Incorporating fossils into hypotheses of insect phylogeny

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Abstract:

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.

Keywords:

Fossils, phylogeny, divergence time estimation, morphology, diversification, extinction.

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 favour 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 [Fig 1, Top][e.g.,6-19,20**,21-23,24**] or, more frequently [Fig 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]. 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 [Fig 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, especially when molecular data are included [Fig 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, Guillerme 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 [Fig 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 [Fig 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**].

Phylogenetic Assessment

a) Morphological Analysis

Input Data

A	0110110001
B	0110100101
C	0101100101
D [†]	0?01?1000?...

b) Combined Analysis

A	0110110001	A	AGCTACGTCC
B	0110100101	B	AGCTAGGTCA
C	0101100101	C	AGCTAGGTCA
D [†]	0?01?10001...	D [†]	??????????...

Divergence Dating Estimation

c) Node Dating

A	AGCTACGTCC
B	AGCTAGGTCA
C	AGCTAGGTCA...

D[†]

Minimum age calibration of node *

d) "Total Evidence" Tip Dating

A	0110110001	A	AGCTACGTCC
B	0110100101	B	AGCTAGGTCA
C	0101100101	C	AGCTAGGTCA
D [†]	0?01?10001...	D [†]	??????????...

D[†]

Age of terminal D acts as calibration

Variation among taxa in abundance of stem- 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- or stem- group lineage. This designation is not trivial: in node-dating analyses, crown- 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- 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- 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 attributes of stem- 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 [Fig 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: 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 [71,11]. 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.

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Figure Captions:

Fig 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.

Fig 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 $D^{\dagger}$ represent alternative user-inputed calibration constraints given the same fossil: lognormal, uniform, normal.

References:

1. Raup DM. **The role of extinction in evolution.** *Proc Natl Acad Sci USA*, **91**[15], 1994: 6758-6763.
2. Gauthier J, Kluge AG, Rowe T. **Amniote phylogeny and the importance of fossils.** *Cladistics*, **4**[2], 1988: 105-209.
3. Eernisse DJ, Kluge AG. **Taxonomic congruence versus total evidence, and amniote phylogeny inferred from fossils, molecules, and morphology.** *Mol Phylogenet Evol*, **10**[6], 1993: 1170-1195.
4. Lewis PO. **A likelihood approach to estimating phylogeny from discrete morphological character data.** *Syst Biol*, **50**[6], 2001:913-925.
5. Bybee SM, Ogden TH, Branham MA, Whiting MF. **Molecules, morphology and fossils: a comprehensive approach to odonate phylogeny and the evolution of the odonate wing.** *Cladistics*, **24**[4], 2008: 477-514.
6. Lee MSY, Oliver P, Hutchinson MN. **Phylogenetic uncertainty and molecular clock calibrations in legless lizards [Pygopodidae, Gekkota].** *Mol Phylogenet Evol*, **50**, 2009: 661–666.
7. Ware JL, Grimaldi DA, Engel MS. **The effects of fossil placement and calibration on divergence times and rates: an example from the termites [Insecta: Isoptera].** *Arthropod Struct Dev*, **39**[2], 2010: 204-219.
8. Pyron RA. **A likelihood method for assessing molecular divergence time estimates and the placement of fossil calibrations.** *Syst Biol*, **59**, 2011:185–194
9. Wood HM, Matzke NJ, Gillespie RG, Griswold CE. **Treating fossils as terminal taxa in divergence time estimation reveals ancient vicariance patterns in the palpimanoid spiders.** *Sys Biol*, **28** 2012: sys092.
10. Wood HM, Matzke NJ, Gillespie RG, Griswold CE. **Treating fossils as terminal taxa in divergence time estimation reveals ancient vicariance patterns in the palpimanoid spiders.** *Syst Biol*, **62** 2013:264–284.
11. Ronquist F, Klopstein S, Vilhelmsen L, Schulmeister S, Murray DL, Rasnitsyn AP. **A total-evidence approach to dating with fossils, applied to the early radiation of the Hymenoptera.** *Syst Biol*, **61**[6], 2012:973-999.
12. Slater GJ, Harmon LJ. **Unifying fossils and phylogenies for comparative analyses of diversification and trait evolution.** *Meth Ecol Evol*, **4**[8], 2013:699-702.
13. Schrago CG, Mello B, Soares AE. **Combining fossil and molecular data to date the diversification of New World Primates.** *J Evol Biol*, **26**[11], 2013:2438-46.

