**High extinction rates and non-adaptive radiation explains patterns of low diversity
and extreme morphological disparity in North American blister beetles (Coleoptera,
Meloidae)**

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Abstract

Untangling the relationship between morphological evolution and lineage diversification is key to explain global patterns of phenotypic disparity across the Tree of Life. Few studies have examined the relationship between high morphological disparity and extinction. In this study, we infer phylogenetic relationships and lineage divergence times within Eupomphini (Meloidae), a tribe of blister beetles endemic to the arid zone of North America, which exhibits a puzzling pattern of very low species richness but wild variation in morphological diversity across extant taxa. Using Bayesian and maximum likelihood inference, we estimate diversification and phenotypic evolutionary rates and infer the time and magnitude of extinction rate shifts and mass extinction events. Our results suggest that Eupomphini underwent an event of ancient radiation coupled with rapid morphological change, possibly linked to the loss of the evolutionary constraint in the elytral shape. Subsequent a high extinction background associated to Miocene-Pliocene transition decimated the diversity within each major clade, resulting in the species-poor genera observed today. Our study supports a connection between high extinction rates and patterns of decoupled phenotypic evolution and lineage diversification, and the possibility of a radiation in the absence of ecological release.

Key words

Body-shape, high extinction rates, Eupomphini, extinction rates, non-adaptive radiation, phenotypic disparity.

1. Introduction

Explaining the mechanisms and factors behind the extraordinary variation in rates of diversification (i.e., unequal species numbers) and phenotypic evolution (i.e., morphological diversity) observed across the Tree of Life, is a major subject in Evolutionary Biology (Darwin, 1859; Erwin, 2007; Eastman et al., 2011; Rabosky and Adams, 2012). Classic evolutionary models propose the existence of a positive correlation between species diversification and phenotypic evolution, with large clades exhibiting more morphological variation than species-poor lineages (*e.g.* Eastel, 1990; Meyer et al., 1990; Pennell et al., 2014). Some recent studies, however, have shown that the amount of phenotypic change is not always correlated with species diversification (Harmon et al., 2003; Slater et al., 2010).

Groups where morphological disparity, i.e., differences in body shape or "bauplan" *sensu* Gould, (1991), is in conflict with the expected levels of species diversity represent ideal models to test the link between lineage diversification (speciation minus extinction rates) and trait evolution (Sanderson and Donoghue, 1996; Rabosky and McCune, 2010; Adams et al., 2009; Lee et al., 2013). Studies on these groups support the long-held tenet that significant morphological change can occur in short time scales (Mayr, 1954; Eldredge and Gould, 1972). Lineage diversification coupled with rapid phenotypic evolution has often been linked to ecological release (Osborn, 1902; Schluter, 2000, 2001; Gavrillets and Vose, 2005), in which the colonization of a new region with different environmental conditions promotes the evolution of novel traits with subsequent speciation (Glor, 2010; Yoder et al., 2010). Other studies, however, have argued that it is the capacity of a lineage to evolve novel phenotypes through different intrinsic mechanisms (genetic, epigenetic, etc.), which triggers species diversification (Adamowicz et al., 2008; Pigliucci, 2008). In this case, diversification is a consequence of intrinsic evolvability ("non-adaptive";

Gittenberger, 1991; Rundell and Price, 2009). Yet, few examples to date have provided support for this hypothesis.

Diversification studies have mainly focused on rapid events of speciation (i.e., species radiations), and the factors driving them such as niche evolution or morphological key innovations (e.g., Lagomarsino et al., 2016). Although extinction is seen as a positive and constructive evolutionary force in paleontology (Raup, 1991; Benton, 2009), difficulties to infer extinction rates from neontological data (Rabosky, 2009) have made it less often the subject of such studies. Recently, the development of likelihood methods to estimate changes in diversification rates and the time and magnitude of mass extinction events from phylogenies containing only extant taxa (Stadler, 2011A; May et al. 2015) has brought renewed attention into extinction. It is now seen as a critical process, responsible for shaping the evolutionary history of individual taxa and regional biotas (Antonelli and Sanmartín, 2011; Condamine and Hilnes, 2015; Sanmartín and Meseguer, 2016). None of these studies, however, have focused on the relationship between extinction rates and phenotypic diversity.

Here, we use the North American desert blister beetles (Meloidae) of the tribe Eupomphini to explore the role of extinction in explaining patterns of low species diversity coupled with extensive morphological variation across extant taxa. Eupomphini is currently represented by only 26 described species, grouped into seven genera (Pinto, 1984). Yet, these 26 species represent an extraordinary level of morphological differentiation in complex anatomical structures within an otherwise relatively morphologically-homogeneous family (Figure 1). Other tribes of Meloidae have much higher species richness but share a generally conservative bauplan. For example, *Epicauta*, within tribe Epicautini, contains more than 370 described species (Pinto and Bologna, 2002), but most

of them exhibit the overall body shape characteristic of blister beetles: an elongate body with long legs, entire or shortened elytra, and wings that are rarely reduced or absent. In contrast, species of Eupomphini, except those within *Eupompha* LeConte 1858 and *Calospasta* LeConte 1862, exhibit strikingly dissimilar morphologies (Figure 2). This diversity is especially marked regarding the shape of elytra and abdomen, and some display also exclusive behavioral traits associated to a specific elytral morphology (Pinto, 1984). In addition to this diversity of body shapes, Eupomphini presents an unusually restricted geographic distribution among blister beetles: it is the only tribe of Meloidae that is restricted to the Nearctic region (Pinto and Bologna, 2002), with all species inhabiting arid and semiarid areas from western Mexico to southwestern USA (Pinto, 1984). Every single genus of this tribe has been reviewed from a taxonomical and ethological approach (Selander, 1964; Pinto, 1972; Pinto, 1975; Pinto 1979; Pinto 1984).

Evolutionary relationships within the tribe Eupomphini have so far been investigated using a large number of morphological and behavioral characters (Pinto, 1979; Pinto, 1984; Pinto and Bologna, 2001), or a limited set of molecular markers (Bologna et al., 2008), but relationships among genera remain poorly resolved. In this study, we use a set of five mitochondrial and nuclear molecular markers to obtain a robust phylogeny covering all seven genera and 85% of species diversity within Eupomphini. We also estimated lineage divergence times and used the resulting timetree as a template to test alternative hypotheses on the link between phenotypic disparity, species diversification and extinction rates. Specifically, we use macroevolutionary models to: (1) estimate changes in rates of speciation and extinction over evolutionary time; (2) estimate the mode and rate of phenotypic evolution by quantifying morphological change in some key traits (elytral and abdominal shape), and (3) relate phenotypic disparity among clades from the origin of the

tribe to the present with shifts in lineage diversification and mass extinction events (Labandeira, 1997; Harmon et al., 2003; Mayhew, 2007; Slater et al., 2010). Additionally, based on our phylogenetic hypothesis, we revise the taxonomy of the non-monophyletic genus *Eupompha* and reconsidered the genus *Calospasta* in agreement with LeConte's original division.

