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Molecular and Morphological Phylogenetics of Weevils (Coleoptera, Curculionoidea): Do Niche Shifts Accompany Diversification?

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Abstract.—The main goals of this study were to provide a robust phylogeny for the families of the superfamily Curculionoidea, to discover relationships and major natural groups within the family Curculionidae, and to clarify the evolution of larval habits and host-plant associations in weevils to analyze their role in weevil diversification. Phylogenetic relationships among the weevils (Curculionoidea) were inferred from analysis of nucleotide sequences of 18S ribosomal DNA (rDNA; ~2,000 bases) and 115 morphological characters of larval and adult stages. A worldwide sample of 100 species was compiled to maximize representation of weevil morphological and ecological diversity. All families and the main subfamilies of Curculionoidea were represented. The family Curculionidae sensu lato was represented by about 80 species in 30 "subfamilies" of traditional classifications. Phylogenetic reconstruction was accomplished by parsimony analysis of separate and combined molecular and morphological data matrices and Bayesian analysis of the molecular data; tree topology support was evaluated. Results of the combined analysis of 18S rDNA and morphological data indicate that monophyly of and relationships among each of the weevil families are well supported with the topology ((Nemonychidae, Anthribidae) (Belidae (Attelabidae (Caridae (Brentidae, Curculionidae))))). Within the clade Curculionidae sensu lato, the basal positions are occupied by mostly monocot-associated taxa with the primitive type of male genitalia followed by the Curculionidae sensu stricto, which is made up of groups with the derived type of male genitalia. High support values were found for the monophyly of some distinct curculionid groups such as Dryophthorinae (several tribes represented) and Platypodinae (Tesserocerini plus Platypodini), among others. However, the subfamilial relationships in Curculionidae are unresolved or weakly supported. The phylogeny estimate based on combined 18S rDNA and morphological data suggests that diversification in weevils was accompanied by niche shifts in host-plant associations and larval habits. Pronounced conservatism is evident in larval feeding habits, particularly in the host tissue consumed. Multiple shifts to use of angiosperms in Curculionoidea were identified, each time associated with increases in weevil diversity and subsequent shifts back to gymnosperms, particularly in the Curculionidae. [18S rDNA; diversification; host associations; larval habits; morphology; phylogenetics; weevils.]

Adaptive radiation explains two related phenomena: taxonomic groups that represent a proliferation of related species marked by distinctive use of a particular resource (or some other dimension of the environment) and related species in such a group that usually differ from one another in small ways, reducing their ecological overlap. Adaptive radiations are thus usually characterized by major shifts in ecological traits, often associated with morphological features that seem to provide entry into such a different set of ecological niches (Futuyma, 1998). Those shifts are also often postulated to explain differences in diversity among lineages (Mitter et al., 1988; Farrell, 1998a). Characterization of the details of such radiations is necessary for study of the potential role of natural selection in diversification, which would inform models of speciation. Here, we report characterization of the rates of evolution of several dimensions of host use by the plant-feeding beetles known as weevils.

Weevils represent one of the most stunning radiations of animals and thus figure prominently among the phenomena to be explained (Mayr, 1963). Collectively, weevils use every plant part and nearly every plant taxon (Anderson, 1995), and yet related species are often similar in host use. Weevils constituting various taxonomic groups feed on plant roots, stems, leaves, flowers, fruits, or seeds. They may be among the first enemies to consume healthy plants or may be specialists on decaying tissues or the dead remains of plants felled by other causes (Farrell et al., 2001; Lanteri et al., 2002). Taxonomic groups of weevils are also often restricted to particular host groups,

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specializing on conifers, cycads, dicots, or monocots or on subsets of these plant taxa, although there are many exceptionally polyphagous species. Because weevils and other herbivores have shifted niches among plant parts and plant taxa innumerable times, studies of the rate and direction of change in these different aspects of host use may reveal repeated patterns. These patterns would suggest further lines of inquiry into the possible ecological and genetic bases that could eventually permit synthesis of evolutionary processes among and within species.

Weevils are classified as the superfamily Curculionoidea, which contains about 60,000 species and 6,000 described genera (Thompson, 1992; Kuschel, 1995). With its sister group Chrysomeloidea, the weevils constitute a radiation of phytophagous insects rivaled in species diversity only by the Lepidoptera (Farrell, 1998a, 1998b). The Curculionoidea is one of the richest groups in terms of potential for insights into the evolution of diversity and remains one of the more challenging taxonomic groups in terms of stability of classification.

To date there have been three cladistic analyses of weevil morphology for the purpose of establishing higher relationships within Curculionoidea (Kuschel, 1995; Marvaldi, 1997; Marvaldi and Morrone, 2000). Kuschel (1995) published a cladogram for families and subfamilies of Curculionoidea, resulting from the analysis of 24 terminals (subfamily groups) plus an outgroup "Chrysomeloidea" and 138 characters from adults (113) and larvae (25). The monophyly of these terminal units was assumed a priori. This phylogeny estimate shows six weevil families: Nemonychidae, Anthribidae (including Urodontinae), Belidae (including Oxycoryninae and Aglycyderinae), Attelabidae (including Rhynchitinae), Brentidae (including Apioninae, Carinae, and Cyladinae), and Curculionidae (this large family classified with only six subfamilies, each resulting from amalgamation of several traditional ones). Marvaldi (1997) assessed higher relationships within the Curculionidae sensu lato based mainly on larval characters, with the main aim of testing monophyly of Kuschel's subfamily Brachycerinae. Marvaldi (1997) analyzed 19 terminals (subfamily groups, of which 13 are smaller units of Brachycerinae) plus an outgroup "Brentidae" and 49 characters from larvae (41), pupae (3), and adults (5). Larvae of ~120 representative species were examined to score the states for each summarized terminal. Results of the cladistic analysis refute the hypothesis of monophyly of Brachycerinae, this being an assemblage of different groups of broad-nosed weevils. In addition, larval and pupal characters support a close relationship between Dryophthorinae and Platypodinae. Marvaldi and Morrone (2000) reviewed larval and adult morphological information and constructed a new data matrix for Curculionoidea. New characters included were those for the larvae of Caridae (May, 1994) and Ocladiinae (Marvaldi, 2000) that were unavailable when Kuschel (1995:19) and Marvaldi (1997) undertook their respective analyses. That family level cladistic analysis of Curculionoidea was based on 100 characters (72 from adults and 28 from larvae), using 13 terminal taxa, previously defined, corresponding to seven families of Curculionoidea of which the largest, Curculionidae, was represented by seven smaller units. The chrysomeloid Palophaginae was used as the outgroup. Although results of the cladistic analysis at the family level were in some respect similar to those of Kuschel (1995), there were some important differences such as the sister group relationship of Nemonychidae and Anthribidae and the Caridae as a distinct family and sister to the clade Brentidae Curculionidae. However, the relationships within Curculionidae sensu stricto (a single terminal) were not assessed.

An earlier (Farrell, 1998a) quantitative phylogenetic analysis of the Phytophaga (Curculionoidea + Chrysomeloidea) was based on 115 nearly complete sequences from the nuclear 18S ribosomal DNA (rDNA) gene, analyzed together with a morphological matrix compiled from the data of Kuschel (1995) and Reid (1995). The weevil families were represented by 45 species in Farrell's study. Here, we expand the taxonomic sampling for both 18S rDNA and morphology to 100 species, including the families Anthribidae and Caridae (formerly not represented) and more representatives for Nemonychidae and particularly for Curculionidae.

The main goals of this study were to provide a robust phylogeny for the families of Curculionoidea, to discover relationships and major natural groups within the

family Curculionidae, and to clarify the evolutionary sequence of shifts in larval habits and host-plant associations for study of their potential role in weevil diversification.

Materials and Methods

Specimens Examined

We obtained samples of 100 species of weevils, selected to maximize morphological and ecological diversity. Weevil taxa used in this study, collecting areas, and GenBank accession numbers are in Table 1. A great proportion of the species sequenced were collected and identified by C. W. O'Brien. Voucher specimens (preserved in ethanol) were deposited in the Farrell laboratory at the Museum of Comparative Zoology, Harvard University. All extant families of Curculionoidea and the main subfamilies are represented: Nemonychidae (Rhinorhynchinae, Cimberidinae), Anthribidae or fungus weevils (Anthribinae), Belidae (Belinae, Oxycoryninae), Attelabidae or leaf-roller weevils (Attelabinae, Rhynchitinae), Caridae (Carinae), Brentidae (Eurhynchinae, Antliarhininae, Apioninae, Cyladinae, Ithycerinae), and Curculionidae sensu lato. The Curculionidae is represented by about 80 species in 30 "subfamilies" in traditional classifications (e.g., Dryophthorinae, Erirhininae, Entiminae or broad-nosed weevils, Curculioninae, Molytinae, Cossoninae, Scolytinae or bark beetles, Platypodinae or ambrosia beetles, etc.; Thompson, 1992). Three outgroup species from the Chrysomeloidea (*Palophagoides vargasorum*, *Prionus laticollis*, and *Dendrobius* sp.) were included. The first two were chosen because they represent two basalmost chrysomeloid families and subfamilies, Megalopodidae (Palophaginae) and Cerambycidae (Prioninae), according to Farrell (1998a). The third, *Dendrobius* sp. (Cerambycinae), was simply selected at random among several possible outgroups.

Molecular Data

Amplification and sequencing of rDNA.—Total genomic DNA was extracted from ethanol-preserved weevil adults (or if necessary larvae) following an adapted version of the "salting out" protocol of Sunnucks and Hales (1996) as modified in Normark (1999). Double-stranded template suitable for sequencing was prepared for 18S rDNA via polymerase chain reaction (PCR) amplification using conserved primer pairs (e–r1138 and f1094–Q) from Sequeira et al. (2000), which amplified two regions with a 100-bp overlap. The temperature profile was 40 cycles of 95°C for 30 sec, 47°C for 60 sec, and 72°C for 90 sec. The PCR products were purified using the QIAquick spin-column purification kit (Qiagen Inc., Valencia, CA, USA). Purified samples were run on 2% agarose gels stained with ethidium bromide to assay the intensity of the product and to check for possible secondary amplification products. The purified PCR product was used as the template for double-stranded sequencing, using the primers used for PCR amplification plus six internal primers (f420, r803, f1403, and r1626) (Sequeira et al., 2000) and primers f876 (CGCGGTGCTCTTCATTGAGTG) and r1439 (CGCTCCACCAACTAAGAAC) designed in Farrell's laboratory by B. Normark (numbers correspond to the position of the 3′ end in the *Tenebrio molitor* sequence for 18S rDNA (GenBank accession X07810). *Taq* DyeDeoxy terminator cycle-sequencing kits were used to prepare the sequencing reactions. Both strands of the 18S rDNA fragments were sequenced following the ABI protocol for automated sequencing, using an ABI Prism 370A sequencer (Perkin Elmer Biosystems, Foster City, CA, USA).

Alignment of ribosomal sequences.—Sequences were edited and aligned by visual inspection using the Sequencher 3.0 (Gene Codes Corp., Ann Arbor, MI) sequence editor. "Conserved" regions of the alignment could be aligned unambiguously by hand, but we could not align 10 small regions to our satisfaction, and these positions (239–245, 251–282, 753–756, 788–881, 1570–1573, 1602–1684, 1689–1692, 1699–1701, 1712–1719, 1722–1725) were excluded from phylogenetic analyses. However, each of these regions were replaced by a single coded character, added to the end of the alignment. The regions are nucleotide sequences of variable length for individual taxa that have no corresponding sequence in the outgroups and in several weevil taxa.

As an additional criterion for exclusion of hypervariable regions, we constructed computer alignments using different alignment parameters in CLUSTAL X (Thompson et al., 1997). Three different gap insertion/extension cost combinations were used: 15/6.6 (default), 10/2, and 10/1.

TABLE 1. Weevil taxa used in this study, collecting area, and GenBank accession numbers (letters beside the accessions correspond to Farrell 1998 (a), Farrell et al., 2001 (b), and Sequeira et al., 2000 (c).