14. Alexandrou MA, Swartz BA, Matzke NJ, Oakley TH. **Genome duplication and multiple evolutionary origins of complex migratory behavior in Salmonidae.** *Mol Phylogenet Evol*, **69**[3], 2013:514-23.
15. Sharma PP, Giribet G. **A revised dated phylogeny of the arachnid order Opiliones.** *Front Genet* **5**, 2014: 255.
16. Tseng, Z. Jack, Xiaoming Wang, Graham J. Slater, Gary T. Takeuchi, Qiang Li, Juan Liu, and Guangpu Xie. **Himalayan fossils of the oldest known pantherine establish ancient origin of big cats.** In *Proc. R. Soc. B*, **281**[1774], 2014: 20132686.
17. Near TJ, Dornburg A, Friedman M. **Phylogenetic relationships and timing of diversification in gonorynchiform fishes inferred using nuclear gene DNA sequences [Teleostei: Ostariophysii].** *Mol Phylogenet Evol*, **80**, 2014:297-307.
18. Slater GJ. **Iterative adaptive radiations of fossil canids show no evidence for diversity-dependent trait evolution.** *Proc Natl Acad Sci USA*, **112**[16], 2015:4897-4902.
19. Larson- Johnson K. **Phylogenetic investigation of the complex evolutionary history of dispersal mode and diversification rates across living and fossil Fagales.** *New Phytol*, **209**[1], 2016:418-435.
20. **Winterton SL, Ware JL. **Phylogeny, divergence times and biogeography of window flies [Scenopinidae] and the therevoid clade [Diptera: Asiloidea].** *Syst Entomol*, **40**, 2015: 491–519. doi: 10.1111/syen.12117
The first combined “total evidence” approach for Diptera, which is among the largest of the insect orders. Winterton and Ware found that incorporating fossils as terminal lineages yielded younger age estimates across the phylogeny.
21. Dembo M, Nicholas JM, Mooers AØ, Collard M. **Bayesian analysis of a morphological supermatrix sheds light on controversial fossil hominin relationships.** *Proc R Soc Lond B Biol Sci*, **282**[1812], 2015: 20150943.
22. Marx FG, Fordyce RE. **Baleen boom and bust: a synthesis of mysticete phylogeny, diversity and disparity.** *R Soc Open Sci*, **2**[4], 2015:140434.
23. Arcila D, Pyron RA, Tyler JC, Ortí G, Betancur-R R. **An evaluation of fossil tip-dating versus node-age calibrations in tetraodontiform fishes [Teleostei: Percomorphaceae].** *Mol Phylogenet Evol*, **82**, 2015:131-45.
24. **Vea IM, Grimaldi DA. **Putting scales into evolutionary time: the divergence of major scale insect lineages [Hemiptera] predates the radiation of modern angiosperm hosts.** *Sci Rep*, **6**, 2016:23487.
Through combined “tip dating” analysis of scale insects (Coccothraupidae: Hemiptera), Vea and Grimaldi generate divergence date estimates for a group of insects with close relationships to their host plants. For this dataset, a combined analysis approach yielded slightly younger estimates than node calibrated analyses.
25. **Misof B, Liu S, Meusemann K, Peters RS, Donath A, Mayer C, Frandsen PB, Ware J, Flouri T, Beutel RG, Niehuis O, Petersen M, Izquierdo-Carrasco F, Wappler T, Rust J, Aberer AJ, Aspöck U, Aspöck H, Bartel D, Blanke A, Berger S, Böhm A, Buckley TR, Calcott B, Chen J, Friedrich F, Fukui M, Fujita M, Greve

C, Grobe P, Gu S, Huang Y, Jermini LS, Kawahara AY, Krogmann L, Kubiak M, Lanfear R, Letsch H, Li Y, Li Z, Li J, Lu H, Machida R, Mashimo Y, Kapli P, McKenna DD, Meng G, Nakagaki Y, Navarrete-Heredia JL, Ott M, Ou Y, Pass G, Podsiadlowski L, Pohl H, von Reumont BM, Schütte K, Sekiya K, Shimizu S, Slipinski A, Stamatakis A, Song W, Su X, Szucsich NU, Tan M, Tan X, Tang M, Tang J, Timelthaler G, Tomizuka S, Trautwein M, Tong X, Uchifune T, Walz MG, Wiegmann BM, Wilbrandt J, Wipfler B, Wong TK, Wu Q, Wu G, Xie Y, Yang S, Yang Q, Yeates DK, Yoshizawa K, Zhang Q, Zhang R, Zhang W, Zhang Y, Zhao J, Zhou C, Zhou L, Ziesmann T, Zou S, Li Y, Xu X, Zhang Y, Yang H, Wang J, Wang J, Kjer KM, Zhou X. **Phylogenomics resolves the timing and pattern of insect evolution.** *Science* **346**, 2014: 763-767.

