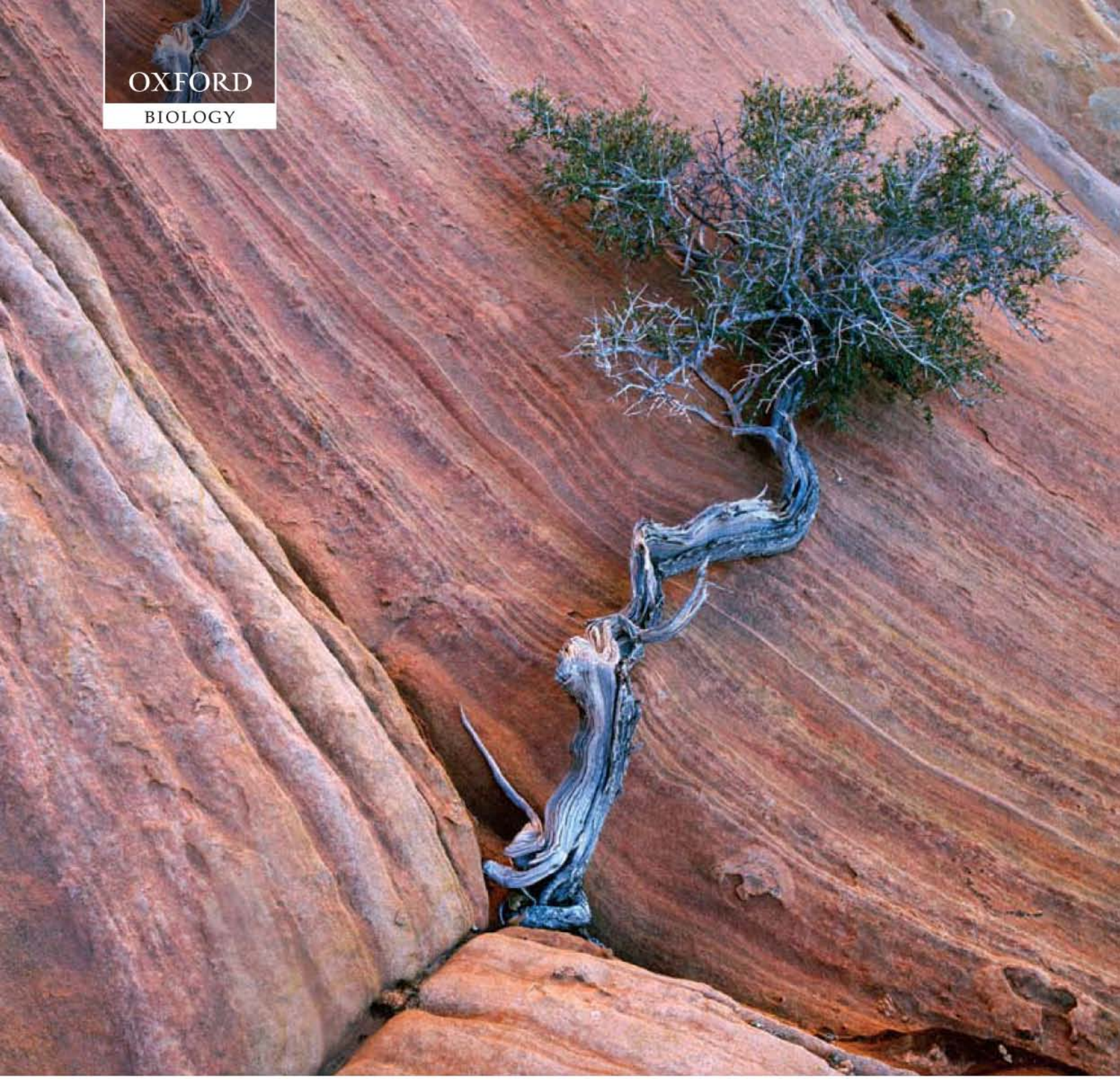

OXFORD
BIOLOGY

the TIMETREE *of* LIFE

edited by **S. BLAIR HEDGES** *and* **SUDHIR KUMAR**

foreword by James D. Watson

Beetles (Coleoptera)

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Abstract

Beetles are placed in the insect Order Coleoptera (~350,000 described species). Recent molecular phylogenetic studies define two major groups: (i) the Suborders Myxophaga and Archostemata, and (ii) the Suborders Adephaga and Polyphaga. The time of divergence of these groups has been estimated with molecular clocks as ~285–266 million years ago (Ma), with the Adephaga–Polyphaga split at ~277–266 Ma. A majority of the more than 160 beetle families are estimated to have originated in the Jurassic (200–146 Ma). However timing and patterns of beetle diversification, including the role of flowering plants in beetle diversification, remain under investigation.

