Non-adaptive radiation and mass extinction 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. Evolutionary theory posits that species diversification is coupled with phenotypic evolution. 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 rampant 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 mass extinction events associated to climatic oscillations 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, extinction rates, Eupomphini, mass extinction events, 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; Gavrilets 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";

67 Gittenberger, 1991; Rundell and Price, 2009). Yet, few examples to date have provided
68 support for this hypothesis.
69 Diversification studies have mainly focused on rapid events of speciation (i.e.,
70 species radiations), and the factors driving them such as niche evolution or morphological
71 key innovations (e.g., Lagomarsino et al., 2016). Although extinction is seen as a positive
72 and constructive evolutionary force in paleontology (Raup, 1991; Benton, 2009),
73 difficulties to infer extinction rates from neontological data (Rabosky, 2009) have made it
74 less often the subject of such studies. Recently, the development of likelihood methods to
75 estimate changes in diversification rates and the time and magnitude of mass extinction
76 events from phylogenies containing only extant taxa (Stadler, 2011A; May et al. 2015) has
77 brought renewed attention into extinction. It is now seen as a critical process, responsible
78 for shaping the evolutionary history of individual taxa and regional biotas (Antonelli and
79 Sanmartín, 2011; Condamine and Hilnes, 2015; Sanmartín and Meseguer, 2016). None of
80 these studies, however, have focused on the relationship between extinction rates and
81 phenotypic diversity.
82 Here, we use the North American desert blister beetles (Meloidae) of the tribe
83 Eupomphini to explore the role of extinction in explaining patterns of low species diversity
84 coupled with rampant morphological variation across extant taxa. Eupomphini is currently
85 represented by only 26 described species, grouped into seven genera (Pinto, 1984). Yet,
86 these 26 species represent an extraordinary level of morphological differentiation in
87 complex anatomical structures within an otherwise relatively morphologically-
88 homogeneous family (Figure 1). Other tribes of Meloidae have much higher species
89 richness but share a generally conservative bauplan. For example, *Epicauta*, within tribe
90 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 genus *Eupompha* LeConte 1858, exhibit strikingly dissimilar morphologies (Figure 2), especially 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).

Evolutionary relationships within the tribe Eupomphini have so far been investigated using morphological and behavioral characters (Pinto, 1979; Pinto, 1984), or a limited set of molecular markers (Bologna and Pinto, 2001), and 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).

115

116 **2. Materials and methods**

117 2.1. *Taxon sampling*

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

129

130 2.2. *DNA sequencing*

131 A tissue sample of each specimen was obtained from thoracic muscle of the hind coxa.
132 Total genomic DNA was extracted using the "DNA Easy extraction Kit" (Qiagen®),
133 following the protocol described the manufacturer. We generated sequences for five gene
134 markers with different molecular evolutionary rates to obtain phylogenetic resolution at
135 different phylogenetic levels. For the mitochondrial (mtDNA) compartment, we sequenced
136 650 bp of the cytochrome oxidase I (*coxI*) marker, and 784 bp of the 16S ribosomal gene.
137 For the nuclear compartment, we sequenced 306 bp of the histone H3 gene, 722 bp of 18S
138 ribosomal marker, and 598 bp of the 28S nuclear ribosomal gene. These markers were

139 selected based on their proven efficacy in previous phylogenetic studies of beetles, in
140 particular for the superfamily Tenebrionoidea (Baselga et al., 2011; Gunter et al., 2014) and
141 the family Meloidae (Bologna et al., 2008; Alcobendas et al., 2008; Percino-Daniel et al.,
142 2013).
143 The primers and PCR protocols employed in this study are listed in Table S2.
144 Amplification was carried out in a total volume of 15µl, with 3µl of PCR buffer, 0.1-0.2 µl
145 of MgCl2, 0.2 µl of each primer and 0.1µl of MyTaq polymerase (BioLine©), 3µl of DNA
146 template and 8.3µl of ddH2O. Unpurified PCR products were sent for sequencing to the
147 genomics unit at IB UNAM. Sequences of *coxI* and H3 were manually aligned, whereas
148 16s, 18s and 28s sequences were aligned based on their secondary structure models, which
149 were obtained through the online program ViennaRNA Package version 2.0, available at
150 the Institute for Theoretical Chemistry, University of Vienna (http://rna.tbi.univie.ac.at).
151
152 2.3. *Phylogenetic and relaxed molecular clock analyses*
153 Concatenated Bayesian analyses were performed with MrBayes version 3.2.6 (Ronquist et
154 al., 2012). Selection of the best substitution model for each marker was carried out in
155 jModeltestet version 2.7.1 (Posada and Crandall, 1998) under the Akaike Information
156 Criterion correction (AIC). The free software PartitionFinder version 1.1.1 (Lanfear, 2012)
157 was used to determine the optimal partition scheme for the examined markers. A total of
158 nine unlinked partitions were selected: COIpos1, COIpos2, COIpos3, H3pos1, H3pos2,
159 H3pos3, 28s, 18s, 16s. The evolutionary models selected for each analysis and partition
160 with their best-fit model, are listed in Table S3.
161 MrBayes analysis consisted of two simultaneous runs of 100 million generations
162 each, sampling trees every 10,000 generations. Mixing and convergence among runs was

