THE ROYAL SOCIETY

doi 10.1098/rspb.2000.1292

Evolutionary assembly of the conifer fauna: distinguishing ancient from recent associations in bark beetles

Andrea S. Sequeira*, Benjamin B. Normark and Brian D. Farrell

Museum of Comparative Zoology, Harvard University, 26 Oxford Street, Cambridge, MA 02138, USA

Several shifts from ancestral conifer feeding to angiosperm feeding have been implicated in the unparalleled diversification of beetle species. The single largest angiosperm-feeding beetle clade occurs in the weevils, and comprises the family Curculionidae and relatives. Most authorities confidently place the bark beetles (Scolytidae) within this radiation of angiosperm feeders. However, some clues indicate that the association between conifers and some scolytids, particularly in the tribe Tomicini, is a very ancient one. For instance, several fragments of Gondwanaland (South America, New Caledonia, Australia and New Guinea) harbour endemic Tomicini specialized on members of the formerly widespread and abundant conifer family Araucariaceae. As a first step towards resolving this seeming paradox, we present a phylogenetic analysis of the beetle family Scolytidae with particularly intensive sampling of conifer-feeding Tomicini and allies. We sequenced and analysed elongation factor 1α and nuclear rDNAs 18S and 28S for 45 taxa, using members of the weevil family Cossoninae as an out-group. Our results indicate that conifer feeding is the ancestral host association of scolytids, and that the most basal lineages of scolytids feed on *Araucaria*. If scolytids are indeed nested within a great angiosperm-feeding clade, as many authorities have held, then a reversion to conifer feeding in ancestral scolytids appears to have occurred in the Mesozoic, when *Araucaria* still formed a major component of the woody flora.

Keywords: coevolution; Curculionoidea; Coleoptera; elongation factor 1α; plant–insect interactions; rDNA

1. INTRODUCTION

There is increasing appreciation that some important aspects of the diversity and structure of ecological communities may reflect historical as well as ahistorical processes (Ricklefs & Schluter 1993). While ecological studies of insect–plant community structure have accumulated over the past several decades (Strong *et al.* 1984; Basset & Novotny 1999), an increasing number of palaeontological and phylogenetic studies reveal that some aspects of insect–plant associations reflect the geological ages in which these associations arose (Labandeira *et al.* 1994; Farrell 1998). In particular, some of the insects that presently attack the oldest extant vascular plant groups, the conifers and cycads, have apparently been doing so since the early Mesozoic, long before the diversification of the angiosperms that dominate most modern forests (Farrell 1998). Nevertheless, angiosperms are host to the majority of insect species. A recent study reveals a correlation between the shift to angiosperms and an increase in insect species diversity (Farrell 1998). However, this diversity increase could result from different causes. It could simply reflect the larger biomass angiosperms afford attacking insects, or it could be a consequence of the greater number of angiosperm species, *per se* (Farrell 1998).

While it seems obvious that the study of angiosperm-feeding insects will be crucial to distinguish the reasons underlying their diversity, it may also be illuminating to study conifer- (and cycad-) associated lineages. In particular, one might contrast lineages that have recently colonized conifers (or cycads) and angiosperms, respectively, with others that are among the original associates of these plant groups. Younger groups, presumably early in the process of adaptation, might provide insights into the effects of the differences between these host groups on herbivore speciation and diversification.

Bark beetles in the family Scolytidae include the most prominent and well-known conifer-feeding insects (due to their sometimes devastating impact on coniferous forests), while many groups (in fact, the majority) attack angiosperms instead (Wood & Bright 1992). The architecture of the bark beetles' intricate gallery systems, excavated by both adults and larvae as they feed, is known to be diagnostic of species (or close relatives). Early Cretaceous trace fossils constitute the earliest (though not undisputed) fossil evidence of bark beetles (Brongniart 1877; Jarzembowski 1990) and strongly resemble galleries bored by the extant genus *Tomicus*, supporting an early Mesozoic origin of the tribe Tomicini (Wood, cited in Boucot 1990). This favours the idea that some associations between conifers and bark beetles are ancient, possibly arising in the early Mesozoic (Bright & Stock 1982). The Tomicini is part of the subfamily Hylesininae, thought to contain the more primitive, conifer-feeding lineages of the Scolytidae, while the largely angiosperm-associated Scolytinae is more diverse in number of species, feeding modes and mating systems (Wood & Bright 1992; Jordal *et al.* 2000). An early origin of the Tomicini is also suggested by the association of several tomicine genera with the conifer genus *Araucaria*, which has widely distributed Jurassic fossil records (Boureau 1949; Sukh & Zeba 1976; Stockey 1978, 1980, 1982), and by tomicine beetle and host assemblages that are endemic to various fragments of

*Author for correspondence (asequeira@oeb.harvard.edu).

Received 2 May 2000 *Accepted* 30 August 2000

Gondwanaland: South America, Australia, New Guinea and New Caledonia.

The hypothesis that *Araucaria* and its specialized fauna of bark beetles are ancient associates is hardly surprising, since there is evidence that some other beetles currently associated with *Araucaria* have been host specific since the Mesozoic (Kuschel & Poinar 1993; Farrell 1998), including weevils thought to be closely related to bark beetles (Morrone 1997). However, a Mesozoic, primitively conifer-associated origin of the Tomicini (and by extension, of the Scolytidae) presents a paradox in light of current estimates of weevil phylogeny. This is because the bark-beetle family Scolytidae is thought to be nested deep within a primitively angiosperm-feeding clade of weevils (Kuschel 1995; Marvaldi 1997; Farrell 1998). Moreover, it is not clear that the Tomicini are a basal lineage even within the Scolytidae; one major authority has portrayed them as having a relatively derived morphology when compared to some angiosperm feeders in the Hylesininae subfamily (Wood 1986).

