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Running in circles in phylomorphospace: host environment constrains morphological diversification in parasitic wasps

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Understanding phenotypic diversification and the conditions that spur morphological novelty or constraint is a major theme in evolutionary biology. Unequal morphological diversity between sister clades can result from either differences in the rate of morphological change or in the ability of clades to explore novel phenotype ranges. We combine an existing phylogenetic framework with new phylogenomic data and geometric morphometrics to explore the relative roles of rate versus mode of morphological evolution for a hyperdiverse group: cryptine ichneumonid wasps. Data from genomic ultraconserved elements confirm that cryptines are divided into two large clades: one specialized in the use of hosts that are deeply concealed under hard substrates, and another with a much more diversified host range. Using a phylomorphospace approach, we show that both clades have experienced similar rates of morphological evolution. Nonetheless, the more specialized group is much more restricted in morphospace occupation, indicating that it repeatedly evolved morphological change through the same morphospace regions. This is in agreement with our prediction that host use imposes constraints in the morphospace available to lineages, and reinforces an important distinction between evolutionary stasis as opposed to a scenario of continual morphological change restricted to a certain range of morphotypes.

1. Introduction

One of the most common patterns observed in biology is that various groups of organisms show differing degrees of morphological variation, with some lineages displaying astounding diversity in form while others remain largely conserved [1,2]. Recognizing the conditions that spur either morphological novelty or constraint is essential to our understanding of the evolutionary process and resulting biodiversity patterns. It is widely recognized that certain functional demands (e.g. feeding or movement specializations) can constrain morphological evolution towards a given 'optimal' morphotype [3–5]. On the other hand, limited morphological disparity can also result from other factors such as phylogenetic inertia [6–8] or clade-specific developmental and physiological constraints [9–11]. Hence, understanding the potential role of ecological aspects in shaping phenotypes requires rigorous examination of morphological disparity patterns within an evolutionary framework.

Explanations for unequal levels of morphological diversification among lineages tend to focus either on clade age, that is, the time available for morphological diversification to take place [12,13]; the rate of morphological change across clades [14,15]; or unequal efficiency of morphological diversification, including hypotheses about 'key innovations' [16–18] and various ecological or organismal constraints [2,19,20]. In terms of quantitative data, such constraints can be understood as the limitation of the morphospace available to lineages

with a given ecological trait or life-history strategy [21–23]. Investigations of which areas of the morphospace are occupied by individual lineages when compared to the universe of 'possible' morphotypes has a long tradition in evolutionary biology (e.g. [24–26]). Nonetheless, our understanding of the ecological traits driving such limitations is still in its infancy [27,28].

Using sister clades as study systems presents an excellent opportunity to test hypotheses of morphological diversification because both lineages depart from an initially equal state. This makes for a suitable experiment to assess the relative impacts of rate versus mode of evolution in the phenotypic outcomes of two clades. Herein, we treat 'rate' as the overall phenotypic rate of change across time, and 'mode' as the distribution of this change across the morphospace. The corollary is that unequal levels of morphological diversification among sister clades could have been achieved by: either (i) differing magnitudes of evolution rate; or (ii) differing abilities between clades to efficiently explore the morphospace [29]. These scenarios can be assessed by projecting a phylogeny into a multivariate morphospace (a phylomorphospace) in order to infer both the magnitude of shape change and the patterns of morphospace occupation across clades. When two sister clades show both contrasting life-history strategies and different levels of morphological disparity, a promising avenue is available for testing the role of ecological constraints in diversification. Furthermore, highly speciose groups are particularly appropriate for investigating patterns of morphological evolution, especially in groups with high rates of morphological homoplasy, as the high number of independent state transitions allow for statistically powerful analyses and maximize explanatory power [30].

In this study, we focus on the hyperdiverse, cosmopolitan wasp lineage Cryptini (Hymenoptera, Ichneumonidae, Cryptinae); with 250 genera and over 2400 species [31], cryptines are one of the most speciose groups of wasps [32,33], with an astounding level of morphological homoplasy [34,35]. Most species of Cryptini are ectoparasitoids that attack pupae or prepupae of moths, beetles or other wasps, although some species attack flies, antlions (Neuroptera, Myrmeleontidae) or spider egg sacs. These hosts tend to be concealed to various degrees, from relatively weak substrates such as leaf rolls, twigs and vines to deeply secluded environments such as hardened clay nests and wood [36].

