

Incorporating fossils into hypotheses of insect phylogeny

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Fossils represent stem and crown lineages, and their inclusion in phylogenetic reconstruction influences branch lengths, topology, and divergence time estimation. In addition, paleontological data may inform trends in morphological evolution as well as biogeographic history. Here we review the incorporation of fossils in studies of insect evolution, from morphological analyses to combined 'total evidence' node dating analyses. We discuss challenges associated with fossil based phylogenetics, and suggest best practices for use in tree reconstruction.

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Introduction

The fate of every species is to go extinct. This results in a mixture of crown lineages [which have living representatives currently] and stem lineages [for which there are no extant representatives] in the fossil record. How to best incorporate such fossils into insect phylogenetic hypotheses has been a matter of debate. Those in favor of incorporating fossils in phylogenetic studies argue that although fossilization is rare, the vast majority of insect species have gone extinct [1] and therefore fossil taxa offer otherwise unknowable insight into the morphological, biogeographic, and temporal history of extant lineages. Initially, phylogenetic analyses that included fossils were based on morphology-only datasets [2], making their incorporation relatively straightforward. The advent of molecular-based phylogenetic and comparative analysis has made the incorporation of fossil data into combined datasets less clear. So-called 'total-evidence' methods of integrating molecular and morphological data arose with the aim of addressing incongruence between datasets while allowing fossils to impact phylogenetic reconstruction [3]. However, these combined analysis

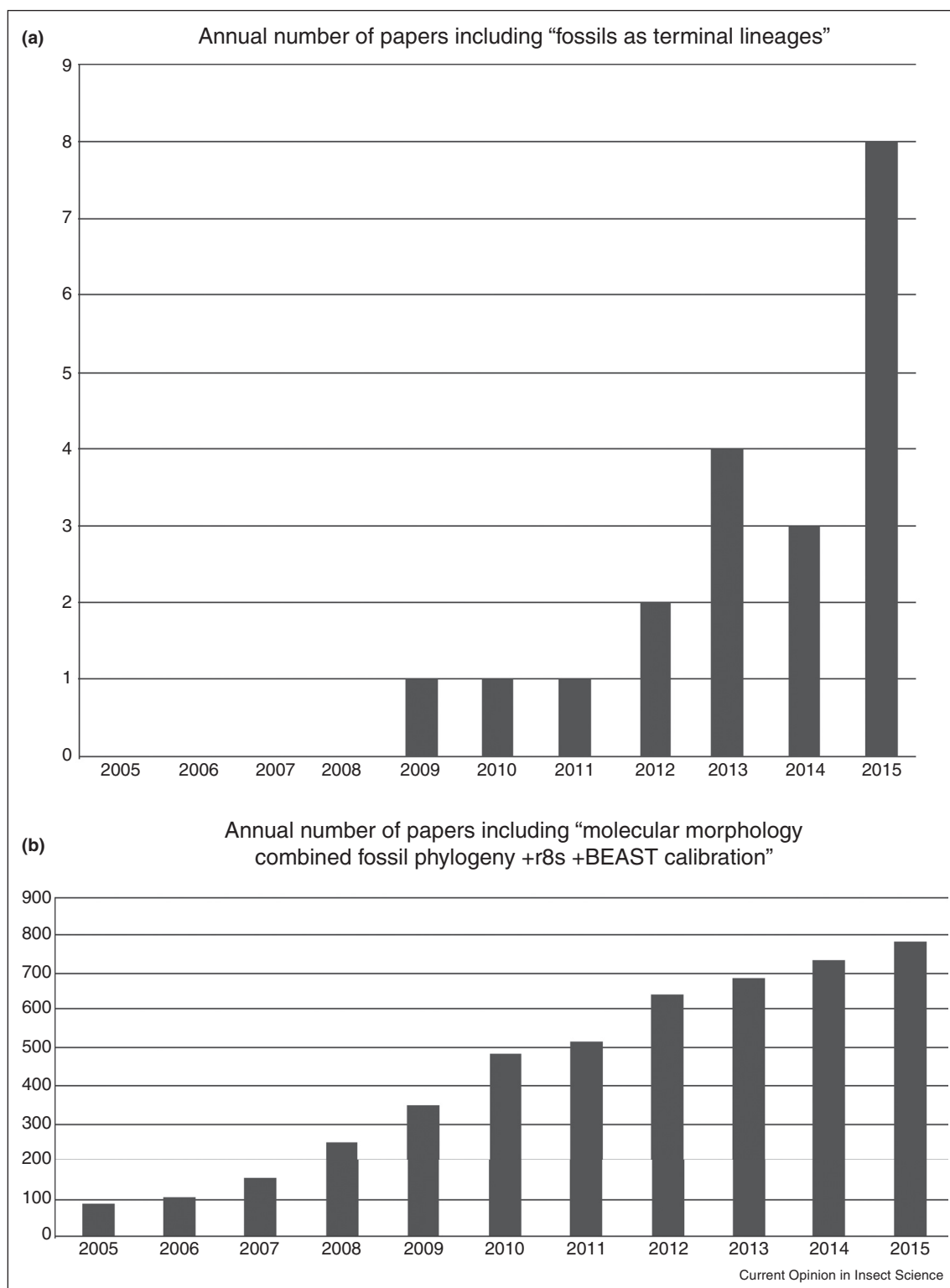
techniques were challenging, as initial model-based procedures prohibited simultaneous optimization of molecular and morphological characters. In the last decade, after implementation of the mk model by several software applications [4], fossils have become increasingly common in combined molecular and morphological phylogenetic datasets (e.g. [5]). In addition, divergence time ['dating'] estimates are now widely considered integral information when interpreting the evolution of organisms from a phylogenetic perspective; these estimates rely on fossil calibrations to estimate node ages. In these dating studies, fossils may be stem or crown, treated as terminal lineages [Figure 1, Top] (e.g. [6–19,20[•],21–23,24^{••}]) or, more frequently [Figure 1, Bottom] used as node calibrations modeled with uniform or non-uniform distributions (e.g. [25^{••},26–29]). For groups with poor fossil records, secondary calibrations [i.e., dates from prior studies, biogeographic constraints] have become more commonly used when estimating divergence times, which is problematic (see [30^{••}] for review; briefly, node ages calibrated by secondary calibrations were found to be younger and give spurious estimates of precision].

