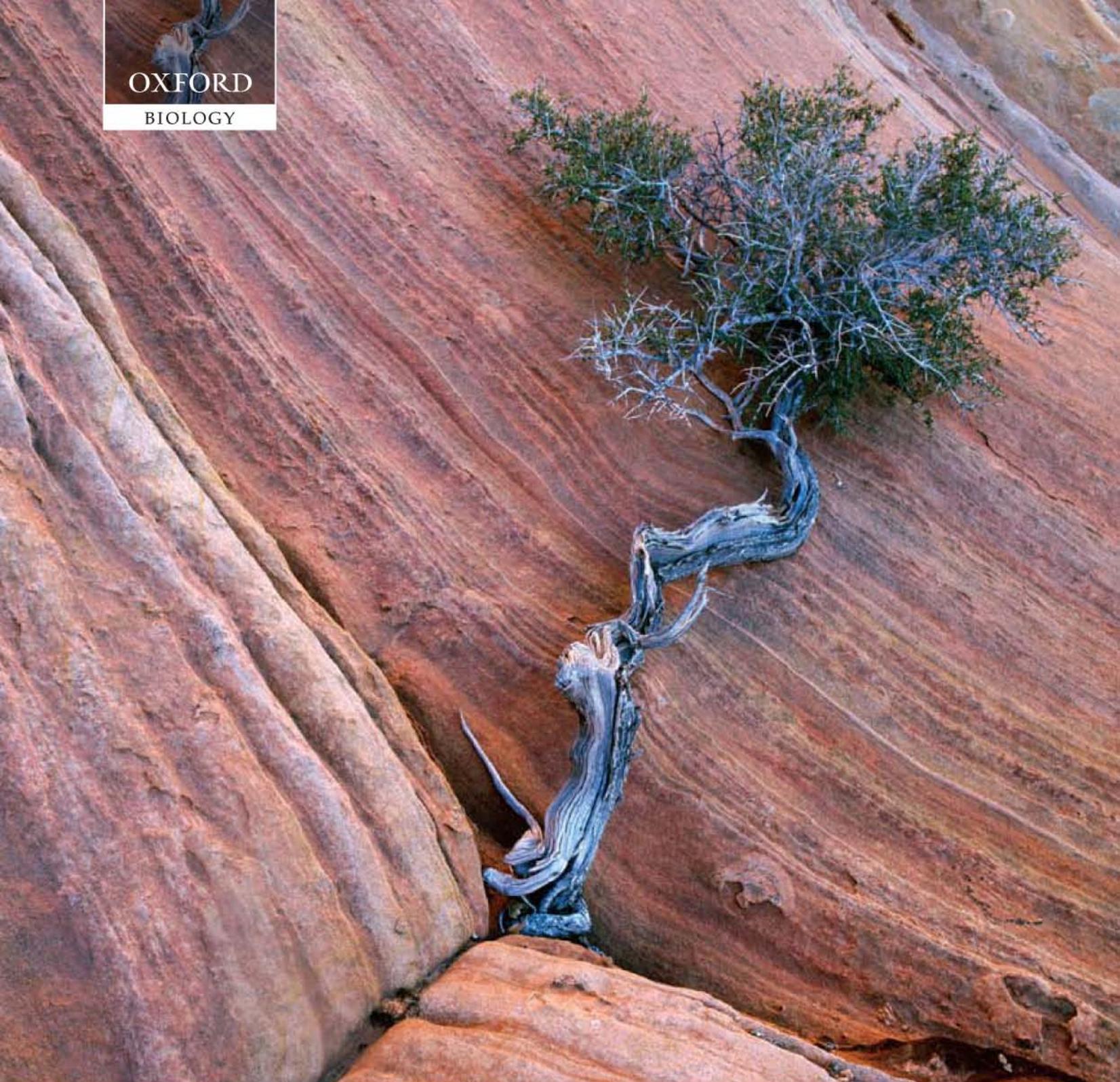# the TIMETREE of LIFE

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# Holometabolous insects (Holometabola)