The most comprehensive phylogeny of Cryptini to date, using a worldwide taxonomic sampling and multi-gene molecular data [34], revealed an intriguing evolutionary pattern, with most species that attack deeply concealed hosts concentrated in a single clade, the *Gabunia* genus group. This large (greater than 200 species), worldwide lineage comprises cryptines that parasitize almost exclusively wood-boring beetles (Cerambycidae and Buprestidae) or mud-nesting solitary wasps (Vespidae, Crabronidae, Sphecidae). In that phylogeny, the *Gabunia* group was recovered (albeit with low bootstrap support) as sister to all remaining Cryptini (henceforth *Cryptus* group or *Cryptus* clade), which collectively exploit a much larger assemblage of hosts, including species from at least 78 families of Coleoptera, Diptera, Hymenoptera, Lepidoptera, Neuroptera and Aranae [31]. Therefore, within Cryptini we find two sister clades of clearly contrasting biological and taxonomic diversity.

If variety in host use is associated with higher morphological disparity, one would expect the species from the *Cryptus* group to have occupied a much larger volume of the morphospace than its biologically conserved sister group, either owing to unequal rates of morphological diversification or to different modes of morphospace exploration. Herein, we combine an existing phylogenetic framework, newly generated phylogenomic data and geometric morphometrics to: (i) confirm the placement of the *Gabunia* group as the earliest divergent lineage within Cryptini; (ii) quantify morphological diversity in the *Gabunia* and *Cryptus* groups; and (iii) test the relative roles of rate versus mode of morphological diversification in generating such a disparity.

2. Material and methods

(a) Phylogenetic tree

The phylogenetic framework was extracted from recent revisionary work on Cryptinae [34], a maximum-likelihood tree based on molecular data from seven genes and 109 morphological characters, including 308 species from 181 genera of Cryptini and 62 outgroup taxa (figure 1; electronic supplementary material, file S1). Taxon sampling is roughly proportional to extant diversity across Cryptini, with 32 terminals from the *Gabunia* group (from 242 described species) and 276 from the *Cryptus* group (from circa 2150 known species). Taxa were selected to cover the broadest morphological diversity for each clade, usually with one representative per genus. Since the placement of the *Gabunia* group as sister to all other Cryptini had relatively poor support (bootstrap 54) in that previous study, we used a phylogenomic approach based on ultraconserved elements (UCEs; [37,38]) to confirm this placement in order to validate downstream analyses. A total of 92 species were included in this dataset, including representatives of 10 genera from the *Gabunia* group, 61 from the *Cryptus* group and 21 outgroup taxa. Laboratory protocols follow methods that have been optimized for Hymenoptera [38], using an RNA bait library [39] that targets 2590 UCE loci (see the electronic supplementary material, appendix A1 for a detailed description). Sequencing reads were cleaned, trimmed, assembled and had UCE loci extracted using the Phyluce v1.5 pipeline [40], which incorporates Illumiprocessor [41] and Trinity *de novo* assembler v. r2013-02-25 [42]. UCE loci were then aligned using MAFFT v. 7.130b [43] and trimmed with Gblocks v. 0.91b [44,45]. Alignments were filtered to include only loci available for at least 50% of the taxa, resulting in an alignment including 1474 loci and 405 082 bp of sequence data (electronic supplementary material, file S2 and see appendix A1 for additional detail on matrix preparation). Maximum-likelihood analyses were run using RAxML v. 8.2 [46], with the dataset partitioned by locus and using the GTR+ Γ+I substitution model, with 100 rapid bootstrap replicates. Trees were ultrametricized using the penalized likelihood criterion under a model of autocorrelated rate changes as implemented in the function 'chronos' of the *ape* package in R [47].