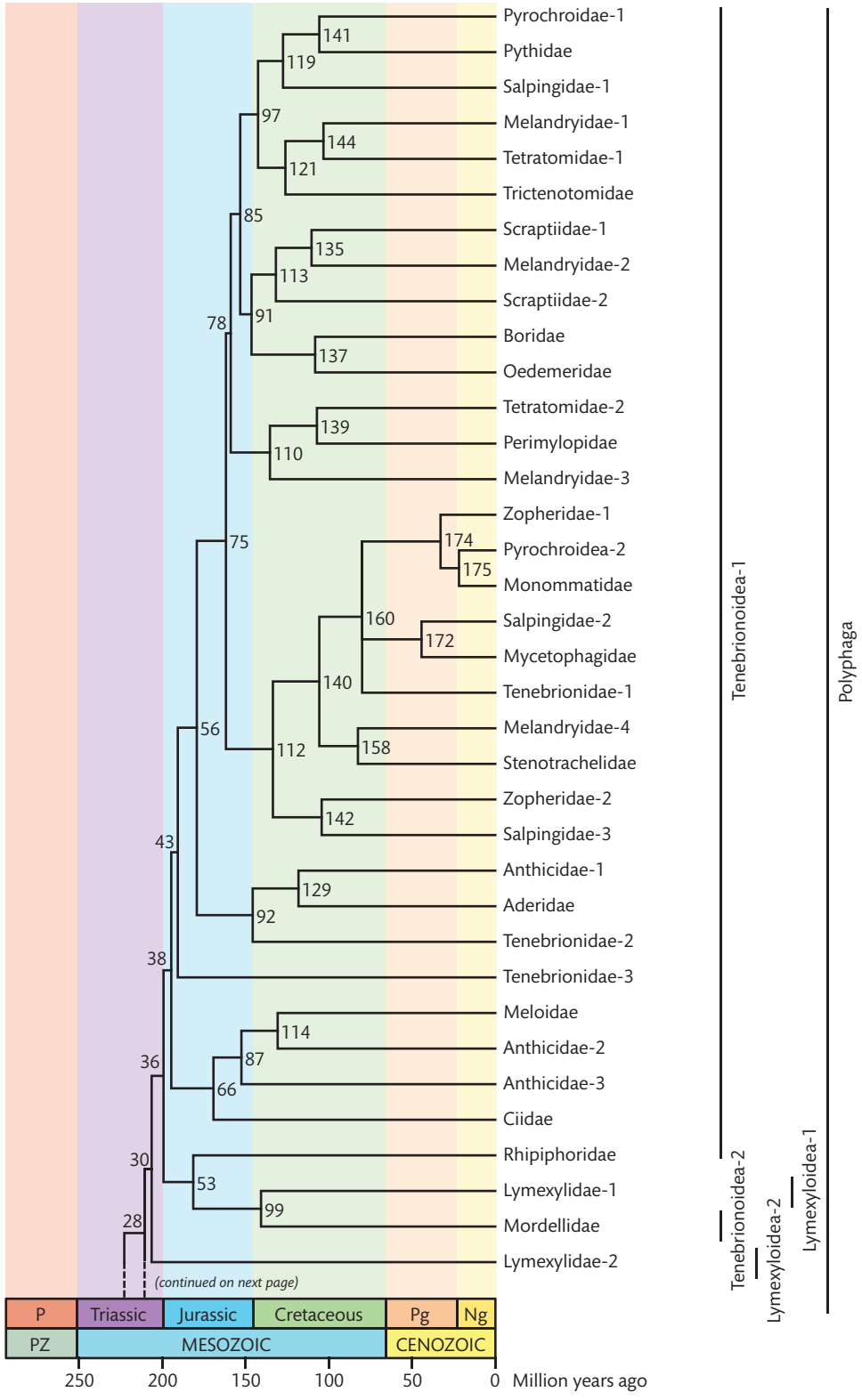
Beetles (Order Coleoptera) comprise the largest order of animals. With an impact magnified by their ubiquity, the ecological importance of beetles lies chiefly in the numerous roles they play with plants and fungi, by facilitating reproduction and decomposition, and by directly consuming plant and fungal tissues—and other consumers. Indeed, beetles are part of the food web in nearly every non-marine habitat. Approximately 350,000 living species have been named. Some estimates of the total number of living beetle species are in the millions. Adult beetles can be distinguished from other insects by a suite of features that suit them to a cryptic lifestyle, perhaps most notably, forewings hardened to form elytra. Elytra protect the hindwings and body from mechanical damage, predators, parasites, pathogens, excessive water loss, and other factors thought to constrain habitat use and longevity in other insects (Fig. 1; ref. *1*).

Present-day workers recognize four suborders of living beetles: Adephaga (~35,000 species; ground beetles, tiger beetles, whirligigs, wrinkled bark beetles, and others), Archostemata (~35 species; reticulated beetles, telephone-pole beetles, and others), Myxophaga (~65 species; minute bog beetles, skiff beetles, and others), and Polyphaga (~315,000 species; checkered beetles, click beetles, fireflies, ladybird beetles, leaf beetles, longhorn beetles, metallic wood-boring beetles, rove beetles, scarabs, soldier beetles, weevils, and others) (*2, 3*). The most recent higher-level classification for living beetles recognizes 16 superfamilies and 168 families (*4, 5*).

Members of the Suborder Adephaga are largely predators, Archostemata feed on decaying wood (larvae) and pollen (adults), and Myxophaga are aquatic or semi-aquatic and feed on green and/or blue-green algae (*6*). Polyphaga exhibit a diversity of habits, but most species feed on plants or dead and decaying plant parts (*1–3*). The earliest known fossil Archostemata are from the late Permian (*7*), and the earliest unequivocal fossil Adephaga and Polyphaga are from the early Triassic (*1*). Myxophaga are not known from the fossil record, but extinct possible relatives are known from the Permian (e.g., *8, 9*).

Fig. 1 Representative families from the four beetle suborders, clockwise from top left: Micromalthidae (Suborder Archostemata; Photo credit: A. Wild), Cicindelidae (Suborder Adephaga; credit: P. Naskrecki); Hydroscaphidae (Suborder Myxophaga; credit: D. Maddison), and Chrysomelidae (Suborder Polyphaga; credit: P. Naskrecki).

D. D. McKenna and B. D. Farrell. Beetles (Coleoptera). Pp. 278–289 in *The Timetree of Life*, S. B. Hedges and S. Kumar, Eds. (Oxford University Press, 2009).



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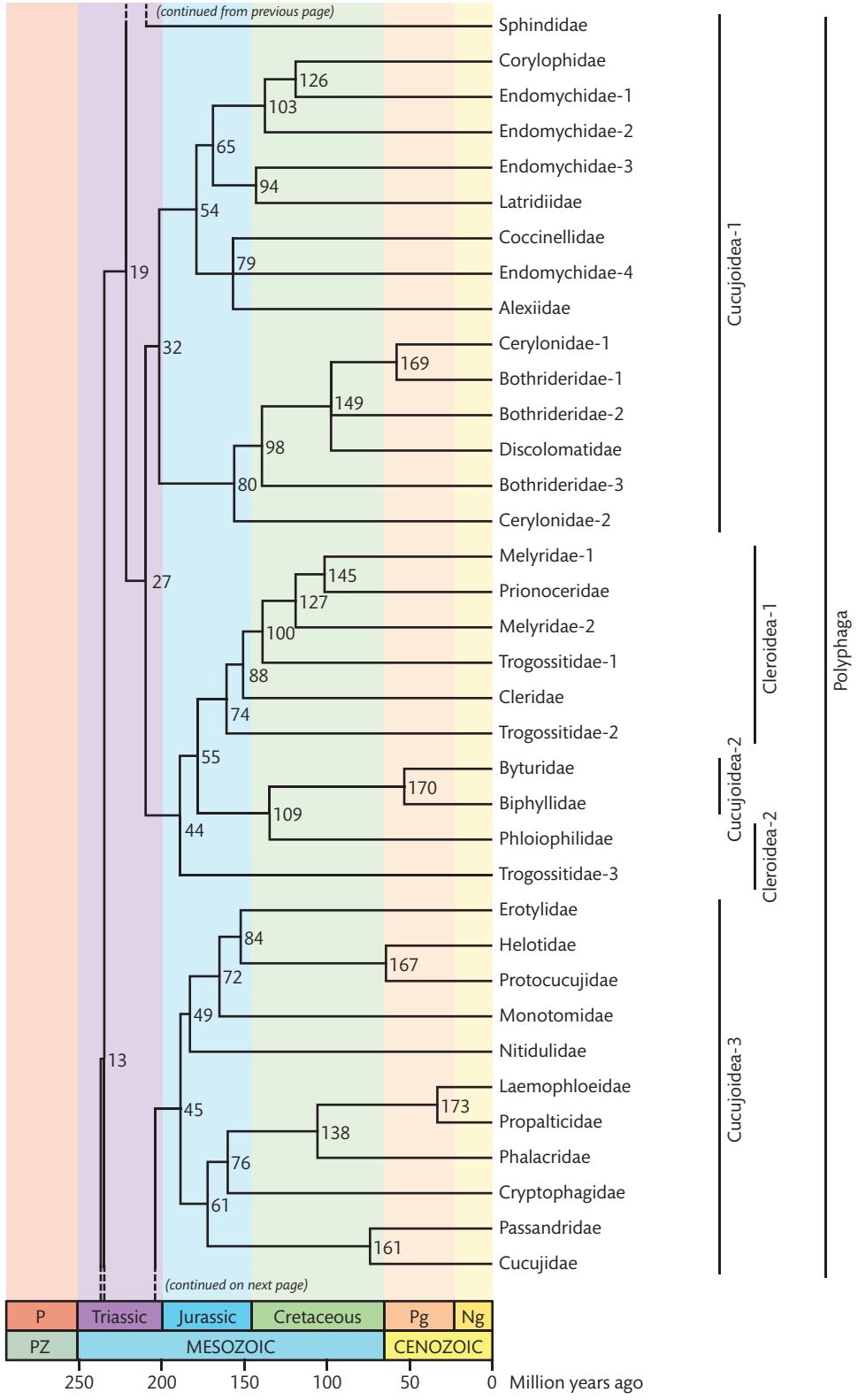