Resolution of the seeming paradox between the fossil evidence supporting an ancient association between the Tomicini and conifers and the strong suggestions that they (and the family Scolytidae) may be highly derived members of an angiosperm-feeding weevil lineage may require molecular phylogenetic analyses at two levels: within the Scolytidae, and between these and other weevil groups. Here we begin the first of these analyses and focus on the placement of the Tomicini and putative relatives in the tribes Hylesinini and Hylastini to ascertain whether associations with *Araucaria* and other conifers are basal in the Scolytidae. To achieve this end, we have produced and analysed partial DNA sequences of three nuclear genes: protein coding elongation factor 1α (ef-1α) and two ribosomal fragments (rDNA), 18S and 28S.

2. MATERIAL AND METHODS

(a) *Samples*

Bark beetles were collected from colonized hosts. A total of 45 terminal taxa were included from ten out of the 11 tribes for the subfamily Hylesininae, with extensive sampling in the conifer-feeding tribe Tomicini, and from seven out of the 14 tribes in the subfamily Scolytinae (Wood & Bright 1992) (table 1). Members of two different tribes from the weevil subfamily Cossoninae (Cossonini and Araucarini), the putative sister group of Scolytidae (Kuschel 1966; Thompson 1992; Marvaldi 1997), were included as out-groups.

DNA was extracted from individual beetles preserved in ethanol, following Sunnucks & Hales (1996) with the modifications described in Normark (1999). DNA for six of the *Dendroctonus* species was kindly provided by Scott Kelley (University of Colorado).

(b) *Amplification and sequencing*

Polymerase chain reaction (PCR) and cycle sequencing were used to obtain partial sequences of three genes: ef-1α (895 bp of coding sequence), 18S (1863 bp) and 28S (823 bp), which include the D2 and D3 expansion segments (Michot & Bachelleire 1987). PCR reactions (50 µl) typically contained 10 pM of each primer (table 2), 0.8 mM dNTPs, Qiagen PCR buffer with additional $MgCl_2$ to a final concentration of 2 mM (18S and 28S) or 2.5 mM (ef-1α) and 1.25 units Qiagen (Valencia, CA, USA) Taq DNA polymerase. Using the primer pairs in table 2 for 18S and 28S regions, and primers efs149 and efa1043 from Normark *et al.* (1999) for ef-1α, amplification conditions were adjusted for each of the three regions. For 18S, the temperature profile was 40 cycles of 95 °C for 30 s, 47 °C for 60 s and 72 °C for 90 s. The temperatures and times for amplification for 28S were 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 (PE Biosystems, CA, USA) using the primers in table 2 for 18S and 28S and efs466, efs701, efa754, efa923 and the amplification primers from Normark *et al.* (1999) for ef-1α. Both strands of the PCR product were sequenced in an ABI 370A automatic sequencer.

(c) *Sequence alignment*

All sequences were compiled using Sequencher 3.1 (Genecodes Corporation, Ann Arbor, MI). Clustal X (Aladdin Systems, Inc., Heidelberg, Germany) was used to align both 18S and 28S fragments using default gap costs, and alignments were then subject to eye inspection. The 18S alignment produced a matrix of 1963 positions. Three hypervariable regions of *ca.* 40 bp each were excluded from the analysis as they could not be unambiguously aligned, producing a final matrix of 1842 characters.

The 28S alignment produced a matrix of 891 bp, which displays two 300 bp regions of great homology. The more variable region with ambiguous alignment was culled and 823 positions were included in the analysis with few gaps.

For ef-1α, evidence of two loci that differ in intron–exon structure has been found in other members of the subfamily Scolytinae (Normark *et al.* 1999). However, within the Hylesininae only in *Hylastes* could both copies be amplified. For this study, we used only one of the two putative loci (with one intron in the fragment sequenced, between coding positions 753 and 754). The sequence of the intron itself was omitted from the phylogenetic analysis. All sequences have been submitted to GenBank under accession numbers AF308304–AF308348 (18S), AF308349–AF308395 (28S), and AF308396–AF308432, AF308513 (ef-1α).

(d) *Phylogenetic analysis*

Phylogenetic analysis was performed by maximum parsimony (MP) using version 4.0b2a of PAUP (Swofford 2000). Each data set was analysed separately and then combined in a total-evidence matrix (3560 characters). All substitutions were weighted equally and gaps were treated as missing data. The heuristic searches used 100 random-addition sequence starting trees. For bootstrapping and incongruence testing (incongruence length difference test, Farris *et al.* 1995), 100 replications, each with 20 random-addition starting trees, were used also as implemented in PAUP 4. We also applied the Templeton (1983) test to test the fit of each of our sets of characters to the alternate topologies according to the method described in Larson (1994), where we used the Wilcoxon signed ranks to determine whether each of the data sets differed significantly in the degree of support for the most parsimonious trees from the other data sets

Table 1. *Host associations of genera sampled (from Wood 1986; Wood & Bright 1992)*