(b) Morphospace data collection and analyses

Geometric morphometric analyses were conducted to test for constraints to morphological evolution, specifically to the shape of the mesosoma in cryptine wasps. The mesosoma was chosen because: (i) it is a good proxy for overall body shape; (ii) it is not articulated, a premise for geometric morphometrics; and (iii) it is straightforward to image, avoiding biases from orientation and parallax effects. Selected anatomical landmarks (figure 2*a*) were placed on standardized photographs of female specimens (see the electronic supplementary material, appendix A1 for details and files S3 and S4 on the Dryad Digital Repository: https://doi.org/10.5061/dryad.41r1q12 [48] for images and landmark data). Subsequent analyses were performed using the R packages *ape* [47], *geomorph* [49], *phytools* [50] and *smatr* [51]. Landmarks were

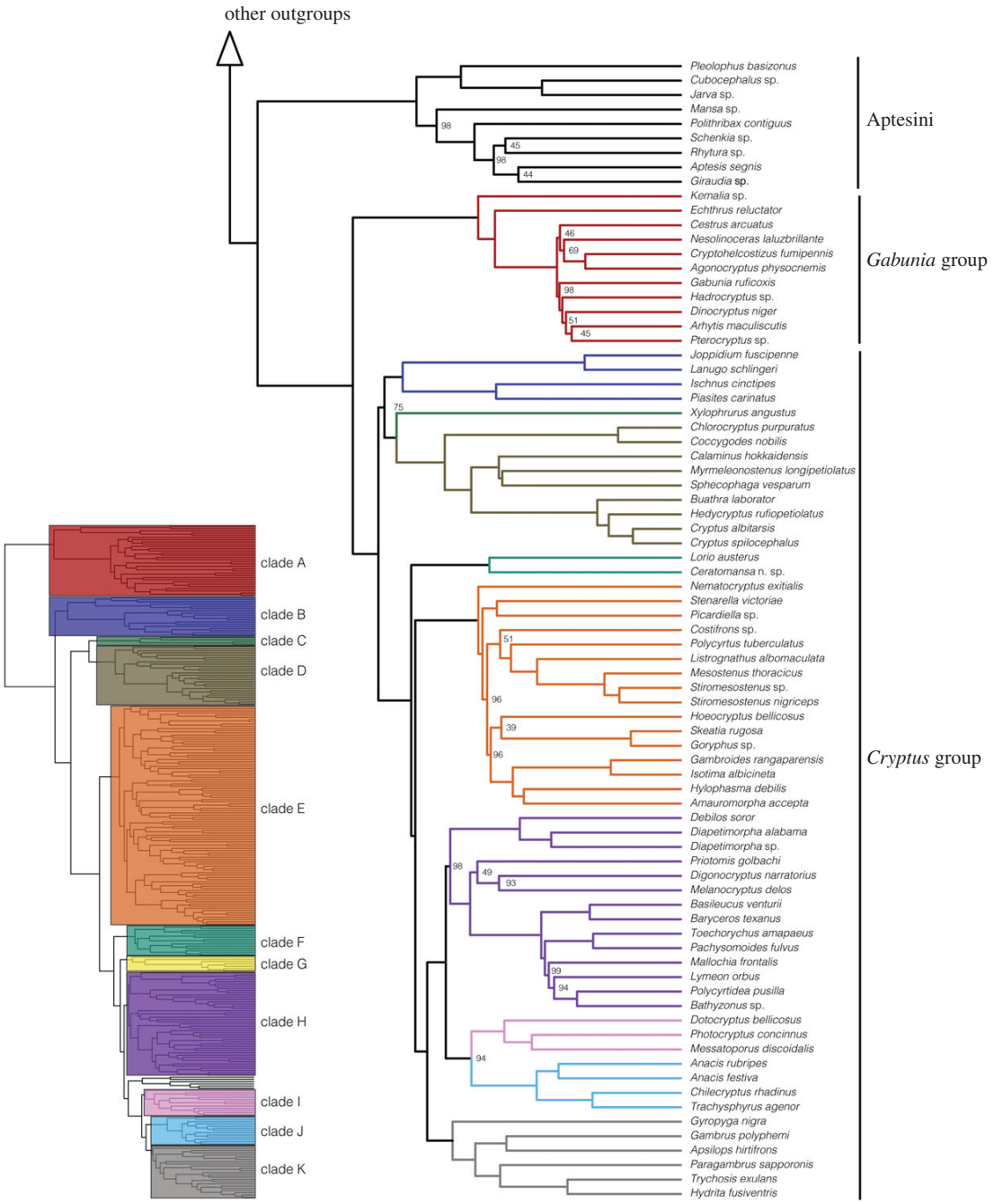

Figure 1. Maximum-likelihood phylogeny obtained from analysis of genomic ultraconserved element (UCE) data for Cryptini. Branches are colour coded to correspond to the main clades identified in a recent, comprehensively sampled phylogeny based on Sanger-sequencing [34] (tree on left side modified from Santos 2017; copyright by John Wiley and Sons, used with permission). Nodes without numbers indicate bootstrap values of 100%.

aligned and superimposed using a generalized Procrustes analysis [52], removing information such as size and orientation to focus purely on geometric shapes (figure 2*b*). Mean shapes were then calculated for each taxon and visualized as a principal component analysis (PCA) on the coordinates projected into the linear tangent space. We kept only the 10 principal components (PCs) with positive eigenvalues, hereafter referred to as 'morphospace' [53].