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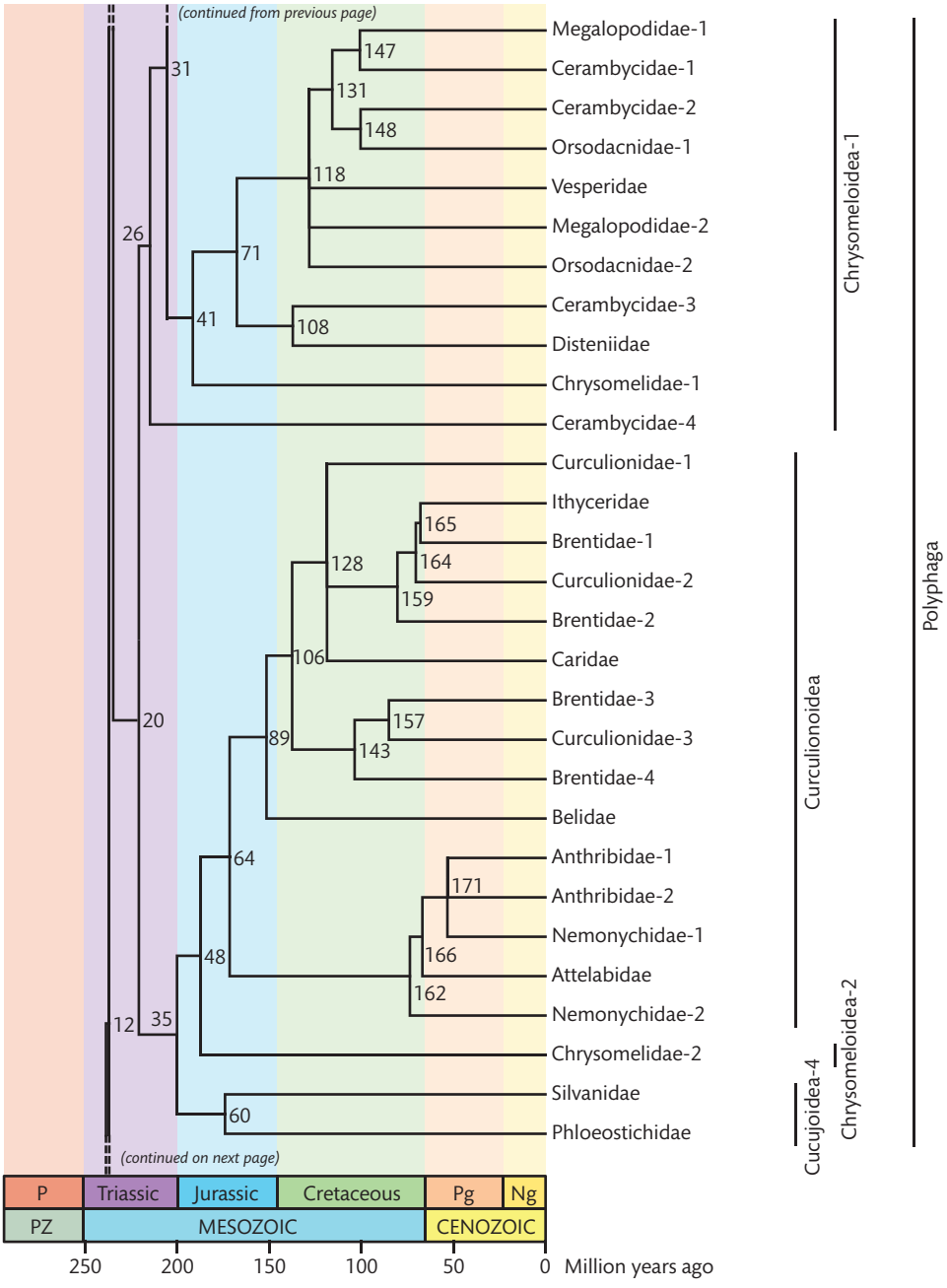