				percentage of species feeding on each host group[b]				
subfamily	tribe[a]	genus	number of species	An	C + A	Cu	Pin	Ar
Hylesininae	Tomicini***†	*Xylechinosomus*	8	—	—	—	—	100
1150 spp.		*Hylurgonotus*	4	—	—	—	—	100
74 genera		*Sinophloeus*	1	—	—	—	—	100
		Pseudohylesinus	11	—	—	—	100	—
		Dendroctonus	19	—	—	—	100	—
		14 genera	119	20	—	—	50	30
	Hylastini***	*Hylurgops****	22	—	—	—	100	—
		*Hylastes****	34	—	—	—	100	—
		three genera	58	—	—	—	100	—
	Hylesinini**	*Hylesinus***	39	100	—	—	—	—
		Hylesinopsis	41	100	—	—	—	—
		Alniphagus	3	100	—	—	—	—
		12 genera	167	100	—	—	—	—
	Polygraphini	*Polygraphus**	96	47	—	—	—	53
		eight genera	149	52	—	2	46	—
	Bothrosternini	*Cnesinus*	101	100	—	—	—	—
		five genera	131	100	—	—	—	—
	Hyorrhynchini	*Sueus*	2	100	—	—	—	—
		three genera	15	100	—	—	—	—
	Phloeosinini***	*Chramesus*	89	100	—	—	—	—
		Pseudochramesus	12	100	—	—	—	—
		Phloeoditica	4	100	—	—	—	—
		*Phloeosinus***	62	18	—	73	9	—
		12 genera	221	63	2	30	4	1
	Diamerini	*Strombophorous*	31	100	—	—	—	—
		Sphaerotrypes	47	97	—	—	3	—
		seven genera	137	97	—	—	3	—
	Phloeotribini**	*Phloeotribus***	108	94	—	—	6	—
		two genera	111	95	—	—	5	—
	Hypoborini	*Chaetophloeus*	24	100	—	—	—	—
		Liparthrum	35	97	—	—	3	—
		eight genera	61	97	—	—	3	—
	Prixosomini		22	100	—	—	—	—
Scolytinae	Cryphalini**	*Hypothenemus*	175	98	—	—	2	—
4028 spp.		24 genera	693	90	1.5	0.5	8	
137 genera	Ipini	*Orthotomicus*	13	—	—	7	93	—
		Pityokteines	9	—	—	—	100	—
		six genera	194	34	0.5	0.5	65	—
	Xyloctonini	*Ctonoxylon*	29	100	—	—	—	—
		five genera	81	97	3	—	—	—
	Crypturgini	*Aphanarthrum*	24	100	—	—	—	—
		six genera	45	72	—	—	28	—
	Ctenophorini**	*Scolytodes***	180	99	1	—	—	—
		four genera	215	99	1	—	—	—
	Xyloterini***	*Trypodendron****	14	50	—	—	50	—
		Xyloterinus	1	—	100	—	—	—
		three genera	23	63	6	—	31	—
	Scolytoplatypodini	*Scolytoplatypus*	36	75	25	—	—	—
			36	75	25	—	—	—
	other tribes[c]**	63 genera	3016	88	—	—	12	—

[a] Asterisks indicate the oldest nominate fossil reported for the taxon: ***Eocene; **Oligocene; *Miocene; †Cretaceous trace fossils are included (Schedl 1947; Larsson 1978; Jarzembowski 1990; Boucot 1990; Bright & Poinar 1994).

[b] Each column represents a taxon or combination of taxa. An, angiosperms; C + A, both conifers and angiosperms (in this case most of the records are for ambrosia beetles, which feed on symbiotic fungi and not on the woody tissue itself); Cu, Cupressaceae and/or Podocarpaceae; Pin, Pinaceae; Ar, Araucariaceae. Numbers refer to percentage of species recorded as feeding exclusively on that taxon across only those species for which some host is known.

[c] Seven Scolytinae tribes not included in the study. The percentages are expressed as the average among all unstudied tribes over the number of species with recorded hosts.

Table 2. *Primers used for amplification and sequencing*

locus	name[a]	alias	use[b]	sequence	reference
18S rDNA	f116	e	a, s	ctggttgatcctgccacgt	Hamby & Zimmer 1988
	f420	f420	s	ggcgacgcatctttcaaatgtctg	this study
	f1094	f1094	a, s	ggatcgtcgcaagacggacagaag	this study
	f1403	f1403	s	cggaaggattgacagattgagag	this study
	r803	r803	s	ccaccggcaggacgtctc	this study
	r1094	P	a, s	cgctttcgtaaacggtt	Hamby & Zimmer 1988
	r1138	r1138	a, s	cgccttcgaacctctaac	this study
	r1626	r1626	s	ggcatcacagacctgttattgctcaatctc	this study
	r1856	Q	a, s	cagcgaggatggctaactta	Hamby & Zimmer 1988
28S rDNA	S3690	S3660	a, s	gagagttmaasagtacgtgaaac	Dowton & Austin 1998 (28S forward)[c]
	A4053	A1	s	tcckgtkttcaagacggggtc	Whiting *et al.* 1997 (28S a)[c]
	S4072	S1	s	gacccgtcttgaamcamgga	Whiting *et al.* 1997 (28S a)[c]
	A4221	A160	s	cgcctcttctcgcaatgaga	this study
	A4285	A247	a, s	cctgacttcgtcctgaccaggc	this study
	A4394	A335	a, s	tcggarggaaccagctacta	Whiting *et al.* (28S b)[c]

[a] Names refer to direction. f, forward; r, reverse; S, sense; A, antisense. Numbers refer to the position of the 3′ end of the primer in the *Tenebrio molitor* sequence for 18S (GenBank X07810) and in the *Drosophila melanogaster* sequence for 28S (GenBank M21017).
[b] Use refers to amplification (a) and sequencing (s).
[c] Primers from the literature that were modified for this study, with the original published name in brackets.

by comparing each pair of two tree files in MacClade 3.06 (Maddison & Maddison 1996).

AutoDecay 4.0 (Eriksson 1998) was used to create the constraint trees for the nodes from the combined MP tree. Decay indexes (Bremer 1994) were calculated from the runs performed in PAUP using heuristic searches with 100 random additions. We performed searches for the combined data set and each separate data set individually, imposing the monophyly of groups proposed from morphological data (Wood 1982, 1986) as constraints to test whether any of the resulting trees were significantly longer than our MP trees. We constrained to monophyly each of the following nodes individually: Tomicini, Phloeosinini, Polygraphini + Hypoborini, Hylesinini and the subfamily Hylesininae. Also, we tested the monophyly of the conifer-feeding Tomicini + Hylastini. To compare our MP trees with the constrained trees we used Templeton's Wilcoxon signed-ranks test and the Kishino–Hasegawa test as implemented in PAUP 4.0.