The magnitude of shape evolution for the *Gabunia* versus *Cryptus* groups was compared using 10 000 iterations of the 'compare.evol.rates' function in *geomorph*, based on the distances between species in the morphospace after phylogenetic transformation [54]. Significance was tested by comparing the rate-ratios observed for the two groups against the expectations of phenotypic disparity under a constant-rate Brownian motion process. The analyses were conducted using both the more inclusive, Sanger-sequencing based tree and the UCE phylogeny. The extension of morphospace occupation was assessed in each clade by measuring the sum of the variances. Significance of the observed discrepancy in variance was tested by simulating morphological evolution under a constant rate Brownian motion process, using the function 'fastBM' in *phytools*, iterated 10 000 times. In order to generate simulations that realistically approximate the variance structure of real cryptine data, we used the standard deviation values of each PC to calibrate the 'sig2' parameter, describing the instantaneous variation of the Brownian motion process.

We computed morphometric branch lengths as the Euclidean distances in the morphospace between the nodes of the phylogeny of Santos [34]. Each internal node was an ancestral shape

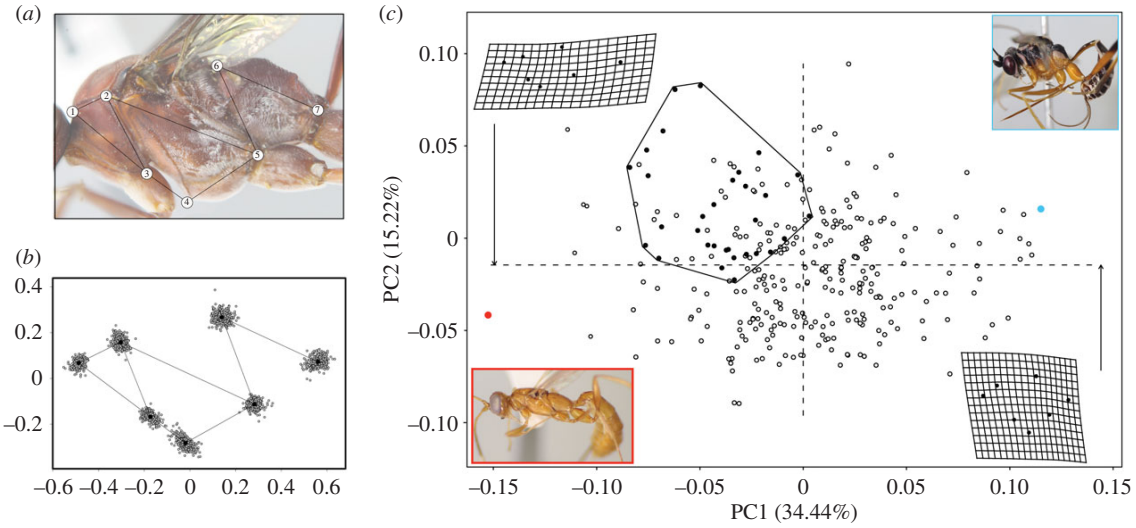

Figure 2. Summary of data and results for geometric morphometric analyses. (*a*) Landmarks used for the geometric morphometrics. (*b*) Set of all landmarks for all studied species aligned by a generalized Procrustes analysis; black dots indicate the mean position for each landmark. (*c*) Two principal components (PC) of the shape space for the mesosoma, plotted over a Procrustes-aligned tangent space. Red dots show the distribution of species of the *Gabunia* group in the cryptine morphospace. Objects in each extremity of the *x*-axis are deformation grids representing the mean shapes at the extreme of the first PC. The pictured specimens are extremes of morphological disparity in the *x*-axis, *Mallochia strigosa* (left) and *Myrmeleonostenus* sp. (right). The polygon delimits the morphospace occupied by species of the *Gabunia* clade. (Online version in colour.)

estimated using the *fastAnc* function of *ape*. In order to test whether magnitude of morphometric change was predicted by the amount of molecular evolution, we compared the branch lengths in the morphospace to the corresponding branch lengths in the molecular phylogeny with a linear regression. We also tested for a difference in the slope between the two clades using the function 'slope.com' from *smatr*.