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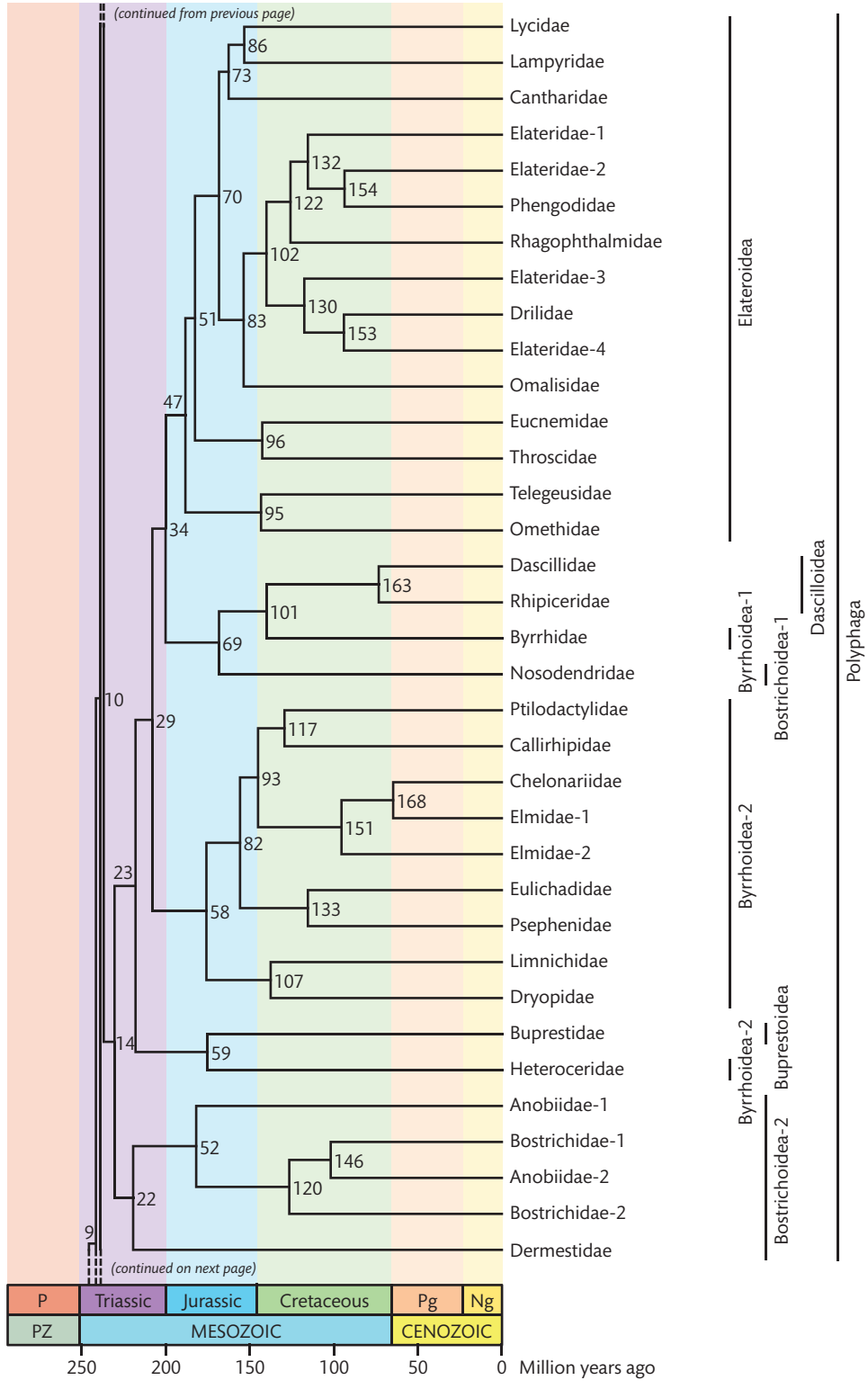
Adephaga are distinctive in having abdominal sternite II divided by the metacoxae. Distinctive features of Archostemata include fusion of the labrum to the head capsule, fine scales on the elytra (absent in *Crowsoniella*, *Micromalthus*, and *Sikhotealinia*), and rolling of the tips of the wings (not folding) when tucking under the elytra (a similar mechanism is found in Myxophaga). Myxophaga are highly specialized for aquatic life. Distinctive features include respiration via a plastron (adults), and tracheal gills (larvae). Pupation occurs inside the last larval exuvium in flowing water. Polyphaga are distinctive in having presternal cervical sclerites, and the propleura internalized (*5, 10*).

While relationships among the four beetle suborders have long been controversial (*8, 11, 12*), an arrangement in which the Archostemata diverge earliest, Myxophaga



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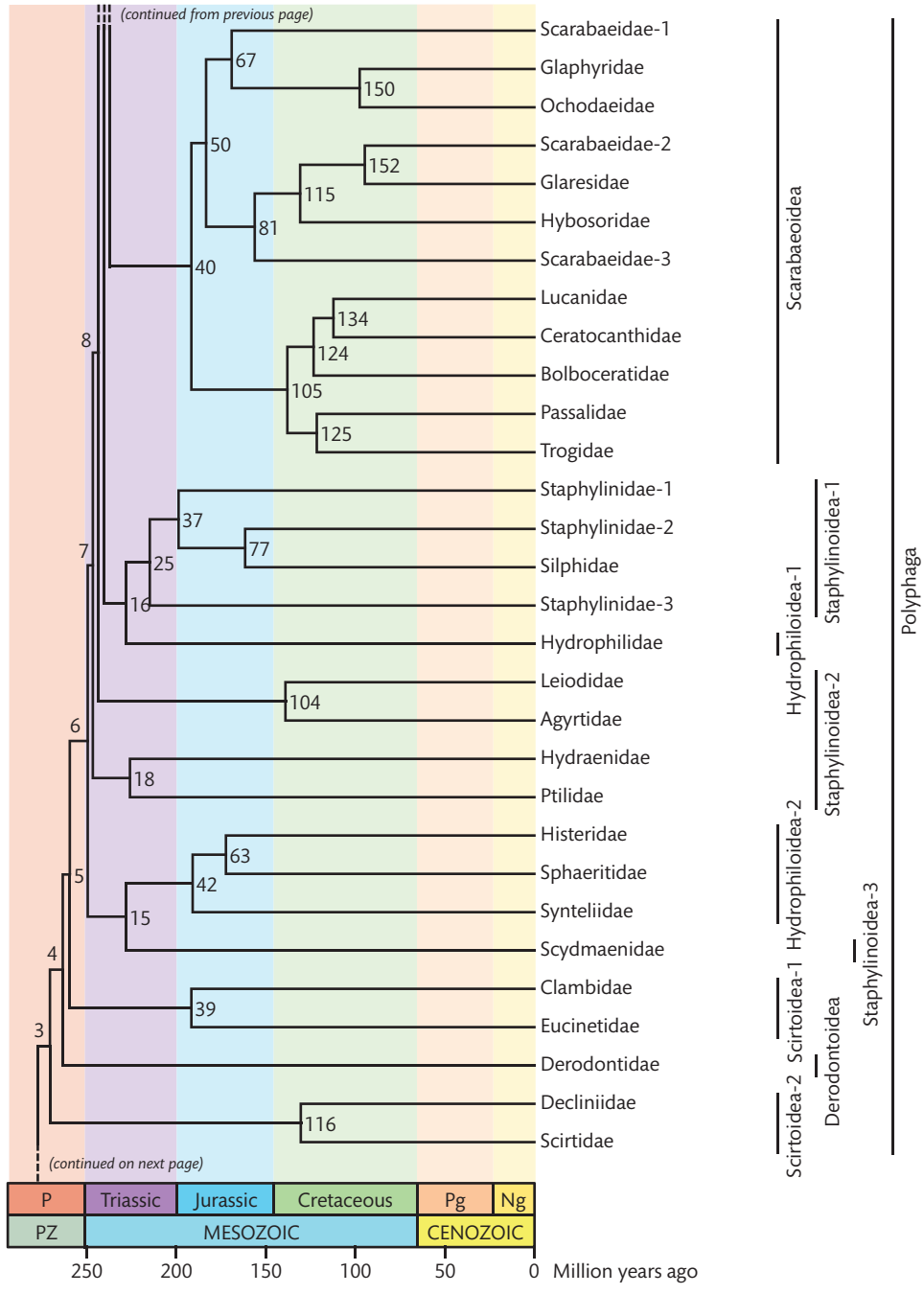

Fig. 2 *Continues*

and Polyphaga are united as closest relatives, and Adephaga is the closest relative of the group comprising Myxophaga and Polyphaga, has emerged as the prevailing hypothesis based on morphology (*13, 14*). Characters supporting this hypothesis include reduced segmentation in the legs of larval Myxophaga and Polyphaga, and rearrangements of the thorax and associated musculature in Adephaga, Myxophaga, and Polyphaga (*10*).