Ethanol collection code	Family	Subfamily/tribe/subtribe	Species	From	GenBank accession
NE01	Nemonychidae	Rhinorhynchinae/Mecomacerini	*Mecomacer* sp.	Argentina: Neuquen Prov.	AF250069a
NRH01		Rhinorhynchinae/Mecomacerini	*Rhynchitomacerinus kuscheli*	Chile: Malleco	AF389026
DOR02		Cimberidinae/Doydirhynchini	*Doydirhynchus austriacus*	Germany: Freiburg	AF389025
ANT02	Anthribidae	Anthribinae/Platystomini	*Toxonotus cornutus*	USA: Florida	AF389028
AB01		Anthribinae/Ptychoderini	*Ptychoderes nebulosus*	Panama	AF389027
BE01	Belidae	Belinae/Belini	*Rhinotia* sp.	Australia: Queensland	AF250066a
AL01		Oxycoryninae/Allocorynini	*Rhopalotria* sp.	Panama: Las Cruces	AF250067a
OX03		Oxycoryninae/Oxycorynini	*Oxycraspedus cornutus*	Chile: Malleco	AF250068a
ATP01	Attelabidae	Attelabinae/Attelabini	*Attelabus analis*	USA: Florida	AF250064a
ATA01		Attelabinae/Apoderini	*Apoderus giraffa*	Madagascar	AF250065a
RTR01		Rhynchitinae/Rhynchitini	*Merhynchites bicolor*	USA	AF250062a
RCA01		Rhynchitinae/Auletini	*Auletobius cassandrae*	USA: Florida	AF250063a
CA01	Caridae	Carinae	*Caenominurus topali*	Chile	AF389029
BRT01	Brentidae	Antliarhininae	*Antliarhis zamiae*	S. Africa	AF250061a
APA01		Apioninae	*Apion* sp.	USA: Florida	AF250060a
CYC01		Cyladinae	*Cylas formicarius*	USA: Florida	AF515703
BRE01		Eurhynchinae	*Aporhina australis*	Papua New Guinea	AF389030
IY01		Ithycerinae	*Ithycerus noveboracensis*	Canada: Ontario	AF389032
OCL01	Curculionidae	Ocladiinae/Ocladiini	*Ocladius obliquesetosus*	S. Africa: Transvaal	AF389033
DRS01		Dryophthorinae/Stromboscerini	Gen. sp.	Uganda	AF389034
RPT03		Dryophthorinae/Litosomini (=Sitophilini)	*Sitophilus granarius*	USA	AF389038
RPP01		Dryophthorinae/Sphenophorini	*Sphenophorus venatus*	USA: Florida	AF250071a
RH11		Dryophthorinae/Sphenophorini	*Trigonotarsus rugosus*	Australia: Queensland	AF389035
RPH		Dryophthorinae/Orthognathini	*Rhinostomus niger*	Madagascar	AF389036
RPR02		Dryophthorinae/Rhynchophorini	*Rhynchophorus cruentatus*	USA	AF389037
ERN01		Erirhininae/Stenopelmini	*Penestes* sp.	Panama	AF250072a
ERN02		Erirhininae/Stenopelmini	*Stenopelmus rufinasus*	USA: Florida	AF389039
ERN04		Erirhininae/Stenopelmini	*Lissorhoptrus longipennis*	USA: Florida	AF389040
ERN08		Erirhininae/Tanysphyrini	*Tanysphyrus lemnae*	USA: Massachusetts (intr. from Europe)	AF389041
BAB01		Baridinae/Madarini	*Ampeloglypter ampelopsis*	USA	AF389048
BAC01		Baridinae/Madopterini	*Odontocorynus* sp.	USA: Florida	AF250078a
BAC02		Baridinae/Madopterini	*Sibariops concinna*	USA: Florida	AF389049
POS01		Entiminae/Sitonini	*Sitona hispidulus*	USA: Florida	AF250087a
ENR01		Entiminae/Entimini/Trachyphloeina	*Callirhopalus bifasciatus*	USA: Georgia	AF250079a
OHY01		Entiminae/Entimini/Eustylina	*Diaprepes abbreviatus*	USA: Florida	AF250080a
ENM01		Entiminae/Entimini/Eudiagogina	*Eudiagogus rosenschoeldi*	USA: Georgia	AF250081a

POR01	Entiminae/Entimini/Eustylina	*Exophthalmus* sp.	Panama	AF250082a
POA01	Entiminae/Entimini/Naupactina	*Naupactus peregrinus*	USA: Florida (intr. from S. Am.)	AF250083a
OHO01	Entiminae/Entimini/Otiorhynchina	*Otiorhynchus sulcatus*	USA: Massachusetts (intr. from Europe)	AF250084a
PON01	Entiminae/Entimini/Naupactina	*Asynonychus cervinus*	USA: Florida (intr. from S. Am.)	AF250085a
POP01	Entiminae/Entimini/Polydrosina	*Polydrusus sericeus*	USA: Texas	AF250086a
POT02	Entiminae/Entimini/Tanymecina	*Tanymecus* sp.	USA	AF250088a
ENC01	Entiminae/Entimini/Cylydrorhinini	*Cylydrorhinus* sp.	Chile	AF389050
ENN01	Entiminae/Entimini/Tropiphorina	*Leptopius* sp.	Australia	AF389051
POB02	Entiminae/Entimini/Geonemina	*Lachnopus atramentarius*	Dominican Republic	AF389052
AY01	Amycterinae/Amycterini	*Talaurinus subvittatus*	Australia: Queensland	AF389054
HI01	Hipporhininae/Hipporhinini	*Bronchus* sp.	S. Africa: Ungni Valley	AF389056
ARA03	Aterpinae/Aterpini	*Chrysolopus spectabilis*	Australia: Queensland	AF389053
GO01	Gonipterinae/Gonipterini	*Oxyops vitiosa*	Australia	AF250090a
GO04	Gonipterinae/Gonipterini	*Gonipterus* sp.	Australia: Queensland	AF389055
RRL01	Rhythirininae/Listroderini	*Listronotus cryptop*s	USA: Florida	AF250089a
RRL03	Rhythirininae/Listroderini	*Listroderes bruchi*	Argentina: Mendoza Prov.	AF389057
ANA01	Anthonominae	*Anthonomus eugenii*	USA: Florida	AF250091a
CM01	Camarotinae	*Camarotus* sp.	Panama	AF250092a
CTP01	Ceutorhynchinae/Phytobiini	*Phytobius* sp.	China	AF250094a
CTP02	Ceutorhynchinae/Phytobiini	*Rhinoncus longulus*	USA: Florida	AF389061
CTN01	Ceutorhynchinae/Cnemogonini	*Auletes nebulosus*	USA: Florida	AF389060
CUC01	Curculioninae/Curculionini	*Curculio niveopictus*	Australia: Queensland	AF389059
ERD01	Derelominae	*Perelleschus carludovicae*	Panama	AF250095a
DED01	Derelominae	*Araucarietius viridans*	Argentina: Neuquen Prov.	AF389062
DED02	Derelominae	*Eisingius chusqueae*	Chile: Malleco	AF389063
GYG	Mecininae	*Gymnetron tetrum*		AF250096a
OTO01	Otidocephalinae	*Myrmex floridanus*	USA	AF250097a
TAT01	Rhamphinae/Tachygonini	*Tachygonus lecontei*	USA: Florida	AF250093a
CRC07	Cryptorhynchinae/Cryptorhynchini	*Rhyephenes humeralis*	Chile: Malleco	AF389064
CRC09	Cryptorhynchinae/Cryptorhynchini	*Pseudomopsis inflata*	Dominican Rep.: San Pedro	AF389065
CLC01	Lixinae/Lixini	*Lixus* sp.		AF250098a
CHC01	Molytinae/Cholini	*Cholus tessellatus*	Panama	AF250099a
CRI02	Molytinae/Conotrachelini	*Conotrachelus nenuphar*	USA: Florida	AF250100a
MOH01	Molytinae/Hylobiini/Epistrophina	*Epistrophus* sp.	Panama	AF250101a
MOH04	Molytinae/Hylobiini/Hylobiina	*Marshallius* sp.	Panama	AF250102a
HYH01	Molytinae/Hylobiini/Hylobiina	*Pachylobius picivorus*	USA: Florida	AF250103a
MOH02	Molytinae/Hylobiini/Hylobiina	*Calvertius tuberosus*	Chile: Malleco	AF389070
MOX01	Molytinae/incertae sedis	*Tranes lyteroides*	Australia	AF389069
ERB01	Bagoinae/Bagoini	*Bagous myriophyllae*	China	AF389066
ERB02	Bagoinae/Bagoini	*Bagous americanus*	USA: Florida	AF389067

(Continued on next page)

TABLE 1. Weevil taxa used in this study, collecting area, and GenBank accession numbers (letters beside the accessions correspond to Farrell 1998 (a), Farrell et al., 2001 (b), and Sequeira et al., 2000 (c). *(Continued)*

Ethanol collection code	Family	Subfamily/tribe/subtribe	Species	From	GenBank accession
MOT04		Bagoinae/Bagoini	Gen. sp.	Botswana: Okavango Delta	AF389068
HYH04		Hyperinae/Hyperini	*Hypera postica*	USA: Florida	AF389058
ZYZ01		Conoderinae/Zygopini	*Macrocopturus cincticollis*	Belize: Orange Walk	AF389071
ZYP01		Conoderinae/Piazurini	*Piazurus maculipes*	Panama	AF250104a
COC01		Cossoninae/Cossonini	*Cossonus* sp.	USA: California	AF308348c
COR01		Cossoninae/Rhyncolini	*Stenancylus* sp.	Argentina: Salta Prov.	AF375246b
COU02		Cossoninae/Araucariini	*Araucarius minor*	Argentina: Neuquen Prov.	AF308304c
CPF01		Cossoninae/	*Phylloplatypus pandani*	Bonin Islanda, W. Pacific	AF389042
HLH05		Scolytinae/Hylesinini/Hylastina	*Hylurgops* sp.		AF308317c
HLY01		Scolytinae/Hylesinini/Hylastina	*Hylastes porculus*	USA: Maryland	AF308339c
HLT03		Scolytinae/Hylesinini/Tomicina	*Hylurgonotus tuberculatus*	Argentina: Neuquen Prov.	AF308328c
HLT01		Scolytinae/Hylesinini/Tomicina	*Dendroctonus pseudotsugae*	USA: Colorado	AF308327c
HLR03		Scolytinae/Hylesinini/Phloeotribina	*Phloeotribus puberulus*	USA: Washington	AF308325c
HLO02		Scolytinae/Hylesinini/Hypoborina	*Liparthrum nigrescens*		AF308323c
SCS01		Scolytinae/Scolytini/Scolytina	*Scolytus multistriatus*	USA: Colorado	AF375261b
SCI02		Scoytinae/Scolytini/Ipina	*Ips grandicollis*	Canada	AF389043
SCI05		Scoytinae/Scolytini/Ipina	*Pityogenes* sp.	USA: Wisconsin	AF389045
SCH02		Scolytinae/Scolytini/Corthylina	*Pityophthorus* sp.	Argentina: Salta Prov.	AF389044
SCX03		Scolytinae/Scolytini/Xyloterina	*Trypodendron lineatum*	USA	AF250076a
SCD04		Scolytinae/Scolytini/Dryocoetina	*Dryocoetes alni*	Norway	AF389046
TST01		Platypodinae/Tesserocerini	*Notoplatypus elongatus*	Australia	AF389047
TST02		Platypodinae/Tesserocerini	*Chaetastus montanus*	Uganda	AF375248b
PLP02		Platypodinae/Platypodini	*Platypus* sp.	Puerto Rico	AF250077a
PLP05		Platypodinae/Platypodini	*Austroplatypus incompertus*	Australia	AF375244b

Saturation levels for the more conserved and hypervariable regions due to multiple substitutions were identified by plotting transition/transversion (ts/tv) ratios versus the overall distance and by comparing these ratios with the ts/tv expected values based on the base composition as described by Holmquist (1983) (not shown). Hypervariable regions (in all alignments) present a pattern of asymptotic divergence that suggests saturation due to multiple sustitutions in these sites; therefore, the exclusion from the analysis is also backed by this result. The two extremes of the gene (positions 1–50 and 2055–2153) were excluded to avoid excessive missing data for several taxa.

Gap treatment.—The recommendations of Lutzoni et al. (2000) and Kjer et al. (2001) were helpful in dealing with regions containing gaps. Length heterogeneous regions containing insertions and/or deletions were evaluated separately (Kjer et al., 2001:786). Ten regions (positions 239–245, 251–282, 753–756, 788–881, 1570–1573, 1602–1684, 1689–1692, 1699–1701, 1712–1719, 1722–1725) that were of equivocal alignment were categorized as indels. Indel regions of variable length apparently were phylogenetically informative in some lineages. Therefore, we eliminated the nucleotide characters from the analysis but coded each unique combination of nucleotides in these variable regions with a different symbol. The implementation of this coding method was not problematic when using PAUP* because we identified up to 25 states, not exceeding the maximum of 32 states per character allowed by the program.

A molecular data matrix (2,153 positions after alignment) for 100 species of Curculionoidea plus 3 outgroup chrysomeloid species was constructed (for GenBank accession numbers, see Table 1). In this study, 55 of the weevil sequences are new and were added to the 45 available from Farrell (1998a). Outgroup sequences for the chrysomeloids also came from Farrell (1998a) and are in GenBank under accession numbers AF267418 (*Palophagoides*), AF267413 (*Prionus*), and AF267403 (*Dendrobius*). The final alignment of 1,761 included positions (1,771 positions considering indel characters) yielded 312 potentially informative characters. The data matrix with the alignment (including the character sets for data removal) is available on the TREEBASE website (www.herbaria.harvard.edu/treebase/), submission number S764.

Morphological Data

In previous cladistic analyses the terminal units were families or subfamily groups. In the present study, the terminal units are 100 representative species. We compiled a new and enlarged morphological data set and scored the states for most species, using specimens from the samples sequenced. In some cases (e.g., if the larva was not described), we assumed the state found in a closely related, often congeneric, species (see Appendix 1). For the sake of clarity the morphological data are grouped into larval and adult characters and described briefly in Appendix 1. The morphological data matrix of 115 characters (37 from larvae and 78 from adults) is shown in Table 2 (http://systematicbiology.org).

Biological Data

The variation in host associations and larval habits embraced by the sampling of species and lineages is summarized and coded in Table 3. The phylogeny estimate was used to trace the evolution of these features. Optimization of these traits (which were not part of the phylogenetic analyses, to avoid circularity) was performed using PAUP* 40b.4a (Swofford, 1998).

Phylogenetic Analyses

Although we believed that the best estimate in this study would most likely be obtained from the combined analysis, separate phylogenetic analyses of the data sets (molecular and morphology) were also performed for comparative purposes. Separate and combined analyses were conducted under the maximum-parsimony optimality criterium. The molecular data set was also analyzed using model-based Bayesian inferences.

Molecular data analysis using Bayesian inference.—Modeltest 3.06 (Posada, 2001) was used to select the most likely model for the 18S rDNA data set. All searches were performed with Mr. Bayes 2.01 (Huelsenbeck, 2001). Bayesian searches were run with four simultaneous chains for 400,000 generations, sampling every 100 generations and applying temperatures of 1, 0.5, and 0.3, which influence the rate of switching between chains.