2. Materials and methods

2.1. Taxon sampling

A total of 72 specimens belonging to 22 of the 26 currently recognized species of Eupomphini were examined, covering about 85 % of the known species richness in the tribe. Six of the seven genera of Eupomphini were represented by all of their species, while eight of the 12 described species of *Eupompha* (from now on included in two separate genera, *Eupompha* and *Calospasta*) were included in our data set. We also added eight representative species of the other tribes of Meloinae (Lyttni, Mylabrini, Pyrotini, Epicautini and Meloini), as well as one species of the sister subfamily Nemognathinae (*Zonitis flava* Fabricius 1775) to root our phylogeny (Bologna et al., 2008). All taxa included in this study, their locality and GenBank accession and voucher numbers are provided in Table 1. All specimens are deposited at the Museo Nacional de Ciencias Naturales, Madrid, Spain (MNCN-CSIC), and the Colección Nacional de Insectos of the Instituto de Biología, Universidad Nacional Autónoma de México, Mexico (IB UNAM).

2.2. DNA sequencing

A tissue sample of each specimen was obtained from thoracic muscle of the hind coxa. Total genomic DNA was extracted using the “DNA Easy extraction Kit” (Qiagen®),

following the protocol described the manufacturer. We generated sequences for five gene markers with different molecular evolutionary rates to obtain phylogenetic resolution at different phylogenetic levels. For the mitochondrial (mtDNA) compartment, we sequenced 650 bp of the cytochrome oxidase I (*coxI*) marker, and 784 bp of the 16S ribosomal gene. For the nuclear compartment, we sequenced 306 bp of the histone H3 gene, 722 bp of 18S ribosomal marker, and 598 bp of the 28S nuclear ribosomal gene. These markers were selected based on their proven efficacy in previous phylogenetic studies of beetles, in particular for the superfamily Tenebrionoidea (Baselga et al., 2011; Gunter et al., 2014) and the family Meloidae (Bologna et al., 2008; Alcobendas et al., 2008; Percino-Daniel et al., 2013).

The primers and PCR protocols employed in this study are listed in Table S2. Amplification was carried out in a total volume of 15µl, with 3µl of PCR buffer, 0.1-0.2 µl of MgCl₂, 0.2 µl of each primer and 0.1µl of MyTaq polymerase (BioLine©), 3µl of DNA template and 8.3µl of ddH₂O. Unpurified PCR products were sent for sequencing to the genomics unit at IB UNAM. Sequences of the five genes were manually aligned; ribosomal markers 16s, 18s and 28s were aligned based on their secondary structure models, which were obtained through the online program ViennaRNA Package version 2.0, available at the Institute for Theoretical Chemistry, University of Vienna (<http://rna.tbi.univie.ac.at>).

2.3. Phylogenetic and relaxed molecular clock analyses

Bayesian analyses were performed with MrBayes version 3.2.6 (Ronquist et al., 2012) using a concatenated data set. Selection of the best substitution model for each marker was carried out in jModeltest version 2.7.1 (Posada and Crandall, 1998) under the Akaike Information Criterion correction (AIC). The free software PartitionFinder version 1.1.1

(Lanfear, 2012) was used to determine the optimal partition scheme for the examined markers. A total of nine unlinked partitions were selected: CsOIpos1, COIpos2, COIpos3, H3pos1, H3pos2, H3pos3, 28s, 18s, 16s. The evolutionary models selected for each analysis and partition with their best-fit model, are listed in Table S3.

MrBayes analysis consisted of two simultaneous runs of 100 million generations each, sampling trees every 10,000 generations. Mixing and convergence among runs was evaluated by checking the average standard deviation of split frequencies and the EES values and Potential Scale Reduction Factor (PSRF) for each parameter. A majority consensus tree was reconstructed after discarding the first 20,000 sampled trees as burn-in. This dataset contained 55 terminal taxa and 3059 nucleotide positions; in a few cases, we merged two specimens of the same population as a single terminal taxon to reduce the amount of missing data in the dataset.

To estimate lineage divergence times within Eupomphini, we used Bayesian relaxed molecular clocks implemented in BEAST version 1.8.2 (Drummond et al. 2012). The analysis used the concatenated mitochondrial-nuclear dataset partitioned by gene but including just one sample per species. We used as reference the relationships obtained in MrBayes with support $PP = 1$, constraining the monophyly of the following nodes:

Cysteodemus + *Tegrodera*, *Phodaga* + *Pleuropasta* and *C. elegans* + *C. imperialis* + *C. viridis* + *C. edmundsi*. Molecular clocks were unlinked across genes, using an uncorrelated lognormal relaxed clock with the mean and standard deviation of substitution rates (subst/site/Ma) for the COI, 16S, and 28S markers following Papadopoulou et al. (2010). The *ucl.d.mean* parameter for the *coxI* marker was assigned a lognormal distribution in real space, with initial value: 0.0168, Log(Mean): 0.0168, Log(Stdev): 0.0018; the *ucl.d.mean* for 16S: lognormal distribution in real space, with 0.0054 as initial value, Log(Mean):

0.0054, Log(Stdev): 0.0009; *ucl.d.mean* for 28S: lognormal in real space, with initial value (0.0006), Log(Mean): 0.0006, Log(Stdev): 0.0003. For the remaining markers we used uninformative priors: the *ucl.d.mean* parameters for 18S and H3 were assigned a gamma distribution in real space, with initial value (0.01), shape parameter (0.01), scale (100), and Offset (0).