Until recently, phylogenetic analyses of molecular data were largely focused on subgroups of the Order Coleoptera, for example, individual superfamilies or families. A few such studies, all employing DNA

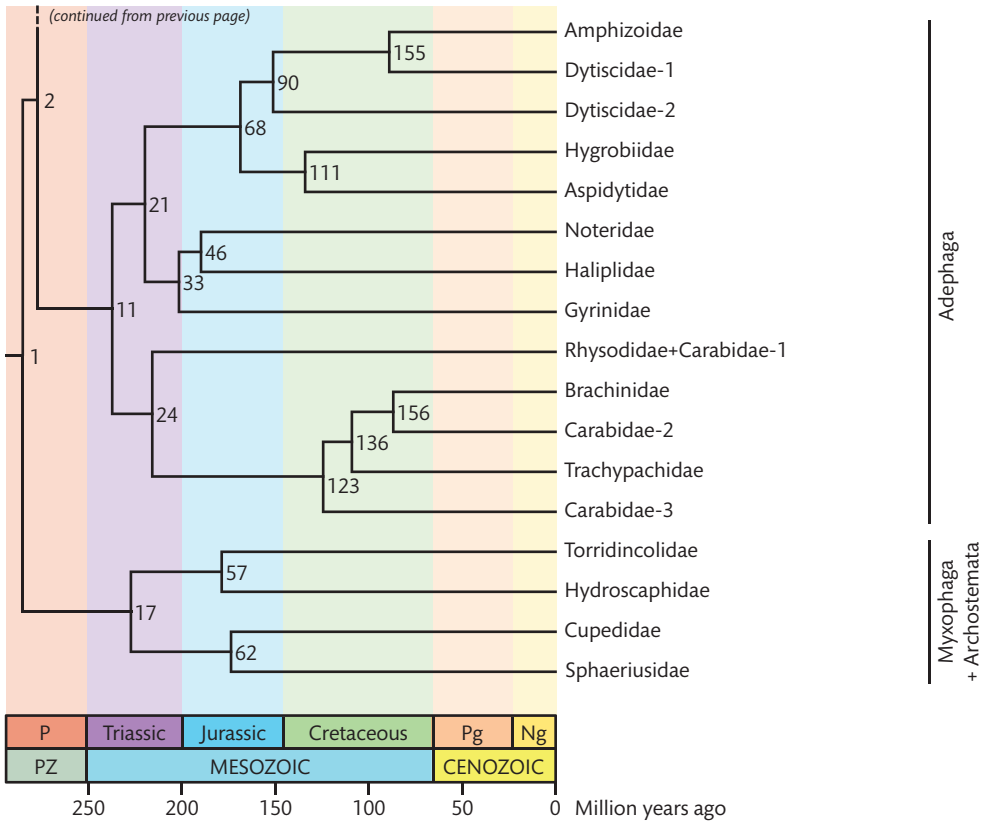

Fig. 2 A timetree of beetles (Coleoptera). Divergence times are shown in Table 1. *Abbreviations*: Ng (Neogene), P (Permian), Pg (Paleogene), PZ (Paleozoic), P (Permian). Codes for paraphyletic and/or polyphyletic families (*sensu 4*, *5*) are as follows: Anobiidae-1 (Anobiinae; Dorcatominae; Gibbiinae; Mesocoelopodinae; Ptilininae; Ptininae; Xyletininae), Anobiidae-2 (Dryophilinae), Anthicidae-1 (Ischaliinae), Anthicidae-2 (Anthicinae), Anthicidae-3 (Eurygeniinae), Anthribidae-1 (Anthribinae; Choraginae), Anthribidae-2 (Urodontinae), Bostrichidae-1 (Bostrichinae; Dinoderinae), Bostrichidae-2 (Lyctinae), Bothrideridae-1 (Teredinae), Bothrideridae-2 (Anommatinae), Bothrideridae-3 (Xylariophilinae), Brentidae-1 (Nanophyinae), Brentidae-2 (Apioninae), Brentidae-3 (Cycladinae), Brentidae-4 (Brentinae), Carabidae-1 (Cicindelinae; Gehringiinae; Harpalinae; Loricerinae; Migadopinae; Omophroninae; Paussinae; Scaritinae; Trechinae), Carabidae-2 (Siagoninae), Carabidae-3 (Elaphrinae), Cerambycidae-1 (Parandrinae), Cerambycidae-2 (Spondylidinae), Cerambycidae-3 (Lepturinae; Necydalinae), Cerambycidae-4 (Cerambycinae; Lamiinae; Prioninae), Cerylonidae-1 (Euxestinae), Cerylonidae-2 (Ceryloninae), Chrysomelidae-1 (Bruchinae; Chrysomelinae; Criocerinae; Cryptocephalinae; Donaciinae; Eumolpinae; Galerucinae; Lamprosmatinae; Sagrinae), Chrysomelidae-2 (Cassidinae; Hispinae), Curculionidae-1 (Curculioninae; Cossoninae; Dryophthorinae; Platypodinae), Curculionidae-2 (Brachycerinae), Curculionidae-3 (Scolytinae), Dytiscidae-1 (Hydroporinae), Dytiscidae-2 (Agabinae, Colymbetinae Copelatinae; Coptotominae; Lancetinae) (Dytiscinae; Laccophilinae), Elateridae-1 (Cebrioninae; Elaterinae; Thylacosterninae), Elateridae-2 (Cardiophorinae), Elateridae-3 (Dendrometrinae; Denticollinae), Elateridae-4 (Agrypninae), Elmidae-1 (Elminae), Elmidae-2 (Larainae), Endomychidae-1 (Holoparamecinae), Endomychidae-2 (Leiestinae), Endomychidae-3 (Endomychinae; Lycoperdininae), Endomychidae-4 (Anamorphinae), Lymexlidae-1 (Hylecoetinae; Melittommatinae), Lymexlidae-2 (Lymexylinae), Megalopodidae-1 (Zeugophorinae), Megalopodidae-2 (Palophaginae), Melandryidae-1 (Melandryinae; Hypulinae), Melandryidae-2 (Osphyinae), Melandryidae-3 (Hallomeninae), Melandryidae-4 (Eustrophinae), Melyridae-1 (Dasytinae; Malachiinae; Melyrinae), Melyridae-2 (Rhadalinae), Nemonychidae-1 (Rhinorhynchinae), Nemonychidae-2 (Doydirhynchinae), Orsodacnidae-1 (Orsodacninae), Orsodacnidae-2 (Aulacoscelidinae), Pyrochroidae-1 (Pedilinae; Pyrochroinae), Pyrochroidae-2 (Agnathinae), Salpingidae-1 (Salpinginae), Salpingidae-2 (Prostominiinae; Trogocryptinae), Salpingidae-3 (Othniinae), Scarabaeidae-1 (Cetoniinae; Dynastinae; Melolonthinae; Orphninae; Rutelinae), Scarabaeidae-2 (Scarabaeinae), Scarabaeidae-3 (Aphodiinae), Scraptiidae-1 (Anaspidinae), Scraptiidae-2 (Scraptiinae), Staphylinidae-1 (Euaesthetinae; Osoriinae; Oxyporinae; Oxytelinae; Paederinae; Phloeocharinae; Piestinae; Pseudopsinae; Staphylininae; Steninae), Staphylinidae-2 (Tachyporinae), Staphylinidae-3 (Aleocharinae; Glypholomatinae; Habrocerinae; Megalopsidiinae; Micropeplinae; Omalinae; Proteininae; Scaphidiinae), Tenebrionidae-1 (Pimeliinae), Tenebrionidae-2 (Lagriinae), Tenebrionidae-3 (Alleculinae; Coelometopinae; Diaperinae; Phrenapatinae; Tenebrioninae), Tetratomidae-1 (Penthinae), Tetratomidae-2 (Tetratominae), Trogossitidae-1 (Peltinae), Trogossitidae-2 (Lophocaterinae), Trogossitidae-3 (Trogossitinae), Zopheridae-1 (Zopherinae; Usechinae), Zopheridae-2 (Colydiinae).