TABLE 3. Biological data. Character list and data matrix. Information on the host plants and larval habits was gathered from the literature and/or the collection data of the specimens studied. The term "dicots" is herein applied to the major clade of flowering plants (true dicotyledons) that together with the monocots constitute the clade euangiosperms (see Qiu et al., 1999, and references therein). The latter is distinct from earliest angiosperm lineages such as the water lilies, herein referred as basal angiosperms. The four characters were scored as follows: (1) major host taxon used: 0 = Pteridophyta; 1 = conifers; 2 = cycads; 3 = basal angiosperms (i.e., Nymphaceae); 4 = monocots; 5 = Dicots. (2) Larval habit inside or outside of host: 0 = endophagous or internal feeding; 1 = external feeding concealed (i.e., in soil or mud); 2 = external feeding exposed (i.e., on aerial plant parts); 3 = combined internal/external concealed; 4 = combined internal/external exposed. (3) Tissue consumed by larvae: 0 = stem, twig, trunk; 1 = leaf; 2 = root; 3 = fruit or flower bud; 4 = seed; 5 = male strobili, pollen sacs; 6 = female strobili, ovules, or seeds; 7 = male strobili, vegetative tissues; 8 = female strobili, vegetative tissues; 9 = fungi. (4) State of host plant tissue at moment of consumption: 0 = living, healthy; 1 = dying, decaying.

	Characters		Characters
Taxon	1234	Taxon	1234
Dendrobius	5000	*Ampeloglypter*	5000
Palophagoides	1050	*Sibariops*	5000
Prionus	10?1	*Odontocorynus*	5000
Doydirhynchus	1050	*Callirhopalus*	5120
Mecomacer	1050	*Diaprepes*	5120
Rhynchitomacerinus	1050	*Cylydrorhinus*	5120
Ptychoderes	5091	*Leptopius*	5120
Toxonotus	5091	*Eudiagogus*	5120
Oxycraspedus	1081	*Exophthalmus*	5120
Rhopalotria	2071	*Naupactus*	5120
Rhinotia	5001	*Otiorhynchus*	5120
Apoderus	5091	*Asynonychus*	5120
Attelabus	5091	*Lachnopus*	5120
Auletobius	5091	*Polydrusus*	5120
Merhynchites	5031	*Sitona*	5120
Caenominurus	1060	*Tanymecus*	5120
Antliarhinus	2060	*Chrysolopus*	5020
Apion	5040	*Talaurinus*	4320
Aporhina	5000	*Gonipterus*	5210
Cylas	5020	*Bronchus*	5320
Ithycerus	5020	*Listronotus*	4400
Ocladius	4000	*Oxyops*	5210
Stromboscerini	?001	*Listroderes*	5210
Trigonotarsus	4001	*Hypera*	5410
Rhinostomus	4001	*Anthonomus*	5030
Rhynchophorus	4001	*Curculio*	5030
Sitophilus	4040	*Camarotus*	5010
Sphenophorus	4020	*Tachygonus*	5010
Stenopelmus	0?00	*Auletes*	5010
Lissorhoptrus	4320	*Rhinoncus*	5000
Tanysphyrus	4010	*Phytobius*	?010
Penestes	?000	*Araucarietius*	1070
Stenancylus	?001	*Eisingius*	1070
Cossonus	1001	*Perelleschus*	4040
Araucarius	1001	*Gymnetron*	5030
Phylloplatypus	4010	*Myrmex*	5000
Dendroctonus	1011	*Rhyephenes*	1001
Liparthrum	1001	*Pseudomopsis*	?001
Phloeotribus	5001	*Bagous myriophyllae*	50?0
Hylurgonotus	1001	*Bagous americanus*	3010
Hylastes	1001	Bagoini	?0?0
Hylurgops	1001	*Lixus*	5020
Ips	1001	*Cholus*	4000
Pityophthorus	?001	*Conotrachelus*	5030
Pityogenes	1001	*Epistrophus*	1000
Trypodendron	1091	*Marshallius*	5000
Dryocoetes	5001	*Calvertius*	1001
Scolytus	5001	*Tranes*	2071
Platypus	5091	*Pachylobius*	1001
Austroplatypus	5091	*Piazurus*	500?
Notoplatypus	5091	*Macrocopturus*	500?
Chaetastus	5091		

The burning or stationarity generation was determined by plotting generations versus log likelihoods (Ln L); all trees below that stationarity level were discarded. The selected model was the general time reversible model (GTR) (Rodriguez et al., 1990), estimating the proportion of invariable sites and the shape of the gamma parameter.

Characteristics of the molecular data set.—The model that best describes the substitution types for the 18S rDNA data set is a simplification of the GTR model. The proportion of invariant sites was 0.3919, and the estimated shape of the gamma parameter was 0.4295. The ts/tv ratio was 1.4. The substitution rate calculated for the data set excluding hypervariable regions (1,761 included positions) was 0.4272 (SD-0.0163). The proportion of parsimony informative sites in the ingroup was 17.14%. A smaller proportion was found for another beetle group, the Chrysomeloidea (10.67%), from Farrell's (1998a) data matrix expanded.

Molecular, morphological, and combined data analyses using parsimony.—The 18S molecular data matrix and the mophological matrix were analyzed separately and together. The phylogenetic analyses were parsimony based using PAUP* 4.0b4a for Macintosh (Swofford, 1998). The molecular matrix was also analyzed with NONA 2.0 (Goloboff, 1998). The program WINCLADA 0.9 (Nixon, 1999b) was used to convert the matrix in nona format and also to visualize trees and character optimization. The program MacClade 3.0 (Maddison and Maddison, 1992) was used for combining data matrices. Separate and combined analyses were performed with all characters equally weighted and treated as unordered; gaps were treated as explained above.

The molecular data matrix (1,771 included characters, 1,761 positions plus 10 indel characters) was analyzed using the ratchet method (Nixon, 1999a) as implemented in PAUP*, by means of 15 pct (default proportion of weighted characters in the search). The molecular data matrix excluding the indel characters (1,761 positions) was also analyzed using the ratchet method with NONA, with the commands "hold/1; nixwts 180 50;". Analysis of the morphological data matrix (115 characters) was carried out using a heuristic search: 1,000 random addition sequence (RAS) replicates, two trees held at each step during stepwise addition, tree bisection-reconnection (TBR) branch swapping, and no upper limit of MaxTrees. Analysis of the combined data matrix (1,886 characters) was performed using a heuristic search: 5,000 RAS replicates, two trees held at each step during stepwise addition, TBR branch swapping, and no upper limit of MaxTrees.

Support to tree topology was evaluated by means of bootstrap values (also jackknife for the combined) as measured by PAUP* using the "faststep" option.

RESULTS

Data

Sequences were submitted to GenBank (see Table 1 for accession numbers). NEXUS files used in phylogenetic analyses and resulting trees are available on the TREEBASE website under accession number S764.

Separate Analyses of the 18S rDNA Data Set under Bayesian and Parsimony Optimality Criteria

Bayesian inference of phylogenetic relationships.—The Bayesian topology obtained (Fig. 1) shows >50% estimated posterior probability for the monophyly of the superfamily Curculionoidea and for several family groups within it: Nemonychidae (excluding *Doydirhynchus*) + Anthribidae, Belidae, Attelabidae (excluding *Auletobius*), and a clade comprising Caridae, Brentidae, and Curculionidae. Within this latter clade there is support for a partial grouping within Brentidae and for several subfamilies of Curculionidae (e.g., Bagoinae, Ceutorhynchinae, Baridinae, Gonipterinae, and Dryophthorinae excluding Stromboscerini). Other groups supported by Bayesian estimation are partial groupings within Cossoninae, Derelominae, Entiminae, Scolytinae, and Platypodinae. Figure 1 shows 31 nodes with >50% probability that are recovered in the combined tree, although there are also some "unexpected" relationships with high probability (i.e., *Caenominurus* + *Stenopelmus*, *Cylas* + *Pachylobius*). Relationships between subfamilies in the speciose subfamily Curculionidae were not resolved by the molecular data, neither by the Bayesian (Fig. 1) nor the parsimony (Fig. 2) estimation.

Parsimony analysis of the 18S rDNA data set.—Parsimony analysis of the molecular data matrix (1,771 included characters)

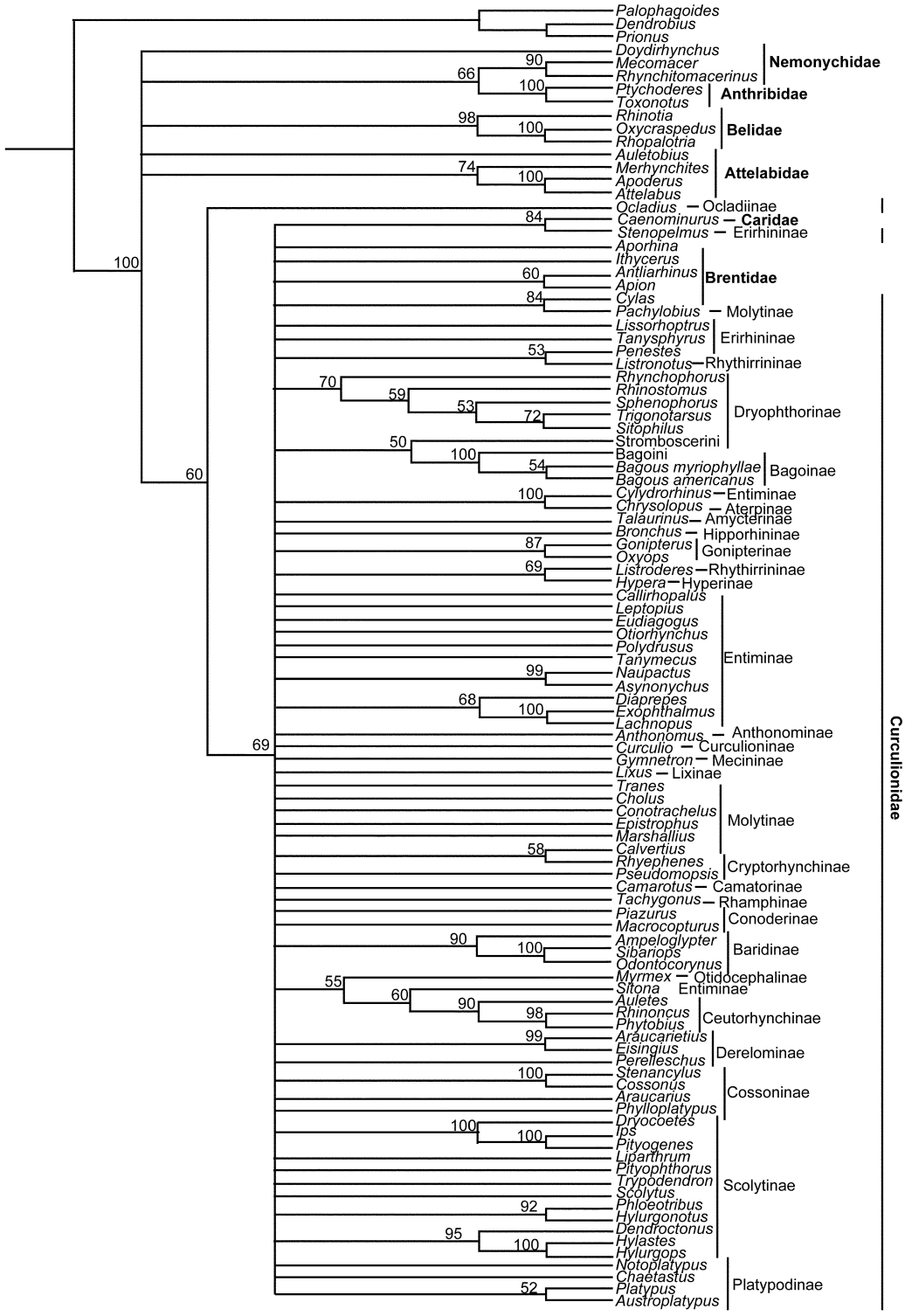

FIGURE 1. Majority rule consensus tree from the Bayesian analysis of the 18S rDNA data set (3,800 trees). The topology obtained is the same for runs with all three temperature settings and burning generations all around 20,000 (or 200 trees). Searches were run with four simultaneous chains for 400,000 generations, sampling every 100 generations (GTR, empirical base frequencies, estimating proportion of invariable sites, and shape of gamma parameter). Numbers above branches indicate Bayesian posterior probabilities of nodes.

FIGURE 2. Strict consensus of 29 MPTs obtained from molecular (18S rDNA) parsimony analysis. Numbers above branches are bootstrap values (>50%).

resulted in 29 most-parsimonious trees (MPTs) (2,119 steps, consistency index [CI] excluding uninformative characters = 0.35, retention index [RI] = 0.43), the strict consensus of which is presented in Figure 2. Other search strategies performed (as for the combined analysis below) resulted in trees that are one to two steps longer than the MPTs obtained with the parsimony ratchet method.

The analysis of the molecular data matrix excluding the indel characters (1,761 positions) resulted in 49 MPTs of 2,008 steps, the strict consensus of which (not shown) show the same well-supported (bootstrap >50%) nodes as the ones resulting from the analysis of the matrix of 1,771 characters. Some but not all of these MPTs agree with results from morphology in the close relationship between Nemonychidae and Anthribidae and Caridae as sister group to Brentidae + Curculionidae. There was a spurious attraction between two attelabines and some curculionid divergent sequences that was rectified when indel characters were included (Fig. 2).

The 21 nodes that are well supported (>50%) in the 18S parsimony cladogram (Fig. 2) are in agreement with the relationships proposed by morphology (Fig. 3) and in the combined tree (Fig. 4). In total, the 18S parsimony tree (Fig. 2) has 31 nodes that were also recovered in the combined tree (Fig. 4). Although the 18S data (Fig. 2) also propose relationships that differ from the morphological cladogram (Fig. 3), none of these ("unexpected") nodes are highly supported (values <50%), thus resulting in weakly supported topological incongruence.