If host use has an impact on body shape, and if species from the *Gabunia* group are constrained around a body shape optimum for that ecology, then the body shape of species from the *Cryptus* group that attack deeply concealed hosts should also tend towards that optimum. Hence, such lineages should have moved closer to the mean of the *Gabunia* group than other species from the *Cryptus* clade. To test this hypothesis, we computed the distances from each tip of the *Cryptus* clade to the mean of the *Gabunia* clade in the morphospace, as well as the four convergence metrics (C1, C2, C3 and C4) implemented in the R package 'convevol' [55]. We then used a *t*-test to compare the distances and convergence metrics based on host type (see the electronic supplementary material, appendix A1 for details).

3. Results

(a) Ultraconserved element phylogeny

Maximum-likelihood analyses of the phylogenomic UCE dataset (figure 1; electronic supplementary material, file S5) confirmed the placement of the *Gabunia* group as sister to all other Cryptini, with high support (bootstrap 100). The tree topology for Cryptini very closely resembles the one recovered in previous analyses [34] in terms of the composition of main clades, providing further confidence in the more inclusive phylogenetic framework used in the phylomorphospace analyses.

(b) Morphospace variation

The variation in mesosoma of Cryptini and their related taxa was well distributed among the morphospace axes (dimensions). The first dimension accounted for 34.44% of the variation among samples, and primarily described the overall aspect ratio of the mesosoma, opposing taxa with short and stout mesosoma to elongated, dorsoventrally depressed species (figure 2*c*). The second dimension accounted for 15.22% of the variation and described primarily how obliquely sloped was the mesosoma across different species (e.g. the displacement of landmarks 2–3 and 5–6 on the *x*-axis). The percentage of variation explained fell rapidly for the following dimensions, from 12.37% in PC3 to 2.10% in PC10, with the remaining four dimensions (PC11–14) accounting for infinitesimal amounts (less than 0.001%). Overall, the results indicate that members of the *Gabunia* group seem to be mostly restricted to one of the quadrants of the two-dimensional PCA graph of the cryptine morphospace, and occupy that particular area more densely than species of the *Cryptus* group (figure 2*c*).

(c) Constrained evolution in the *Gabunia* group

Comparison of morphological evolution rates (table 1) showed only a modest rate difference between the *Gabunia* and *Cryptus* groups ($M_G = 0.060910$, $M_C = 0.078616$). While the observed rate for the *Cryptus* group was 29.1% higher, that difference was clearly non-significant when compared to simulations ($p = 0.321$): a constant-variance Brownian motion process could have easily generated this difference in magnitude of morphological change (figure 3*a*). Similar results were found when the analyses were repeated using the tree based on UCE data ($M_G = 0.001365$, $M_C = 0.001887$), with a rate-ratio of 1.382 and still far from statistical significance ($p = 0.749$). The implication is that the two clades have probably experienced similar rates of morphological change.

At the same time, species of the *Gabunia* group do occupy a distinctly restricted region of the morphospace; the variance for the *Cryptus* group was consistently higher when considering the sum of the diagonal of the variances for each clade ($V_G = 0.003536$, $V_{NG} = 0.\ 005036$). Such an amount of discrepancy in morphospace occupation (42.5%) was not observed in any