The birth-death model with incomplete taxon sampling (Stadler, 2009) was used as a tree prior to account for the effect of extinction and taxon sampling on tree topology and branch lengths. The analysis was run for 100 million generations, discarding the first 10,000 generations as burn-in. Inspection of the trace plots and effective sample sizes in Tracer 1.8.0 (Drummond and Rambaut, 2007) was used to assess the convergence and mixing of the MCMC runs. All analyses were run in the web public resource CIPRES Science Gateway version 3.3 (Miller et al., 2010). After discarding the burn-in, the remaining trees were employed to build a maximum-clade credibility (MCC) tree with 95% high-posterior density (HPS) credibility intervals of ages using TreeAnnotator version 1.8.2 included in the BEAST package. Phylogenetic trees were visualized with FigTree version 1.4.2 (<http://tree.bio.ed.ac.uk/software/figtree/>).

2.4. *Tempo and mode of species diversification*

Diversification analyses were based on the MCC timetree, pruned to leave just one specimen per species to avoid bias in speciation rate estimates. We first plotted the number of lineages through time with the function *ltt.plot* in the R package *ape* (Paradis et al., 2004) to visually inspect the diversification trajectory; we plotted both the BEAST MCC tree and a random sample of 650 trees from the Bayesian MCMC posterior distribution to reflect uncertainty in divergence time estimates. We then statistically evaluated the fit of

the MCC tree to alternative birth-death models: rate-constant diversification, density-dependent diversification, and discrete time-variable diversification using the whole-tree likelihood algorithms implemented in the R package *TreePar* (Stadler, 2011B). This method accounts for the effect on the tree topology of incomplete taxon sampling - by incorporating the sampling fraction of the extant taxa - and the "pull-of-the-present" - an artifact of the effect of extinction on recent lineage diversification (Stadler, 2011C). The function *bd.shift.optim* was used to detect tree-wide rate shifts: changes in speciation and extinction rates that affect all clades in the tree simultaneously at discrete points in time. In particular, we estimated by maximum likelihood the time of the rate shift and the magnitude of the rate of diversification ($r = \text{speciation } (\lambda) - \text{extinction } (\mu)$), and the extinction fraction or turnover ($\epsilon = \mu / \lambda$) before and after the change. Potential mass extinction events (MEEs) - defined as a sampling event that removes part of the standing diversity at a given point in time (parameter ρ) - were also estimated using the option `MEE=TRUE`; this constrains the magnitude of diversification and turnover to be equal before and after the MEE, since discrete rate shifts in diversification cannot be distinguished from MEE sampling events by likelihood methods (Stadler, 2011C; Sanmartín and Meseguer, 2016). We tested four alternative models using likelihood-ratio tests to select the best model at 95% confidence level. 1) a pure birth (Yule) model without conditioning on a particular value of speciation rate but, conditioned on survival of extant taxa ($N=26$); 2) a birth-death model (BD), where extinction and speciation rates are estimated as constant; 3) an episodic birth-death model with one, two or three rate-shifts (BD-RS), assuming no mass extinction events (`ME=FALSE`); and 4) a mass extinction model (`ME=TRUE`), estimating the intensity of the mass extinction (ρ) for one, two or three events. For all models, we set the sampling fraction to 0.85 to account for incomplete taxon sampling (22

out of 26 species) and used a grid of discrete time at every 0.2 Ma intervals to detect potential rate shifts in the episodic birth-death models.

Alternatively, we explored the power of the Bayesian Inference framework to estimate simultaneously the timing and magnitude of changes in diversification rates and mass extinction events. We used the CoMET model (Compound Poisson Process of Mass Extinction, May et al., 2015) implemented in the R package TESS (Höhna, 2013), which uses reversible model jumping algorithms to estimate MEEs while accounting for shifts in speciation and extinction rates as nuisance parameters. As in TreePar, CoMET implements an episodic, stochastic-branching process, where speciation and extinction rates are constant between MEEs or rate-shift events (May et al. 2015). The analyses consisted of two chains of 10 millions of iterations, a sampling frequency of 100 and a minimum number of effective sample size of 1000. The shape of the prior distributions for speciation and extinction rates was estimated from the data using the argument "empiricalHyperPriors = TRUE". We set the sampling probability (sampling fraction) to 0.85, and used default values in TESS for the initial speciation rate = 2.0, initial extinction rate = 1.0, and number of expected rate changes and MEEs = two. Mixing and convergence of the two chains was assessed by estimating MCMC diagnostics in TESS (Höhna, 2013) - the Rubin-Gelman statistic and ESS values (> 500) - and by comparing posterior density plots between chains.

2.5. Morphological disparity patterns and character evolution

To explore variation in levels of morphological disparity over time and across clades, we used a set of four morphological characters that vary extensively in Eupomphini:

- 1) Elytral volume: we adjusted the elytra to an ellipse form, and calculated the volume measuring the three radii of an ellipse (width, length and depth), and then using the volume formula: $V = (4\pi/3)r_1r_2r_3$
- 2) Abdominal volume: we adjusted the abdomen to an elliptical form as above.
- 3) Elytral amplitude: we measured the angle formed by two lines; the first line was measured from the insertion point of the elytra with the prothorax to the medium point of the elytral curve. The second one was measured from the medium point of the elytral curve to the posterior apex of the elytra.
- 4) Elytral convexity: we measured the angle formed by two lines; one from the insertion point of the elytra with the prothorax to the posterior apex of elytra, and the second one from the insertion point of the elytra with the prothorax to the medium point of the elytral curve.

A single representative male specimen was measured for each species, we were not interested in measuring intraspecific variation. All characters were measured with the specimen in lateral view (Figure 3). The measurements were obtained with the program TPS version 1.14 (Rohlf, 2002), and all values log-transformed before the analysis.

We fitted various likelihood models for continuous character evolution to our dataset using the function *fitContinuous* in the R package *geiger* (Harmon et al. 2008): Brownian Motion (BM) (Felsenstein, 1973), Ornstein-Uhlenbeck model (OU) (Butler and King, 2004), Early-burst model (EB) (Harmon et al. 2010) and White-noise model (WH). The AIC test with the obtained likelihood scores was used to select the best-fit model. We then performed a morphological-disparity-through-time analysis (Harmon et al., 2003) in *geiger* to compare levels of phenotypic disparity within the tribe, among clades, and through time. For each character, the function *dtm* (*disparity-through-time*) was used to

estimate variation in morphological disparity using the average pairwise Euclidean distances between species (Harmon et al., 2003). Values near zero imply that most of the variation is partitioned among subclades, while subclades contain relatively little variation. Conversely, values near one suggest that disparity is high within subclades relative to the total disparity across the entire phylogeny, and that subclades are likely to overlap extensively in morphological space (Harmon et al., 2003). Finally, we calculated the Morphological Disparity Index (MDI), comparing phenotypic disparity simulated under a Brownian motion model - the best-fit model selected by the AIC test - against observed phenotypic disparity among and within subclades relative to total disparity at all time steps in the phylogeny (Rowe et al., 2011).