3. RESULTS

(a) *The combined analysis*

Results of the analysis of the combined matrix are presented in figure 1. The most parsimonious reconstruction of the host relationships of Scolytidae indicates ancestral association with the genus *Araucaria*. The three *Araucaria*-feeding genera of the tribe Tomicini (*Hylurgonotus*, *Xylechinosomus* and *Sinophloeus*) are present in the two basal-most branches of the scolytid tree. The internode separating the *Araucaria*-feeding genera and the genus *Polygraphus* from the rest of the Scolytidae has a bootstrap support of 75 and a decay index of six. The third offshoot of the main lineage of Scolytidae is dominated by the Pinaceae-feeding genera of Tomicini and Hylastini (and also includes the angiosperm-feeding genus *Hylesinus*). The remainder of the tree consists of a well-supported clade (bootstrap 80, decay 6) that contains almost all of the angiosperm-feeding lineages, representing the great majority of scolytid diversity at the level of tribes, genera and species, and including most of the tribes of Hylesininae and the entire subfamily Scolytinae. This large, primarily angiosperm-feeding clade includes one predominantly conifer-feeding tribe, the Ipini, plus a number of lineages with small groups of species associated with conifers (table 1).

(b) *Separate analyses of ef-1α, 18S and 28S*

Independent analyses (see letters for each gene region used in figure 1) resolve relationships and provide support at different levels; the nuclear ribosomal 28S region resolves mainly intergeneric and intertribal relationships but not within the angiosperm-feeding clade; protein coding ef-1α provides better resolution mainly at lower levels (within genera); 18S alone does not resolve intertribal relationships but provides support for groupings provided by other genes (e.g. Hylastini and *Dendroctonus*).

Partition homogeneity tests indicate that there is significant incongruence among the three data sets ($p = 0.04$). However, when taxa with incongruent positions in the separate analysis (four out of 32 genera: *Sueus*, *Alniphagus*, *Hylesinus*, *Polygraphus*) are excluded, either one at a time or all together, the incongruence between all three data sets decreases and is non-significant (p ranges from 0.65 to 0.08). Two out of the six Templeton's Wilcoxon signed-ranks tests of individual data sets provide support for the most parsimonious trees given by the other data sets and showed significant difference in support only between 18S and ef-1α. The main topological incongruence was the position of *Hylesinus*: in the most parsimonious 18S trees *Hylesinus* was placed within the angiosperm-feeding clade as basal to the other Hylesinini genus *Hylesinopsis*, whereas ef-1α placed *Hylesinus* with the conifer-feeding Tomicini genera, as in the combined analysis. When performing the Templeton test for 18S and ef-1α excluding *Hylesinus*, the differential support is non-significant ($p > 0.5$). The only data set that individually supports the sister-group relationship between Pinaceae-associated beetles and angiosperm feeders, and one origin within

