

## TECHNICAL RESPONSE

## INSECT PHYLOGENOMICS

# Response to Comment on “Phylogenomics resolves the timing and pattern of insect evolution”

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Tong *et al.* comment on the accuracy of the dating analysis presented in our work on the phylogeny of insects and provide a reanalysis of our data. They replace log-normal priors with uniform priors and add a “roachoid” fossil as a calibration point. Although the reanalysis provides an interesting alternative viewpoint, we maintain that our choices were appropriate.

**W**e welcome reanalyses of our data (1), yet we disagree with several key points raised by Tong *et al.* (2). Regarding priors, they state that “uniform priors...better [reflect]... the uncertainty in fossil evidence.” Although priors can greatly influence node-age estimates, uniform priors are not necessarily “conservative” either; more testing is needed for large data sets. Perhaps our priors were restrictive (as priors may be). Our log-normal priors affected our posterior estimates. For example, the internode leading to Polyneoptera in our figure 1 (1) appears longer than the corresponding internode in our maximum-likelihood trees, and this was likely influenced by our priors.

An informative discussion of the influence of priors on node ages is welcome, as are comments on our presentation. It is not a matter of controversy that priors can influence posterior estimates, but what needs to be demonstrated here is that the priors suggested by Tong *et al.* yield more reliable results. Indeed the source(s) of differences between their results and those in our

paper remain unclear. To permit a more direct comparison, multiple analyses are necessary, using the same software and with the additional calibration point suggested by Tong *et al.*, first using our priors and then using their uniform priors.

Tong *et al.* used MCMCTREE, which tends to estimate older ages with broader confidence intervals than BEAST (used in our paper), especially at the root (3). The error bars in Tong *et al.* are very narrow, which illustrates a difference that we find puzzling. Additionally, MCMCTREE requires a user-specified soft maximum constraint at the root of the tree. Tong *et al.* do not indicate whether this was the same as ours. It is also unclear how thoroughly they evaluated convergence among the runs of their analyses. Two, and preferably more, chains should have been used to evaluate convergence. These data [i.e., analyses with several priors, detailed confidence intervals (CIs) for each node, and convergence values] are vital in an evaluation of the accuracy of node ages.

According to our estimates, stem dictyopterans extend back to only 287 million years ago (Ma). Tong *et al.* are particularly critical of this conclusion, noting that the “diversification of this clade...after the Permian...is inconsistent with the presence of fossil roachoid representatives from the Carboniferous.” Indeed, if Carboniferous “roachoids” are shown to be dictyopterans, then our dates are underestimates. Any ambiguity is in classifying these fossils. The origin of Polyneoptera may extend back to 335 Ma, considering our CIs. Therefore, if “roachoid” fossils are not stem dictyopterans, but rather, generalized polyneopterans, then our estimates fit the fossil record (our age estimates range from 307 to 195 Ma).

Adding different fossils to an analysis may change the results. The synapomorphies for Bashkirian “roachoids” mentioned by Tong *et al.* have been interpreted in contradictory ways by different (and even the same) authors. They have not been evaluated in a formal phylogenetic analysis, so their placement is unclear. Following strict criteria (4), all of our calibration points were selected in light of modern phylogenetic analyses. We excluded fossils whose phylogenetic placement is highly speculative or founded only on older catalogs or compendia. Much of the disagreement between Tong *et al.* and our work stems from whether a particular “roachoid” fossil is, or is not, a stem dictyopteran. If we reject the use of tenuously identified fossils as calibration points, perhaps we can use our data to shed some light on the identity of these fossils. Our dating analysis suggests that these fossils are not stem dictyopterans.

Tong *et al.* are also critical of our findings on the timing of the origin of lice. Of the five papers reporting the discovery of a “fossil louse,” only one (5) describes an unambiguously placed Cenozoic phthirapteran. We calculate the origin of extant Phthiraptera to have coincided with the radiations of modern bird and mammal orders—the contemporary hosts of parasitic lice. Tong *et al.* do not differentiate between the origin of parasitic lice with their extant diversification. We agree that our conclusion that parasitic lice did not evolve on feathered nonavian dinosaurs (theropods) might have been premature. It is difficult to tell the difference between feathered nonavian dinosaurs and early birds (which are also theropod dinosaurs). Feathered nonavian dinosaurs could have existed until the end of the Cretaceous. Therefore, our estimated split between book lice and stem lineage representatives of parasitic lice between 102 and 116 Ma is compatible with the hypothesis that the earliest parasitic lice fed on feathered nonavian dinosaurs. Regardless, our work finds that modern lice diversified after the Cretaceous. What the stem lineage fed upon is currently only speculation.

Tong *et al.* state that Lepidoptera and Diptera appeared 100 million years earlier than we estimate. Their comparison refers to the crown group, but our figure 2 (nodes 86 and 100) shows that the timing of the origin of the stem lineage of both orders encompasses their estimates (1). Indeed, analyses of Triassic dipteran fossils from Virginia (6) indicate that crown-group Diptera is older than our estimates. Clearly, the origin of Diptera is in need of further analyses. One strength of our analysis is that many of our node-age estimates are within the range of known fossils. Detailed phylogenetic analyses of known fossil groups can now potentially be used to falsify our results and/or further focus paleontological and molecular research.

In conclusion, we disagree with Tong *et al.*'s premise that they used fewer assumptions. They implemented strong assumptions about the identity of fossils and the understudied influence of node-age priors. The effect of priors was not thoroughly tested and should be more thoroughly

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evaluated. Most of Tong *et al.*'s results confirm our results, and differences can be explained by opposing opinions or analytical choices that are not directly comparable. We described clear and consistent, repeatable justifications for our choices. What would be required for an improved critique would be a thorough analysis regarding the use of priors and a consistent usage of criteria justifying placement of fossils that we excluded.

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