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## Abstract

**The Holometabola includes 11 orders that represent the
vast majority of insect diversity (~850,000 species). Recent
molecular and morphological treatments support holom-
etabolan monophyly, confirm the monophyly of the major
orders, and provide new evidence to place the orders in a
phylogeny. Estimates of divergence time based on molecu-
lar evidence suggest an origin of the Holometabola within
the Carboniferous, 359–299 million years ago, Ma, but
definitive fossils first appear in the Permian, 299–280 Ma.
The molecular timetree reveals striking parallel radiations
of insect lineages throughout the Mesozoic (251–66 Ma).**

The insect clade Holometabola (~850,000 species)
includes 11 living orders that together comprise the vast
majority of all insect diversity and therefore also represent
a significant fraction (>60%) of all terrestrial animals
(*1*). Holometabola includes the four largest orders of
insects: Coleoptera (beetles, Fig. 1), Hymenoptera (bees,
ants, and wasps), Diptera (true flies), and Lepidoptera
(moths and butterflies), as well as the Neuroptera
(lacewings), Megaloptera and Raphidioptera (dobson-
flies and alderflies), Trichoptera (caddisflies), Mecoptera
(scorpionflies), Siphonaptera (fleas), and Strepsiptera
(twisted-wing insects). The name of the group reflects
their defining characteristic—they undergo complete
metamorphosis. Their life history is divided into discrete
developmental stages, including a distinct larval (feed-
ing) and pupal (quiescent) stage. The major developmen-
tal, morphological, and behavioral modifications that led
to the holometabolous larva are thought to have arisen
through extension of the prenymphal stage of hemi-
metabolous insects (*2, 3*). Metamorphosis from larval to
adult morphology occurs in the pupal stage where the
larval structures are broken down and adult features

(legs, wings, antennae, genitalia) then develop from spe-
cialized internal regions of subcuticular epidermal cells
called imaginal discs (*4*). The larval cuticle is reduced or
entirely lost and an adult cuticle is newly formed. The
internal development of the wings is denoted in the other
common name of the group, the Endopterygota. Despite
its huge diversity, there are relatively few holometabolan
lineages that contain exceptionally large numbers of spe-
cies. Developmental specialization clearly played a major
role in the expansion of holometabolan life histories, but
the hyperdiversity of major clades of beetles, flies, moths,
and wasps are most often attributed to independent, lin-
eage-specific radiations enabled by unique combinations
of trophic, life history, morphological adaptations, and
the expansion of terrestrial plant communities (*2, 5–9*).
For a more comprehensive perspective on insect diver-
sity, fossil history, and evolution, see Grimaldi and Engel
(*2*). Here, we review evidence on the phylogeny and
divergence times of holometabolous insects.

Phylogenetic classifications of Holometabola based on
morphological features divide the group into two major
subclades, the Neuropteroidea, which includes Cole-
optera + the Neuropterida (Neuroptera, Megaloptera,
Rhaphidioptera), and the Mecopterida (= Panorpida),



**Fig. 1** A holometabolous beetle larva (Coleoptera:
Chrysomelidae, *Zygogramma* sp.) from Arizona, USA. Photo
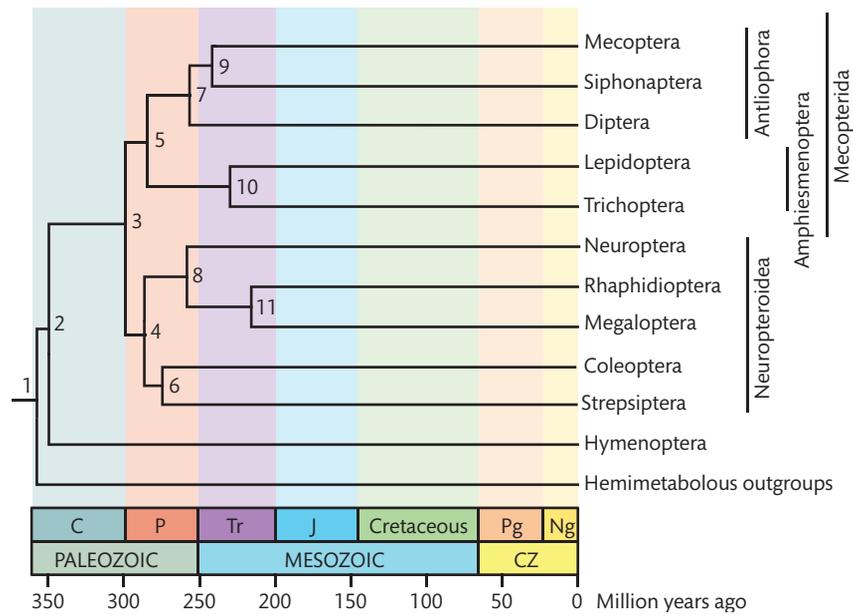credit: A. Wild.