Table 1. Divergence times (Ma) and their confidence/credibility intervals (CI) among Coleoptera.

Timetree			Timetree			Timetree		Timetree		Timetree	
Node	Ref. (*18*)		Node	Ref. (*18*)		Node	Ref. (*18*)	Node	Ref. (*18*)	Node	Ref. (*18*)
(Fig. 2)	Time	CI	(Fig. 2)	Time	CI	(Fig. 2)	Time	(Fig. 2)	Time	(Fig. 2)	Time
1	285.0	–	36	199.2	–	71	167.6	106	137.5	141	105.7
2	277.0	–	37	198.7	–	72	166.4	107	137.5	142	104.3
3	270.5	273–268	38	194.6	–	73	162.5	108	137.1	143	103.6
4	263.4	–	39	191.5	–	74	162.0	109	136.1	144	103.1
5	259.4	–	40	191.4	–	75	161.7	110	135.5	145	102.8
6	249.4	–	41	191.3	–	76	161.4	111	134.1	146	101.6
7	246.3	–	42	190.8	200–181	77	161.3	112	133.4	147	100.8
8	243.3	–	43	190.7	–	78	159.1	113	131.8	148	100.3
9	240.0	–	44	190.4	–	79	158.2	114	130.7	149	98.8
10	238.4	–	45	190.2	–	80	157.5	115	130.7	150	97.7
11	237.2	240–234	46	189.5	–	81	156.1	116	130.5	151	95.5
12	236.7	–	47	188.1	210–166	82	155.7	117	129.4	152	94.8
13	236.2	244–228	48	187.6	–	83	153.6	118	128.4	153	94.0
14	230.2	–	49	184.2	–	84	153.5	119	127.6	154	93.7
15	228.0	–	50	183.3	–	85	153.4	120	126.5	155	88.8
16	227.8	–	51	182.4	–	86	153.4	121	126.1	156	86.9
17	227.0	229–225	52	181.7	–	87	152.6	122	125.7	157	85.1
18	225.7	–	53	181.5	–	88	152.0	123	124.3	158	82.4
19	222.8	–	54	180.3	–	89	151.5	124	123.0	159	80.3
20	220.9	–	55	179.7	–	90	150.9	125	121.4	160	80.1
21	219.8	224–216	56	179.3	–	91	146.4	126	120.5	161	75.3
22	219.4	–	57	178.4	–	92	145.8	127	120.4	162	73.5
23	217.7	–	58	175.9	–	93	145.0	128	118.7	163	73.1
24	215.7	–	59	174.9	–	94	144.3	129	118.3	164	70.4
25	214.8	–	60	173.9	–	95	143.3	130	117.4	165	67.9
26	214.6	–	61	173.7	–	96	142.5	131	115.8	166	66.7
27	211.0	–	62	173.5	–	97	142.5	132	115.5	167	65.8
28	210.7	–	63	172.1	–	98	140.8	133	115.3	168	64.8
29	208.0	–	64	171.5	199–144	99	140.5	134	112.2	169	59.3
30	206.5	–	65	170.3	–	100	140.5	135	110.5	170	54.5
31	205.4	–	66	169.4	–	101	140.1	136	109.1	171	53.4
32	202.9	214–191	67	169.0	–	102	139.9	137	108.2	172	44.3
33	201.4	–	68	168.5	–	103	139.1	138	107.3	173	34.5
34	200.1	–	69	168.3	–	104	138.9	139	107.0	174	32.9
35	200.0	–	70	168.3	–	105	137.9	140	105.9	175	21.8

Notes: Divergence time estimates are from an analysis of *18S rRNA*, mitochondrial *16S rRNA*, and *cytochrome oxidase subunit I* (*18*). Divergence times with 95% confidence intervals are from the original timetree and those without confidence intervals were estimated from a "recreated" tree provided by Hunt *et al.* (*18*). Note that the original timetree published in Hunt *et al.* (*18*) was not available.

sequences from the small subunit ribosomal RNA (*18S rRNA*) (*14–18*), sampled broadly enough to determine at least a subset of subordinal relationships. These studies, which varied considerably in density of taxon sampling, all found Adephaga and Polyphaga to be closest relatives, and Archostemata alone or in combination with Myxophaga as the closest relative of all other beetles. A notable exception is a study by Hughes *et al.* (*19*), who used expressed sequence tags to generate phylogenetic markers (66 genes; matrix 28.6% complete) for 14 beetles, including representatives of all four suborders. When the resulting trees were rooted with Archostemata, the remaining suborders were resolved in the arrangement (Adephaga, (Myxophaga, Polyphaga)). This arrangement agrees with the prevailing hypothesis based on morphology; however, their supertree analyses favored an arrangement that placed Myxophaga within Polyphaga.

In late 2007, Hunt *et al.* (*18*) published the most extensively taxon-sampled molecular phylogeny for Coleoptera to date, and the first molecular timetree covering the entire order. Their study was based on nearly complete 18S rRNA sequences representing all suborders, series, superfamilies, and >80% of families, with DNA sequences from mitochondrial *16S rRNA* and cytochrome oxidase subunit I for nearly half of the taxa. They analyzed a 340-taxon subset of their 1880-taxon data set using Bayesian inference, fixed the age of the ingroup at 285 Ma in an "all compatible" version of the resulting consensus tree, and applied seven fossil age constraints to calibrate and date internal nodes using penalized likelihood. Higher-level molecular timetrees (molecular trees calibrated with fossils) previously had been available only for the beetle superfamilies Chrysomeloidea (*20, 21*; long-horn beetles, leaf beetles, and allies) and Curculionoidea (*20*) (Table 1).

