
Continued.

Taxa	CO1	18S	eno2I	ef1	eno1ni	28S	Locality
Hypothenemus sp.	AF375324	AF375255	—	—	—	—	Argentina: Salta
Hypothenemus sp.	AF187110 *3*	AF308340 *5*	—	AF186658 *3*	AF375342	AF308388 *5*	Uganda
Scolytogenes sp.	—	AF375260	—	AF375270	—	—	Singapore
Crypturgini							
Aphanarthrum sp.	AF187131 *3*	—	—	AF186681 *3*	AF375332	—	Uganda
Coleobothrus germeauxi	AF375311	AF375245	—	AF375375	AF375333	—	Uganda
Crypturgus borealis	AF187130 *3*	—	—	AF186680 *3*	—	—	Canada: BC
Ctenophorini							
Scolytodes sp.	—	AF308432 *5*	—	AF308432 *5*	—	—	Costa Rica
Dryocoetini							
Coccotrypes advena	AF187116 *3*	—	—	AF186664 *3*	—	—	Uganda
Coccotrypes cardamomi	AF375308	—	—	AF259869 *4*	—	—	Japan
Coccotrypes cyperi	AF375309	—	—	AF259863 *4*	—	—	Costa Rica
Coccotrypes dactyliperda	AF187111 *3*	—	AF375282	AF186659 *3*	—	AF375300	Argentina: Salta
Coccotrypes gedeanus	AF375310	—	—	AF259867 *4*	—	—	Borneo
Dryocoetes affaber	AF187113 *3*	—	—	AF186661 *3*	—	—	USA: NH
Dryocoetiops coffeae	AF187122 *3*	—	—	AF188670 *3*	—	—	Sarawak
Ozopemon brownei	—	—	—	AF259870 *4*	—	—	Sarawak
Thamnurgus lobeliae	AF187117 *3*	—	—	AF186665 *3*	—	—	Uganda
Thamnurgus senecionis	AF187114 *3*	—	—	AF186662 *3*	—	—	Uganda
Taphrorychus bicolor	AF375330	—	—	—	AF375347	—	Norway
Ipini							
Acanthotomicus tanganyikaensis	AF187126 *3*	—	—	AF186674 *3*	—	—	Uganda
Ips perturbatus	AF187127 *3*	—	AF375287	AF186675 *3*	—	—	Canada
Orthotomicus caelatus	AF187124 *3*	AF308342 *5*	—	AF188672 *3*	—	—	USA: WI
Pityogenes hopkinsi	AF187128 *3*	—	AF375288	AF186676 *3*	AF375343	—	USA: MD
Pityokteines minutus	AF187125 *3*	AF308343 *5*	AF375286	AF186673 *3*	AF375344	AF308390 *5*	Canada
Micracini							
Hylocurus femineus	AF187108 *3*	—	—	AF186678 *3*	—	—	USA: AZ
Micracis carinulatus	AF187107 *3*	AF375257	AF375289	AF186677 *3*	—	AF375303	USA: AZ
Miocryphalus sp.	—	AF375258	—	AF375268	—	—	Uganda
Scolytoplatypodini							
Scolytoplatypus spp.	—	AF308344 *5*	—	—	—	AF30839 *5*	Uganda/Japan
Scolytini							
Scolytus multistriatus	AF375329	AF375261	—	—	—	—	
Scolytus unispinosus	—	AF375262	—	—	AF375346	AF375306	Canada
Xyleborini							
Amasa versicolor	AF187146 *3*	—	—	AF186696 *3*	—	—	Borneo
Arixyleborus medius	AF187145 *3*	—	—	AF186695 *3*	—	—	Borneo
Cnestus suturalis	AF187144 *3*	—	—	AF186694 *3*	—	—	Borneo
Cyclorhipidion pruinosum	AF375317	—	—	AF259883 *4*	—	—	Borneo
Dryocoetoides cristatus	AF187137 *3*	—	AF375291	AF186687 *3*	—	—	Uganda
Dryocoetoides cristatus	AF375319	—	—	AF375277	—	—	Uganda
Eccoptopterus spinosus	AF187136 *3*	—	—	AF186686 *3*	—	—	Uganda
Euwallacea validus	AF375320	—	—	AF259878 *4*	—	—	USA: MD
Premnobius cavipennis	AF187139 *3*	—	AF375292	AF186689 *3*	—	—	Uganda
Webbia quattuordecimspinatus	AF375331	—	—	AF259882 *4*	—	—	Borneo
Xyleborinus intersetosus	AF187134 *3*	—	—	AF186684 *3*	—	—	Costa Rica
Xylosandrus cf. zimmermanni	AF187135 *3*	AF375263	AF375290	AF186685 *3*	—	—	Argentina: Salta
Xyloctonini							
Ctonoxylon flavescens	AF187129 *3*	AF308345 *5*	—	AF186679 *3*	AF375340	AF308292 *5*	Uganda

APPENDIX
Continued.

Taxa	CO1	18S	eno2I	ef1	eno1ni	28S	Locality
Xyloterini							
Indocryphalus pubipennis	—	AF375256	—	AF375276	—	—	Japan
Trypodendron lineatum	AF187132 *3*	AF250076 *2*	—	AF186682 *3*	—	AF308394 *5*	
Xyloterinus politus	AF187133 *3*	AF308347 *5*	—	AF186683 *3*	—	AF308395 *5*	USA: NH
Platypodinae							
Dinoplatypus pseudocupulatus	—	AF375248	—	AF375267	—	AF375302	Borneo
Platypus sp.	AF375327	AF375259	—	AF375265	—	AF375304	Puerto Rico
Australoplatypus incompertus	—	AF375243	—	AF375266	—	AF375298	Australia: NSW
Chaetastus montanus	—	AF375244	AF375293	AF375278	—	AF375299	Uganda