insects are the most diverse group of animals on Earth, occupying a broad range of niches across ecosystems. However, many insect groups have a poor fossil record, meaning that the early evolution of hexapod orders remains shrouded in mystery (1). For this reason, studies of insect diversification, the timing of evolutionary innovations, and the arthropod phylogeny rely on molecular date estimates. This places considerable importance on the estimate obtained by Misof et al. (2).

Here, we examine the robustness of the date estimates reported by Misof et al. (2). The accuracy of evolutionary date estimates relies on several factors, including the estimation of the phylogenetic tree topology, branch lengths, and the calibrating information (3). The analysis of genome-scale data means that there is relatively little uncertainty in the estimates of the tree topology and branch lengths (4). However, the choice of calibrations will affect the accuracy of molecular date estimates.

The molecular-clock analysis of Misof et al. (2) was calibrated with a range of fossils throughout the insect phylogeny. These fossils were chosen on the basis of criteria relating to the confidence in their phylogenetic placement and isotopic dating (5). However, some key polyneopteran fossils were omitted from the analysis. For example, numerous “roachoid” fossils from the late Carboniferous period (~315 million years ago (Ma)) are widely considered more closely related to Dictyoptera (cockroaches, termites, and mantids) than to insects of any other order (1, 6, 7). The close relationship between these stem group dictyopteran roachoids and extant Dictyoptera was inferred on the basis of synapomorphies that include pronotum shape, tegminous forewings, and wing venation (8). Misof et al. estimated an origin of 250 Ma for the clade comprising Dictyoptera, Phasmatoidea (stick insects), Embioptera (webspinners), Grylloblattodea (ice crawlers), Mantophasmatodea (gladiators), and Orthoptera (crickets and katydids), corresponding to node 130 in figure 1 in (2). The diversification of this clade is thus presumed to have occurred after the Permain mass extinction, but this is inconsistent with the presence of fossil roachoid representatives from the Carboniferous.

We also investigated the dating analysis of Misof et al. Although they followed sound criteria for choosing their fossil calibrations, their approach to implementing these calibrations was less conservative. For 20 nodes in the insect phylogeny, the fossil evidence was summarized in the form of a log-normal prior on the age of the clade to which the fossil was assigned. As with all calibration priors, this is a quantitative statement of the relationship between the age of a clade and the timing of its earliest appearance in the fossil record (8). This relationship is typically very difficult to quantify, even for groups with multiple fossils in the Carboniferous (~315 Ma) or older. Although they followed sound calibration schemes, Misof et al. (2) and others (9) have noted the difficulty of assigning fossils to specific nodes and the challenges of interpreting their meaning.

Date estimates were obtained from each data set using Markov chain Monte Carlo (MCMC) sampling. We drew a total of 20,000 samples from the posterior, sampling every 50th step after a discarded burn-in of 100,000 steps. If this did not yield an effective sample size of at least 200 for each parameter, we doubled the number of MCMC steps until we reached sufficient sampling. Two independent replicates of the analysis converged on the same date estimates; we present the results of only one analysis here.

Fig. 1. Bayesian estimates of the insect evolutionary time scale using three different calibration schemes. (A) Calibration scheme of Misof et al., which comprised 37 age constraints and 20 log-normal priors. In place of the latter, we used uniform priors with soft bounds (13) chosen to match the 95% highest prior densities of the log-normal calibrations used by Misof et al. (B) Conservative calibration scheme. All calibrations were treated as soft minimum-age constraints, with soft maximum-age constraints as shown in table S9 of (2). (C) Conservative calibration scheme with additional constraint determined on the basis of multiple Carboniferous (~315 My old) roachoid fossils, corresponding to node 125 in figure 1 of (2). All node times represent the mean estimates from 105 data metapartitions identified by Misof et al. Shading indicates the variation in date estimates for corresponding nodes in the three trees; the error bars indicate 95% credibility intervals for these nodes.