The first transcriptomic phylogenetic reconstruction of Insecta, which resolved insect relationships among orders. Fossils were implemented as calibration points, to recover the node ages across Insecta. Age estimates were largely in congruence with the fossil record. Debate ensued after this publication, largely stemming from the author's decision to not include controversial stem roachoid fossils, the inclusion of which would have resulted in much older ages of Polyneoptera. Kjer et al. (2015) responded to criticisms regarding fossil choice.

26. Dabert M, Proctor H, Dabert J. **Higher-level molecular phylogeny of the water mites [Acariformes: Prostigmata: Parasitengonina: Hydrachnididae].** *Mol Phylogenet Evol*, **101**, 2016: 75-90.
27. Krishnankutty SM, Dietrich CH, Dai W, Siddappaji MH. **Phylogeny and historical biogeography of leafhopper subfamily Iassinae [Hemiptera: Cicadellidae] with a revised tribal classification based on morphological and molecular data.** *Syst Entomol*, **41**[3], 2016: 580-595.
28. McCulloch GA, Wallis GP, Waters JM. **A time-calibrated phylogeny of southern hemisphere stoneflies: Testing for Gondwanan origins.** *Mol Phylogenet Evol*, **96**, 2016:150-160.
29. Morita SI, Bayless KM, Yeates DK, Wiegmann BM. **Molecular phylogeny of the horse flies: a framework for renewing tabanid taxonomy.** *Syst Entomol*, **41**[1], 2016: 56-72.
30. **Schenk JJ. **Consequences of secondary calibrations on divergence time estimates.** *PloS one*, **11**[1], 2016: e0148228.
In this study, the authors evaluate the effect of using node ages from prior studies as calibration points for datasets for which the organisms do not have an appropriate fossil calibration. Their results suggest strongly that secondary fossil calibrations should not be used in the absence of fossil calibrations, and that doing so results in spurious assumptions of precision.
31. Near TJ, Meylan PA, Shaffer HB. **Assessing concordance of fossil calibration points in molecular clock studies: an example using turtles.** *Am Nat*, **165**, 2005:137–146.
32. Ho SYW, Saarma U, Barnett R, Haile J, Shapiro B. **The effect of inappropriate calibration: three case studies in molecular ecology.** *PLoS ONE* **3**, 2008: e1615.

33. Ho SYW, Phillips MJ. **Accounting for calibration uncertainty in phylogenetic estimation of evolutionary divergence times.** *Syst Biol*, **58**, 2009:367–380.
34. Ksepka, DT, Benton, MJ, Carrano, MT, Gandolfo, MA, Head, JJ, Hermesen, EJ, Joyce, WG, Lamm, KS, Patané, JSL, Phillips, MJ, Polly, PD, Ware, JL, Warnock, RCM, van Tuinen, M, Parham, JF. **Synthesizing and databasing fossil calibrations: divergence dating and beyond.** *Biol Lett*, 2011: DOI: 10.1098/rsbl.2011.0356.
35. Parham JF, Donoghue PC, Bell CJ, Calway TD, Head JJ, Holroyd PA, Inoue JG, Irmis RB, Joyce WG, Ksepka DT, Patané JS. **Best practices for justifying fossil calibrations.** *Syst Biol*, **61**[2], 2011: 346-359.
36. Ksepka DT, Parham JF, Allman JF, Benton MJ, Carrano MT, Cranston KA, Donoghue PC, Head JJ, Hermesen EJ, Irmis RB, Joyce WG. **The Fossil Calibration Database—A new resource for divergence dating.** *Syst Biol*, **64**[5], 2015:853-9.
37. Hennig W [Ed]: *Die stammesgeschichte der Insekten*. Waldemar Kramer; 1969.
38. Hennig W [Ed]: *Insect phylogeny*. John Wiley & Sons Ltd.; 1981.
39. Simpson GG [Ed]: *Principles of animal taxonomy*. Columbia University Press; 1961.
40. Huelsenbeck JP. **When are fossils better than extant taxa in phylogenetic analysis?** *Syst Biol*, **40**[4], 1991: 458-469.
41. Donoghue MJ, Doyle JA, Gauthier J, Kluge AG, Rowe T. **The importance of fossils in phylogeny reconstruction.** *Annu Rev Ecol Syst*, 1989: 431-460.
42. Lee MS. **Convergent evolution and character correlation in burrowing reptiles: towards a resolution of squamate relationships.** *Biol J Linn Soc*, **65**[4], 1998: 369-453.
43. Wiens JJ. **Missing data, incomplete taxa, and phylogenetic accuracy.** *Syst Biol*, **52** 2003:528–538.
44. Wiens JJ. **Paleontology, phylogenomics, and combined-data phylogenetics: can molecular data improve phylogeny estimation for fossil taxa?** *Syst Biol*, **58** 2009:87–99.
45. Wiens JJ. **Can incomplete taxa rescue phylogenetic analyses from long-branch attraction?.** *Syst Biol*, **54**[5] 2005:731-42.
46. Manos PS, Soltis PS, Soltis DE, Manchester SR, Oh S-H, Bell CD, Dilcher DL, Stone DE. **Phylogeny of extant and extinct Juglandaceae inferred from the integration of molecular and morphological data sets.** *Syst Biol*, **56**, 2007:412–430.
47. Matallón S. **Using fossils to break long branches in molecular dating: a comparison of relaxed clocks applied to the origin of angiosperms.** *Syst Biol*, **59** 2010:384–399.
48. Nylander JA, Ronquist F, Huelsenbeck JP, Nieves-Aldrey J. **Bayesian phylogenetic analysis of combined data.** *Syst Biol*, **53**[1], 2004: 47-67.
49. **Guillerme T, Cooper N. **Effects of missing data on topological inference using a Total Evidence approach.** *Mol Phylogenet Evol*, **94**, 2016:146-58.