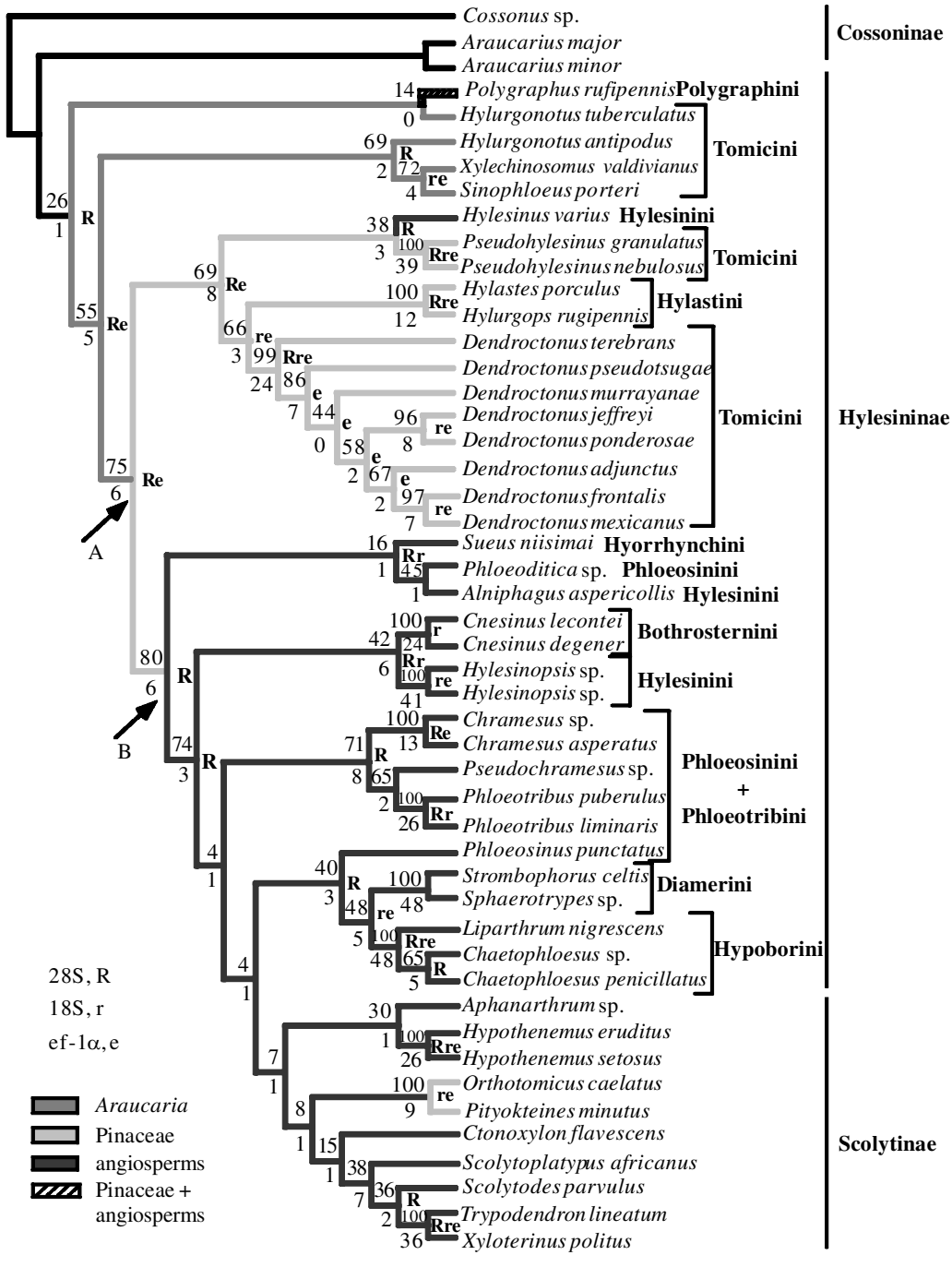

Figure 1. One of four most parsimonious trees from the combined analysis of ef-1α, 18S and 28S (3560 characters: 1842 18S, 823 28S, 895 ef-1α), 992 informative (220 18S, 423 28S, 349 ef-1α). Length = 6285; CI (informative characters only) = 0.388; RI = 0.468. Numbers above the internal branches indicate bootstrap support for that branch from the combined analysis. Support for each node by the analysis of independent data sets is indicated by letters adjacent to each node (R, 28S; r, 18S; e, ef-1α nucleotides). Decay indexes for each branch in the combined analysis are below each internal branch. Patterns on the branches in the in-group indicate the host-plant group on which the bark-beetle genus feeds. Host associations are not coded for the out-group (solid bars). Bars beside taxon names indicate tribe, subfamily and family classification after Wood (1986).

this group to feeding on angiosperms (nodes A and B in figure 1) is 28S. Node A is also supported by ef-1α and conflicts only slightly with the 18S data (the cost of imposing the node as a constraint on the 18S matrix is two steps); node B conflicts slightly with both ef-1α (two steps) and 18S (one step). Combining the data sets increases the bootstrap values for both nodes (node A has a bootstrap of 65 for 28S alone and 68 for ef-1α; node B has a bootstrap of 70 for 28S). This increase in bootstrap support can be ascribed to the combination of weak signals generating a stronger signal in the combined analysis (Olmstead & Sweere 1994).

(c) *Tests of nodes that conflict with morphology-based hypotheses*

When testing the nodes of tribal relationships suggested by previous morphological studies (Wood 1982, 1986), the Templeton's and Kishino–Hasegawa's tests both permit

the hypothesis of monophyly to be rejected at $\alpha = 0.01$ for the combined data set and each individual data set for the following tribes: Tomicini, Phloeosinini, Polygraphini + Hypoborini, Hylesinini, the subfamily Hylesininae and the conifer-feeding Tomicini + Hylastini.

4. DISCUSSION

(a) *Scolytid diversification*

The association with conifers of the most basal scolytid clades strongly implies that scolytids were originally conifer feeding, as are many other phytophagous beetle clades (Farrell 1998).

There is a stepped increase in diversity from the basal *Araucaria*-feeding genera (24 species) to the Pinaceae-feeding clade (213 species), to the angiosperm-feeding clade (over 5100 species) (Wood & Bright 1992). Thus the angiosperm-feeding clade is 23 times more diverse than its conifer-feeding sister lineage (table 1), consistent with the general pattern of diversity increase across six other origins of angiosperm associations in phytophagous beetles (Farrell 1998). One potential difference, however, between this shift to angiosperms and other angiosperm shifts in beetles is that use of angiosperms within the Scolytidae may actually represent a reversal that is much more recent than other angiosperm associations. This will be the case if the conifer-associated scolytid ancestor itself constituted a shift to conifers within a lineage of angiosperm-associated weevils (see § 4(b)). The alternative possibility that this scolytid association with angiosperms is homologous with the angiosperm association of their weevil ancestor would be supported by monophyly of a group comprised of the conifer-feeding Tomicini + Hylastini (which could then be most parsimoniously interpreted as a single reversal to conifer feeding). However, the grouping of Tomicini + Hylastini is rejected by our monophyly tests.

(b) *Relict Mesozoic communities and weevil diversification*

Our results indicate that the scolytid species that attack the ancient tree genus *Araucaria* are the most ancient lineages in this beetle family. Seed cones of *Araucaria* occur in the Jurassic fossil beds (Stockey 1978, 1980, 1982), and the ancient origin of the genus is reflected in its relict distribution (Pole 1994; Macphail 1997; Setoguchi *et al.* 1998). This pattern of species-poor *Araucaria*-feeding beetle lineages basal to a large group of mostly angiosperm-feeding lineages is seen in a number of other beetle groups, including Palophaginae within Megalopodidae, Mecomacerini within Nemonychidae, Oxycoryninae within Belidae and Araucariini within Cossoninae (Kuschel 1983; Morrone 1997; Farrell 1998).

The interpretation that most of these other beetle and *Araucaria* associations have been retained since the early Mesozoic is supported by evidence from Jurassic fossils, Gondwanan distributions and their phylogenetic positions at the bases of their respective groups (Farrell 1998). However, this interpretation is less straightforward for Araucariini (within Cossoninae) and for the *Araucaria*-feeding Tomicini considered here. This is because the placement of the Scolytidae and Cossoninae, widely regarded as sister taxa (and therefore as subfamilies), has been within the predominantly angiosperm-feeding family Curculionidae (Crowson 1967; Thompson 1992; Kuschel 1966, 1995; May 1993; Lyal 1995; Lyal & King 1996; Marvaldi 1997; Farrell 1998; but see Morimoto 1976; Wood 1986). How can we reconcile the results of this study with those of previous classifications that place Scolytidae within an angiosperm-feeding clade? Alternative hypotheses correspond to different time-frames: (i) Early Mesozoic, the Scolytidae may be currently misplaced and could actually be more basal in the Curculionoidea than currently supposed (Wood 1986; Morimoto 1976); (ii) Late Mesozoic, an ancestor of Scolytidae may have reverted to feeding on Araucariaceae, perhaps late in the Cretaceous during angiosperm diversification but when Araucariaceae were still a major component of the woody flora; or (iii) Tertiary, basal scolytid lineages may have colonized Araucariaceae more recently, after these trees had assumed their present, relict distribution; (iv) for completeness, we note that it is possible (though unlikely) that basal Araucariaceae-feeding lineages of the angiosperm-associated curculionoid families Brentidae, Attelabidae, Dryophthoridae and Curculionidae may have gone extinct (perhaps coincident with the extinction of most of the araucariad flora), leading to an erroneously early estimate of the shift to angiosperms in the curculionid lineage (Farrell 1998). While the position of Scolytinae (Scolytidae in the present paper) within Curculionidae is supported by several morphological studies and will not be discussed here, current molecular and morphological studies of higher weevil phylogeny will test hypothesis 1. For the present we will argue that moderately late Mesozoic association between *Araucaria* and scolytids (hypothesis 2) is more likely than a more recent, Tertiary association.

