Supplemental information

Reply to Phillips et al.

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1. Exclusion of Third Codon Positions and Estimation of Divergence Times

Phillips et al. [S1] criticize that the 3rd codon positions (CPs) of the mitochondrial protein-coding genes are highly saturated in our phylogeny, and that this resulted in an underestimation of deep divergences relative to shallow divergences. To further explore this we have performed a reanalysis run in BEAST v1.7.2 [S2] using our original dataset and assumptions [S3] but excluding 3rd CPs, to test if their exclusion influences the estimation of divergence times. Following reanalysis, estimates do not differ substantially (Fig. 1), with the exclusion of 3rd CPs generating a divergence time of 72.3 mya (50.5-97.3 95% highest posterior distribution, HPD) for the node of importance. On the other hand, saturation of 3rd CPs does become a more serious issue when deeply divergent amphipod outgroups are included, as in the case for the analyses of Phillips et al. [S1].

2. Models of Rate Variation

A posterior simulation-based analog of Akaike's information criterion through Markov chain Monte Carlo (AICM) [S4] indicates that single uncorrelated lognormal rate distributions (UCLD) per codon site are superior to random local clocks models (RLC) (single lognormal distributions for 1st and 2nd CPs vs. RLC for 1st and 2nd, AICM 114862.08 vs. 114961.55; single lognormal distributions for 1st, 2nd and 3rd CPs vs. RLC for 1st, 2nd and 3rd CPs, AICM 250011.79 vs. 250208.53). Notwithstanding, we computed divergences, either including or excluding 3rd CPs, and assuming a random local clock model. This resulted in a decrease of divergence times approaching the minimum HPD values obtained in other analyses (Fig. 1), that are nevertheless still compatible with our previous estimates as well as our biological interpretation of the latter.
3. Calibration Issues and Outgroups

In addition to the sequences of the two amphipods we obtained (Pseudoniphargus daviui and Bahadzia jaraguensis), Phillips et al. add other non-metacrangonyctid amphipod outgroups to our original alignment, namely Genbank mitochondrial sequences from Caprella mutica and C. scaura [S1]. They also include sequences of two isopods (Armadillidium vulgare and Ligia oceanica), two decapods (Penaeus monodon and Scylla tranquebarica) and a hoplocarid (Squilla mantis). They then apply an inferred date of separation of the Subclasses Eumalacostraca (to which the Amphipoda, Isopoda and Decapoda, among others, belong) and Hoplocarida derived from fossil information to calibrate their tree.

We argue that the use of the divergence between these two crustacean Subclasses as a calibration point, based on fossil dating, largely drives the discrepancy between their divergence date estimate and ours. We consider that such calibrations may be biased due to the inappropriate placing of the Phreatoicidean fossil (see main text and Fig. S1). We have commented already in the main section on the condition of Hesslerella, a Palaeozoic fossil isopod assigned to the suborder Phreatoicidea [S5]. The fact that it is not included in any living family does not invalidate its phreatoicidean condition, and we do not find any basis in the literature to consider it as a "stem isopod" as Phillips et al. do in their reply (see [S6]). In addition, recent mitogenomic data certainly supports Phreatoicidea at a basal position within the Isopoda [S7]. Surprisingly, Phillips et al. did not include in their analyses the mitogenome of Eophreatoicus, a member of the Phreatoicidea and thus of the lineage of isopods with the oldest fossil record [S6-S8]. We have performed a preliminary phylogenetic analysis including Eophreatoicus plus all isopodan and amphipodan mitogenomes available in nucleotide databases. In this analysis, we have used the node leading to Eophreatoicus (assigning it the age of Hesslerella) to calibrate the tree. This analysis produced age estimates for the metacrangonyctids that were compatible with our initial calculations based on palaeogeographical considerations.

Both hoplocaridans and eucarids would represent in principle useful taxa to provide deep calibrations given their old fossil record and high number of mitogenomes available. We think, however, that the inclusion of more distant outgroups other than isopods (the only peracarids aside
amphipods for which mitogenomes are currently available), could produce considerable overestimations in deep-age calibrations and these are by no means "directly scalable" as Phillips et al. assume in their reply. Peracarids display rather high mitochondrial nucleotide substitution rates compared to both hoplocaridans and eucarids (Decapoda+Euphausiacea) [S9], and consequently long tree branches. This has been related to the high number of gene rearrangements affecting the mitogenome of peracarids such as changes in strand bias, replication origins and inversion of genes [S7]. This makes mitogenome-based deep phylogenies of Crustacea unreliable, and particularly for estimation of divergence times.

Most of the available amphipod fossils preserved in Eocene Baltic amber dated at 54–40 million years (my) can be assigned to modern genera within the freshwater families Niphargidae (*Niphargus*) and Crangonyctidae (*Synurella*) [S10], a feature that lends credence to the extreme persistence (i.e., at geological time scales) of *Metacrangonyx* lineages. Nevertheless, since the age of the known amphipod fossils does not extend to deeper calibration times, we used two relatively recent palaeogeographic events that affected the Moroccan High-Atlas (37.2–25.0 mya) and the Balearic Islands in the Mediterranean (16–5.5 mya) to calibrate our tree [S3]. Using these calibrators we obtained an average long-term pairwise sequence divergence of 10.9% per million years for *Metacrangonyx* [S3], a rate that would increase by at least 4-fold assuming the fossil calibration proposed by Phillips et al. [S1].

References


Figure S1. Pancrustacean phylogenetic tree.

Simplified tree depicting the major Crustacean relationships and the relevant clades mentioned in the text. Also shown are the phylogenetic position of Hesslerella according to Phillips et al. and according to more accurate taxonomic interpretations.