- Guillerme and Cooper explore the impact of missing data on simultaneous analysis of molecular and morphological data. In doing so, the authors suggest that missing morphological data for extant taxa may have the greatest impact on accurate phylogenetic reconstruction.
50. **O'Reilly JE, dos Reis M, Donoghue PC. **Dating Tips for Divergence-Time Estimation.** *Trends Genet*, **31**[11], 2015: 637-650.
This study was transformative, as it evaluated how fossil calibration uncertainty affected divergence time estimate uncertainty. The authors suggest that both node and tip calibrations can and should be used in concert when applicable for a potential increase in accuracy and precision.
 51. **O'Reilly JE, Donoghue PCJ. **Tips and nodes are complementary not competing approaches to the calibration of molecular clocks.** *Biol Lett* **12**[4], 2016: 20150975.
This study builds upon O'Reilly et al. (2015), in evaluating the impact of combining tip calibrations and node calibration when estimating divergence time. As in O'Reilly et al., (2015), the authors suggest that both node and tip calibrations can and should be used in concert when applicable for a potential increase in accuracy and precision.
 52. Carle FL. **The wing vein homologies and phylogeny of the Odonata: a continuing debate.** *Societas Internationalis Odonatologica Rapid Communications*, **4**, 1982:1–66.
 53. Li JK, Nel A, Zhang XP, Fleck G, Gao MX, Lin L, Zhou J., **A third species of the relict family Epiophlebiidae discovered in China [Odonata: Epiproctophora].** *Syst Entomol*, **37**[2], 2012: 408-412.
 54. Carle FL. **A new Epiophlebia [Odonata: Epiophlebioidea] from China with a review of epiophlebian taxonomy, life history, and biogeography.** *Arthropod Systematics & Phylogeny*, **70**[2], 2012: 75-83.
 55. Grimaldi D, Engel MS [Eds]. **Evolution of the Insects.** 2005; Cambridge University Press.
 56. Legendre F, Nel A, Svenson GJ, Robillard T, Pellens R, Grandcolas P. **Phylogeny of Dictyoptera: dating the origin of cockroaches, praying mantises and termites with molecular data and controlled fossil evidence.** *PloS one*, **10**[7], 2015:e0130127.
 57. Tong KJ, Duchêne S, Ho SY, Lo N. **Comment on “Phylogenomics resolves the timing and pattern of insect evolution”.** *Science*, **349**[6247], 2015:487-487.
 58. Kjer KM, Ware JL, Rust J, Wappler T, Lanfear R, Jermiin LS, Zhou X, Aspöck H, Aspöck U, Beutel RG, Blanke A, Donath A, Flouri T, Frandsen PB, Kapli P, Kawahara AY, Letsch H, Mayer C, McKenna DD, Meusemann K, Niehuis O, Peters RS, Wiegmann BM, Yeates DK, von Reumont BM, Stamatakis A, Misof B. **INSECT PHYLOGENOMICS. Response to Comment on Phylogenomics resolves the timing and pattern of insect evolution,** *Science*. **349**[6247], 2015:487. doi: 10.1126/science.aaa7136.