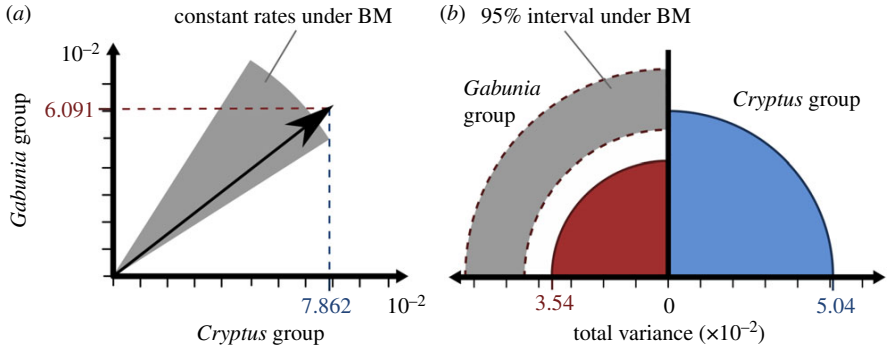

Figure 3. Phylomorphospace statistical results. (*a*) Comparison of the rates of morphological evolution for the two clades. The grey area delimits 95% of the rate-ratios as simulated under a constant-rate Brownian motion (BM) model for the two groups. The arrow illustrates the rate-ratio observed empirically. (*b*) Morphospace occupation in the two investigated clades as measured by the sum of the variances in the *Gabunia* (red) and *Cryptus* (blue) groups. The dotted lines delimit the 95% interval computed from 10 000 iterations of a random assemblage from the *Cryptus* group with sample size equal to that of the *Gabunia* group. (Online version in colour.)

Table 1. Outcomes of phylomorphospace tests for the hypothesis that the *Gabunia* clade is constrained in its morphological evolution. (G, *Gabunia* genus group clade; C, *Cryptus* group, its sister clade. *M*, magnitude of morphological change, proportional to morphological branch lengths in the morphospace or to rates of morphological evolution. *V*, variance among samples within each group. *p*, *p*-values generated through simulations under a constant rate Brownian motion process.)

clade	*M*	M_C/M_G	*p*-value	*V*	V_C/V_G	*p*-value
Gabunia group	0.060910	1.2907	0.321	0.0035366	1.425	<0.0001
Cryptus group	0.078616			0.0050366		

of the 10 000 simulation replicates, in which the maximum observed difference was of 29.4% (figure 3*b*).

Morphometric branch lengths are highly variable within the cryptine tree, and were generally positively correlated with molecular branch lengths, with high statistical significance ($p < 0.00001$; electronic supplementary material, figures S1–S2). The morphometric variation explained by molecular branch lengths was considerable ($R^2 = 0.570$), indicating that the rate of molecular evolution impacts the morphological branch lengths but is not the only factor influencing phenotypic change. In fact, the slope of the regression was clearly higher for the *Cryptus* than for the *Gabunia* group (8.137 versus 4.807; $p < 0.00001$), suggesting that, given the same amount of molecular evolution, the latter clade experiences generally lower morphological change (electronic supplementary material, figure S2).

These results strongly support the idea that the morphology of the *Gabunia* clade is constrained, inhibiting its species to occupy certain areas in the morphospace (figure 4). The similar rates of morphological change suggest that both groups have gone through equivalent 'walks' through the morphospace. However, the highly disparate end results for the two clades in terms of morphological diversity indicate that members of the *Cryptus* clade were more efficient in occupying novel areas of the morphospace, while members of the group have repeatedly evolved similar morphologies.

All four convergence metrics indicate that species in the *Cryptus* clade that attack deeply concealed hosts ($n = 40$) have, in general, moved closer towards the mean shape of the *Gabunia* group compared to species that do not attack deeply concealed hosts ($p = 0.0018$ for C1, 0.0131 for C2 and C4, and 0.0120 for C3). Likewise, those species were significantly closer to the mean of the *Gabunia* group ($p < 0.00001$; electronic supplementary material, figure S3). This result suggests that species with similar ecology tend to gravitate towards the same shape optimum. It provides further evidence that morphospace occupation within the *Gabunia* group is at least partially linked to the use of deeply concealed hosts.

4. Discussion

(a) Observed pattern and possible causes

Our results support the idea that the *Gabunia* group and the remaining Cryptini are indeed sister clades that experienced differing modes of morphological evolution. The rate of morphological change was similar in both clades, fitting well the expectation of a constant rate Brownian motion model. In addition, while the overall magnitude of change in the *Cryptus* group was slightly higher, the method of reconstruction of morphological evolution has probably underestimated the rate of the *Gabunia* group: because the phylomorphospace is generated by minimizing the morphological changes along the tree, branch lengths will be more biased in a clade with high homoplasy such as the *Gabunia* group. Hence, with similar evolutionary rates, the *Cryptus* clade has explored a much greater volume of the morphospace. By contrast, members of the *Gabunia* group distributed their proportionally equal evolutionary change through a much more restricted set of morphotypes. The discrepancy in morphological variance observed across the two clades was higher than in 10 000 simulations under Brownian motion using the ultrametric tree, suggesting that the difference is owing to evolutionary constraints rather than chance or unequal crown group ages.