**Fig. 2** A timetree of the holometabolous insects. Divergence times are shown in Table 1. *Abbreviations*: C (Carboniferous), CZ (Cenozoic), J (Jurassic), Ng (Neogene), P (Permian), Pg (Paleogene), and Tr (Triassic).

including Lepidoptera, Trichoptera, Diptera, Mecoptera, and Siphonaptera. Hymenoptera and Strepsiptera have been placed in various positions, the former often placed as closest to the Mecopterida and the latter traditionally placed either within or closest to Coleoptera (*10, 11*). The consensus view is that most morphological features of the Hymenoptera and the Strepsiptera are too highly modified to unequivocally resolve their phylogenetic positions. Additional widely accepted groupings are the Amphiesmenoptera (Lepidoptera + Trichoptera) (*8*) and the Antliophora (Diptera + Mecoptera including Siphonaptera) (*12*). Morphological evidence also supports a close relationship between the Mecoptera and the Siphonaptera (*11*). The most comprehensive review of the morphological evidence for holometabolan relationships is that of Kristensen (1999; *8*) and was further evaluated in light of emerging alternative phylogenetic hypotheses by Beutel and Pohl (*12*). New perspectives on specific character systems such as sclerites, muscle insertions, and functional features of the wing-base (*13*), and mouthparts (*14*), as well as new paleontological findings and interpretations (*2, 15*) continue to add to the evidence on relationships.

Molecular analyses of holometabolan phylogeny have primarily relied on 18S ribosomal DNA, and the results have been highly dependent on taxon sampling, alignment, and methods of analysis. Previous molecular studies, most using rDNA, have recovered a monophyletic Neuropterida (*16, 17*), Neuropteroidea (*16, 18, 19*), Amphiesmenoptera (*16, 17, 19–21*), Mecopterida (*20, 21*) and, most provocatively, Halteria (Strepsiptera + Diptera) (*17, 19*). Two recent phylogenomic projects, with limited taxon sampling but large numbers of genes, addressed the placement of the Hymenoptera; mitochondrial genomes provided evidence that Hymenoptera is the closest relative of Mecopterida (*22*), while combined analysis of 185 nuclear genes strongly supports placement of the Hymenoptera as the earliest branching lineage, the closest relative of all other Holometabola (*23*).

The most current molecular study by Wiegmann *et al.* (submitted) is the first to include both nuclear genes (*AATS, CAD, TPI, SNF, PGD,* and *RNA POLII*) and representative taxa from all holometabolan orders. Their findings support traditional morphological hypotheses (Neuropteroidea + Mecopterida including Amphiesmenoptera + Antliophora) and Savard *et al.*'s (*23*) early branching position for Hymenoptera. Additionally, multiple nuclear genes provide evidence for the placement of the enigmatic Order Strepsiptera as the closest relative of Coleoptera. These results add to the compounding and conflicting evidence for the placement of Strepsiptera—the most controversial issue

**Table 1.** Divergence times (Ma) and their credibility/confidence intervals (CI) among holometabolous insects, based on Wiegmann *et al.* (submitted).