Whether for chronogram or phylogenetic reconstruction, the use of fossils has often been contentious due to a lack of agreement about how to use stem and crown fossils, in particular when considering mixed types of data (e.g. [31–36,73^{••}74]). Here we review the use of fossils in past phylogenetic datasets, discuss current methodology, and the challenges facing future dataset analyses.

Traditional fossil treatments in phylogenetics: 'total evidence' analyses

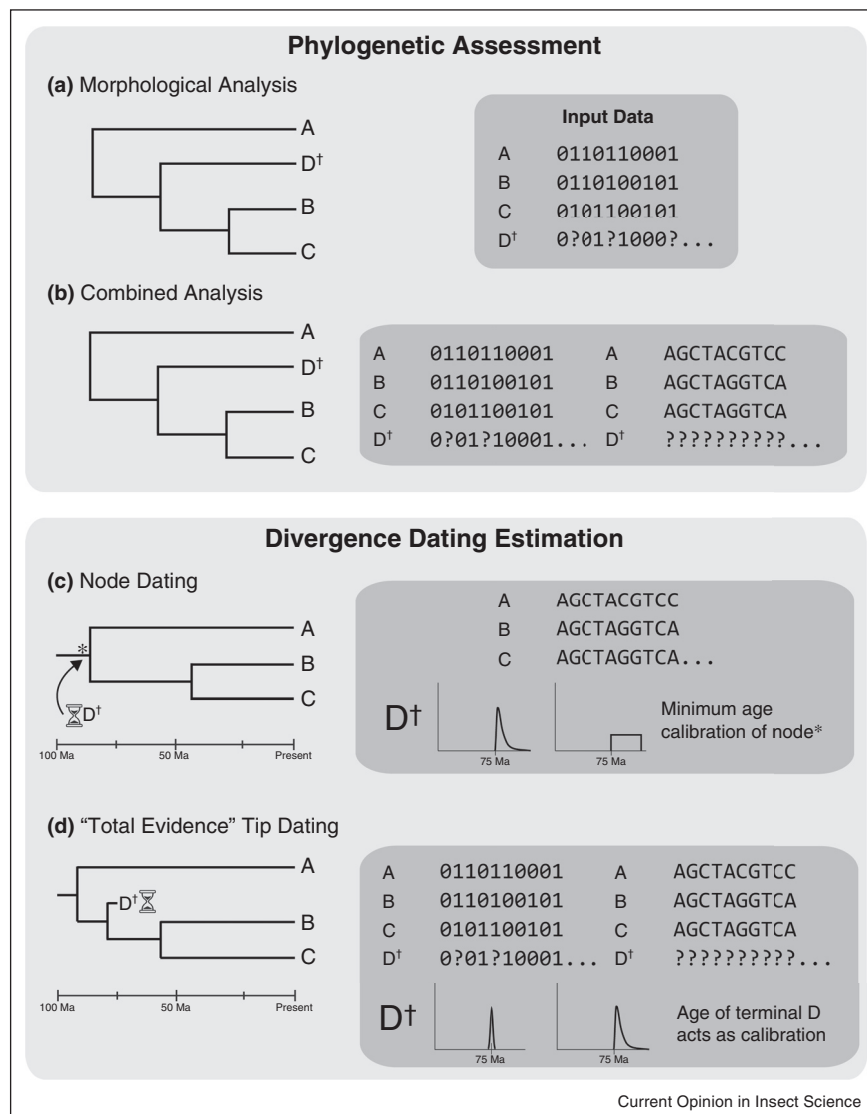
Hennig [37,38] incorporated fossils in a phylogenetic treatment of insects, based on morphological synapomorphies [Figure 2a]; this was the first phylogenetic evaluation of extant and living insect lineages. When reconstructing evolutionary relationships among insects, authors have argued that fossils provide vital information about character polarity (e.g. [39]). Several have argued further that fossil inclusion may reduce long-branch attraction [2,40]. Donoghue *et al.* [41] tested the effect of fossils on amniote and seed-plant morphological phylogeny, and suggested that fossil inclusion may result in topological differences. Similarly, Lee [42] found that potentially incorrect relationships resulted when fossils were omitted from phylogenetic reconstructions of lizards. Further, the results from Wiens [43] support the inclusion of even incomplete fossils in phylogenetic reconstruction despite some level of missing data,