163 evaluated by checking the average standard deviation of split frequencies and the EES
164 values and Potential Scale Reduction Factor (PSRF) for each parameter. A majority
165 consensus tree was reconstructed after discarding the first 20,000 sampled trees as burn-in.
166 This dataset contained 55 terminal taxa and 3059 nucleotide positions; in a few cases, we
167 merged two specimens of the same population as a single terminal taxon to reduce the
168 amount of missing data in the dataset.

169 To estimate lineage divergence times within Eupomphini, we used Bayesian relaxed
170 molecular clocks implemented in BEAST version 1.8.2 (Drummond et al. 2012). The
171 analysis used the concatenated mitochondrial-nuclear dataset partitioned by gene but
172 without internal (codon) partitions. Molecular clocks were unlinked across genes, using an
173 uncorrelated lognormal relaxed clock with the mean and standard deviation of substitution
174 rates (subst/site/Ma) for the COI, 16S, and 28S markers following Papadopoulou et al.
175 (2010). The *ucld.mean* parameter for the *coxI* marker was assigned a lognormal distribution
176 in real space, with initial value: 0.0168, Log(Mean): 0.0168, Log(Stdev): 0.0018; the
177 *ucld.mean* for 16S: lognormal distribution in real space, with 0.0054 as initial value,
178 Log(Mean): 0.0054, Log(Stdev): 0.0009; *ucld.mean* for 28S: lognormal in real space, with
179 initial value (0.0006), Log(Mean): 0.0006, Log(Stdev): 0.0003. For the remaining markers
180 we used uninformative priors: the *ucld.mean* parameters for 18S and H3 were assigned a
181 gamma distribution in real space, with initial value (0.01), shape parameter (0.01), scale
182 (100), and Offset (0).

183 The birth-death model with incomplete taxon sampling (Stadler, 2009) was used as
184 a tree prior to account for the effect of extinction and taxon sampling on tree topology and
185 branch lengths. The analysis was run for 100 million generations, discarding the first
186 10,000 generations as burn-in. Inspection of the trace plots and effective sample sizes in

187 Tracer 1.8.0 (Drummond and Rambaut, 2007) was used to assess the convergence and
188 mixing of the MCMC runs. All analyses were run in the web public resource CIPRES
189 Science Gateway version 3.3 (Miller et al., 2010). After discarding the burn-in, the
190 remaining trees were employed to build a maximum-clade credibility (MCC) tree with 95%
191 high-posterior density (HPS) credibility intervals of ages using TreeAnnotator version 1.8.2
192 included in the BEAST package. Phylogenetic trees were visualized with FigTree version
193 1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/).
194
195 2.4. *Tempo and mode of species diversification*
196 Diversification analyses were based on the MCC timetree, pruned to leave just one
197 specimen per species to avoid bias in speciation rate estimates. We first plotted the number
198 of lineages through time with the function *ltt.plot* in the R package *ape* (Paradis et al.,
199 2004) to visually inspect the diversification trajectory. We then statistically evaluated the fit
200 of the MCC tree to alternative birth-death models: rate-constant diversification, density-
201 dependent diversification, and discrete time-variable diversification using the whole-tree
202 likelihood algorithms implemented in the R package *TreePar* (Stadler, 2011B). This
203 method accounts for the effect on the tree topology of incomplete taxon sampling - by
204 incorporating the sampling fraction of the extant taxa - and the "pull-of-the-present" - an
205 artifact of the effect of extinction on recent lineage diversification (Stadler, 2011C). The
206 function *bd.shift.optim* was used to detect tree-wide rate shifts: changes in speciation and
207 extinction rates that affect all clades in the tree simultaneously at discrete points in time. In
208 particular, we estimated by maximum likelihood the time of the rate shift and the
209 magnitude of the rate of diversification (r = speciation (λ) - extinction (μ)), and the
210 extinction fraction or turnover ($\varepsilon = \mu / \lambda$) 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); 4) a mass extinction model (ME=TRUE), estimating the intensity of the mass extinction (ρ) for one, two or three events; and 5) an intermediate episodic birth-death model allowing both MEEs and rate shifts, where the rho parameter values estimated in (4) are used as fixed values for the sampling fraction (survival probability) in the past with ME =FALSE. 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: $V = (4\pi/3)r1r2r3$