Hunt *et al.* (*18*) recovered Adephaga and Polyphaga as closest relatives, themselves closest to Myxophaga plus Archostemata. This arrangement is consistent with most other molecular phylogenetic studies. They reported average ages and 95% confidence intervals (CIs) for 13 selected clades in their published timetree [Adephaga (237.2 ± 2.63 Ma), Bostrichiformia (219.4 ± 11.19 Ma)], the cerylonid series of families in Cucujoidea (flat bark beetles, flower beetles, ladybird beetles; 202.9 ± 11.44 Ma), Cucujiformia (236.2 ± 7.47 Ma), Curculionoidea; weevils (171.5 ± 27.06 Ma), Elateriformia (217 ± 10.92 Ma), Elateroidea; click beetles and allies (188.1 ± 22.23 Ma), Histeroidea; clown beetles (190.8 ± 9.42 Ma), Hydradephaga; diving beetles and whirligigs (219.8 ± 3.89 Ma), Hydrophiloidea (175.4 ± 23.36 Ma), Myxophaga + Archostemata (227.0 ± 1.68 Ma), Nitidulidae (sap-feeding beetles; 129.7 ± 12.34 Ma), and Polyphaga (270.5 ± 2.26 Ma)]. Because only these 13 age estimates and corresponding CIs were published (*18*), and because their published timetree lacks names for terminal taxa, timing and patterns of diversification across much of the tree, for example, for most superfamilies and all families but Nitidulidae, remain difficult to interpret.

To help clarify divergence times for family-level and higher groupings, we obtained a "recreated" version of the Hunt *et al.* (*18*) timetree (their Figure 3) from the authors. This tree has the same topology as the published version and names for terminal taxa, but node ages estimated from the timetree differ by at least 5 Ma from the published version for seven of the aforementioned 13 nodes for which average ages were published (perhaps on account of this being a "recreated" tree) (*18*). These differences were greatest in Cucujiformia. Nonetheless, we reduced the timetree to family-level taxa (when possible), and obtained estimated ages for all nodes based on their relative positions in the timetree (Table 1). Age estimates reported without CIs are our own best estimates based on the timetree and corresponding timescale provided by the authors (unless otherwise noted).

Based on these data, the Adephaga–Polyphaga split was estimated as ~277 Ma. The Suborder Adephaga comprised two well-supported clades, the aquatic Hydradephaga and the terrestrial Geadephaga (215.7 Ma; ground beetles and tiger beetles). Overall, relationships within Adephaga were similar to those found by other authors using molecular data (*22, 23*), and reconstructed divergence times are compatible with the fossil record (*13*).

Within the Suborder Polyphaga, five series of families are traditionally recognized (*4, 5*); Bostrichiformia (Superfamilies Bostrichoidea and Derodontoidea), Cucujiformia (Chrysomeloidea, Cleroidea, Cucujoidea, Curculionoidea, Lymexyloidea, and Tenebrionoidea), Elateriformia (Buprestoidea, Byrrhoidea, Dascilloidea, Elateroidea, and Scirtoidea), Scarabaeiformia (Scarabaeoidea), and Staphyliniformia (Hydrophiloidea and Staphylinoidea). The Superfamilies Derodontoidea (Family Derodontidae) and Scirtoidea (Families Clambidae, Decliniidae, Eucinetidae, and Scirtidae) occupied the basal nodes in Polyphaga. This arrangement conflicts with the traditional placement of Derodontoidea in the Series Bostrichiformia and Scirtoidea in the Series Elateriformia (*4*), but is consistent with other recent studies (*16, 17*). Derodontoidea and Scirtoidea exhibit several pleisiomorphic morphological features that

support their placement at the base of Polyphaga, including paired dorsal ocelli, mesocoxal cavities partly closed by the metepisterna, a transverse metasternal suture, a trilobed aedeagus, and six free Malphigian tubules (*8*). The Series Staphyliniformia comprised a paraphyletic grade near the base of Polyphaga. Scarabaeiformia (~191.4 Ma) appeared within Staphyliniformia. Elateriformia minus Scirtoidea was found to be the closest relative of Bostrichiformia minus Derodontoidea. Cucujiformia was strongly supported as monophyletic.

The Superfamilies Buprestoidea (~142.5 Ma; metallic wood-boring beetles), Dascilloidea (~73.1 Ma), and Elateroidea were each found to be monophyletic. Byrrhoidea was polyphyletic, with the Byrrhidae (moss beetles) resolved separately from a clade comprised of the remaining families. Lymexyloidea (ship-timber beetles) was polyphyletic and appeared near the base of Tenebrionoidea. The Families Biphyllidae and Byturidae appeared within Cleroidea (checkered beetles and allies), an arrangement previously suggested by other authors (e.g., *4*) based on morphology. Cucujoidea was polyphyletic, with Sphindidae as the closest relative of Tenebrionoidea plus Lymexloidea, and Silvanidae and Phloeostichidae as the closest relatives of a clade comprised of the chrysomelid Subfamily Hispinae and a monophyletic Curculionoidea. The Superfamily Chrysomeloidea and the Family Chrysomelidae (leaf beetles and long-horn beetles) were therefore polyphyletic. Traditionally, Chrysomeloidea and Chrysomelidae are thought to be monophyletic, and Chrysomeloidea is thought to be closest to Curculionoidea (e.g., *20, 21*, *24*).

While direct comparisons are difficult due to differences in taxon sampling and resolution, all lineages of Chrysomeloidea sampled by Hunt *et al.* (*18*), including both lineages of their polyphyletic Family Chrysomelidae (Chrysomelidae-1; ~191.3 Ma, Chrysomelidae-2; ~187.6 Ma) are estimated to have originated before 100 Ma (Table 1). This is in contrast to a recent study (*21*) employing *18S, 28S rRNA,* and mitochondrial *16S rRNA*, which places the origin of Chrysomelidae at 73–79 Ma (95% CI; 63–86 Ma), and argues that the Family Chrysomelidae radiated in the Cenozoic, long after the Jurassic to early Cretaceous origin and middle Cretaceous diversification (e.g., see *25*) of their (predominantly) angiosperm host plants. Note that the estimated timing of origin of most chrysomeloid lineages in Hunt *et al.* (*18*) are at least loosely consistent with the aforementioned timing of angiosperm diversification (*25*). The questions remain open, therefore, as to whether and how the origin and diversification of angiosperms influenced diversification in the largely herbivorous Superfamily Chrysomeloidea (>50,000 species), and other groups of beetles—e.g., Curculionoidea (>60,000 species).

We recently completed an analysis of *18S rRNA* sequence data with the dual goals of reconstructing higher-level relationships and divergence times in Coleoptera. While overlapping in part with the data set analyzed by Hunt *et al.* (*18*), our methods of vetting data, alignment, and fossil calibration to form a timetree differ sufficiently from Hunt *et al.* (*18*) to warrant mention. Further, the resulting timetree permits at least casual comparison with the Hunt *et al.* (*18*) timetree.