Figure 1



Comparison of publication rates for alternative divergence time estimation protocols. **Top** total papers per year found on Google Scholar, which include 'fossils as terminal lineages' in text. **Bottom** total annual papers on Google Scholar including the terms 'molecular morphology combined fossil phylogeny calibration' which utilized the phylogenetic programs r8s or BEAST.

Figure 2



Summary of common methods for fossil integration. **(a)** Traditional phylogenetic placement of fossil and extant taxa through optimization of morphological data. **(b)** Combined analysis which includes both molecular and morphological data in tree construction. **(c)** Node dating divergence time estimation that includes fossil taxa only to calibrate certain nodes. The placement of calibrations, which implicitly assumes a phylogenetic interpretation of fossils, is at the discretion of the investigator. **(d)** More recent tip dating approach which calibrates the age of the tree based on the placement of fossil taxa determined through combined analysis. Note: for subfigures **(c)** and **(d)**, the probability densities for D[†] represent alternative user-inputted calibration constraints given the same fossil: lognormal, uniform, normal.

especially when molecular data are included [Figure 2b] [44]; combined analyses with fossils have been suggested to reduce long branch attraction even with the proportion of missing data is high [45]. Model-based analysis of combined datasets including fossil and extant taxa in a morphological matrix with molecular characters became more common during the first decade of the 21st century (e.g. [46,47]; see [48] for a discussion of the importance of morphology in placing fossils via Bayesian analyses of combined datasets). Recently, Guillaume and Cooper

[49**] evaluated the effect of missing morphological data in combined 'total evidence' simulated datasets; they accounted for missing data in the fossil record and sampling effects in simulated morphological and molecular datasets using both maximum likelihood and Bayesian analyses. In their study, missing data from fossil and living taxa affected the topologies in different ways, as did phylogenetic reconstruction methods, suggesting that missing morphological data for extant taxa was most detrimental to recovering the 'best' tree topology. Taken

together, these studies suggest that fossils are valuable in phylogenetic analyses, and may improve topologies, but that missing data for living taxa and low numbers of morphological characters may limit the positive impact that fossils might have on tree topology and branch length estimation.