Date estimates were obtained from each data set using Markov chain Monte Carlo (MCMC) sampling. We drew a total of 20,000 samples from the posterior, sampling every 50th step after a discarded burn-in of 100,000 steps. If this did not yield an effective sample size of at least 200 for each parameter, we doubled the number of MCMC steps until we reached sufficient sampling. Two independent replicates of the analysis converged on the same date estimates; we present the results of only one analysis here.
Fig. 2. Bayesian estimate of the insect evolutionary time scale using the 37 fossil minimum-age constraints of Misof et al. (black circles) and an additional roachoid fossil–based constraint in the polyneopteran clade (black star). Horizontal blue bars denote 95% credibility intervals of node-time estimates. Major clades are labeled on the right of the tree. First appearances of notable clades are shown below the time scale.
with detailed fossil records. In fact, the parameterization of the log-normal priors by Misof et al. embodies an expectation that the age of each calibrated node is only 7.4 million years (My) older than the earliest fossil appearance of any of its descendants. Moreover, the prior density allows only a 2.5% probability of the node being >19.7 My older than the earliest fossil. The potential effect of using log-normal priors for their calibrations is that the ages of nodes are more likely to be underestimated. A more conservative approach, using uniform priors, provides a better reflection of the uncertainty in fossil evidence.

We reanalyzed the genomic data of Misof et al. with a Bayesian dating approach in MCMCTREE (9), which is able to use an approximate likelihood calculation to reduce computational burden (10). This allowed us to investigate the effects of different calibration treatments, which was not possible using the computationally intensive approach employed by Misof et al. First, we emulated their analysis by matching their implementation of 37 fossil-based calibrations, of which 20 were specified as highly restrictive age priors (Fig. 1A). We then reanalyzed the data using less restrictive uniform priors across the 37 nodes. This calibration scheme yielded older estimates of evolutionary divergence times (Fig. 1B). Finally, we added a calibration within the polyneopteran clade (node 125 of figure 1 in (2)) on the basis of late Carboniferous roachoid fossils (from ~315 Ma (6, 7) that had not been included in the original analysis. The inclusion of this calibration led to a further increase in the estimates of node times, whether the calibrations were treated as highly restrictive age priors (results not shown) or as less restrictive uniform priors (Fig. 1C).

Our revised estimate of the insect evolutionary time scale calls for reinterpretation of some of the conclusions drawn by Misof et al. First, the origin of Polyneoptera is estimated at ~380 Ma [95% confidence interval (CI) 367 to 408 Ma], ~80 My earlier than in the original analysis (Fig. 2). Our estimate is more consistent with the widespread increase in fossils allied with Polyneoptera during the Carboniferous (1, 7). Our estimate is consistent with the hypothesized close relationship between several Carboniferous fossils and particular polyneopteran groups, such as oedischoids and Orthoptera (1).

We infer that parasitic lice evolved ~120 Ma (95% CI 87 to 166 Ma), compared with the estimate of ~56 Ma by Misof et al. Our estimate is congruent with the hypothesis that parasitic lice evolved on feathered theropod dinosaurs (11). Finally, we estimate the ages of the megadiverse orders Diptera (flies) and Lepidoptera (butterflies and moths) at ~266 and ~263 Ma, respectively. These are ~100 My earlier than those of Misof et al. Our estimates are consistent with the fossil record (1, 6, 12) and challenge the hypothesis that these two orders diversified contemporaneously with angiosperms.

Our results demonstrate the effect of relaxing some of the assumptions made by Misof et al. and provide alternative scenarios for the time scale of insect evolution. We expect that the insect evolutionary time scale will continue to be revised in the light of fossil discoveries, developments in molecular-clock methods, and new data sets of the caliber generated by Misof et al. This will lead to ongoing improvement of our understanding of this important group of animals.

REFERENCES AND NOTES

ACKNOWLEDGMENTS
This work was supported by Australian Research Council Discovery Project Grants DP1097265 to N.L. and DP110100383 to S.Y.W.H.

22 December 2014; accepted 8 June 2015
10.1126/science.aaa5460
Comment on "Phylogenomics resolves the timing and pattern of insect evolution"
K. Jun Tong, Sebastián Duchêne, Simon Y. W. Ho and Nathan Lo

Science 349 (6247), 487.
DOI: 10.1126/science.aaa5460

Use of this article is subject to the Terms of Service