3. Results

3.1. *Phylogenetic relationships in Eupomphini*

The Bayesian phylogram derived from the concatenated dataset (Figure 4) recovered with “high” support values (PP > 0.95, Alfaro et al. 2003) the monophyly of Eupomphini (PP = 1). All ingroup genera were recovered as monophyletic with high support (PP > 95), except for representatives of former *Eupompha* (see taxonomic comments). These were instead recovered as a grade of two separate well supported clades at the base of the tribe. The first of these clades, *Calospasta* (PP= 1), is sister to the remaining species of Eupomphini, and comprises two species pairs: *Calospasta edmundsi* Selander 1953 + *C. viridis* Horn 1883 (PP= 1), and *C. elegans* (LeConte 1851) + *C. imperialis* Wellman 1912 (PP= 1). The second clade, *Eupompha*, (PP= 0.98) showed *Eupompha fissiceps* (LeConte 1858) as sister to the clade formed by *E. histrionica* (Horn 1891) and *E. schwarzi* (Wellman 1909) + *E. sulciphrons* (Champion 1892) (PP= 0.88). Other relationships were recovered with

“moderate” Bayesian clade support (> 0.70 , Alfaro et al., 2003). The *Eupompha* clade is sister to a clade including all remaining species of the tribe (PP = 0.88), all of which have some degree of elytral deformation. Within this clade (PP = 0.89), *Phodaga* LeConte 1858 and *Pleuropasta* Wellman 1909 are sister genera (PP= 1) and sister to a clade containing two subclades, one with *Megetra* LeConte 1859 + *Cordylospasta* Horn 1875 (PP= 0.66), and the other one with *Cysteodemus* LeConte 1851 + *Tegrodera* LeConte 1851 (PP= 1).

3.2. Divergence time estimates

Figure 5A shows the MCC tree obtained from the BEAST analysis with mean age estimates and 95% HPD credibility intervals for age estimates. The topology was totally congruent with the phylogenetic hypothesis based on the MrBayes concatenated analysis. The origin of Eupomphini was dated during the Early Miocene (Mean 19.91, 95% HPD 16.15-21.72 Mya). An Middle Miocene origin was estimated for the clade whose members have elytral deformation (Mean 14.89, 95% HPD 13.56-18.06 Mya). For most genera, the most recent common ancestor (MRCA) was dated as originating during the Late Miocene (*Cordylospasta*, *Megetra*, *Pleuropasta*, *Phodaga* and *Cysteodemus*), between 8.19 and 5.41 Mya. The youngest MRCA estimate belongs to *Tegrodera*, which appears to have originated during the Lower Pleistocene (Mean 1.4, 95% HPD 0.74-4.15 Mya). The oldest MRCA for genera correspond to *Eupompha*, whose origins are placed in the Early Miocene between 17.53 Mya (95%HPD 12.12-18.08). Origin of MRCA for *Calospasta* was dated in the Middle Miocene at 13.69 Mya (95%HPD 11.3-18.02).

3.3. Diversification analyses

The LTT plot showed a sigmoidal shape, with initial accumulation in the number of lineages, followed by a slowdown and a slightly uplift towards the present (Figure 5A right). This is confirmed by the *TreePar* analyses, summarized in Table 2 and Figure 5B. Likelihood ratio tests supported an episodic model with one rate shift (BD-1-S p -value = 0.99) against the constant-rate Yule and BD models. BD-2-S and BD-3-S were not significant better than BD-1-S (p -value = 0.86, p -value = 0.77 respectively). Thus, the pattern of diversification in Eupumphini shows a several decreases in net diversification rates, started with a value of $r_0 = 0.15$, following by a brutal decreased to a value of $r_{-1} = 0.02$. Conversely, we observed an increase over time in the background extinction rate, starting with $\varepsilon_0 = 3.2e-07$; then rising to $\varepsilon_{-1} = 0.01$. The time of this shift was estimated at $t = 5.35$ Mya (Figure 5B right).

Models with the ME = TRUE were not significant better than the BD-1S (Table 2) discarding mass extinction events as driver of resultant topology.

Results from CoMET showed a similar pattern (Figure 5A left), though uncertainty in parameter estimation (represented by the 95% HPD) was high: there is an initially high net diversification rate between 20-15 Ma ($r \sim 0.11$), which rapidly decreases towards the present ($r \sim 0.03$); this is concurrent with an increase in the background extinction rate from an initial value $\varepsilon \sim 0.25$, followed by an increase over time that peaks towards the present ($\varepsilon \sim 0.5$). Figure 5B (left) shows the Bayes Factor comparisons for the timing of MEE events ("mass extinction times"): CoMET detects that relative-extinction rate starts to increase at c. 5 Mya until the present, with a substantial Bayes Factor ($\ln BF = 1.9$ (May et al., 2015)). Figures S1-S4 show the MCMC diagnostics and plots of the other parameters estimated by CoMET: speciation and extinction rates, rate shifts, and MEE time estimates.

3.4. *Morphological disparity patterns and character evolution*

Our comparison of continuous trait evolutionary models in *geiger* selected the Brownian motion as the best-fit model for all characters, except for Elytral amplitude where White-Noise model was selected (Table S3). The traits elytral and abdominal volume were estimated to evolve at a rate of 0.15 for both characters, whereas elytral convexity and amplitude evolved with a slower rate of 0.01 and 0.001, respectively.

Disparity-through-time (DTT) plots were similar across all four morphological traits, with morphological diversity being higher at the first two thirds of the phylogeny, indicating that the disparity was equally distributed through subclades. There is a sharp decrease of MDI values at c. 5 Mya, suggesting that the disparity is pronounced among subclades but poor at intraclade level (Figure 6). The Morphological Diversity Index (MDI) was negative for abdominal volume, elytral convexity and elytral amplitude (-0.13, -0.9, -0.1, respectively); MDI for elytral volume was 0.008 (Figure 6).