Fossils as terminal lineages or calibration priors in divergence time estimation

Datasets including fossil and extant taxa have been used for phylogenetic reconstruction and, more recently, divergence time estimation. Most commonly, fossils are utilized to ‘calibrate’ nodes on a phylogenetic tree, effectively acting as a link between relative branch lengths based on molecular evolution and real, geologic time [Figure 2c]. In addition to ‘total evidence’ phylogenetic reconstruction discussed above, it is now possible to calibrate divergence date analyses by including fossils as terminal taxa in ‘tip dating’ approaches [Figure 2d]. Among the criticisms for using ‘total evidence’ approaches in dating studies is a critique of use of the *mk* model to model phenotypic data. Despite this criticism, however, it has become fashionable to include fossil lineages, coded for morphology, in combined datasets because of potential reductions in the errors associated with the fossil calibration step (but see [50**] for discussion). Including fossils as terminal lineages can impact the topology of extant lineages, which may reduce node age error. Terminal fossils may reduce divergence time error associated with misapplication of fossil calibrations, and may further influence extinction rate estimates [7]. The first attempts to use fossils as terminal lineages in a combined ‘total evidence’ analysis for divergence time estimation were undertaken by Lee *et al.* [6], Ware *et al.* [7] and Pyron [8]. Each study incorporated fossils as terminal lineages, with Lee *et al.* [6] and Pyron [8] running straightforward dating analyses and Ware *et al.* [7] comparing the divergence time estimates recovered using fossils as terminal lineages to those recovered using fossils as uniform node priors. O’Reilly *et al.* [50**,51**] proposed best practices for total evidence dating, and suggested a method for incorporating fossil age uncertainty into divergence time estimation priors. Briefly, they argue that fossils should not be used simply as point estimates, as fossil preservation bias and the myriad of measurements used to estimate fossil ages [e.g., lithographic, magnetostratigraphic data] inherently result in error around a given age [i.e. resulting in a minimum and maximum age for a given fossil]. Rather, they suggest a method for modeling the uncertainty in fossil ages, which they suggest may reduce the perceived advantage [in terms of node age error reduction] of ‘total evidence’ studies. Finally, there are initial results that suggest it may be desirable to use both tip and node dating in conjunction as a means of mitigating the drawbacks of each method alone [50**,51**].

Variation among taxa in abundance of stem-group and crown-group lineages

Whether fossils are important components in phylogenetic and divergence time analyses is an inconsequential debate for those working on insect orders with poor fossil records, as a dearth of fossils for genera, such as *Polythore*, a damselfly with only a handful of *incertae sedis* fossil representatives, preclude their inclusion in such analyses. However, for many taxa, there are an abundance of fossils, and for these groups the challenge is often to distinguish whether a fossil belongs to a crown-group or stem-group lineage. This designation is not trivial: in node-dating analyses, crown-affiliation or stem-affiliation will impact which node is calibrated, ultimately altering inferred divergence estimates. Crown groups are monophyletic taxa, living or extinct, which all possess synapomorphies for the group, except in cases of derived loss. Stem lineages are more closely related to the crown group than they are to extant sister groups, but lie outside of the crown group itself; stem taxa may possess some crown group synapomorphies, but by definition lack all characters necessary for crown group placement. Crown-group or stem-group placement should be informed by morphological characters, not previously obtained divergence estimates. Cockroaches [Blattodea] and Dragonflies/Damselflies [Odonata] both are rich in stem-group and crown-group fossils. Dragonflies and damselflies comprise over 6000 extant species, and there are large numbers of crown fossils for several families; for the monogeneric family Epiophlebiidae, however, only four crown species exist with the remaining species in the family belonging to Jurassic stem lineages (see [52], for review). Epiophlebiidae have suffered from near complete extinction, with the four extant species found currently only in Asia (the Himalayas, China and Japan [53,54]). *Epiophlebia* has rarely been incorporated in divergence time estimation analyses, but it would be an excellent taxon to use to test the effect of including stem fossils on estimates of extinction. Blattodea comprise the termites and cockroaches, whose oldest fossil is *Bassaitermes* (140 Ma [55]). Within the cockroaches, there is controversy regarding the status of putative Bashkirian ‘roachoid’ fossils, which have been omitted from several studies due to their uncertain status (e.g. [25**,56]) yet included in others (e.g. [57]; but see [58]). This debate is of considerable importance, given that the inclusion of stem roachoid fossils changes the ages of the Polyneoptera and within it, the Dictyoptera, dramatically influencing interpretations of the evolution of sociality (Polyneoptera age [25**]: 377–231 Ma; 57: 408–367 Ma).