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. 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 *dtt* (*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) showed significant support for the monophyly of Eupomphini (PP = 1) and Epicautini as its sister group (PP = 96). All ingroup genera were recovered as monophyletic with strong support (PP > 95), except *Eupompha*. Members of this genus were instead recovered as a grade of two separate clades at the base of the tribe. The first of these clades was sister to the remaining species in Eupomphini, and comprises two species pairs: *Eupompha edmundsi* (Selander 1953) + *E. viridis* (Horn 1883) (PP= 1), and *E. elegans* (LeConte 1851) + *E. imperialis* (Wellman 1912). The second clade (PP= 0.88) 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). This second *Eupompha* clade was sister to a clade including all remaining species of the tribe (PP = 0.89), all of which have some degree of elytral deformation. Within this clade, *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 congruent with the phylogenetic hypothesis based on the MrBayes concatenated analysis. The origin of Eupomphini was dated during the Early Miocene (Mean 17.88, 95% HPD 15.24-20.53

307 Mya). Also, an Early Miocene origin was estimated for the clade whose members have
308 elytral deformation (Mean 16.08, 95% HPD 12.81-17.20 Mya). For most genera, the most
309 recent common ancestor (MRCA) was dated as originating during the Late Miocene
310 (*Cordylospasta, Megetra, Pleuropasta, Phodaga* and *Cysteodemus*), between 7.84 and 5.8
311 Mya. The youngest MRCA estimate belongs to *Tegrodera*, which appears to have
312 originated during the Late Pleistocene (Mean 1.58, 95%HPD 0.76-2.94 Mya). The oldest
313 MRCAs correspond to the two non-sister clades of *Eupompha*, whose origins are placed in
314 the Middle Miocene between 13.66 Mya (95%HPD 10.93-16.45) and 13.53 Mya (95%HPD
315 10.51-16.74).
316
317 3.3. *Diversification analyses*
318 The LTT plot showed a sigmoidal shape, with initial accumulation in the number of
319 lineages, followed by a slowdown and a final uplift towards the present (Figure 5A right).
320 This is confirmed by the *TreePar* analyses, summarized in Table 1 and Figure 5B.
321 Likelihood ratio tests supported an episodic model with two rate shifts against the constant-
322 rate Yule and BD models. The pattern of diversification shows an increase over time in the
323 background extinction rate, starting with $\varepsilon_0 = 0.99$; then rising to $\varepsilon_1 = 1.81$ at $t_1 = 5.77$ Mya,
324 and finally peaking at $t_2 = 1.97$ Mya with a very high turnover of $\varepsilon_2 = 7.56$ (Figure 5B
325 right). Conversely, the net diversification rate started with a value of $r_0 = 0.24$, then
326 decreased to a negative value of $r_1 = -1.02$, and showed a slight recovery towards the
327 present ($r_2 = 0.0006$). When the ME = TRUE option was used (modeling mass extinction
328 events as sampling events in the past), a model with one MEE was selected as the best-fit
329 model (Table 1). The survival probability - the fraction of existing lineages that survived
330 the mass extinction event and went to the next diversification rate period - was inferred as ρ