4. Discussion

4.1. *Phylogenetic relationships in Eupomphini*

Previous phylogenetic studies had suggested the monophyly of the tribe Eupomphini either based on morphological and molecular information from two gene markers (16S and ITS2) (Pinto, 1984; Bologna and Pinto, 2001; Bologna et al., 2008). Our phylogeny - based on a much larger sample of markers - supports the monophyly of the tribe (PP > 0.95), and confirms the two morphological and behavioral adult synapomorphies proposed by Pinto (1984): adults with ventral blade of claws shorter than dorsal blade and largely adnate to it, and cleaning of antennae using only forelegs, not mouthparts.

Similarly, all genera within Eupomphini excepting former *Eupompha* were recovered as monophyletic with significant support. For almost a century, species of *Eupompha* were placed into two separate genera (*Calospasta* and *Eupompha*) (LeConte, 1862), though they were subsequently synonymized by Selander (1954). In his revision of the genus, Pinto (1979) proposed two informal sections defined by morphological characters of the first larval and adult stages. These two sections corresponded with LeConte's (1862) original division of *Calospasta* and *Eupompha*. In our phylogenetic tree, the two clades grouping the species of *Eupompha* and *Calospasta* also correspond with the sections described by Pinto (1979).

Based on the above information, we propose the formal division of *Eupompha* into two genera. The name *Calospasta* LeConte 1862 is reestablished for the section 1 of Pinto (1979), since it contains its type species, *C. elegans*. *Calospasta* is represented by six species: *Calospasta decolorata* Horn, 1894, *Calospasta elegans* (LeConte 1851), *Calospasta imperialis* Wellman 1912, *Calospasta viridis* Horn 1883, *Calospasta edmundsi* Selander 1953, and *Calospasta vizcaina* (Pinto 1983). Members of *Calospasta* are morphologically characterized by having an asymmetrical third segment of the maxillary palpi in the first larval stage (Pinto, 1979). *Eupompha*, on the other hand, corresponds to Pinto's (1979) Section 2, which contains its type species, *E. fissiceps*. The genus *Eupompha* now comprises *E. histrionica*, *E. schwarzi*, *E. terminalis* Selander 1957, *E. sulciphrons*, *E. fissiceps* and *E. wenzeli* (Skinner 1904); members of *Eupompha* are characterized by presenting a robust and short spine of the aedeagus (Pinto, 1979).

Phylogenetic relationships among genera obtained in this study do not correspond well with those proposed by Pinto (1984) and Bologna and Pinto (2001), based on morphological characters. The only point of agreement is the sister relationship between

Pleuropasta and *Phodaga*: species of these two genera share the pronotal disk somewhat inflated and bilobed at its basis (Pinto, 1984).

4.2. *Non-adaptive radiation and dramatic extinction explain patterns of low diversity and rampant phenotypic disparity in Eupomphini*

All lineages of Eupomphini share a common geographic range, and most of them overlap over a large portion of their ranges (Mohave, Sonora, and Chihuahua deserts) (Pinto, 1984). They mostly inhabit arid and semiarid zones within the physiographic "Basin and Range" province in Mexico and the USA. This province underwent a period of intense geological activity from 24 to 12 Mya, which ended with its separation from the Colorado Plateau. Initial diversification in Eupomphini (25-18 Mya, Figure 5) might have been promoted by the emergence of new landscapes - arid and semi-arid habitats - after this event, as suggested in other studies based on different animal groups (i.e., Avise, 1998; Knowles, 2000; Bryson et al., 2013). Although the opening of new habitat opportunities is an almost straight forward explanation for the taxic diversification of the group, it is questionable that the wide morphological heterogeneity shown by Eupomphini was the solely result of ecological adaptive pressures over a common ancestral bauplan. Markedly divergent evolutionary trends among close relatives sharing a common desert habitat (i.e. the unusual development of elytra in *Cysteodemus*, vs their reduction in *Megetra*), is unlikely if selection pressures drive morphological change. If local selection pressures were driving phenotypic diversification, we could expect a quite homogeneous phenotype outcome with minor morphological change across lineages in response to local variant desert conditions (as shown for example in *Berberomeloe*, a genus of Lyttini subjected to local selection for more than 8 Ma in the Iberian and Northern African semi-arid

landscapes; Percino-Daniel et al., 2013; Bravo et al., 2018). Though we cannot discard this explanation, our diversification and disparity analyses suggest a different type of scenario, in which diversification is associated to rapid morphological evolution in the absence of ecological release, a "non-adaptive" radiation *sensu* Gittenberger (1991). Both *TreePar* and CoMET (Figure 5A-B) inferred a pattern of net diversification rate that decreases over time, concomitant with an increase in background extinction rates that peaks towards the present.

The pattern of lineage radiation detected here was paralleled by a similar pattern of morphological variation in the four body-shape traits studied. A high initial disparity is observed, indicating that subclades contained early a substantial proportion of the total morphological variation, as expected in a radiation. This proportion then decreases abruptly at c. 5 My the present, with most of the variation partitioned among the extant genera and little variation within them as the morphological space becomes saturated (Figures 5B, 6). We also estimated a negative MDI value for all characters excepting the elytral amplitude (Figure 6), which is also considered evidence of a morphological radiation (Harmon et al., 2003; Cantalapiedra et al. 2017).

What could have promoted this initially rapid morphological evolution? The outcome of the evolutionary process is limited by evolutionary constraints (Alberch, 1982; Gould, 1989; Arnold, 1992). Some constraints arise in the epigenetic interactions involved during the developmental process, limiting drastically the possibilities of morphological change and restricting the set of possible bauplans (Alberch, 1982). The extreme diversification of elytral shape observed within the derived "elytral deformation" clade of Eupomphini (i.e., grouping all genera except *Eupompha* and *Calospasta*) (Figures 1, 2), suggests that a probable developmental disturbance affected the common ancestor of this

clade. A general disturbance involving the loss of the evolutionary constraint responsible for the relationship between elytral and abdominal shape in Meloidae would result in a dramatic extension of the available morphospace. This morphospace widening would open the gate for wild exploratory morphological experiments, visualized in the form of a fast radiation of morphotypes among which the extant generic forms are included (Figure 1). Further developmental studies are needed to confirm the hypothesis that the loss of evolutionary constraints in body shape drove the rapid phenotypic evolution of Eupomphini.

These process requires the existence of an evolutionary radiation followed by rapid morphological differentiation. Rapid lineage divergence contributes to the maintenance of ancestral polymorphisms among the incipient clades, and results in a loss of phylogenetic signal and poorly resolved internal nodes (Whitfield and Lockhart, 2007); this is observed in the pattern of lineage accumulation in the tribe, with low support values at the deepest nodes compared to the tip clades, and a topology with short internal versus long external branches (Fig. 4). Additionally, a fast-evolutionary rate was estimated for elytral and abdominal volume traits under the BM model, suggesting that the new forms produced along the morphological radiation were an array of morphotypes that changed almost randomly across lineages.