59. Barden P, Grimaldi DA. **Adaptive radiation in socially advanced stem-group ants from the Cretaceous.** *Curr Biol*, **26**[4], 2016: 515-521.
60. Perrichot V, Wang B, Engel MS. **Extreme morphogenesis and ecological specialization among Cretaceous basal ants.** *Curr Biol*, **26**[11], 2016: 1468-1472.
61. Engel MS, Barden P, Riccio ML, Grimaldi DA. **Morphologically specialized termite castes and advanced sociality in the Early Cretaceous.** *Curr Biol*, **26**[4], 2016: 522-530.
62. Blagoderov V, Grimaldi D. **Fossil Sciarioidea [Diptera] in Cretaceous ambers, exclusive of Cecidomyiidae, Sciaridae, and Keroplatidae.** *Am. Mus. Nov.* 2004:1-76.
63. Archibald SB, Cover SP, Moreau CS. **Bulldog ants of the Eocene Okanagan Highlands and history of the subfamily [Hymenoptera: Formicidae: Myrmeciinae].** *Ann Entomol Soc Am*, **99**[3], 2006: 487-523.
64. Krishna K, Grimaldi DA, Krishna V, Engel MS. **Treatise on the Isoptera of the world.** *Bull Am Mus Nat Hist* **377**, 2013: 1-2704.
65. Wright AM, Hillis DM. **Bayesian analysis using a simple likelihood model outperforms parsimony for estimation of phylogeny from discrete morphological data.** *PloS one* 9[10], 2014: e109210.
66. Warnock RC, Parham JF, Joyce WG, Lyson TR, Donoghue PC. **Calibration uncertainty in molecular dating analyses: there is no substitute for the prior evaluation of time priors.** *Proc R Soc Lond B Biol Sci*, **282**, 2014: 20141013. doi:10.1098/rspb.2014.1013 pmid:25429012
67. ****Gavryushkina A, Heath TA, Ksepka DT, Stadler T, Welch D, Drummond AJ. Bayesian total evidence dating reveals the recent crown radiation of penguins.** *arXiv preprint* 2015: arXiv:1506.04797.
By applying a Bayesian fossilised birth-death model that incorporates molecular and morphological data, Gavryushkina and colleagues explore the impact on divergence estimation of allowing a fossil to be sampled as a direct ancestor, instead of solely as a terminal taxon. In doing so, the authors suggest that crown-group penguins diversified more recently than others had previously estimated.
68. ****Kraemer MM, Kraemer AS, Stebner F, Bickel DJ, Rust J. Entrapment bias of arthropods in Miocene amber revealed by trapping experiments in a tropical forest in Chiapas, Mexico.** *PloS one*. **10**[3] 2015:e0118820.
Through comparison of various insect trapping techniques in a modern ecosystem with the faunal composition of an amber deposit in Chiapas, Mexico, Solórzano Kraemer and colleagues demonstrate inherent biases in fossil preservation. Ultimately, the preservation of taxa trapped in fossil resins is dependent on behavioral and ecological traits, and common fossil insects may not necessarily have been common in paleoenvironments.
69. Engel MS, Grimaldi DA. **New light shed on the oldest insect.** *Nature*, **427**[6975], 2004: 627-630.
70. Shear WA, Kukalová-Peck J. **The ecology of Paleozoic terrestrial arthropods: the fossil evidence.** *Can J Zool*, **68**[9], 1990:1807-1834.

71. Wiens JJ, Kuczynski CA, Townsend T, Reeder TW, Mulcahy DG, Sites JW. **Combining phylogenomics and fossils in higher-level squamate reptile phylogeny: molecular data change the placement of fossil taxa.** *Syst Biol*, **59**[6], 2010: 674-688.
72. Schmidt AR, Jancke S, Lindquist EE, Ragazzi E, Roghi G, Nascimbene PC, Schmidt K, Wappler T, Grimaldi DA. **Arthropods in amber from the Triassic Period.** *Proc Natl Acad Sci USA*, **109**[37], 2012:14796-14801.
73. **Kraemer MMS, Kraemer AS, Stebner F, Bickel DJ, Rust J. **Entrapment bias of arthropods in Miocene amber revealed by trapping experiments in a tropical forest in Chiapas, Mexico.** *PloS one*, **10**[3], 2015: p.e0118820.
74. Ksepka DT, Ware JL, Lamm KS. **Flying rocks and flying clocks: disparity in fossil and molecular dates for birds.** *Proc R Soc Lond B Biol Sci*, **281**[1788], 2014: 20140677.