A relatively early origin of Scolytidae is suggested by fossil galleries in conifer bark from the early Cretaceous (Brongniart 1877; Wood, cited in Jarzembowski 1990). The proposed ancestral host taxon, the conifer family Araucariaceae, was a major component of the woody flora throughout the Mesozoic (Stockey 1982; Stockey *et al.* 1992; Hill 1995) and still had a worldwide distribution in the early Cretaceous (Schultze 1966; Erasmus 1976; del Fuego 1991; Barale 1992; Nissenbaum & Horovitz 1992; Alvin *et al.* 1994; Stockey *et al.* 1994; Meijer 1997). There are unsubstantiated reports (Whalley, cited in Jarzembowski 1990) of scolytids from the early–mid-Cretaceous *Araucaria*-derived Lebanese amber from which fossils of the *Araucaria*-associated weevil family Nemonychidae are also known (Kuschel & Poinar 1993). However, the oldest well-documented scolytid fossils are adult beetles in Eocene Baltic amber, most of which belong to the extant Pinaceae-associated genera *Hylastes* and *Hylurgops* of the tribe Hylastini (Schedl 1947; Larsson 1978). This provides further evidence for the hypothesis of an ancient association between conifers and scolytids, since Hylastini is nested well within the Tomicini in our analysis. The timing is also consistent with the plant fossil record. Although there are Jurassic Pinaceae fossils (Harris 1979), it was not until the late Cretaceous that the family Pinaceae was well established and the genus *Pinus* was diverse and widespread (Miller 1977, 1988; Millar 1993; Savard 1994).

A Gondwanan distribution of *Araucaria*-feeding lineages in Tomicini provides further evidence of an early origin

and an ancient association with *Araucaria*. In addition to the South American genera discussed here, the tribe also contains two *Araucaria*-feeding genera (*Hyludrectonus* and *Pachycotes*) restricted to Australia, New Zealand and Papua New Guinea. A planned molecular study of the phylogenetic affinities of these genera will provide a critical test of the hypotheses advanced here.

The hypothesis of a recent colonization of *Araucaria* by basal Tomicini (hypothesis 3) implies that at least one lineage of scolytids colonized *Araucaria* after it had already (by the Eocene) decreased markedly in abundance and diversity and been reduced to a relict distribution (Setoguchi *et al.* 1998). That the colonizing lineage was one of the oldest lineages of bark beetles, is, according to this view, a coincidence. Instead, the phylogenetic evidence presented here suggests that the ancient and depauperate lineages of Tomicini that today breed in *Araucaria* are surviving remnants of the fauna of Mesozoic *Araucaria* forests. If scolytids are indeed nested within a great angiosperm-feeding clade, as many authorities have held, then a reversion to conifer feeding in ancestral scolytids appears to have occurred later in the Mesozoic, when *Araucaria* still formed a major component of the woody flora.

We thank T. Atkinson, B. Ayers, H. Goto, B. Jordal, L. Kirkendall, S. Kelley, S. Kuhnholz, M. Lombardero, C. O'Brien, R. Rabaglia, M. Reid, A. Schumate and S. Wood for providing specimens and identifications; B. O'Meara and P. Hollstein for technical support; and B. Jordal and A. Marvaldi for helpful discussions. This research was supported by United States Department of Agriculture National Research Initiative Grant 97-35302-4226 to B.D.F. and by the Putnam Expedition Fund of the Museum of Comparative Zoology. A.S.S. was supported by postdoctoral grants from Fundacion Antorchas and CONICET (Argentina).

REFERENCES

Alvin, K. L., Watson, J. & Spicer, R. A. 1994 A new coniferous male cone from the English Wealden and a discussion of pollination in the Cheirolepidiaecae. *Palaeontology* **37**, 173–180.

Barale, G. 1992 Nouveaux restes d'Araucariacées dans les calcaires lithographiques du Crétacé inférieur du Montsec (Espagne). In *Organisation Internationale de Paléobotanique: IVeme conference; résumés des communications* (ed. D. Pons & J. Broutin), p. 12. Villeneuve d'Asca, France: Organisation Française de Paléobotanique.

Basset, Y. & Novotny, V. 1999 Species richness of insect herbivore communities on *Ficus* in Papua New Guinea. *Biol. J. Linn. Soc.* **67**, 477–499.

Boucot, A. J. 1990 *Evolutionary paleobiology of behaviour and coevolution*. New York: Elsevier.

Boureau, E. 1949 *Dadoxylon* (Araucarioxylon) *teixeirae* n. sp., bois fossile du jurassique superieur portugais. In *Comunicações dos Servicos Geólogicos de Portugal*, pp. 187–194. Lisbon: Servicos Geólogicos de Portugal.

Bremer, K. 1994 Branch support and tree stability. *Cladistics* **10**, 295–304.

Bright, D. E. & Poinar, G. O. 1994 Scolytidae and Platypodidae (Coleoptera) from Dominican Republic amber. *Annls Entomol. Soc. Am.* **87**, 170–194.