Analysis of the morphological data set.—Parsimony analysis of the morphological data matrix (115 characters) was carried out using a heuristic search with PAUP*: 1,000 RAS replicates, two trees held at each step during stepwise addition, TBR branch swapping, and no upper limit of MaxTrees. The search resulted in 984 MPTs (230 steps, CI excluding uninformative characters = 0.59, RI = 0.93), the strict consensus of which is presented in Figure 3.

The morphological tree (Fig. 3) has 32 nodes (24 with support >50%) that were also recovered in the combined tree (Fig. 4).

Combined analysis of 18S rDNA and morphology data.—Heuristic searches retained four MPTs (2,456 steps, CI excluding uninformative characters = 0.36, RI = 0.60), the strict consensus of which is depicted in Figure 4, showing bootstrap and jackknife values. One of the MPTs is presented in Figure 5, and the morphological apomorphies defining each node are listed in Appendix 2.

The combined 18S + morphology tree (Fig. 4) shows an improvement in resolution with respect to those trees from separate analyses, with a larger number of well-supported clades (42 nodes with bootstrap and jackknife values >50% plus 4 nodes with at least one of those values >50%). Not all clades found in the combined tree (Fig. 4) were present in the ones from the individual 18S (Fig. 2) and morphological (Fig. 3) data sets. This result suggests the existence of phylogenetic signal (hidden by homoplasy in results of separate analyses) that emerged after combining the data partitions.

Weevil Relationships

Results of the combined analysis show that monophyly of weevil families and their interrelationships are in general well supported (Fig. 4); only the taxon Brentidae is supported by values of <50%. The combined analysis of 18S rDNA plus morphology data resolved groups at higher levels, but most relationships within the Curculionidae are relatively weakly supported, as indicated by bootstrap/jackknife values (Fig. 4) and will require further study.

The combined cladogram shows a close relationship of Anthribidae and Nemonychidae, as also suggested by results of the morphological analysis and in some but not all trees from the 18S separate analysis. These two weevil groups share similar oviposition structures (Thompson, 1992: figs. 170–172; Howden, 1995) and setiferous patches (Oberprieler, 1999). Although fossil Nemonychidae are known from Jurassic beds (Kuschel, 1983; Zherikhin and Gratshev, 1995), fossils attributable to Anthribidae are known only from the Middle Cretaceous (Fig. 5; Zherikhin, 1993), consistent with their use of angiosperms and angiosperm-dependent ascomycetes and basidiomycetes but implying the possible paraphyly of Nemonychidae. More extensive sampling of these two families may resolve the history of host use. Results of both separate (Figs. 1–3) and combined (Fig. 4) analyses support an enlarged concept of the Belidae including Belinae and Oxycoryninae.

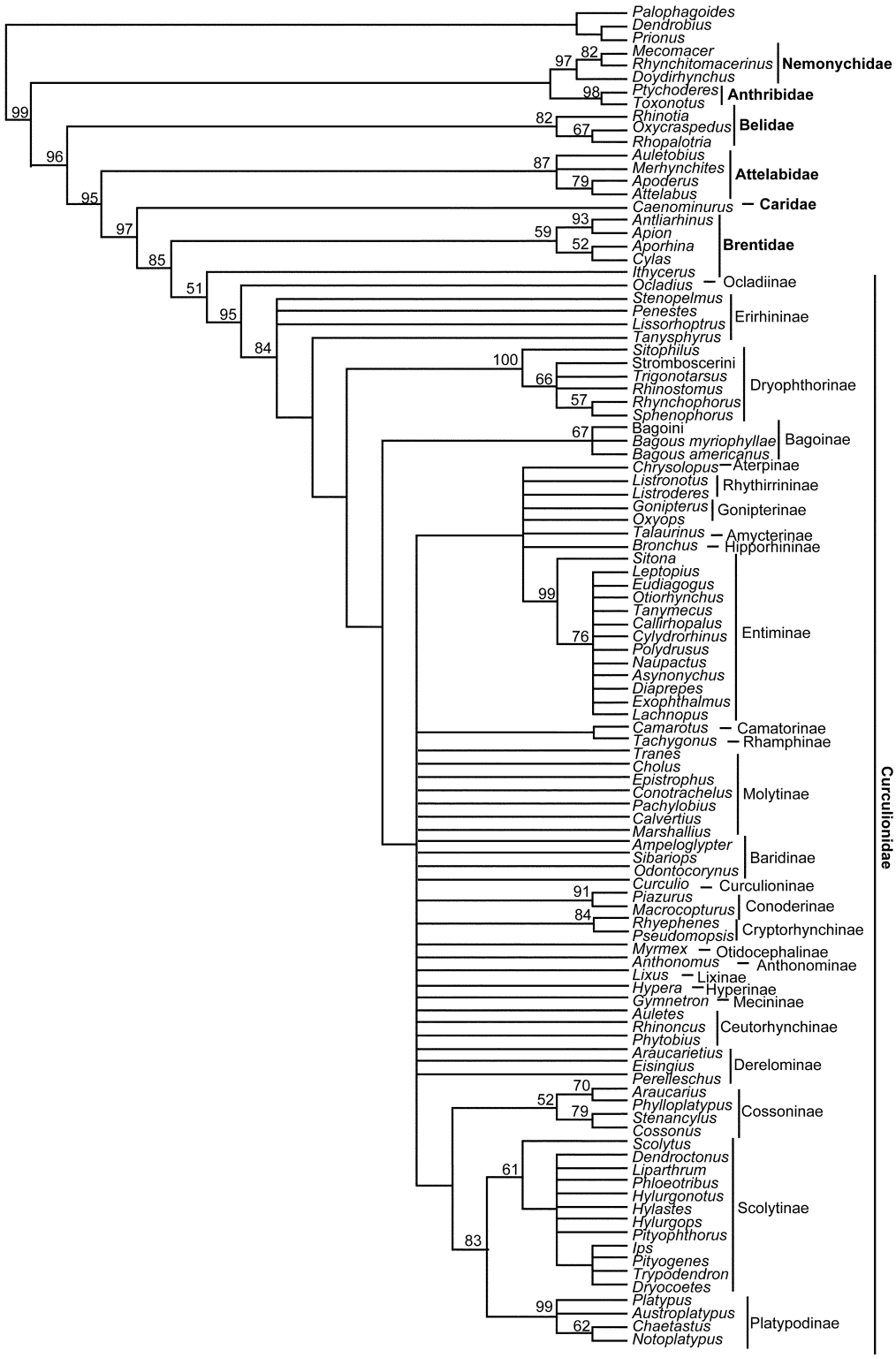

FIGURE 3. Strict consensus of 984 MPTs obtained from the morphological parsimony analysis. Numbers above branches are bootstrap values (>50%).

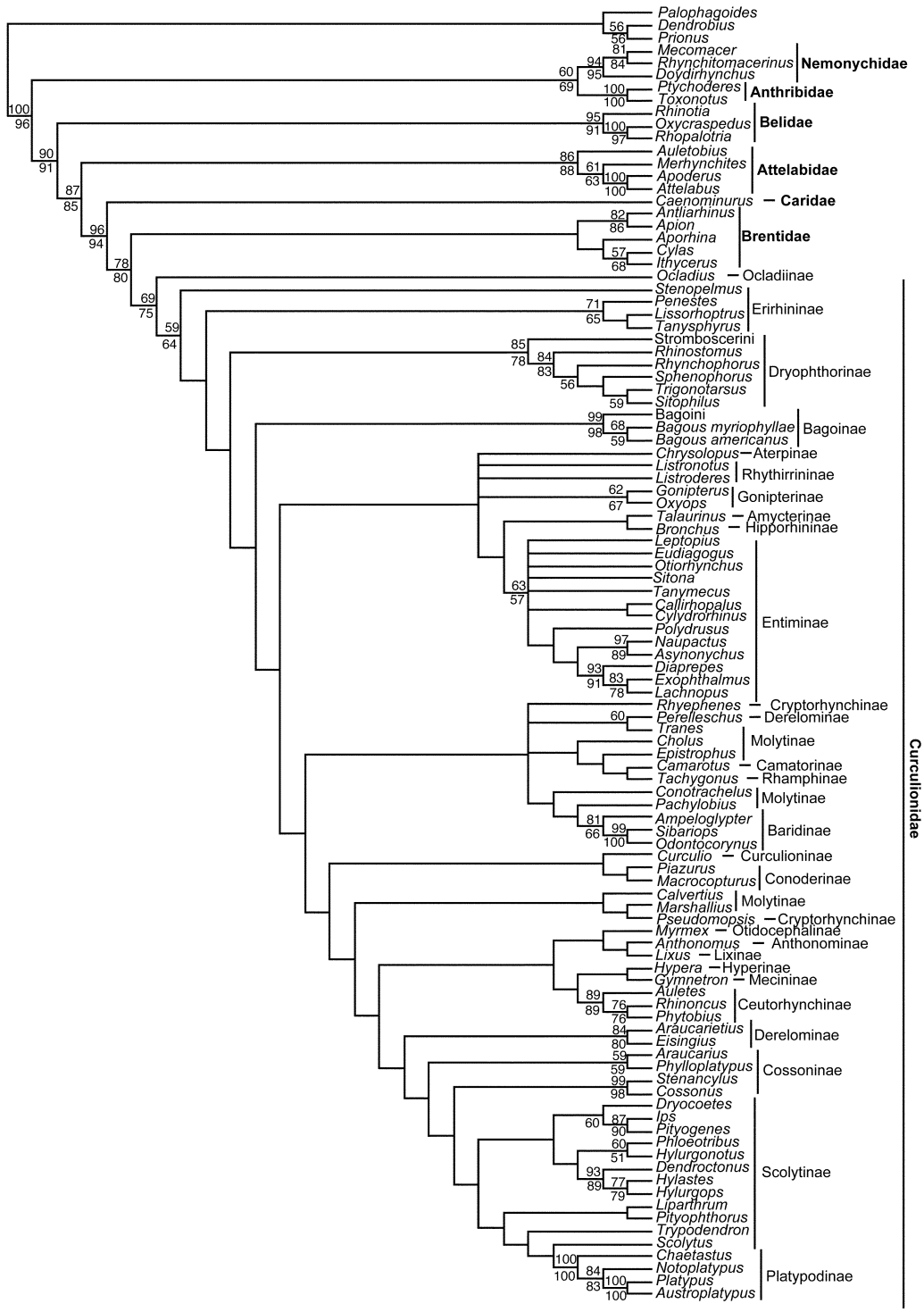

FIGURE 4. Strict consensus of four MPTs obtained from the combined 18S rDNA + morphology parsimony analysis. Numbers above and below branches are bootstrap and jackknife values (>50%), respectively.