Evolutionary radiations are expected to show a characteristic trajectory, which corresponds to a density-dependent model for lineage diversification (Etienne et al., 2012) and an "early-burst" model for trait evolution (Harmon et al. 2008), in which initially rapid rates are followed by a slowdown towards the present. Interestingly, the diversification trajectory of Eupomphini does not fit any of these models (Figure 6, Table S3). The reason is that the morphological radiation in Eupomphini was followed by historically high

extinction rates, resulting in a tree with initially rapid evolution (17 to 15 Mya), followed by a subsequent slowdown in the rate of diversification as the morphological space became saturated (10 to 5 Mya), and a slightly uplift in the last 2 Mya (Figure 5A, B). Both *TreePar* and CoMET estimated increasingly high background extinction rates, in the Miocene-Pliocene transition (c. 5 Mya, Fig. 5A,B). At this time, the proportion of plants with C3 metabolism changed dramatically towards C4 plants, affecting the atmospheric temperature and the CO₂ proportion, and causing a deep desertification and the extinction of several living groups (Cerling et al., 1997; Ehleringer et al., 1997; MacFadden et al., 1999; Krause et al., 2008). These events would have extirpated lineages from all clades within Eupomphini, resulting in the deep divergences and extremely different morphotypes observed today across living genera (e.g., *Cysteodemus* and *Tegrodera*).

5. Conclusions

Changes in speciation and extinction rates have been considered key factors to explain phylogenetic, temporal, and spatial variation in species richness across organisms (Glor, 2010; Paradise, 2011; Morlon et al., 2011; Rabosky, 2014). Our results suggest that phenotypic evolvability – in this case the loss of the evolutionary constraint for elytral shape in Meloidae shortly after the initial divergence of Eupomphini – acted as a trigger driving morphological diversification and accelerated speciation (non-adaptive radiation) in this tribe. They also highlight the role played by historically high extinction rates, driven by abiotic factors such as climate change, to explain the evolutionary riddle posed by groups in which phenotypic disparity is decoupled from patterns of lineage diversification.

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791

792 **Table 1.** List of specimens included in this study, their localities, DNA voucher and
793 GenBank accession numbers for the molecular markers examined.
794

Voucher	Taxon	Locality	CoxI	16S	18S	28S	H3
KRN023	<i>Cordylospasta fulleri</i>	USA: California: Inyo Co.: 3 mi NE Big Pine, al inicio de la Death Valley Rd. hacia Saline Valley. 1204m.					
KRN111	<i>Cordylospasta fulleri</i>	USA: California: Inyo Co.: 3 mi NE Big Pine, al inicio de la Death Valley Rd. hacia Saline Valley. 1204m.					
KRN01	<i>Cordylospasta opaca</i>	USA: California: San Bernardino Co.: Summit Valley Rd., a 4 km del cruce con la Hwy. 138. 1148m.					
KRN112	<i>Cordylospasta opaca</i>	USA: California: San Bernardino Co.: Summit Valley Rd., a 4 km del cruce con la Hwy. 138. 1148m.					
KRN024	<i>Cysteodemus armatus</i>	MÉXICO: Baja California Norte: 14 km al O de Mexicali, cerca del cementerio.					
KRN02	<i>Cysteodemus armatus</i>	MÉXICO: Baja California Norte: 12 km al OSO de Mexicali.					
KRN025	<i>Cysteodemus armatus</i>	MÉXICO: Baja California Norte: Municipio Mexicali: Ejido Luchadores del Desierto, en el NO de la Laguna Salada. 0m.					
KRN026	<i>Cysteodemus wislizeni</i>	USA: New Mexico: Sierra Co.: 4 mi. E Hillsboro, 1593m.					
KRN03	<i>Cysteodemus wislizeni</i>	USA: New Mexico: Sierra Co.: 5 mi. N Truth or Consequences					
KRN28	<i>Cysteodemus wislizeni</i>	USA: New Mexico: Cibola Co.: 13 mi. E Laguna, 1702m.					
KRN030	<i>Calospasta elegans</i>	USA: California: Riverside Co.: Desviación de la Hwy. 371 hacia Hemmet, unas 4 mi al N de Aguanga. 871m.					
KRN113	<i>Calospasta elegans</i>	USA: California: Riverside Co.: Desviación de la Hwy. 371 hacia Hemmet, unas 4 mi al N de Aguanga. 871m.					
KRN040	<i>Calospasta elegans</i>	USA: California: Inyo Co.: 5 km N Little Lake cerca de Fossil Falls. 1025m.					
KRN021	<i>Calospasta elegans</i>	USA: California: Inyo Co.: Haiwee Reservoir North. 1191m.					
KRN114	<i>Calospasta elegans</i>	USA: California: Inyo Co.: Haiwee Reservoir North. 1191m.					
KRN038	<i>Calospasta elegans</i>	USA: California: Inyo Co.: Orilla Oeste de Owens Lake. 1100m.					
KRN115	<i>Calospasta elegans</i>	USA: California: Inyo Co.: Orilla Oeste de Owens Lake. 1100m.					
KRN029	<i>Calospasta elegans</i>	USA: California: Inyo Co.: 5 km N Little Lake cerca de Fossil Falls. 1025m.					
KRN054	<i>Eupompha fissiceps</i>	USA: New Mexico: Hidalgo Co.: 19 mi SW Lordsburg					
KRN121	<i>Eupompha fissiceps</i>	USA: New Mexico: Hidalgo Co.: 19 mi SW Lordsburg					
KRN043	<i>Eupompha fissiceps</i>	USA: New Mexico: Grant Co.: 10 mi N Hachita, 1364m.					