331 = 0.03, indicating that 97% of extant diversity went extinct at 1.97 Mya (Figure 5B right).
332 A second, older mass extinction event was detected at 9.37 Mya with survival probability ρ
333 = 0.155, but this model was not significantly better than the 1-MEE model (Table 1; Figure
334 5B left).
335 Results from CoMET showed a similar pattern (Figure 5A left), though uncertainty
336 in parameter estimation (represented by the 95% HPD) was high: there is an initially high
337 net diversification rate between 20-15 Ma (r ~0.15), which rapidly decreases towards the
338 present (r ~0.05); this is concurrent with a decrease in the background extinction rate from
339 an initial value ε ~0.12, followed by an increase over time that peaks towards the present (ε
340 ~0.43).. Figure 5B (left) shows the Bayes Factor comparisons for the timing of MEE events
341 ("mass extinction times"): CoMET detects one MEE at c. 2 Mya), with Bayes Factor (lnBF)
342 = 1; other (non-significant) MEEs are detected at 5 Mya and at close to the start of the
343 phylogeny (c. 17 Mya). Figures S6-S9 show the MCMC diagnostics and plots of the other
344 parameters estimated by CoMET: speciation and extinction rates, rate shifts, and MEE time
345 estimates.
346
347 3.4. *Morphological disparity patterns and character evolution*
348 Our comparison of continuous trait evolutionary models in *geiger* selected the Brownian
349 motion as the best-fit model for all characters (Table S4), which was also the null model
350 used in the MDI analyses (below). The traits elytral and abdominal volume were estimated
351 to evolve at a rate of 0.172 and 0.177, respectively, whereas elytral convexity and
352 amplitude evolved with a slower rate of 0.012 and 0.001, respectively.
353 Disparity-through-time (DTT) plots were similar across all four morphological
354 traits, with morphological diversity being higher at the first two thirds of the phylogeny,

355 indicating that the disparity was equally distributed through subclades. In the last third of
356 the phylogeny, there is a sharp decrease of values, suggesting that the disparity is
357 pronounced among subclades but poor at intraclade level (Figure 6). The Morphological
358 Diversity Index (MDI) was negative for abdominal volume, elytral convexity and elytral
359 amplitude (-0.10, -0.10, -0.07, respectively); MDI for elytral volume was 0.03 (Figure 6).
360 The MDI test thus rejected the Brownian model (BM) as the model of trait evolution, albeit
361 with no significant *p*-values; likelihood and AIC scores are summarized in Table S4.
362
363 **4. Discussion**
364 4.1. *Phylogenetic relationships in Eupomphini*
365 Previous phylogenetic studies had suggested the monophyly of the tribe Eupomphini based
366 on a limited taxon sampling and using morphological and molecular information from two
367 gene markers (16S and ITS2) (Pinto, 1984; Bologna and Pinto, 2001). Our phylogeny -
368 based on a much larger sample of markers - supports the monophyly of the tribe (PP >
369 0.95), and confirms the two morphological and behavioral adult synapomorphies proposed
370 by Pinto (1984): adults with ventral blade of claws shorter than dorsal blade and largely
371 adnate to it, and cleaning of antennae using only forelegs, not mouthparts.
372 Similarly, all genera within Eupomphini excepting *Eupompha* were recovered as
373 monophyletic with significant support. Species of *Eupompha* have originally being placed
374 (LeConte, 1862) into two separate genera (*Calospasta* and *Eupompha*), though they were
375 subsequently synonymized by Selander (1954). In his revision of the genus, Pinto (1979)
376 proposed two informal sections defined by morphological characters of the first larval and
377 adult stages. These two sections corresponded with LeConte's original division. In our

phylogenetic tree, the two clades grouping the species of *Eupompha* also correspond with the sections described by Pinto (1979).

Based on the above information, we propose the 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. *Eupompha s. str.*, on the other hand, corresponds to Pinto's (1979) Section 2, which contains its type species, *E. fissiceps*. This genus now comprises *E. histrionica*, *E. schwarzi*, *E. terminalis* Selander 1957, *E. sulciphrons*, *E. fissiceps* and *E. wenzeli* Skinner, 1904; and they differ from members of *Calospasta* by differences in male genitalia as indicated by Pinto (1979).

Phylogenetic relationships among genera obtained in this study do not correspond well with those proposed by Pinto (1984) 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*

Eupomphini are distributed within the physiographic "Basin and Range" province in Mexico. 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 for other animal groups (i.e., Avise, 1998; Knowles, 2000; Bryson et al., 2013). Though we cannot discard this explanation - we did not analyze climatic niches - 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. However, whereas CoMET recovers the signal of the initial radiation, showing a high diversification rate at the onset of the phylogeny (Fig. 5A left), *TreePar* does not (Fig. 5B). This can be explained by the different inferential framework. *TreePar* uses a maximum likelihood *greedy* algorithm in which the time of one rate shift is estimated and fixed before estimating the time of the next rate shift and cannot estimate simultaneously both tree-wide rate shifts and MEEs (Stadler, 2011A,B). CoMET uses a hierarchical Bayesian approach and MCMC to jointly estimate the posterior distribution of rate shifts and MEEs, and thus has a higher statistical power to detect these events than *TreePar* (May et al. 2015).