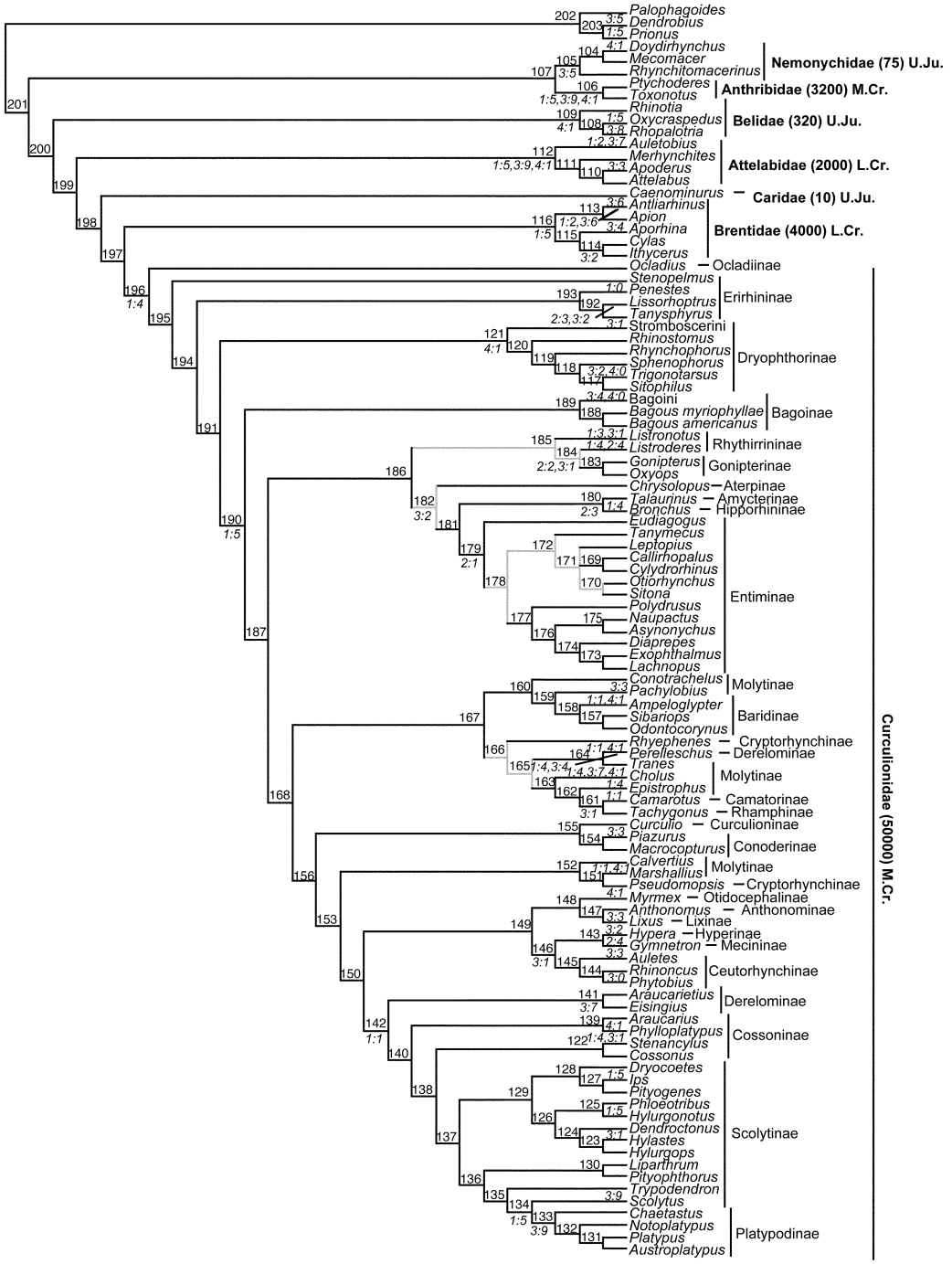

FIGURE 5. Phylogenetic hypothesis for the weevils. This is one of four MPTs from the combined 18S rDNA + morphology analysis. Gray lines mark branches that are common to some but not all of the alternative solutions (collapsed nodes in the consensus tree of Fig. 4). Numerals above branches are node numbers corresponding to the morphological apomorphies listed in Appendix 2. Curculionoid family diversity is expressed as approximate number of extant species and probable age as indicated by the oldest known fossil (U.Ju. = Upper Jurassic; L.Cr. = Lower Cretaceous; M.Cr. = Middle Cretaceous). Biological information is mapped in italics below branches, with character numbers as follows: (1) Major host taxon used: 0 = Pteridophyta; 1 = conifers; 2 = cycads; 3 = basal angiosperms (i.e., Nymphaceae); 4 = monocots; 5 = dicots. (2) Larval habit inside or outside of host: 0 = endophagous or internal feeding; 1 = external feeding concealed (i.e., in soil or mud); 2 = external feeding exposed (i.e., on aerial plant parts); 3 = combined internal/external concealed; 4 = combined internal/external exposed. (3) Tissue consumed by larvae: 0 = stem, twig, trunk; 1 = leaf; 2 = root; 3 = fruit or flower bud; 4 = seed; 5 = male strobili, pollen sacs; 6 = female strobili, ovules, or seeds; 7 = male strobili, vegetative tissues; 8 = female strobili, vegetative tissues; 9 = fungi. (4) State of host plant tissue at moment of consumption: 0 = living, healthy; 1 = dying, decaying.

The basal position of the Belidae suggested (weakly) by the separate 18S rDNA parsimony analysis is refuted when morphological characters are added or under Bayesian inference but remains as an interesting hypothesis to be tested with the use of additional genes. The fossil evidence (Fig. 5) shows that Belidae were present in the Jurassic (Zherikhin and Gratshev, 1995), which is in accordance with the basal dichotomy of Curculionoidea (Fig. 4) leading to Nemonychidae + Anthribidae and Belidae + remaining families.

A monophyletic Attelabidae was recovered in the combined analysis (Fig. 4), but the placement of *Auletobius* is ambiguous in results of molecular data alone (18S). The oldest fossils (Fig. 5) attributable to Attelabidae are from late Lower Cretaceous (Gratshev, 1998) to Middle Cretaceous (Kuschel et al., 1994), but the phylogenetic placement of the family would predict that older fossils may be found.

The phylogenetic position of *Car* and its allies (Caridae) has been enigmatic for a long time. Different authors have included *Car* and related taxa in different families, e.g., Attelabidae (Crowson, 1955), Apionidae (Wibmer and O'Brien, 1986), Belidae (Thompson, 1992; Zherikhin and Gratshev, 1995), Curculionidae (Kuschel et al., 1994), and Brentidae (Kuschel, 1995), whereas others considered them to be a distinct family (e.g., Zimmerman, 1994a). Results of the combined cladistic analysis (Fig. 4) support placement of Caridae (here represented by *Caenominurus topali*) as sister taxon of the clade Brentidae + Curculionidae. This placement is also present in the morphology tree (Fig. 3) and was recovered in some but not all 18S rDNA parsimony trees. The Caridae (Fig. 5) are known from Late Jurassic deposits (Arnoldi, 1977; Gratshev and Zherikhin, 1999) and were abundant in the Lower Cretaceous (Kuschel et al., 1994). The original concept of Brentidae was widened by several authors (Morimoto, 1976; Kuschel, 1990, 1995; Thompson, 1992) to include Eurhynchinae, Antliarhininae, Cyladinae, Apioninae, and Nanophyinae (and also Carinae; Kuschel, 1995). Exclusion of Carinae is supported by the present study.

The combined analysis (Fig. 4) places the monotypic (and enigmatic) genus *Ithycerus* in the Brentidae, in accordance with Oberprieler (2000), but independent analyses of morphology and molecules do not support this grouping. This finding can be regarded as a possible example of phylogenetic signal that emerges when the data are combined. The oldest described fossil of a brentid is an eurhynchine from Middle to Upper Cretaceous (Kuschel et al., 1994), but several middle Lower Cretaceous brentid fossils (Fig. 5), attributable to eurhynchines and nanophyines, were described by V. Zherikhin (R. Oberprieler, 2000 in litt.).

The limits and definition of a monophyletic family Curculionidae have always been problematic. Changes in the suprageneric taxa included in this family have resulted because of taxa of ambiguous or equivocal placement (such as *Ithycerus*) and more importantly because of the more or less inclusive concept of Curculionidae adopted by various authors (see references in Marvaldi and Morrone, 2000).

The results of the present study (Fig. 4) clearly establish the family Curculionidae as the sister group to the Brentidae and permit identification of Curculionidae as a monophyletic group (see Fig. 5 and Appendix 2 for larval and adult apomorphies defining the Curculionidae). The oldest described fossil of a curculionid (Fig. 5) is *Cretulio nucula* from late Lower Cretaceous deposits (Zherikhin, 1993). Its tentative placement in Erirhininae is consistent with the placement of erirhinines among the most basal curculionids in our cladogram (Fig. 4). Thus, the Curculionidae and their sister group Brentidae date to the Lower Cretaceous, near the origin of angiosperms.

Curculionids classified in Ocladiinae, Erirhininae and Dryophthorinae, which retain the primitive orthocerous type of male genitalia (Morimoto, 1962a; Kuschel, 1971; Thompson, 1992), occupy basal positions in the phylogeny estimate (Fig. 4). Although sequences were not available for representatives of two small groups with orthocerous-type genitalia (Brachycerinae and Cryptolarynginae), the morphological characters suggest they are among the basal members of the Curculionidae. Evidence for a close relationship between Brachycerinae sensu stricto and Ocladiinae is provided by both adult (Thompson, 1992) and larval (Marvaldi, 2000) morphology. Larvae of the Cryptolarynginae remain unknown, but adult morphology suggests a close relationship to Ocladiinae or Erirhininae (Marvaldi

and Morrone, 2000; R. Oberprieler, 2000 in litt.). The Erirhininae in the strict sense of Kuschel (1971; see also Alonso-Zarazaga and Lyal, 1999) are difficult to delimit. According to our study, there are no clear larval or adult synapomorphies to justify their monophyly, and when molecular data are added, they appear to be paraphyletic (Fig. 4). However, monophyly of the Dryophthorinae is strongly supported, and they probably represent an independent offshoot sister to the remaining Curculionidae.

Curculionidae sensu stricto, with the derived gonatocerous type of male genitalia (node 190 in Fig. 5; see Appendix 2 for apomorphies defining this clade), is the largest group of weevils, in agreement (except for the inclusion of Platypodinae) with the restricted concept of Curculionidae proposed by Thompson (1992) and Zimmerman (1993, 1994a, 1994b). Some of the curculionid "subfamilies" were recovered in the combined cladogram (Fig. 4), with high support values found for their monophyly (e.g., Bagoinae, Entiminae, Baridinae, Ceutorhynchinae, Platypodinae). Other "subfamilies" appear to be polyphyletic (e.g., Molytinae, Derelominae) or paraphyletic (e.g., Cossoninae, Scolytinae) in the combined analysis, but these determinations are weakly supported and further evidence is required.

Support for the monophyly of Platypodinae is present in both separate and combined parsimony analyses, and their inclusion in Curculionidae is clear (Figs. 2–4). The exact position of the platypodines within that family, however, remains enigmatic. The Platypodinae have been considered by several authors as a distinct family, mainly because unique adult morphological characters (e.g., Calder, 1989, 1990; Thompson, 1992; Lyal and King, 1996) were interpreted as providing none or equivocal evidence of relationship to any other group of weevils. However, the larval characters naturally place them within Curculionidae (May, 1993) and suggest a close relationship of Platypodinae with Dryophthorinae (Marvaldi, 1997). The combined molecular and morphological analysis (Fig. 4) indicates that the Platypodinae are best included in the Curculionidae as proposed by Crowson (1955) and Kuschel (1995), but their condition as apomophic derivatives of the Scolytinae (Kuschel et al., 2000; Farrell et al., 2001) is only weakly supported by the present analysis and mainly determined by adult morphology, without any clear larval synapomorphy (Figs. 3–5).

Biological Data and Phylogeny

We used a delayed (deltran, slow) optimization on one of the MPTs from the combined analysis (Fig. 5) to analyze the evolution of host-plant associations and larval habits. The accelerated (acctran, fast) optimization was not preferred because it proposes an origin of association with dicot angiosperms in the ancestor of Curculionoidea (with reversals to gymnosperm feeding in Nemonychidae, Belidae, and Caridae), which the fossil record indicates to have existed in the Jurassic, before the origin of euangiosperms.

DISCUSSION

Collectively, weevils have colonized virtually every plant group and every plant part, but particular lineages often show strong conservatism in the evolution of host use. Weevil lineages that are classified at ranks from subfamilies to groups of genera are primarily associated with one of the major vascular plant groups: cycads, conifers, monocots, or dicots. The earliest weevils, represented today by the depauperate families Nemonychidae, Belidae, and Caridae (see Fig. 5), continue associations with gymnosperms (i.e., conifers) formed in the Mesozoic, dating back at least to the late Jurassic. The phylogenetic evidence that these three weevil families are older than angiosperms is corroborated by their occurrence as Jurassic fossils and their disjunct relictual distributions, primarily in the Southern Hemisphere. Thus phylogenetic, fossil, and biogeographic evidence suggest that the association of Nemonychidae and Belidae with strobili of conifers has been conserved for $\geq$200 million years (Farrell, 1998a).

The present study provides continued support for the association of increased diversity with shifts from gymnosperms to angiosperm hosts (Fig. 5; Farrell, 1998a). The phylogeny estimate (Fig. 5) permits identification of multiple origins of associations with monocots and dicots and several further shifts to conifers and cycads. Thus, although some weevils are among the original conifer feeders, other weevil groups associated with these plants (particularly within

Curculionidae) seem nested well within clades of angiosperm feeders, representing shifts back to gymnosperms.

Monocot Use

Several major weevil lineages (herein represented by species in the Ocladiinae, Erirhininae, and Dryophthorinae) that appear primarily associated with monocots have a basal phylogenetic position in the clade Curculionidae (Fig. 5). The early association with monocots, plants otherwise little used by weevils outside the family Curculionidae, characterizes some 2,300 species and suggests an early role played by the monocots in the diversification of the Curculionidae, as suggested by Reid (2000) for its sister group the Chrysomeloidea (Table 4). The proximity of these lineages on the phylogeny estimate suggests a common origin of monocot feeding that is comparable to that characterizing the common ancestor of the subfamilies Criocerinae, Hispinae, Bruchinae, and Donaciinae of the Chrysomelidae (Farrell, 1998a; Reid, 2000; Farrell and Sequeira, in prep.). Like the weevils, these chrysomelid subfamilies each specialize on different monocot groups. The Brachycerinae and Criocerinae are mainly associated with Liliaceae; the Ocladiinae, Dryophthorinae, and Hispinae are mostly associated with the grasses, palms, and gingers; and the Erirhininae and Donaciinae largely use the aquatic monocot families (Kuschel, 1971, 1995; Farrell, 1998a; Reid, 2000; Farrell and Sequeira, in prep.).

The phylogeny estimate for the curculionids, together with evidence from weevil fossils, suggests that these weevils may approach the early Cretaceous age of the monocots. The monocots constitute an early offshoot from relatively basal angiosperms, according to recent molecular and phylogenetic studies (Qiu et al., 1999, and references therein), with oldest known fossils dating from the Middle to Upper Cretaceous (Gandolfo et al., 1998), an age coincident with that of the Curculionidae (Fig. 5) and with the monocot—associated chrysomelid subfamilies (Wilf et al., 2000). Monocot diversification thus may have facilitated the early diversification of curculionids and chrysomelids, although the greatest diversity of each beetle group is associated with dicots.

Larval Habits and Host Tissues Consumed

The larvae of Curculionoidea are primitively endophagous, feeding inside host tissues. Like the endophagous longhorn beetle family Cerambycidae, weevil larvae have lost the development of legs (Crowson, 1955; Stehr, 1991; Marvaldi, 1997; Farrell, 1998a). Larval endophagy is obviously associated with adult rostrum development and oviposition behavior. Most weevils use the rostrum to place eggs inside larval substrates, but the adults of Scolytinae and Platypodinae tunnel deep inside tree trunks and branches for adult feeding and oviposition. Adults of broad-nosed weevils, such as those in the large subfamily Entiminae, do not use the rostrum for oviposition and resemble the chrysomelid subfamily Eumolpinae in that the larvae feed on roots from adjacent positions in the soil. Although relationships among broad-nosed weevils are still weakly resolved, the larval habit of Amycterinae may be transitional between the primitive endophagous habit of most weevils and burrowing in soil for root feeding in the Entiminae. Newly hatched larvae of Amycterinae are internal root feeders, whereas older larvae feed from an external position protected by an earthen cell open to the root (Howden, 1987). Construction of earthen cells for feeding has also been reported for other broad-nosed weevils in Thecesterninae (McClay and Anderson, 1985), in some Rhytirrhininae (Scott and Way, 1989), and in Lixinae (O'Brien and Marshall, 1987). Thus, even the external root feeders are still endophagous in the sense that they live concealed inside the

TABLE 4. Independent contrasts of sister groups not associated versus associated with early monocots.