Bright, D. E. & Stock, M. W. 1982 Taxonomy and geographic variation. In *Bark beetles in North American conifers: a system for the study of evolutionary biology* (ed. J. B. Mitton & K. B. Sturgeon), pp. 46–73. Austin, TX: University of Texas Press.

Brongniart, C. 1877 Perforations observees dans deux morceaux de bois fossile. *Annls Soc. Entomol. France* **7**, 215–220.

Crowson, R. A. 1967 *The natural classification of the families of Coleoptera*. Hampton, Middlesex, UK: Classey Ltd.

del Fuego, G. M. 1991 Una Araucariaceae fertil del Cretacico de la Provincia de Santa Cruz; Argentina. *Ameghiniana* **28**, 203.

Dowton, M. & Austin, A. D. 1998 Relationships among the microgastroid wasps (Hymenoptera: Braconidae): combined analysis of 16s and 28s rDNA genes and morphological data. *Mol. Phylogenet. Evol.* **10**, 354–366.

Erasmus, T. 1976 A new species of *Dammaroxylon* Schultze-Motel, *D. Natalense* sp. nov. from the Cretaceous of Natal, South Africa. *Palaeontologia Africana* **19**, 135–139.

Eriksson, T. 1998 AutoDecay v. 4.0. Department of Botany, Stockholm University, Stockholm (program distributed by the author).

Farrell, B. D. 1998 'Inordinate fondness' explained: why are there so many beetles? *Science* **281**, 555–559.

Farris, J. S., Källersjö, M., Kluge, A. C. & Bult, C. 1994 Testing significance of incongruence. *Cladistics* **10**, 315–319.

Hamby, R. K. & Zimmer, E. A. 1988 Ribosomal RNA sequences for inferring phylogeny within the grass family (Poaceae). *Plant Syst. Evol.* **160**, 29–37.

Harris, T. M. 1979 *The Yorkshire Jurassic flora. 5. Coniferales*. London: British Museum.

Hill, R. S. 1995 Conifer origin, evolution and diversification in the Southern Hemisphere. In *Ecology of the southern conifers* (ed. N. J. Enright & R. S. Hill), pp. 10–29. Cambridge University Press.

Jarzembowski, E. A. 1990 A boring beetle from the Wealden of the Weald. In *Evolutionary paleobiology of behaviour and coevolution* (ed. A. J. Boucot), pp. 373–376. New York: Elsevier.

Jordal, B. H., Normark, B. B. & Farrell, B. D. 2000 Evolutionary radiation of an inbreeding haplodiploid beetle lineage (Curculionidae, Scolytinae). *Biol. J. Linn. Soc.* **71**. (In the press.)

Kuschel, G. 1966 A cossonine genus with bark-beetle habits, with remarks on relationships and biogeography (Coleoptera: Curculionidae). *N. Z. J. Sci.* **9**, 3–29.

Kuschel, G. 1983 Past and present of the relict family Nemonychidae (Coleoptera: Curculionidae). *GeoJournal* **7**, 499–504.

Kuschel, G. 1995 A phylogenetic classification of Curculionoidea to families and subfamilies. *Mem. Entomol. Soc. Wash.* **14**, 5–33.

Kuschel, G. & Poinar, G. O. 1993 *Libanorhinus succinus* gen. & sp.n (Coleoptera; Nemonychidae) from Lebanese amber. *Entomol. Scand.* **24**, 143–146.

Labandeira, C. C., Dilcher, D. L., Davis, D. R. & Wagner, D. L. 1994 Ninety-seven million years of angiosperm–insect association: paleobiological insights into the meaning of coevolution. *Proc. Natl Acad. Sci. USA* **91**, 12 278–12 282.

Larson, A. 1994 The comparison of morphological and molecular data in phylogenetics systematics. In *Molecular ecology and evolution: approaches and applications* (ed. B. Schierwater, B. Streit, G. P. Wagner & R. R. DeSalle), pp. 371–390. Basel, Switzerland: Birkhäuser Verlag.

Larsson, S. G. 1978 *Baltic amber: a palaeobiological study*. Klampenborg, Denmark: Scandinavian Science Press Ltd.

Lyal, C. H. C. 1995 The ventral structures of the weevil head. *Mem. Entomol. Soc. Wash.* **14**, 35–51.

Lyal, C. H. C. & King, T. 1996 Elytro-tergal stridulation in weevils (Insecta: Coleoptera: Curculionoidea). *J. Nat. Hist.* **30**, 703–773.

Macphail, M. K. 1997 Comment on Pole (1994): The New Zealand flora—entirely long distance dispersal? *J. Biogeogr.* **24**, 113–117.

Maddison, W. P. & Maddison, D. R. 1996 *MacClade 3.06*. Sunderland, MA: Sinauer Associates, Inc.

Marvaldi, A. 1997 Higher level phylogeny of Curculionidae (Coleoptera: Curculionoidea) based mainly on larval characters, with special reference to broad-nosed weevils. *Cladistics* **13**, 285–312.

May, B. M. 1993 Larvae of Curculionoidea (Insecta: Coleoptera): a systematic overview. *Fauna N. Z.* **28**, 1–221.

Meijer, J. J. F. 1997 Wood remains from the Late Cretaceous Aachen formation. In *Proceedings of the 4th European palaeobotanical and palynological conference* (ed. G. F. W. Herngreen), pp. 131–137. Haarlem, Netherlands: Nederlands Instituut voor Toegepaste Geowetenschappen TNO.

Michot, B. & Bachelleire, J. P. 1987 Comparisons of large subunit rRNAs reveal some eukaryotic specific elements of secondary structure. *Biochimie* **69**, 11–23.

Millar, C. I. 1993 Impact of the Eocene on the evolution of *Pinus* L. *Annls Mo. Bot. Gard.* **80**, 471–498.

Miller, C. N. 1977 Mesozoic conifers. *Bot. Rev.* **43**, 217–280.