KRN044	<i>Eupompha fissiceps</i>	USA: New Mexico: Grant Co.: 10 mi N Hachita, 1364m.
KRN108	<i>Eupompha fissiceps</i>	USA: New Mexico: Grant Co.: 10 mi N Hachita, 1364m.
KRN045	<i>Eupompha fissiceps</i>	USA: New Mexico: Luna Co.: Rd.26, 4-10 mi NE Deming,
KRN042	<i>Calospasta imperialis</i>	MÉXICO: Sonora: 3 km al E de San Luis del Río Colorado. 43m.
KRN06	<i>Calospasta imperialis</i>	MÉXICO: Baja California Norte: 2 km al NE del Ejido Mérida, unos 8 km al SO de Los Algodones (Vicente Guerrero). 28m.
KRN020	<i>Eupompha imperialis</i>	MÉXICO: Baja California Norte: 12 km al OSO de Mexicali.
KRN019	<i>Eupompha sulciphrons</i>	MÉXICO: Guerrero: Mexcala
KRN018	<i>Calospasta viridis</i>	USA: New Mexico: Valencia Co.: ca. 2 mi. al W de Los Lunas
KRN135	<i>Calospasta viridis</i>	USA: New Mexico: Valencia Co.: ca. 2 mi. al W de Los Lunas
KRN059	<i>Eupompha viridis</i>	USA: New Mexico: Luna Co.: 2 mi N Deming, 1322m
KRN07	<i>Megetra cancellata</i>	USA: New Mexico: Cibola Co.: 13 mi. E Laguna, 1702m
KRN133	<i>Megetra cancellata</i>	USA: New Mexico: Luna Co.: Rd.418, (exit 69), 5-10 mi W Deming 1350msnm 14-VIII-2006
KRN129	<i>Megetra cancellata</i>	MÉXICO: San Luis Potosí: 8 km al N de Cedral, 1766 m
KRN08	<i>Megetra punctata</i>	USA: New Mexico: Grant Co.: 2 mi al E de Separ, 1372m
KRN09	<i>Megetra vittata</i>	USA: New Mexico: McKinley Co.: 8-11 mi. E Pinedale, 2221m
KRN010	<i>Phodaga alticeps</i>	USA: California: Inyo Co.: Death Valley Rd. hacia Saline Valley, unas 6 mi E-NE Big Pine. 1362m.
KRN118	<i>Phodaga alticeps</i>	USA: California: Inyo Co.: Death Valley Rd. hacia Saline Valley, unas 6 mi E-NE Big Pine. 1362m.
KRN052	<i>Phodaga alticeps</i>	MÉXICO: Sonora: 3 km al E de San Luis del Río Colorado. 43m.
KRN014	<i>Phodaga alticeps</i>	MÉXICO: Baja California Norte: Municipio Mexicali: Ejido Luchadores del Desierto, en el NO de la Laguna Salada. 0m.
KRN050	<i>Phodaga marmorata</i>	USA: New Mexico: Grant Co.: 2 mi al E de Separ, 1372m
KRN120	<i>Phodaga marmorata</i>	USA: New Mexico: Grant Co.: 2 mi al E de Separ, 1372m
KRN107	<i>Phodaga marmorata</i>	USA: New Mexico: Luna Co.: 5-10 mi W Deming, 1350m
KRN015	<i>Phodaga marmorata</i>	USA: New Mexico: Luna Co.: 5-10 mi W Deming, 1350m
mel06166	<i>Phodaga marmorata</i>	USA: Arizona: Cochise Co.: 2 mi al E de McNeal, en Davisn Rd.1299m. 12-VIII-2006
KRN047	<i>Pleuropasta mirabilis</i>	USA: California: Inyo Co.: Haiwee Reservoir North. 1191m.
KRN119	<i>Pleuropasta mirabilis</i>	USA: California: Inyo Co.: Haiwee Reservoir North. 1191m.
KRN048	<i>Pleuropasta mirabilis</i>	MEXICO: Sonora: 3 km al E de San Luis del Río Colorado. 43m.
KRN049	<i>Pleuropasta mirabilis</i>	MEXICO: Baja California Norte: 12 km al OSO de Mexicali.
KRN051	<i>Pleuropasta reticulata</i>	USA: New Mexico: Hidalgo Co.: Granite Gap, 1294 m
KRN011	<i>Tegrodera erosa</i>	USA: California: Riverside Co.: Desviación de la Hwy. 371 hacia Hemmet, unas 4 mi al N de Aguanga. 871m.
KRN012	<i>Tegrodera erosa</i>	USA: California: Riverside Co.: Diamond Valley, R3, 2 mi. al S de Hemmet. 512m.
KRN013	<i>Tegrodera latecincta</i>	USA: California: Inyo Co.: 7 mi. NE Olancha, Hwy. 190, orilla SE del Owens Lake. 1112 msnm.
KRN053	<i>Tegrodera latecincta</i>	USA: California: Inyo Co.: Rudolph Rd., 7.5 mi. al NE de Bishop, Hwy. 6. 1272m.
KRN088	<i>Eupompha histrionica</i>	USA: California: Riverside Co. Mouth of the Box Canyon E of Mecca; March 26, 2005; W.B. Warner
KRN089	<i>Eupompha histrionica</i>	USA: California: Riverside Co. Mouth of the Box Canyon E of Mecca; March 26, 2005; W.B. Warner
KRN090	<i>Eupompha histrionica</i>	USA: California: Riverside Co. Mouth of the Box Canyon E of Mecca; March 26, 2005; W.B. Warner
KRN092	<i>Eupompha edmundsi</i>	USA: Utah: Wayne Co. Sr24, 7 mi N. Hanksville 28-V-2014
KRN093	<i>Calospasta edmundsi</i>	USA: Utah: Wayne Co. Sr24, 7 mi N. Hanksville 27-V-2014

KRN096	<i>Tegrodera aloga</i>	USA: Arizona: Mesa E Regina St. April 2014
KRN097	<i>Tegrodera aloga</i>	USA: Arizona: Mesa E Regina St. April 2014
KRN098	<i>Tegrodera aloga</i>	USA: Arizona: Mesa E Regina St. April 2014
KRN100	<i>Tegrodera aloga</i>	USA: Arizona: Mesa E Regina St. April 2014
KRN101	<i>Tegrodera aloga</i>	USA: Arizona: Mesa E Regina St. April 2014
KRN131	<i>Eupompha schwarzi</i>	USA: Arizona: Yuma Co., 1-8 at Telegraph Pass; iii.29.2003; Encelia, Bebbia & mallow fls.; W.B. Warner
MEL038	<i>Epicauta stigmata</i>	MEXICO: Querétaro: 1 km al E de Bellavista del Río, 1964 m, 10-X-2009, M. García-París & N. Percino
mel05073a	<i>Lytta vesicatoria</i>	SPAIN: Ourense: A Acea (Baños de Molgas) 0605583/467522, 489m
mel04015	<i>Lagorina sericea</i>	SPAIN: Cádiz: 3 km al S de Benalup de Sidonia
mel04255	<i>Meloe mediterraneus</i>	SPAIN: Cádiz: Puerto Real
mel06161a	<i>Pyrota akhurstiana</i>	USA: Arizona: Cochise Co.: Willcox, N32°14'68.2''/W109°50'27.4'', 1265 m
mel04190	<i>Zoonitis flava</i>	SPAIN: Guadalajara: Canales de Molina
mel06156	<i>Epicauta tenella</i>	USA: California: Needles, San Bernardino Co. 9-VIII- 2006, MGP, JLR, CS

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796 **Table 1.** List of specimens included in this study, their localities, DNA voucher and
797 GenBank accession numbers for the molecular markers examined.