	Not associated		Associated	
Comparison	Group	No. taxa	Group	No. taxa
1	Brentidae	4,000	Curculionidae	50,000
2[a]	Orsodacninae–Aulacoscelinae	26	Remaining Chrysomelidae	37,000

[a]Host plants and species numbers from Jolivet and Hawkeswood (1995:xi, 21).

substratum (Marvaldi, 1997). Being totally legless, larvae of Curculionidae are mostly endophagous feeders, although some instances of larval ectophagy have evolved, e.g., as exposed external feeders on leaves in Gonipterinae (*Gonipterus* and *Oxyops*), Rhytirrhininae (*Listroderes*), or Hyperinae (*Hypera*). This development is in sharp contrast with the habit of larvae in the comparably diverse family Chrysomelidae, which have retained legs and where ectophagy (external leaf feeding) is predominant. Predicted consequences of leaf feeding are higher overall rates of parasitism (Hawkins, 1994), and external feeding insects show fewer instances of interspecific competition than do internal feeders (Denno et al., 1995). Contrasts of sister groups that differ in larval feeding mode should provide insights into the possible macroevolutionary consequences of the presumably different selective regimes to which these larvae are subject.

Feeding inside coniferous tissues, probably branches and trunks, appear to be the ancestral larval habit in Curculionoidea. Pollen feeding in male strobili of conifers, as it occurrs in Nemonychidae, is also the larval habit of basal chrysomeloids (Palophaginae) and of the basal lineages of the Hymenoptera and Lepidoptera (Kuschel and May, 1990, 1996; Farrell, 1998a, 1998b), which suggests that these relatively protein-rich resources were instrumental in fostering the origins of herbivory in these three holometabolous orders (Farrell, 1998b).

Although most curculionoids feed on tissues of vascular plants, dependence on fungi, fungus-modified host tissues, or fungus-riddled wood has evolved in the Anthribidae, Attelabidae, in some Brentidae, and in Scolytinae and Platypodinae, typically enabling use of a broad array of host-plant groups (Holloway, 1982; Beaver, 1989; May, 1993; Oberprieler, 1999; Farrell et al., 2001).

Compared to specialists on conifer and cycads, the angiosperm feeding weevils occupy a larger array of larval niches (Fig. 5), including stem/trunk boring, root feeding, folivory, leaf mining, and seed and fruit feeding, although it is not clear whether these differences reflect greater average disparity (i.e., given the greater numbers of both hosts and weevils). Larval feeding habits clearly are highly conservative; species with similar habits (strobilus feeders, root feeders, leaf miners, aerial leaf eaters, seed feeders) usually appear grouped together or in close proximity (Fig. 5). Some of these feeding habits are apparently irreversible (e.g., feeding on leaves or seeds), whereas stem and trunk boring frequently give rise to use of other tissues.

Larvae in several groups of weevils develop on host tissues that are not living, raising the issue of whether such associations should be expected to evolve as associations with obviously living, and thus defended, plant parts (Anderson, 1995). Our phylogeny estimate (Fig. 5) shows that development in dying tissues characterizes most basal weevils except Nemonychidae (i.e., Anthribidae, Belidae, Attelabidae). This finding suggests that angiosperm colonization by these weevils is coupled with breeding in decaying tissues, whereas consumption of living tissues of angiosperms occurrs remarkably in the brentid–curculionid clade.

CONCLUSIONS

An important aspect of this study is the compilation of a comprehensive body of evidence on weevil relationships by including DNA sequences and morphology of both larva and adult stages for exemplars from every weevil extant family, with multiple representatives from the major subclades. Combination of the molecular data with morphology provides resolution not achieved by separate analyses. The estimate of curculionoid relationships allows a more stable higher classification of the superfamily. Also, the limits of the largest family, Curculionidae, now are defined more clearly and the Curculionidae is shown confidently to be monophyletic. Perhaps because the curculionid clade went through an explosive radiation, further resolution of their phylogenetic relationships requires a much more extensive sampling of characters (more genes and new morphological data) and taxa.

Several aspects of host use are highly conservative, and groups with similar life histories emerged in the combined analysis. Association with conifers is ancestral and likely formed in the Jurassic or earlier, and multiple shifts to angiosperms, coupled with shifts in larval feeding habits, are associated with increases in diversity. The present study shows that niche shifts in host-plant associations and in larval feeding habits accompany diversification of weevils, today numbering

more than 60,000 living weevil species. Certainly, this interpretation also should be the subject of retesting when a more refined phylogenetic picture for the weevils becomes available.

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REFERENCES

ALONSO-ZARAZAGA, M., AND C. H. C. LYAL. 1999. A world catalogue of families and genera of Curculionoidea (Insecta: Coleoptera) (excepting Scolytidae and Platypodidae). Entomopraxis, Barcelona.

ANDERSON, D. M. 1991. Curculionidae (broad sense) (Curculionoidea). Pages 594–612 *in* Immature insects, Volume 2 (F. W. Stehr, ed.). Kendall/Hunt, Dubugue, Iowa.

ANDERSON, R. S. 1995. An evolutionary perspective of diversity in Curculionoidea. Mem. Entomol. Soc. Wash. 14:103–114.

ANDERSON, W. H. 1947. Larvae of some genera of Anthribidae (Coleoptera). Ann. Entomol. Soc. Am. 40:25–35.

ANDERSON, W. H. 1948. Larvae of some genera of Calendrinae (=Rhynchophoridae) and Stromboscerinae (Coleoptera: Curculionidae). Ann. Entomol. Soc. Am. 41:413–437.

ARNOLDI, L. V. 1977. Family Eobelidae L. Arnoldi fam. nov. Tr. Paleontol. Inst. Akad. Nauk SSSR 161:144–176. [In Russian.]

BEAVER, R. A. 1989. Insect–fungus relationships in the bark and ambrosia beetles. Pages 121–143 *in* Insect–fungus interactions (N. Wilding, N. M. Collings, P. M. Hammond, and J. F. Webber, eds.). Academic Press, London.

BROWNE, F. G. 1972. Larvae of the principal world genera of the Platypodinae (Coleoptera: Platypodidae). Trans. R. Entomol. Soc. Lond. 124:167–190.

CALDER, A. A. 1989. The alimentary canal and nervous system of Curculionoidea (Coleoptera): Gross morphology and systematic significance. J. Nat. Hist. 23:1205–1265.

CALDER, A. A. 1990. Gross morphology of the soft parts of the male and female reproductive systems of Curculionoidea (Coleoptera). J. Nat. Hist. 24:453–505.

CROWSON, R. A. 1955. The natural classification of the families of Coleoptera. Lloyd and Co., London. [Reprinted 1967, Classey Ltd., Middlessex.]

DENNO, R. F., M. S. MCCLURE, AND J. R. OTT. 1995. Interespecific interactions in phytophagous insects: Competition reexamined and resurrected. Annu. Rev. Entomol. 40:297–331.

FARRELL, B. D. 1998a. "Inordinate fondness" explained: Why are there so many beetles? Science 281:555–559.

FARRELL, B. D. 1998b. Flowers and insect evolution. Response to B. N. Danforth, J. Ascher, and R. S. Anderson. Science 283:143.

FARRELL, B. D., A. S. SEQUEIRA, B. B. NORMARK, B. H. JORDAL, J. CHUNG, AND B. D. O'MEARA. 2001. Evolution of agriculture in beetles (Curculionidae: Scolytinae and Platypodinae). Evolution 55(10):2011–2027.

FUTUYMA, D. J. 1998. Evolutionary biology, 3rd edition. Sinauer, Sunderland, Massachusetts.

GANDOLFO, M. A., K. C. NIXON, W. L. CREPET, D. W. STEVENSON, AND E. M. FRIIS. 1998. Oldest known fossils of monocotyledons. Nature 394:532–533.

GARDNER, J. C. M. 1932. Immature stages of Indian Coleoptera (11) (Platypodidae). Indian For. Rec. Entomol. 17(3):1–12.

GOLOBOFF, P. 1998. NONA, version 2.0. Published by the author, Tucumán, Argentina. [Available at http://www.cladistics.com.]

GRATSHEV, V. G. 1998. Ulyanidae, an extinct family of weevils (Coleoptera, Curculionoidea). Page 9 *in* Abstracts of the first Paleoentomological Conference. Paleontological Institute of the Russian Academy of Sciences, Moscow.

GRATSHEV, V. G., AND V. V. ZHERIKHIN. 1999. *Gobicar* (Insecta, Coleoptera: Eccoptarthridae), a new Late Jurassic genus of eccoptarthrid weevils from Mongolia. Paleontol. J. 29:112–127.

HAWKINS, B. A. 1994. Pattern and process in host–parasitoid interactions. Cambridge Univ. Press, Cambridge, England.

HOLLOWAY, B. A. 1982. Anthribidae (Insecta: Coleoptera). Fauna of NZ 3:1–264.

HOLMQUIST, R. 1983. Transitions and transversions in evolutionary descent: An approach to undestanding. J. Mol. Evol. 19:134–144.

HOWDEN, A. T. 1987. Notes on the biology of adult and immature Amycterinae (Coleoptera, Curculionidae). Proc. Linn. Soc. N.S.W. 109:91–105.

HOWDEN, A. T. 1995. Structures related to oviposition in Curculionoidea. Mem. Entomol. Soc. Wash. 14:53–100.

HUELSENBECK, J. P. 2001. MrBayes: Bayesian inference of phylogeny. Distributed by the author. Department of Biology, Univ. Rochester, Rochester, New York.

JOLIVET, P., AND T. J. HAWKESWOOD. 1995. Host-plants of Chrysomelidae of the world. Backhuys, Leiden.

KJER, K. M., R. J. BLAHNIK, AND R. W. HOLZENTHAL. 2001. Phylogeny of Trichoptera (caddisflies): Characterization of signal and noise within multiple datasets. Syst. Biol. 50:781–816.

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

KUSCHEL, G. 1971. Entomology of the Aucklands and other islands south of New Zealand: Coleoptera: Curculionidae. Pac. Insects Monogr. 27:225–259.

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

KUSCHEL, G. 1990. Beetles in a suburban environment: A New Zealand case study. Pages 1–118 *in* DSIR plant protection report 3. New Zealand Department of Scientific and Industrial Research, Auckland.

KUSCHEL, G. 1992. Reappraisal of the Baltic amber Curculionoidea described by E. Voss. Mitt. Geol.-Paleontol. Inst. Univ. Hamb. 73:191–215.

KUSCHEL, G. 1994. Nemonychidae of Australia, New Guinea and New Caledonia. Pages 563–637 *in* Zimmerman's Australian weevils, Volume I. CSIRO, Melbourne.

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

KUSCHEL, G., AND B. M. MAY. 1990. Palophaginae, a new subfamily for leaf-beetles, feeding as adult and larva on *Araucaria* pollen in Australia (Coleoptera: Megalopodidae). Invertebr. Taxon. 3:697–719.

KUSCHEL, G., AND B. M. MAY. 1996. Discovery of Palophaginae (Coleoptera: Megalopodidae) on *Araucaria araucana* in Chile and Argentina. NZ Entomol. 19:1–13.

KUSCHEL, G., R. A. B. LESCHEN, AND E. C. ZIMMERMAN. 2000. Platypodidae under scrutiny. Invertebr. Taxon. 14:771–805.

KUSCHEL, G., R. OBERPRIELER, AND J. RAYNER. 1994. Cretaceous weevils from southern Africa, with description of a new genus and species and phylogenetic and zoogeographical comments (Coleoptera: Curculionoidea). Entomol. Scand. 25:137–149.

LANTERI, A. A., A. E. MARVALDI, AND S. SUAREZ. 2002. Gorgojos de la Argentina y sus plantas huéspedes. Tomo I: Apionidae y Curculionidae. Publ. Espec. Soc. Entomol. Argent. 1:1–98.

LUTZONI, F., P. WAGNER, V. REEB, AND S. ZOLLER. 2000. Integrating ambiguously aligned regions of DNA sequences in phylogenetic analyses without violating positional homology. Syst. Biol. 49:628–651.

LYAL, C. H. C. 1995. The ventral structures of the weevil head (Coleoptera: Curculionoidea). Mem. Entomol. Soc. Wash. 14:169–174.

LYAL, C. H. C., AND T. KING. 1996. Elytro-tergal stridulation in weevils (Insecta: Coleoptera: Curculionoidea). J. Nat. Hist. 30:703–773.

MADDISON, W. P., AND D. R. MADDISON. 1992. MacClade: Analysis of phylogeny and character evolution, version 3.0. Sinauer, Sunderland, Massachusetts.

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

MARVALDI, A. E. 1998. Larvae of South American Rhytirrhininae (Coleoptera: Curculionidae). Col. Bull. 52(1):71–89.

MARVALDI, A. E. 2000. Morphologic characters of the immature stages of Ocladius dianthi Marshall (Coleoptera: Curculionidae: Ocladiinae), with phylogenetic implications. Coleopt. Bull. 54:325–331.

MARVALDI, A. E., AND J. J. MORRONE. 2000. Phylogenetic systematics of weevils (Coleoptera: Curculionoidea): A reappraisal based on larval and adult morphology. Insect Syst. Evol. 31:43–58.

MAY, B. M. 1993. Larvae of Curculionoidea (Insecta: Coleoptera): A systematic overview. Fauna NZ 28:1–221.

MAY, B. M. 1994. An introduction to the immature stages of Australian Curculionoidea. Pages 365–755 *in* Zimmerman's Australian weevils, Volume II. CSIRO, Melbourne.