In addition to improving divergence estimations, the incorporation of fossils can alter the perceived evolutionary history of certain groups, particularly as stem lineages, with no living members and thus no means of inclusion within extant-only datasets, can have substantial impact on ancestral trait and area reconstruction. While the

conceptual paragons of stem diversity are probably non-avian dinosaurs, there are insect groups with significant early branching fossil taxa. Among these are early ants, which were diverse, social, and members of lineages distinct from extant groups [59,60], Cretaceous termites with caste specialization [61], many species of fungus gnats [62], and others (see [55]). With respect to biogeographic inference, there are clear examples of relictual distributions revealed only after the discovery of fossil taxa. The termite genus *Mastotermes* and ant subfamily Myrmiciinae, both endemic to Australia today, are known from fossil deposits in the Americas and Europe, suggesting a previous worldwide distribution [63,64]. It is important to note that such distributions are not recovered through analysis of modern biogeographic ranges alone.

What challenges face us?

Several challenges face researchers incorporating fossils in their datasets, such as model choice, fossil selection, and calibration methodology in divergence time estimation. To date, few studies have evaluated the effect of the incorporation of fossils in next generation datasets and biogeographical or diversification rate analyses (see [58] for discussion). Many have argued for or against the use of the mk model, which is based on the 1969 Jukes Cantor model to model phenotypic diversity present in morphological matrices (see [65] for review). It is possible, however, that the benefits of morphological fossil data inclusion may outweigh the costs of the error associated with poor model fit. Future work should aim to modify the mk model to better model reversals, exaptations and covariation; O'Reilly *et al.* [51**] further suggest the use of continuous characters to better characterize the evolution of phenotypes. The most appropriate fossils to include in an analysis varies with time as new fossils are discovered, and as taxonomy or stratigraphic estimates are revised. Hence, fossils choice is not trivial, and it behooves researchers to evaluate their fossil selection based on current knowledge rather than the taxon choice of past studies. Calibration methods in node-dating studies are not universal, with extreme heterogeneity across studies in the use of uniform versus non-uniform priors, fossils as point calibrations, fossils with and without age uncertainty, and the types of programs used to estimate divergence times [66]. Gavryushkina *et al.* [67**] reconstructed the phylogeny of penguins, accounting for the possibility that stem lineages may be direct ancestors of other lineages in their matrix [Fossilized Birth Death modeling, FBD], and found that including stem-fossil penguins greatly improved node age estimates of crown penguins.

Conclusions

The incorporation of fossils in phylogenetic reconstructions has been shown to improve crown group topology and node-age estimates, especially when the unique

Box 1 Challenges and advantages of paleoentomological preservation

As with all fossils, there are biases that influence the composition of preserved insects (e.g. [68**]); fossil deposits are inherently distorted windows into the past. Although there are numerous secondarily preserved taxa (ichnotaxa), the two most common kinds of insect fossils utilized for biogeographic, phylogenetic, and chronological inference are those impressed in rock or trapped in fossilized tree resin [amber] — each with distinct environmental requirements and temporal ranges (reviewed in [55]). The oldest hexapod fossils are silicified remains embedded in chert (Devonian [69]). Impression fossils in general typically are restricted to taxa with a high propensity toward capture in marine and lake sediments [70]. Although such fossils are often partial and wanting in detail, the oldest representatives of numerous insect groups are known from the early Mesozoic or Paleozoic impressions [Coleoptera, Diptera, Hemiptera, Hymenoptera, Lepidoptera, Odonata, and others]. While poor and partial preservation may lead to greater missing data in combined analysis procedures, there is evidence that this may not severely impact phylogenetic topology or divergence estimation [11,71]. Amber, while unmatched in preservational quality and fidelity, is more restrictive in a temporal sense: most amber deposits are dated to the Cenozoic or Cretaceous, and while there is amber from the Triassic, inclusions are exceedingly rare [72]. Therefore, complete amber fossils may lend themselves particularly well to integration in phylogenetic reconstruction in a morphological sense, however, frequently an investigator must focus on older, less complete impression fossils as these are of great value as oldest known calibration points. Nevertheless, the insect fossil record is exceedingly rich, with thousands of described taxa and many tens of thousands of specimens.

attributes of stem-fossils and crown-fossils are considered. There are still debates about aspects of combined fossil + extant taxon analyses, regarding the limited models for morphological data and choices of fossil calibration procedures, for example. With 'total evidence' dating slowly increasing in practice [Figure 1b], phylogenetic reconstructions that incorporate estimates of time will likely become more accurate and reliable. Moreover, the utilization of fossil data will improve the reconstruction of biogeographic patterns, and expand hypotheses detailing the early evolution of groups with extensive fossil histories (Box 1).

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