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Table 2. Comparative table of macroevolutionary models tested to identify the diversification pattern and rate shifts. Best model selected are shown in bold.

Mass Extinction Events = FALSE						Mass Extinction Events = TRUE			
Models/ Parameters	Yule	BD	BD-1-S	BD-2-S	BD-3-S	Models/ Parameters	ME-1	ME-2	ME-3
LH	69.2	69.2	63.8	61.08	58.94	LH	66.07	66.06	65.4
ε^0	—	2.51e-7	1.92e-02	0.01	0.13	ε	4.41e-08	1.26e-08	8.26e-08
ε^{-1}	—	—	3.2e-07	0.99	0.98	r	0.22	0.23	0.31
ε^{-2}	—	—	—	1	0.99	t^0	0.75	0.75	0.75
ε^{-3}	—	—	—	—	1.01	ρ^0	0.23	0.11	0.27
r^0	0.09	0.09	2.20e-02	0.02	0.06	t^{-1}	—	8.35	1.55
r^{-1}	—	—	1.52e-01	2.38e-03	1e-04	ρ^{-1}	—	0.99	0.24
r^{-2}	—	—	—	4.72e-03	1e-04	t^{-2}	—	—	8.35
r^{-3}	—	—	—	—	-0.01	ρ^{-2}	—	—	0.42
t^0	—	—	5.35	5.35	1.55				
t^{-1}	—	—	—	12.95	5.35				
t^{-2}	—	—	—	—	12.95				

Figure legends

Figure 1. Morphological diversity within Meloidae. Habitus *in vivo* from

representative species. The most speciose tribes of blister beetles retain the typical bauplan of the family (A, B, C), or a widespread alternative (D); two genera of Eupomphini (E, F) share the general body plan of the family. A) *Mylabris varians* (tribe Mylabrini c. 700 species, Old World). B) *Epicauta terminata* (tribe Epicautini c. 500 species, almost worldwide). C) *Lagorina sericea* (Lyttni c. 400 species, almost worldwide). D) *Meloe tuccia* (tribe Meloini c. 200 species, mostly Northern Hemisphere). E) *Eupompha elegans* (tribe Eupomphini: *Eupompha*, six species, western North America). F) *Calospasta fissiceps* (tribe Eupomphini: *Calospasta*, six species, western North America).

Figure 2. Morphological diversity within Eupomphini. Habitus *in vivo* from a

representative species of each genus. Genera of Eupomphini (26 species, western North America) (Fig. 1E and 1F) plus A to E, display an astonishing diversity of body shapes, some of them representing markedly divergent evolutionary trends (specially in elytral and abdominal shape) with very little intragenus diversification. A) *Tegrodera latecincta* (*Tegrodera*, three species). B) *Cordylospasta opaca* (*Cordylospasta*, two species). C) *Cysteodemus wislizeni* (*Cysteodemus*, two species). D) *Megetra vittata* (*Megetra*, three species). E) *Phodaga alticeps* (*Phodaga*, two species). F) *Pleuropasta reticulata* (*Pleuropasta*, two species).

Figure 3. Morphological characters used as traits in the phenotypic analysis. A)

Elytral amplitude and convexity. B) Two of the three ratios measured to calculate the abdominal volume. C) Third ratio measured to calculate the abdominal volume.

Figure 4. Molecular phylogeny of Eupomphini. Bayesian tree obtained using MrBayes

based on the concatenated mitochondrial-nuclear data set (COI+28S+18S+16S+H3).

Numbers near branches represent the posterior probabilities of clades. Color shades represent different genera; characteristic morphotype of each genus is represented next to its clade. The MRCA of the "elytral deformation clade" is marked in red.

Figure 5. Lineage divergence times, phenotypic evolution and diversification trajectories in Eupomphini. (A) The chronogram shows mean ages for lineage divergences as estimated in BEAST using Bayesian relaxed clocks; black circles near nodes indicate a posterior probability (PP) > 0.95; gray horizontal bars show 95% HPD values. Left: Variation in net-diversification and turnover rates over time as estimated in CoMET using Bayesian episodic birth-death models. Notice the marked decrease in diversification rates and increase in extinction rates close to the present (B) Disparity through time plot (black line) as estimated in geiger using four morphological characters linked to elytral and abdominal shape; the X-axis represents relative time, with 0 being the origin of the tribe and 1 being the present. Overlaid is the variation in the turnover rate (red line) and net-diversification rate (blue line), as estimated with TreePar using maximum-likelihood episodic birth-death models. Left: Time estimates for mass extinction events as inferred in CoMET using Bayes Factor comparisons.

Figure 6. Disparity through time and morphological disparity index (MDI) for individual morphological characters. The disparity-through-time (DTT) plot for the empirical data is shown as a solid line against the median DTT based on 1.000 simulations of trait evolution under Brownian Motion. Gray area denotes 95% range of simulated data. The morphological disparity index is estimated as the difference between the observed, empirical DTT and that expected under a Brownian motion model of trait evolution (Harmon et al. 2003).

Supplementary material

Table S1. Markers, primer sequences, and protocols used to amplify the gene fragments used in this study.

Table S2. Partition schemes and substitution models used for the phylogenetic analyses according to Partition Finder.

Table S3. Likelihood and AIC scores for morphological evolutionary models tested for all characters.

Figure S1. Effective Sample Sizes for the different parameters estimated with TESS, ESS>500 suggest convergence of the chain.

Figure S2. Rubin-Gelman statistic for the different parameters estimated with TESS; blue dots passed the test, red dots failed the test.

Figure S3. Posterior density plots of the two chains performed to assess convergence of the MCMC

Figure S4. Plots for the other parameters estimated by CoMET: speciation rate, extinction rate, rate shifts, and mass extinction timing.