MAYR, E. 1963. Populations, species, and evolution. Harvard Univ. Press, Cambridge, Massachusetts.

MCCLAY, A. S., AND D. M. ANDERSON. 1985. Biology and immature stages of *Thecesternus hirsutus* Pierce (Coleoptera, Curculionidae) in north-eastern Mexico. Proc. Entomol. Soc. Wash. 87:207–215.

MITTER, C., B. D. FARRELL, AND B. WIEGMANN. 1988. The phylogenetic study of adaptive zones: Has phytophagy promoted insect diversification? Am. Nat. 132:107–128.

MORIMOTO, K. 1962a. Comparative morphology, phylogeny and systematics of the superfamily Curculionoidea of Japan I. J. Fac. Agric. Kyushu Univ. 11:331–373.

MORIMOTO, K. 1962b. Key to families, subfamilies, tribes and genera of the superfamily Curculionoidea of Japan excluding Scolytidae, Platypodidae and Cossoninae. J. Fac. Agric. Kyushu Univ. 12:21–66.

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

NIXON, K. C. 1999a. The parsimony ratchet, a new method for rapid parsimony analysis. Cladistics 15:407–414.

NIXON, K. C. 1999b. WINCLADA, (beta) version 0.9. Published by the author, Ithaca, New York. [Available at http://www.cladistics.com]

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

OBERPRIELER, R. 1999. Systematics and evolution of the cycad-associated weevil genus *Apinotropis* Jordan (Coleoptera: Anthribidae). Afr. Entomol. 7:1–33.

OBERPRIELER, R. 2000. The larvae of the weevil tribe Eurhynchini and the phylogeny of the Brentidae (Coleoptera: Curculionoidea). Invertebr. Taxon 14:755–770.

O'BRIEN, C. W., AND G. B. MARSHALL. 1987. Unusual larval "sand tube" construction in the weevil genus *Cleonidius* in west Texas. Southwest. Entomol. 12:357–360.

POSADA, D. 2001. Modeltest, version 3.06. Department of Zoology, Brigham Young Univ., Provo, Utah.

QIU, Y., J. LEE, F. BERNASCONI-QUADRONI, D. E. SOLTIS, P. S. SOLTIS, M. ZANIS, E. A. ZIMMER, Z. CHEN, V. SAVOLAINEN, AND M. W. CHASE. 1999. The earliest angiosperms: Evidence from mitochondrial, plastid and nuclear genomes. Nature 402:404–407.

REID, C. A. M. 1995. A cladistic analysis of subfamilial relationships in the Chrysomelidae sensu lato (Chrysomeloidea). Pages 559–631 *in* Biology, phylogeny and classification of Coleoptera. Papers celebrating the 80th birthday of Roy A. Crowson, Volume. 2 (J. Pakaluk and S. Slipinski, eds.). Muzeum i Instytut Zoologii PAN, Warszawa, Poland.

REID, C. A. M. 2000. Spilopyrinae Chapuis: A new subfamily in the Chrysomelidae and its systematic placement (Coleoptera). Invetebr. Taxon. 14:837–862.

RODRIGUEZ, F. J, J. L. OLIVER, A. MARIN, AND J. R. MEDINA. 1990. The general stochastic model of nucleotide substitution. J. Theor. Biol. 142:485–501.

SANBORNE, M. 1981. Biology of *Ithycerus noveboracensis* (Forster) (Coleoptera) and weevil phylogeny. Evol. Monogr. 4:1–80.

SCOTT, J. K., AND M. J. WAY. 1989. Biology of *Rhytirrhinus inaequalis* (F.), a weevil associated with *Emex australis* Steinh. (Polygonaceae) in southern Africa. J. Entomol. Soc. South Afr. 52:1–9.

SEQUEIRA, A. S., B. B. NORMARK, AND B. D. FARRELL. 2000. Evolutionary assembly of the conifer fauna: Distinguishing ancient from recent associations in bark beetles. Proc. R. Entomol. Soc. Lond. B 267:2359–2366.

STEHR, F. W. (ed.). 1991. Immature insects, Volume 2. Kendall/Hunt, Dubugue Iowa.

SUNNUCKS, P., AND D. F. HALES. 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. 1998. PAUP*, version 4.0b4a. Sinauer, Sunderland, Massachusetts.

THOMPSON, J. D., T. J. GIBSON, F. PLEWNIAK, F. JEANMOUGIN, AND D. G. HIGGINS. 1997. The CLUSTAL X windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. 25:4876–4882.

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.

VALENTINE, B. D. 1960. The genera of the weevil family Anthribidae north of Mexico. Trans. Am. Entomol. Soc. 86:41–85.

WIBMER, G. J., AND C. W. O'BRIEN. 1986. Annotated checklist of the weevils (Curculionidae sensu lato) of South America (Coleoptera: Curculionoidea). Mem. Am. Entomol. Inst. 39:1–563.

WILF, P., C. C. LABANDEIRA, W. J. KRESS, C. L. STAINES, D. M. WINDSOR, A. L. ALLEN, AND K. R. JOHNSON. 2000. Timing the radiations of leaf beetles: Hispines on gingers from Latest Cretaceous to recent. Science 289:291–294.

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

WOOD, S. L. 1993. Revision of the genera of Platypodidae (Coleoptera). Great Basin Nat. 53:259–281.

ZHERIKHIN, V. V. 1993. Cretaceous insects from the Ulya River Basin (West Okhot Region) Tr. Paleontol. Inst. Akad. Nauk SSSR 252:5–60. [In Russian.]

ZHERIKHIN, V. V., AND V. G. GRATSHEV. 1995. A comparative study of the hind wing venation of the superfamily Curculionoidea, with phylogenetic implications Pages 633–777 *in* Biology, phylogeny and classification of Coleoptera. Papers celebrating the 80th birthday of Roy A. Crowson, Volume 2 (J. Pakaluk and S. Slipinski, eds.). Muzeum i Instytut Zoologii PAN, Warszawa, Poland.

ZIMMERMAN, E. C. 1993. Australian weevils, Volume III. Nanophyidae, Rhynchophoridae, Erirhinidae, Curculionidae: Amycterinae, literature consulted. CSIRO, Melbourne.

ZIMMERMAN, E. C. 1994a. Australian weevils, Volume I. Orthoceri: Anthribidae to Attelabidae. CSIRO, Melbourne.

ZIMMERMAN, E. C. 1994b. Australian weevils, Volume II. Brentidae, Eurhynchidae, Apionidae and a chapter on immature stages by Brenda May. CSIRO, Melbourne.

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APPENDIX 1.
MORPHOLOGICAL DATA

Particularly important publications followed for character interpretation, other than Kuschel (1995) for adults and May (1993, 1994) and Marvaldi (1997) for larvae, are given for each character. The data matrix is provided in Table 2.

Larva

Head

1. Head: (0) extrusible; (1) permanently retracted.
2. Epicranium: (0) without posterior extension; (1) with hyaline posterior extension.
3. Frontal lines: (0) complete, extending to mandibles; (1) incomplete, not extending to mandibles.
4. Antennae: (0) 3- or 2-segmented; (1) 1-segmented.
5. Antennal sensorium: (0) conical, longer than wide; (1) cushionlike, wider than long.
6. Antennal sensorium: (0) circular in apical view; (1) elliptical in apical view.
7. Number of stemmata or eye spots on each side of head capsule: (0) 6; (1) 4; (2) 3; (3) $\leq$2.
8. Frons: (0) with $\leq$5 setae; (1) with >5 setae.
9. Frontal seta 2 (*fs*2): (0) present; (1) absent.
10. Sensillum next to dorsoepicranial seta 2: (0) present; (1) absent.
11. Dorsal epicranial seta 3 (*des3*): (0) on epicranium; (1) on frontal line or on frons.
12. Hypopharyngeal bracon: (0) with sclerome; (1) without sclerome. The bracon is absent in Chrysomeloidea.
13. Head: (0) without postoccipital condyles; (1) with postoccipital condyles.
14. Head: (0) lacking postoccipital lamina with apodemes; (1) with such lamina.
15. Frontoclypeal suture: (0) distinct; (1) effaced. Anthribidae = ? (several species = 0, several others = 1) (Anderson, 1947).
16. Pseudoclypeus formed by the frons produced forward: (0) absent; (1) present.
17. Clypeus: (0) subrectangular; (1) reduced to transverse narrow band (Gardner, 1932; Browne, 1972).
18. Labral scleromes: (0) lateral or tormae; (1) submedian or labral rods (Anderson, 1991).
19. Basal stem uniting labral rods: (0) absent; (1) present.
20. Labrum: (0) with 4 pairs of setae; (1) with $\leq$3 pairs of setae. Reduction in the number of labral setae is frequent within Brentidae, but the maximum number of four setae is found in this family and a transitional condition, with the fourth pair of setae vestigial, is presented by some species, e.g., *Lasiorhynchus barbicornis* (A. Marvaldi, pers. obs.) and *Arrenodes minutus* (Sanborne, 1981: fig. 119).
21. Lateral labral seta: (0) well developed; (1) vestigial or absent.
22. Labral sensilla: (0) 2 basal sensilla; (1) single basal sensillum; (2) sensillum absent; (3) 3 sensilla: single basal sensillum and 2 median sensilla; (4) 4 sensilla: 2 paired basal sensilla and 2 median sensilla. (Sanborne, 1981).
23. Setae on epipharynx and/or maxillae: (0) simple; (1) some branched or tufted (Gardner, 1932; Anderson, 1948; Browne, 1972).
24. Mandibles: (0) with mola; (1) lacking mola.

25. Mandibles: (0) lacking diagonal masticatory ridge; (1) with such ridge.
26. Maxillary articulatory lobes: (0) distinct; (1) absent.
27. Maxilla: (0) with lacinial lobe or spine; (1) without lacinial lobe or spine.
28. Ventral malar setae (vms) on maxilla: (0) 5 vms; (1) 4 vms; (2) the 5th vms close to sensillum present but minute, very short (Marvaldi, 1998).
29. Maxillary palp: (0) 3-segmented; (1) 2-segmented.
30. Maxillary palp: (0) with seta on last segment; (1) lacking seta on last segment.

Thorax

31. Thoracic spiracle: (0) on mesothorax or intersegmental; (1) on prothorax.
32. Legs: (0) present and segmented; (1) vestigial with faint segmentation; (2) absent.
33. Legs: (0) with claws; (1) without claws.

Abdomen

34. Segments: (0) with 2 folds; (1) with 3 or 4 folds.
35. Spiracle on abdominal segment VIII: (0) present; (1) absent.
36. Position of spiracle on abdominal segment VIII: (0) lateral, on pleuron; (1) on dorsum.
37. Abdominal pleura: (0) entire; (1) subdivided in ≥ 2 superimposed lobes.

Adult

Head

38. Gular suture: (0) double; (1) simple (Lyal, 1995).
39. Joint of "subgenal suture" elements with gular suture (head, ventral view): (0) not lining an sclerite; (1) lining a distinct subtriangular sclerite (Wood, 1993; Lyal, 1995).
40. Type of antennae: (0) orthocerous or straight; (1) geniculate. In *Antliarhinus zamiae* although the scape is elongate, the antennae are not functionally geniculate (Thompson, 1992:872).
41. Last (7th) funicular segment: (0) free, distinct from club; (1) added to club (Thompson, 1992:876).
42. Antennal club: (0) indistinct; (1) distinct.
43. Antennal club (segments 9–11): (0) first 2 or all 3 segments loosely articulated; (1) all segments tightly articulated or compact.
44. Clypeolabral suture: (0) distinct; (1) indistinct.
45. Mandible insertion: (0) not lodged in distinct socket at base; (1) inserted in distinct socket at base (Wood, 1986).
46. Mandibular pharyngeal process: (0) absent; (1) present, shorter than mandible; (2) present, about as long or longer than mandible (Morimoto, 1962a).
47. Mandibles, setae: (0) plurisetose, setae over large area; (1) plurisetose, setae on lateral groove; (2) paucisetose (1–4 setae).
48. Mandibular mola: (0) present; (1) absent.
49. Deciduous processes on mandibles: (0) absent; (1) present (Thompson, 1992).
50. Mandibular theca (pupal stage): (0) without setae; (1) with 1 or 2 setae.
51. Maxillary galea and lacinia: (0) distinct; (1) indistinct.
52. Maxillary palpi: (0) elongate, projecting anterolaterad; (1) short, not projecting.
53. Number of segments of maxillary palpi: (0) 4; (1) 3 or 2.
54. Prementum in ventral view: (0) visible; (1) not visible, inflexed over postmentum.
55. Labial palpi: (0) near base or middle of prementum; (1) near apex.
56. Labial palpi: (0) free; (1) concealed in pits or grooves (Thompson, 1992:872, 881; Zimmerman, 1994b:220, 229).
57. Number of segments of labial palpi: (0) 3; (1) 2 or 1 (Zimmerman, 1994a). Attelabidae = 0 (the condition is rather indistinct in Attelabinae because the palps are atrophied); Caridae = 0 (except *Caenominurus* = 1) (Kuschel, 1992).