The pattern of lineage radiation detected here was paralleled by a similar pattern of morphological variation in the four studied body-shape traits. 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 towards 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

426 also estimated a negative MDI value for all characters excepting the elytral amplitude
427 (Figure 6), which is also considered evidence of a morphological radiation (Harmon et al.,
428 2003; Cantalapiedra et al. 2017).
429 What could have promoted this initially rapid morphological evolution? The
430 outcome of the evolutionary process is limited by evolutionary constraints (Alberch, 1982;
431 Gould, 1989; Arnold, 1992). Some constraints arise in the epigenetic interactions involved
432 during the developmental process, limiting drastically the possibilities of morphological
433 change and restricting the set of possible bauplans (Alberch, 1982). The extreme
434 diversification of elytral shape observed within the derived "elytral deformation" clade of
435 Eupomphini (i.e., grouping all genera except for *Eupompha* and *Calospasta*) (Figures 1, 2),
436 suggests that a probable developmental disturbance affected the common ancestor of this
437 clade. A general disturbance involving the loss of the evolutionary constraint responsible
438 for the relationship between elytral and abdominal shape in Meloidae would result in a
439 dramatic extension of the available morphospace. This morphospace widening would open
440 the gate for wild exploratory morphological experiments, visualized in the form of a fast
441 radiation of morphotypes among which the extant generic forms are included (Figure 1).
442 Though further developmental studies are needed to confirm the hypothesis that the loss of
443 evolutionary constraints in body shape drove the rapid phenotypic evolution of
444 Eupomphini, several lines of evidence support this. 1) Rapid lineage
445 divergence contributes to the maintenance of ancestral polymorphism among the incipient
446 clades, and results in a loss of phylogenetic signal and poorly resolved internal clades
447 (Whitfield and Lockhart, 2007); this is observed in the pattern of lineage accumulation in
448 the tribe, with low support values at the deepest nodes compared to the tip clades, and a
449 topology with short internal versus long external branches (Fig. 4). 2) A fast-evolutionary

450 rate was estimated for elytral and abdominal volume traits under the BM model, suggesting
451 that the new forms produced along the morphological radiation were an array of
452 morphotypes that changed almost randomly across taxa.
453 Evolutionary radiations are expected to show a characteristic trajectory, which
454 corresponds to a density-dependent model for lineage diversification (Etienne et al., 2012)
455 and an "early-burst" model for trait evolution (Harmon et al. 2008), in which initially rapid
456 rates are followed by a slowdown towards the present. Interestingly, the diversification
457 trajectory of Eupomphini does not fit any of these models (Figure 6, Table S3). The reason
458 is that the morphological radiation in Eupomphini was followed by historically high
459 extinction rates, resulting in a tree with initially rapid evolution (17 to 15 Mya), followed
460 by a subsequent slowdown in the rate of diversification as the morphological space became
461 saturated (15 to 5 Mya), and a final uplift in the last 5-2 Mya, as expected for diversity
462 rebounding after a mass extinction event (Figure 5A,B). Both *TreePar* and CoMET
463 estimated increasingly high background extinction rates. They also detected a dramatic
464 mass extinction event at around 2 Mya, when more than 97% of extant lineages of
465 Eupomphini went extinct (Figure 5, Table 1); the lack of statistical significance for this
466 event in Bayes Factor comparisons in CoMET is probably due to the small size of our
467 phylogeny (May et al. 2015). This MEE could have been caused by the well-known
468 climatic fluctuations of the Pleistocene (Berger, 1984; Bartlein and Prentice, 1989; Webb
469 and Bartlein, 1992). These environmental changes, with alternating glacial and interglacial
470 cycls, considerably altered the geographical ranges of many groups of organisms, including
471 Coleoptera (Coope, 1979), promoting speciation processes (Mayr and O´Hara, 1985;
472 Baselga et al., 2011), but also limiting speciation in several taxa (Zink and Slowinski
473 1995). *TreePar* and CoMET detected a second, older (albeit non-significant) mass