| | Timetree | |
| --- | --- | --- |
| Node | Time | CI |
| 1 | 355 | 360–334 |
| 2 | 350 | 359–336 |
| 3 | 300 | 315–287 |
| 4 | 286 | 299–274 |
| 5 | 282 | 300–264 |
| 6 | 274 | 285–270 |
| 7 | 256 | 279–234 |
| 8 | 255 | 276–227 |
| 9 | 243 | 270–222 |
| 10 | 230 | 261–190 |
| 11 | 213 | 247–134 |

in holometabolan phylogenetics. Beutel (*12*) recently reviewed arguments surrounding the "Strepsiptera problem" (*24*). The original findings of Whiting and Wheeler (*25*) placed Strepsiptera as the closest relative of Diptera based on *18S rDNA* and initiated a useful debate regarding empirical evidence for spurious grouping by "long-branch attraction" in molecular phylogenetics (*26–30*). Further studies supporting Halteria were based on *28S rDNA*, *18S rDNA*, and morphology; the most convincing morphological evidence being modifications of the wings into halteres, shared by both dipterans and strepsipterans, albeit on different thoracic segments (*19, 27, 31*). Several additional morphological and molecular studies reported evidence refuting the existence of Halteria (*30, 32–34*). The findings of Wiegmann *et al.* (submitted) supporting the close relationship between the Strepsiptera and the Coleoptera are robust and in agreement with traditional morphological hypotheses.

The Holometabola is thought to have originated in the late Carboniferous (*2, 35, 36*), but definitive fossil evidence is lacking until the Permian (~280 Ma), a time when most of the extant orders had their origins (*2, 37*). An insect gall, presumed to be from a member of Holometabola, has been identified from the late Pennsylvanian, 302 Ma, that if accurately diagnosed provides the earliest physical evidence of their existence. A molecular analysis that relied on mitochondrial data (cox1) and maximum likelihood (ML) global and local molecular clocks to date the origin of the insects included

both dipterans and lepidopterans and found the origin of the taxon-limited Holometabola to be 351–338 Ma (*38*). Wiegmann *et al.* (submitted) estimated the divergence times of all holometabolan orders using a Bayesian phylogeny based on multiple nuclear genes, fossil calibrations, and relaxed clock Bayesian methods using the program Multidivtime (*39*). Congruent with the findings of Gaunt and Miles (*38*), multiple nuclear genes placed the origin of the Holometabola at 355 Ma, within the Carboniferous, but earlier than traditional estimates.

The Hymenoptera, as the earliest branching lineage in the phylogeny, has an age of origin nearly equivalent with the age of the divergence of Holometabola from its closest relative (Fig. 2). This date is considerably older than existing fossil estimates, typical of molecular estimates (*39*). The split between the two major subclades Neuropteroidea and Mecopterida took place in the Permian 300 Ma, with the Amphiesmenoptera/Antliophora divergence occurring 284 Ma. The divergence of all orders (excluding the Hymenoptera) appears to have occurred in relatively rapid succession, with dates of origin falling in the range 274–213 Ma, with the earliest being the Coleoptera/Strepsiptera divergence at 274 Ma and the most recent being the split of Rhaphidioptera and Megaloptera at 213 Ma. Though some estimates of ordinal-level divergences do not precisely correspond with traditional ages based on fossils, paleontological evidence is dramatically expanding, and thus, better fossil calibrations coupled with larger samples of genes and taxa as well as improved analytical methods should continue to refine divergence time estimates for the major holmetabolan clades.

Molecular divergence time estimates and fossils agree that the Holometabola had its origins within the Paleozoic. The origination of the orders (excluding Hymenoptera) took place primarily within the Triassic with the primary split (Neuropterida + Mecopterida) occurring at the end of the Permian, and the remaining orders all appearing in the Jurassic. The huge hyperdiverse lineages of the Holometabola that contribute to the group's reputation for evolutionary success (phytophagous and staphylinid beetles, apocritan wasps, cyclorrhaphan flies, and ditrysian Lepidoptera) may owe their species-richness to mid- and late Jurassic developments such as the radiation of angiosperms and the acquisition of specialized morphological innovations, such as a wasp-waist and the fly puparium (*8, 40*). Extreme diversity has made it difficult to resolve phylogenetic relationships among the major orders and conflicting lines of evidence continue to make holometabolan phylogeny one of the most challenging problems in insect phylogenetics.

## Acknowledgment

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