Thorax, elytra, and wings

58. Prothorax: (0) not or only slightly proclinate in lateral view; (1) strongly proclinate, hence head largely concealed in dorsal view (Wood, 1986).
59. Prosternum length: (0) longer than procoxal width; (1) equal or shorter than procoxal width (Kuschel et al., 2000).
60. Notosternal suture: (0) at first transverse, then horizontally cephalad; (1) only transverse, vertically to the notum (Zimmerman, 1994a:6).
61. Sclerolepidia at metepisternal suture: (0) absent; (1) present (Kuschel, 1966:5).
62. Mesepimera: (0) not ascending, not visible from above; (1) ascending between the base of pronotum and elytra (Morimoto, 1962b; Zherikhin and Gratshev, 1995:669).
63. Prosternal channel for reception of rostrum in repose: (0) absent; (1) present, simple; (2) present, with mesosternal receptacle (Thompson, 1992:873, 881; Lyal and King, 1996:739).
64. Strong medial longitudinal ridge on internal surface of metathorax: (0) absent; (1) present (Zherikhin and Gratshev, 1995:647).
65. Elytral punctae: (0) irregularly punctate; (1) aligned to striae.
66. Elytral erect sensory hairs: (0) present; (1) absent.
67. Scutellar striole: (0) present (unless elytra lacking striae); (1) absent on striate elytra (Holloway, 1982).
68. Inferolateral flange of elytron: (0) absent; (1) present.
69. Inferolateral line or carina apicad from flange of elytron: (0) absent; (1) present.
70. Elytral stridulatory file and tergal stridulatory plectrum: (0) absent; (1) present, plectrum as paired tubercules; (2) present, plectrum as a median ridge (Lyal and King, 1996).
71. Radial cross vein (R-m) in hind wings: (0) present; (1) vestigial or absent (several examples figured by Zherikhin and Gratshev, 1995; Zimmerman, 1994a: fig. 340).
72. Number of anal veins in hind wings: (0) 5; (1) 4; (2) ≤ 3.
73. Connection between vein CuA1 and cubitoanal cell(s): (0) present; (1) absent (Reid, 1995, after Kukalová-Peck and Lawrence, 1993; several examples in figures by Zherikhin and Gratshev, 1995).
74. Radial sclerite in hind wing: (0) single; (1) paired, 2 distinct sclerites separated from the stigmal patch; (2) paired, the proximal sclerite very reduced and fused with the stigmal patch (Zherikhin and Gratshev, 1995:768, 771).
75. Point of origin of vein Rr in hind wings: (0) at the radial cell; (1) shifted, placed at anterior portion of

r-m (Zherikhin and Gratshev, 1995: fig. 89, shows condition in Dryophthorinae = 1).

Legs

76. Midcoxal cavity: (0) open laterally to pleurites; (1) closed laterally by meso- and metasternal lobes.
77. Tibial spurs: (0) present; (1) absent or very rudimentary.
78. Grooming area of dense vestiture on protibiae on face opposite to tarsal articulation: (0) absent; (1) present.
79. Socketed spines on protibia: (0) absent; (1) present (Kuschel, 1966; Wood, 1986).
80. Corbel comb or grooming brush on fore tibiae: (0) absent; (1) present, short comb next to tarsal articulation; (2) present, long comb or set of setae on the apical third or more of the tibia (Kuschel, 1966:5; Kuschel et al., 2000).
81. Distal or ascending combs on middle and hind tibiae: (0) present; (1) absent.
82. Apex of tibiae: (0) lacking uncus; (1) with uncus developed in all 3 tibiae; (2) with uncus in fore and middle tibiae, but uncus absent or very rudimentary in 3rd tibia (Thompson, 1992).
83. Tarsite 1: (0) short, about as long as 2 or 3; (1) very elongate, about as long as 2–5 combined (Wood, 1993).
84. Tarsal segment 2: (0) projecting at apical angles; (1) rounded at apical angles.
85. Tarsal groove on dorsal edge of hind tibiae: (0) absent; (1) present.
86. Dorsal and ventral dermal lobes separating tarsal claws: (0) absent; (1) present (Zimmerman, 1993:43).

Abdomen

87. Ventrites: (0) all free, with sutural areas membranous and extendible; (1) last 2 or 3 free, the others fused with sutural areas well pigmented and rigid. In the Anthribidae the Urodontinae have free ventrites, as illustrated by Thompson (1992: fig. 6), but the condition in Anthribinae and Choraginae, although corresponding to state 0 is quite different because the ventrites (except the last one, usually free) are braced or partially fused, with pale nonextendible sutures (Kuschel, 1995:9, 29).
88. Relative position of ventrites 1–3: (0) ventrites 1 and 2 at same level with 3; (1) ventrites 1 and 2 more convex, more protruding than 3 in lateral view ("stepped ventrites" of Oberprieler, 2000).
89. Relative length of ventrites 2 and 3: (0) similar; (1) 3 shorter than 2.
90. Shape of tergites 6 and 7: (0) medially not grooved; (1) medially grooved (one or both) (Valentine, 1960:43).
91. Number of abdominal spiracles: (0) 6 or 7 pairs; (1) 5 pairs.
92. Male pygidium: (0) absent; (1) present (Thompson, 1992:839, 840).
93. Male tergite 8: (0) concealed under tergite 7; (1) exposed beyond tergite 7 (Thompson, 1992:840, 872).
94. Male sternite 8: (0) completely free; (1) fused or articulated to sternite 9 on each side beyond arms.
95. Plate of male sternite 8: (0) undivided; (1) divided to form paired hemisternites (Thompson, 1992).
96. Male tergite 9: (0) completely sclerotized; (1) only laterally sclerotized to completely membranous; (2) desclerotized to a narrow band over sternite 9. Anthribidae = 1 according to Kuschel's (1995) data matrix, but tergite 9 is absent in males of Anthribidae of the Australian Region (Kuschel, 1994:568), and the male genitalia of anthribids of other regions remain almost unknown (Zimmerman, 1994a:40).
97. Bladal part of male sternite 9: (0) membranous; (1) sclerotized.
98. Male genitalia: manubrium (apodeme of tegmen): (0) larger than spiculum gastrale (apodeme of sternite 9); (1) smaller than spiculum gastrale (Thompson, 1992; Zimmerman, 1994a:3).
99. Aedeagal dorsal plate or tectum: (0) similar in size to aedeagal pedon; (1) present but less developed than pedon; (2) absent (dorsal part of the aedeagus entirely membranous and sometimes enfolded by ventral part) (Morimoto, 1962a; Kuschel, 1971; Thompson, 1992; Zimmerman, 1993, 1994a, 1994b). When the tectum is present (states 0, 1) the male genitalia is referred to as being of the orthocerous type; when the tectum is absent (state 2), it is of the gonatocerous type.
100. Tegminal ring, lateroventrally: (0) slender; (1) strong (Kuschel et al., 2000:774).
101. Tegminal dorsal plate (=cap piece or parameral sector of tegmen): (0) large, not bilobed, triangular, or trapezoidal, anterior margin setose; (1) large, bilobed, often articulated with basal piece, apical part hirsute or setose; (2) vestigial, reduced to a pair of delicate asetose lobes, or absent; (3) absent but replaced by the membranes of segment 9 (Morimoto, 1962a:360, 361; Thompson, 1992).
102. Insertion and relative position of aedeagal apodeme in lateral view: (0) dorsal, on line with axis of aedeagal body; (1) lateral or ventral, deflexed from axis of aedeagal body.
103. Aedeagal apodemes: (0) present; (1) vestigial ("median struts" of Morimoto, 1962a:357–359).
104. Apodemal bridge of aedeagus: (0) present; (1) absent (Morimoto, 1962a; Zimmerman, 1993).

Female abdomen

105. Spiculum ventrale or apodeme of female sternite 8: (0) present; (1) vestigial or absent (Thompson, 1992:842).
106. Female tergite 9: (0) sclerotized, at least at margins; (1) completely membranous (Kuschel, 1994:567, 568; Howden, 1995:57, 60, 61, 95).
107. Proximal hemisternites of ovipositor: (0) separated from distal hemisternites; (1) fused to distal hemisternites as 1 body (several examples in Howden, 1995).
108. Spermatheca: (0) falciform, well pigmented; (1) not falciform, very reduced to absent (Calder, 1990).
109. Spermathecal duct and gland: (0) on common atrium outside spermathecal body; (1) contiguous or subcontiguous on spermathecal body; (2) well apart on spermathecal body (Calder, 1990).
110. Number of ovarioles per ovary: (0) $\geq$4; (1) 2 (Calder, 1990).

Alimentary canal

111. Proventricular blades: (0) not developed; (1) well developed (Calder, 1989).
112. Proventricular grinding plates: (0) absent; (1) present as transverse rows of denticles;

(2) present as peglike denticles (Calder, 1989; Kuschel et al., 2000).

113. Number of Malpighian tubules: (0) 6; (1) 4 (examples of adults in Calder, 1989; see also May, 1993, 1994, for larvae).

Nervous system

114. Meso- and metathoracic ganglia of nervous cord: (0) separate; (1) fused (Calder 1989).
115. Last abdominal ganglia of nervous cord: (0) VII–VIII or VI–VIII fused; (1) V–VIII fused (Calder, 1989; Reid, 1995).

Note.—For the following species sequenced (see Table 1), the larval characters were scored as they are present in other related species (in parentheses): *Rhopalotria* sp. (*R. mollis*); *Attelabus analis* (*A. nitens*); *Oxycraspedus cornutus* (known larvae in Oxycoryninae, after May, 1993, 1994); *Caenominurus topali* (*Car condensatus*); *Ocladius obliquesetosus* (*O. dianthi*); *Lissorhoptrus longipennis* (*L. oryzophilus*); *Cylydrorhinus* sp. (*C. farinosus*); *Leptopius* sp. (*L. colossus*); *Talaurinus subvittatus* (known larvae in Amycterinae, after May, 1994); *Epistrophus* sp. (*E. cristulatus*); *Stenancylus* sp. (*Rhyncolus* sp.); *Myrmex floridanus* (*M. laevicollis*, *Micromyrmex asclepia*).

APPENDIX 2.

Morphological apomorphies for the combined cladogram in Figure 5, with character numbers as given in Appendix 1, using a delayed optimization option.

Node/Taxon	Apomorphies	Node/Taxon	Apomorphies
201 (=Curculionoidea)	33:1, 42:1, 45:1, 96:1, 115:1	187	93:1
107 (=Nemonychidae + Anthribidae)	4:1, 27:0, 46:1, 73:1	168	11:0
		150	36:0, 82:0
105 (=Nemonychidae)	7:1, 16:1, 25:1, 47:1, 93:1, 95:1, 96:2	140	13:0, 61:1, 81:1
		138	70:1, 112:1
Doydirhynchus	72:1	122	80:2, 82:1
104	32:1, 65:1	*Cossonus*	93:0
106 (=Anthribidae)	7:2, 8:1, 60:1, 68:1, 72:1, 76:1, 77:1, 90:1, 92:1, 114:1	137	39:1, 45:0, 46:1, 47:1, 58:1, 59:1, 79:1
		Hylurgonotus	70:0
200	12:1, 15:0, 24:1, 26:1, 32:2, 44:1, 46:2, 47:2, 48:1, 52:1, 109:1	128	93:0
		Ips	95:0
		Trypodendron	93:0
109 (=Belidae)	1:1, 78:1, 108:1	134	61:0, 79:0
108	4:1, 7:3, 72:2	*Scolytus*	82:1
Rhopalotria	29:1	133	17:1, 37:1, 58:0, 70:2, 83:1, 95:0, 103:1, 112:0
Rhinotia	42:0, 93:1		
199	18:1, 38:1, 55:1, 68:1, 106:1, 107:1, 114:1	131	14:1, 87:0
		Platypus	23:1, 93:0
112 (=Attelabidae)	2:1, 66:1, 72:1, 87:1, 92:1, 94:1, 95:1, 105:1	*Notoplatypus*	51:0
		Chaetastus	23:1, 51:0
110	45:0, 77:1	139	79:1, 80:2
Attelabus	29:1	146	9:1
198	4:1, 29:1, 51:1, 53:1, 65:1, 67:1, 69:1, 72:2, 76:1, 80:1, 99:1, 101:1	*Gymnetron*	34:0
		145	62:1, 63:1, 64:1
Caenominurus	3:1, 7:1, 32:0, 33:0, 57:1, 91:1	148	82:2
197 (=Brentidae + Curculionidae)	7:3, 22:1, 30:1, 66:1, 71:1, 87:1, 89:1, 110:1	*Anthonomus*	9:1
		Lixus	82:1
116 (=Brentidae)	57:1, 88:1, 93:1, 113:1	152	70:1
113	9:1, 20:1, 22:2, 35:1, 77:1	*Pseudomopsis*	19:1, 36:0, 63:2
Apion	56:1	*Curculio*	9:1, 82:2, 95:0
115	32:0	154	30:0, 62:1, 63:1, 64:1, 81:1
Aporhina	7:2, 34:1, 56:1	167	70:1
Cylas	34:1	158	36:0, 62:1, 64:1
Ithycerus	3:1, 7:2, 43:1, 50:1, 57:0, 84:1, 88:0, 92:1	*Ampeloglypter*	70:0
		Conotrachelus	63:1
196 (=Curculionidae)	3:1, 10:1, 11:1, 31:1, 34:1, 40:1, 43:1, 77:1, 84:1, 109:2	161	9:1, 40:0, 70:0
		Tachygonus	63:1
Ocladius	47:0, 63:1, 93:1	*Perelleschus*	82:0
195	13:1, 20:1, 22:3, 36:1, 74:2, 75:1, 85:1, 105:1, 111:1	*Tranes*	70:0
		Rhyephenes	19:1, 36:0, 63:2
191	74:1, 82:1	186	82:0, 97:1
121	11:0, 14:1, 23:1, 37:1, 41:1, 54:1, 81:1, 86:1, 92:1, 100:1, 101:3	181	36:0
		179	5:1, 6:1, 28:1, 47:0, 49:1, 50:1
120	95:1	*Sitona*	50:0
119	112:2	185	21:1
Sitophilus	11:1, 23:0	*Listroderes*	28:2, 70:1
Sphenophorus	22:4	189	63:1
Rhynchophorus	22:4	*Lissorhoptrus*	41:1
190	95:1, 98:1, 99:2, 101:2, 102:1, 104:1	*Tanysphyrus*	101:2