474 extinction eventin the Miocene-Pliocene transition (c. 5 Mya, Fig. 5A,B), which apparently
475 eliminated c. 90% extant lineages (Table 1). At this time, the proportion of plants with C3
476 metabolism changed dramatically towards C4 plants, affecting the atmospheric temperature
477 and the CO_2 proportion, and causing a deep desertification and the extinction of several
478 living groups (Cerling et al., 1997; Ehleringer et al., 1997; MacFadden et al., 1999; Krause
479 et al., 2008). This event could have extirpated the early branching off lineages within the
480 "elytral deformation" clade, resulting in the deep divergences and extremely different
481 morphotypes observed today across living genera (e.g., *Cysteodemus* and *Tegrodera*).
482
483 **5. Conclusions**
484 Changes in speciation and extinction rates have been considered key factors to
485 explain phylogenetic, temporal, and spatial variation in species richness across organisms
486 (Glor, 2010; Paradise, 2011; Morlon et al., 2011; Rabosky, 2014). Our results suggest that
487 phenotypic evolvability – in this case the loss of the evolutionary constraint for elytral
488 shape in Meloidae shortly after the initial divergence of Eupomphini - acted as a trigger
489 driving morphological diversification and accelerated speciation (non-adaptive radiation) in
490 this tribe. They also highlight the role played by historically high extinction rates, driven by
491 abiotic factors such as climate change, to explain the evolutionary riddle posed by groups in
492 which phenotypic disparity is decoupled from patterns of lineage diversification.
493
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509

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799

800 **Table 1.** List of specimens included in this study, their localities, DNA voucher and
801 GenBank accession numbers for the molecular markers examined.
802

Voucher	Taxon	Locality	CoxI	16S	18S	28S	H3
KRN023	*Cordylospasta fulleri*	USA: California: Inyo Co.: 3 mi NE Big Pine, al inicio de la Death Valley Rd. hacia Saline Valley. 1204m.					
KRN111	*Cordylospasta fulleri*	USA: California: Inyo Co.: 3 mi NE Big Pine, al inicio de la Death Valley Rd. hacia Saline Valley. 1204m.					
KRN01	*Cordylospasta opaca*	USA: California: San Bernardino Co.: Summit Valley Rd., a 4 km del cruce con la Hwy. 138. 1148m.					
KRN112	*Cordylospasta opaca*	USA: California: San Bernardino Co.: Summit Valley Rd., a 4 km del cruce con la Hwy. 138. 1148m.					
KRN024	*Cysteodemus armatus*	MÉXICO: Baja California Norte: 14 km al O de Mexicali, cerca del cementerio.					
KRN02	*Cysteodemus armatus*	MÉXICO: Baja California Norte: 12 km al OSO de Mexicali.					
KRN025	*Cysteodemus armatus*	MÉXICO: Baja California Norte: Municipio Mexicali: Ejido Luchadores del Desierto, en el NO de la Laguna Salada. 0m.					
KRN026	*Cysteodemus wislizeni*	USA: New Mexico: Sierra Co.: 4 mi. E Hillsboro, 1593m.					
KRN03	*Cysteodemus wislizeni*	USA: New Mexico: Sierra Co.: 5 mi. N Truth or Consequences					
KRN28	*Cysteodemus wislizeni*	USA: New Mexico: Cibola Co.: 13 mi. E Laguna, 1702m.					
KRN030	*Eupompha elegans*	USA: California: Riverside Co.: Desviación de la Hwy. 371 hacia Hemmet, unas 4 mi al N de Aguanga. 871m.					
KRN113	*Eupompha elegans*	USA: California: Riverside Co.: Desviación de la Hwy. 371 hacia Hemmet, unas 4 mi al N de Aguanga. 871m.					
KRN040	*Eupompha elegans*	USA: California: Inyo Co.: 5 km N Little Lake cerca de Fossil Falls. 1025m.					
KRN021	*Eupompha elegans*	USA: California: Inyo Co.: Haiwee Reservoir North. 1191m.					
KRN114	*Eupompha elegans*	USA: California: Inyo Co.: Haiwee Reservoir North. 1191m.					
KRN038	*Eupompha elegans*	USA: California: Inyo Co.: Orilla Oeste de Owens Lake. 1100m.					
KRN115	*Eupompha elegans*	USA: California: Inyo Co.: Orilla Oeste de Owens Lake. 1100m.					
KRN029	*Eupompha elegans*	USA: California: Inyo Co.: 5 km N Little Lake cerca de Fossil Falls. 1025m.					
KRN054	*Eupompha fissiceps*	USA: New Mexico: Hidalgo Co.: 19 mi SW Lordsburg					
KRN121	*Eupompha fissiceps*	USA: New Mexico: Hidalgo Co.: 19 mi SW Lordsburg					
KRN043	*Eupompha fissiceps*	USA: New Mexico: Grant Co.: 10 mi N Hachita, 1364m.					
KRN044	*Eupompha fissiceps*	USA: New Mexico: Grant Co.: 10 mi N Hachita, 1364m.					
KRN108	*Eupompha fissiceps*	USA: New Mexico: Grant Co.: 10 mi N Hachita, 1364m.					
KRN045	*Eupompha fissiceps*	USA: New Mexico: Luna Co.: Rd.26, 4-10 mi NE Deming,					