We obtained all Genbank *18S rRNA* sequences available for beetles as of May 2007. To this data set, we added one unpublished sequence of our own, *Prolixocupes* (Archostemata: Cupedidae), effectively doubling the number of Archostemata included in previous studies. All sequences with fewer than 1300 bp of sequence data were excluded from analysis. Genus-level exemplars were randomly selected when duplicates were present, except when sequences differed by more than 100 bp in aligned length, in which case the most complete sequence available was used. Six sequences were excluded due to large numbers of Ns and unusual alignment problems indicative of low-quality data. The final data set consisted of 955 ingroup sequences representing 134 families. Genbank sequences from six neuropteroids were used to root the tree [*Hemerobius* (AF423790); *Myrmeleon* (chimera of U65137 & L10182); *Oliarces* (AF012527); *Phaeostigma* (X89494); *Sialis* (chimera of AY521864 & X89497); and *Mantispa* (chimera of AY620034 & U65189)]. DNA sequences were aligned with Clustal X (*26*) and manually adjusted in MacClade v.4.05 (*27*). An annotated secondary structural alignment for insect 18S (*28*) was used to further refine the alignment. Regions 4, 11A, 14A, and 14B of Kjer (*28*) could not be unambiguously aligned, and were excluded from analysis. The remaining aligned data consisted of 1920 nucleotide positions.

A maximum likelihood (ML) search employing the GTR+I+Γ substitution model and limited to 10^7 generations, was implemented in GARLI v0.951 (*29*). Branch lengths were optimized in PAUP* v.4.03b10 (*30*). In the absence of clocklike molecular evolution, we used nonparametric rate smoothing (*31*) implemented in r8s v.1.71 (*32*) to generate an ultrametric tree from the ML topology (–lnL = –73081.14). Fossils used to calibrate the tree and to date internal nodes included: (i) the oldest unequivocal fossil Hydradephaga (*1*), applied as a minimum constraint of 225 Ma on the

age of Hydradephaga, (ii) the oldest unequivocal fossil Scarabaeidae (*1*), applied as a minimum constraint of 152 Ma on the age of Scarabaeioidea, (iii) the oldest unequivocal fossil Tenebrionidae (*1*), applied as a minimum constraint of 125 Ma on the age of Tenebrionoidea, (iv) the oldest unequivocal fossil Curculionoidea (*1, 33*), applied as a minimum constraint of 152 Ma on the age of Curculionoidea, (v) the oldest unequivocal fossil Staphylinidae (*1*), applied as a minimum constraint of 227.5 Ma on the age of Staphylinoidea, and (vi) the oldest unequivocal fossil Cupedidae (*1*), applied as a minimum constraint of 199.6 Ma on the age of Cupedidae. When the age of a given fossil was not reported in the literature, we used the upper boundary of the subdivision of the global geological record reported as having contained the fossil, as a minimum age constraint. Fossil constraints were applied conservatively, so the resulting nodal age estimates should be considered similarly conservative.

Holometabolous insects are not known from before the Permian (*2*), so we constrained the maximum age of the ingroup to 299.0 Ma, the Carboniferous–Permian boundary. We separately applied each of two alternative maximum constraints on the age of the ingroup, the Carboniferous–Devonian boundary (359.2 Ma), and the Devonian–Silurian boundary (416 Ma), to evaluate the robustness of nodal age estimates to relaxation of this constraint. Nodal age estimates were only minimally affected, so we report our results as a range of ages (and mean) spanning the three age estimates determined for each node of interest.

Based on these analyses, we determined the basal relationships of suborders to be: (Myxophaga + Archostemata, (Adephaga, Polyphaga)). This is in agreement with Hunt *et al.* (*18*) and most other analyses employing 18s rDNA (*14–16*). The placement of Archostemata within Myxophaga should be viewed as tentative due to the limited sampling of Archostemata in all studies to date. Sampling of additional Archostemata (e.g., *Ommatidae* and *Micromalthidae*) and Myxophaga (*Lepiceridae*), experimentation with outgroup taxon sampling, inclusion of data from nuclear protein coding genes, and additional analyses, may help clarify relationships between these two interesting suborders. Overall, relationships within Adephaga were very similar to those obtained by other authors (*15, 17, 18, 22, 23*). While relationships within Polyphaga were not well resolved, they were grossly similar to other studies, for example, obtaining Scirtoidea and Derodontoidea as the earliest branching lineages in the suborder.

The series Cucujiformia, while monophyletic, contained numerous very short internal branches, and relationships within the series were generally not well supported. It should be noted that with few exceptions, the 18S sequences of Cucujiformia and several other higher-level groups within Polyphaga exhibit relatively little overall divergence. As a consequence, 18S should be expected to be minimally informative for such relationships, especially because several of the more highly variable regions of 18S were excluded from this and most other studies. The series Cucujiformia contains nearly half of all beetle families and most beetle species, and most cucujiform beetles feed on plants. Therefore, an accurate reconstruction of relationships and timing and patterns of diversification within the series is critical to our understanding of beetle macroevolution, including the role of angiosperms in beetle diversification. Due to the lack of well-supported resolution at lower levels in Polyphaga and beyond, we did not evaluate divergence times below the subordinal level.

Based on the topology we obtained, and employing the fossil age constraints described herein, we estimate that the split between the clade comprised the Suborders Myxophaga + Archostemata and the clade comprised the Suborders Adephaga and Polyphaga, occurred ~269–265 Ma (mean 266.8 Ma). Hunt *et al.* (*18*) fixed this age at 285 Ma. We determined the Adephaga–Polyphaga split to have occurred ~269–265 Ma (mean 266.4 Ma), just slightly later than Hunt *et al.* (*18*), who estimated this split to have occurred ~277 Ma. These observations suggest that the four living suborders of beetles diverged at a time (Permian) when many other groups of terrestrial organisms, including other insects (*34*), underwent rapid diversification.

The subordinal relationships and divergence times based on the limited numbers and kinds of genes used in the papers reviewed here appear to be robust. However, lower-level relationships and divergence times remain unsettled. Without a well-supported topology at this level, accompanied by dated nodes with confidence intervals, particularly for the most species-rich Cucujiformia, it is difficult to justify detailed evaluation of the timing, causes, and consequences of ecological diversification (e.g., the role of phytophagy, predation, or fungivory). Nonetheless, large-scale molecular phylogenetic studies such as that presented by Hunt *et al.* (*18*) promise the most comprehensive picture to date of the main branching events and their divergence times in the evolution of insects, including the famously diverse beetle order Coleoptera.

Acknowledgments

The authors would like to thank A. Vogler for providing the timetree we used to estimate node ages in Hunt *et al.* (*18*). Support was provided by the U.S. National Science Foundation.

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