Miller, C. N. 1988 The origin of modern conifer families. In *Origin and evolution of gymnosperms* (ed. C. B. Beck), pp. 448–486. New York: Columbia University Press.

Morimoto, K. 1976 Notes on the family characters of Apionidae and Brentidae (Coleoptera) with key to the related families. *Kontyu Tokyo* **44**, 469–476.

Morrone, J. J. 1997 Weevils (Coleoptera: Curculionidae) that feed on *Araucaria araucana* (Araucariaceae) in Southern Chile and Argentina, with an annotated checklist. *Folia Entomol. Mex.* **100**, 1–14.

Nissenbaum, A. & Horovitz, A. 1992 The Levantine amber belt. *J. Afr. Earth Sci.* **14**, 295–300.

Normark, B. B. 1999 Evolution in a putatively ancient asexual aphid lineage: recombination and rapid karyotype change. *Evolution* **53**, 1458–1469.

Normark, B. B., Jordal, B. J. & Farrell, B. D. 1999 Origin of a haplodiploid beetle lineage. *Proc. R. Soc. Lond.* B **266**, 1–7.

Olmstead, R. G. & Sweere, J. A. 1994 Combining data in phylogenetic systematics: an empirical approach using three molecular data sets in the Solanaceae. *Syst. Biol.* **43**, 467–481.

Pole, M. S. 1994 The New Zealand flora—entirely long distance dispersal? *J. Biogeogr.* **21**, 625–635.

Ricklefs, R. E. & Schluter, D. 1993 Species diversity: regional and historical influences. In *Species diversity in ecological communities* (ed. R. Ricklefs & D. Schluter), pp. 350–364. Chicago, IL: University of Chicago Press.

Savard, L., Li, P., Strauss, S. H., Chase, M. W., Michaud, M. & Bousquet, J. 1994 Chloroplast and nuclear gene sequences indicate Late Pennsylvanian time for the last common ancestor of extant seed plants. *Proc. Natl Acad. Sci. USA* **91**, 5163–5167.

Schedl, K. E. 1947 Die Borkenkäfer Baltischen Bernsteins. *Zentralblatt für das Gesamtgebiet der Entomologie* **2**, 12–45.

Schultze, M. J. 1966 Ergebnisse der Forschungsreise Richard Kraeusel's 1954 nach Sued- und Suedwest-Afrika, I, 9; gymnospermen-Hoelzer aus den oberkretazischen Umzamba-Schichten von Ost-Pondoland (S-Afrika). *Senckenbergiana Lethaea* **47**, 279–323.

Setoguchi, H., Osawa, T. A., Pintaud, J. C., Jaffre, T. & Veillon, J. M. 1998 Phylogenetic relationships within Araucariaceae based on RBCL gene sequences. *Am. J. Bot.* **85**, 1507–1516.

Stockey, R. A. 1978 Reproductive biology of Cerro Cuadrado fossil conifers: ontogeny and reproductive strategies in *Araucaria mirabilis* (Spegazzini) Windhausen. *Paleontographica* **166**, 1–15.

Stockey, R. A. 1980 Jurassic araucarian cone from southern England. *Paleontology* **23**, 657–666.

Stockey, R. A. 1982 The Araucariaceae: an evolutionary perspective. *Rev. Palaeobot. Palynol.* **37**, 133–154.

Stockey, R. A., Nishida, H. & Nishida, M. 1992 Upper Cretaceous araucarian cones from Hokkaido: *Araucaria nihongii* sp. nov. *Rev. Palaeobot. Palynol.* **72**, 27–40.

Stockey, R. A., Nishida, H. & Nishida, M. 1994 Upper Cretaceous araucarian cones from Hokkaido and Saghalien: *Araucaria nipponensis* sp. nov. *Int. J. Plant Sci.* **155**, 806–815.

Strong, D. R., Lawton, J. H. & Southwood, T. R. E. 1984 *Insects on plants: community patterns and mechanisms*. Cambridge, MA: Harvard University Press.

Sukh, D. & Zeba, B. 1976 *Araucaria indica* and two other conifers from the Jurassic–Cretaceous rocks of Madhya Pradesh, India. *Palaeobotanist* **25**, 496–508.

Sunnucks, P. & Hales, D. F. 1996 Numerous transposed sequences of mitochondrial cytochrome oxidase I-II in aphids of the genus *Sitobion* (Hemiptera: Aphididae). *Mol. Biol. Evol.* **13**, 510–524.

Swofford, D. L. 2000 *PAUP**. *Phylogenetic analysis using parsimony* (**and other methods*), v. 4. Sunderland, MA: Sinauer Associates, Inc.

Templeton, A. R. 1983 Phylogenetic inference from restriction endonuclease cleavage site maps with particular reference to the evolution of humans and apes. *Evolution* **37**, 221–224.

Thompson, R. T. 1992 Observations on the morphology and classification of weevils (Coleoptera: Curculionoidea) with a key to major groups. *J. Nat. Hist.* **26**, 835–891.

Whiting, M. F., Carpenter, J. C., Wheeler, Q. D. & Wheeler, W. C. 1997 The Strepsistera problem: phylogeny of the holometabolous insect orders inferred from 18s and 28s ribosomal DNA sequences and morphology. *Syst. Biol.* **46**, 1–68.

Wood, S. L. 1982 The bark and ambrosia beetles of North and Central America (Coleoptera: Scolytidae): a taxonomic monograph. *Great Basin Nat. Mem.* **6**, 1–1359.

Wood, S. L. 1986 A reclassification of the genera of Scolytidae (Coleoptera). *Great Basin Nat. Mem.* **10**, 1–126.

Wood, S. L. & Bright, D. E. 1992 A catalog of Scolytidae and Platypodidae (Coleoptera). 2. Taxonomic index. *Great Basin Nat. Mem.* **13**, 1–1533.

As this paper exceeds the maximum length normally permitted, the authors have agreed to contribute to production costs.