KRN042	*Eupompha imperialis*	MÉXICO: Sonora: 3 km al E de San Luis del Río Colorado. 43m.
KRN06	*Eupompha imperialis*	MÉXICO: Baja California Norte: 2 km al NE del Ejido Mérida, unos 8 km al SO de Los Algodones (Vicente Guerrero). 28m.
KRN020	*Eupompha imperialis*	MÉXICO: Baja California Norte: 12 km al OSO de Mexicali.
KRN019	*Eupompha sulciphrons*	MÉXICO: Guerrero: Mexcala
KRN018	*Eupompha viridis*	USA: New Mexico: Valencia Co.: ca. 2 mi. al W de Los Lunas
KRN135	*Eupompha viridis*	USA: New Mexico: Valencia Co.: ca. 2 mi. al W de Los Lunas
KRN059	*Eupompha viridis*	USA: New Mexico: Luna Co.: 2 mi N Deming, 1322m
KRN07	*Megetra cancellata*	USA: New Mexico: Cibola Co.: 13 mi. E Laguna, 1702m
KRN133	*Megetra cancellata*	USA: New Mexico: Luna Co.: Rd.418, (exit 69), 5-10 mi W Deming 1350msnm 14-VIII-2006
KRN129	*Megetra cancellata*	MÉXICO: San Luis Potosí: 8 km al N de Cedral, 1766 m
KRN08	*Megetra punctata*	USA: New Mexico: Grant Co.: 2 mi al E de Separ, 1372m
KRN09	*Megetra vittata*	USA: New Mexico: McKinley Co.: 8-11 mi. E Pinedale, 2221m
KRN010	*Phodaga alticeps*	USA: California: Inyo Co.: Death Valley Rd. hacia Saline Valley, unas 6 mi E-NE Big Pine. 1362m.
KRN118	*Phodaga alticeps*	USA: California: Inyo Co.: Death Valley Rd. hacia Saline Valley, unas 6 mi E-NE Big Pine. 1362m.
KRN052	*Phodaga alticeps*	MÉXICO: Sonora: 3 km al E de San Luis del Río Colorado. 43m.
KRN014	*Phodaga alticeps*	MÉXICO: Baja California Norte: Municipio Mexicali: Ejido Luchadores del Desierto, en el NO de la Laguna Salada. 0m.
KRN050	*Phodaga marmorata*	USA: New Mexico: Grant Co.: 2 mi al E de Separ, 1372m
KRN120	*Phodaga marmorata*	USA: New Mexico: Grant Co.: 2 mi al E de Separ, 1372m
KRN107	*Phodaga marmorata*	USA: New Mexico: Luna Co.: 5-10 mi W Deming, 1350m
KRN015	*Phodaga marmorata*	USA: New Mexico: Luna Co.: 5-10 mi W Deming, 1350m
mel06166	*Phodaga marmorata*	USA: Arizona: Cochise Co.: 2 mi al E de McNeal, en Davisn Rd.1299m. 12-VIII-2006
KRN047	*Pleuropasta mirabilis*	USA: California: Inyo Co.: Haiwee Reservoir North. 1191m.
KRN119	*Pleuropasta mirabilis*	USA: California: Inyo Co.: Haiwee Reservoir North. 1191m.
KRN048	*Pleuropasta mirabilis*	MEXICO: Sonora: 3 km al E de San Luis del Río Colorado. 43m.
KRN049	*Pleuropasta mirabilis*	MEXICO: Baja California Norte: 12 km al OSO de Mexicali.
KRN051	*Pleuropasta reticulata*	USA: New Mexico: Hidalgo Co.: Granite Gap, 1294 m
KRN011	*Tegrodera erosa*	USA: California: Riverside Co.: Desviación de la Hwy. 371 hacia Hemmet, unas 4 mi al N de Aguanga. 871m.
KRN012	*Tegrodera erosa*	USA: California: Riverside Co.: Diamond Valley, R3, 2 mi. al S de Hemmet. 512m.

KRN013	*Tegrodera latecincta*	USA: California: Inyo Co.: 7 mi. NE Olancha, Hwy. 190, orilla SE del Owens Lake. 1112 msnm.
KRN053	*Tegrodera latecincta*	USA: California: Inyo Co.: Rudolph Rd., 7.5 mi. al NE de Bishop, Hwy. 6. 1272m.
KRN088	*Eupompha histrionica*	USA: California: Riverside Co. Mouth of the Box Canyon E of Mecca; March 26, 2005; W.B. Warner
KRN089	*Eupompha histrionica*	USA: California: Riverside Co. Mouth of the Box Canyon E of Mecca; March 26, 2005; W.B. Warner
KRN090	*Eupompha histrionica*	USA: California: Riverside Co. Mouth of the Box Canyon E of Mecca; March 26, 2005; W.B. Warner
KRN092	*Eupompha edmundsi*	USA: Utah: Wayne Co. Sr24, 7 mi N. Hanksville 28-V-2014
KRN093	*Eupompha edmundsi*	USA: Utah: Wayne Co. Sr24, 7 mi N. Hanksville 27-V-2014
KRN096	*Tegrodera aloga*	USA: Arizona: Mesa E Regina St. April 2014
KRN097	*Tegrodera aloga*	USA: Arizona: Mesa E Regina St. April 2014
KRN098	*Tegrodera aloga*	USA: Arizona: Mesa E Regina St. April 2014
KRN100	*Tegrodera aloga*	USA: Arizona: Mesa E Regina St. April 2014
KRN101	*Tegrodera aloga*	USA: Arizona: Mesa E Regina St. April 2014
KRN131	*Eupompha schwarzi*	USA: Arizona: Yuma Co., 1-8 at Telegraph Pass; iii.29.2003; Encelia, Bebbia & mallow fls.; W.B. Warner
MEL038	*Epicauta stigmata*	MEXICO: Querétaro: 1 km al E de Bellavista del Río, 1964 m, 10-X-2009, M. García-París & N. Percino
mel05073a	*Lytta vesicatoria*	SPAIN: Ourense: A Acea (Baños de Molgas) 0605583/467522, 489m
mel04015	*Lagorina sericea*	SPAIN: Cádiz: 3 km al S de Benalup de Sidonia
mel04255	*Meloe mediterraneus*	SPAIN: Cádiz: Puerto Real
mel06161a	*Pyrota akhurstiana*	USA: Arizona: Cochise Co.: Willcox, N32º14’68.2’’/W109º50’27.4’’, 1265 m
mel04190	*Zoonitis flava*	SPAIN: Guadalajara: Canales de Molina
mel06156	*Epicauta tenella*	USA: California: Needless, San Bernardino Co. 9-VIII-2006, MGP, JLR, CS

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

808 **Table 2.** Comparative table of macroevolutionary models tested to identify the
809 diversification pattern and rate shifts.
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Macroevolutionary model	LH	ε^0	ε^{-1}	ε^{-2}	ε^{-3}	r^0	r^{-1}	r^{-2}	r^{-3}	t^0	t^{-1}	t^{-2}
Constant rates												
Yule	114.42					0.16						
Birth-Death	110.56	0.83				0.063						
BD-1-S	98.64	1.85	0.99			-0.79	0.23					
BD-2-S	92.6	7.54	1.85	0.99		-0.0006	-1.03	0.23		0.17	5.77	
BD-3-S	90.68	7.56	1.71	0.99	0.99	-0.001	-0.81	0.13	0.1	0.17	5.77	14.77
MEE's or sampling events												
ME-1-S	96.83	0.38				0.063				1.97 ρ=0.035		
ME-2-S	94.43	0.0024				0.49				1.97 ρ=0.017	9.37 ρ=0.15	
ME-3-S	93.04	$3.3e^{-7}$				0.56				1.57 ρ=0.13	1.97 ρ=0.07	9.37 ρ=0.97
Combined model	96.83	0.83	0.74			0.063	0.15			1.97 ρ=0.03		
Density-dependent cladogenesis	LH	λ	μ	k								
	105.38	0.95	0.26	58								

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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* (Lyttini 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 phylogram obtained in 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. Blue vertical bars indicate the time of the two mass extinction events inferred by TreePar. 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 asses convergence of the MCMC

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