These results raise an obvious question as to the underlying causes for the constrained morphological diversification within

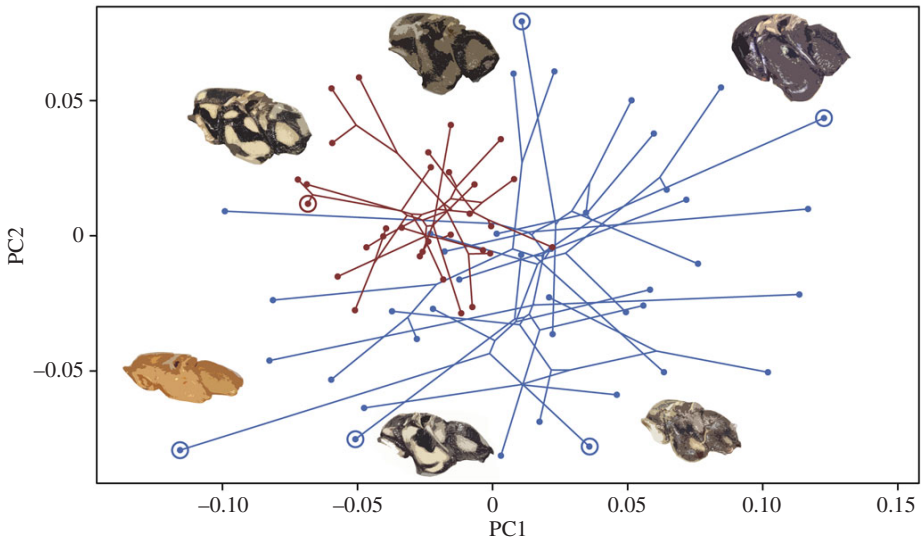

Figure 4. Phylomorphospace projection of the Cryptini phylogeny showing the complete *Gabunia* clade in red and a representative sample of the *Cryptus* group in blue (sample downsized to avoid cramming of the graph). Circled nodes indicate the position of the pictured wasps in the morphospace.

the *Gabunia* clade. Is the limitation in host use enough to explain this phenomenon? It has been suggested that areas in the morphospace not visited by lineages may correspond to implausible or unfeasible phenotypes [1]; however, that is certainly not the case for morphospace regions in our study since they are occupied by other cryptines. This limitation may also be because of unknown, clade-specific developmental constraints acquired by the *Gabunia* group after the split of the two clades, which are yet to be demonstrated. Functional innovations have certainly been shown to generate bursts in cladogenesis and morphological diversification [18,56,57], though several other studies failed to find such a relationship (e.g. [58,59]).

Our analyses show that species in the *Cryptus* group that attack deeply concealed hosts tend to converge towards a body shape closer to the *Gabunia* group than species that attack different hosts (electronic supplementary material, figure S3). Several discrete traits had already been identified as associated with the use of wood-boring or otherwise deeply concealed hosts, and are known both from the *Gabunia* group and from other taxa [32,33,60]. Such purported adaptations include a system for echolocation, or 'vibrational sounding', in which wasps use modified antennal tips to tap the substrate and transmit vibrations that are detected by hypertrophied mechanoreceptors located in their legs (the subgenual organ), informing about the location of the host [61–64]. Several species also show enlarged ovipositor muscles and an ovipositor tip reinforced with a high concentration of metal ion-protein complexes such as manganese, calcium and zinc to drill into the hard substrate [35,65]. Likewise, previous authors noted that wasps with this habit tend to have a 'subcylindric body shape' [32], which is a close description of the patterns generated by the combination of PC1 and PC2 of our shape data (figure 4). While multiple discrete traits have been shown to be correlated with the use of deeply concealed hosts using phylogenetic comparative methods [60,66], the impact on overall body shape has previously been an untested hypothesis.

Parasitic wasps as a whole tend to be specialized towards a type of host, which is thought to impose strong adaptive constraints [36,67]. In fact, host specificity is probably one of the reasons behind the astounding diversity shown by parasitoids, believed by some to comprise the most speciose group of insects in the world [68]. The results of our study corroborate that view by demonstrating how ecological factors related to hosts can have measurable impacts on the evolution and the morphology of these wasps.

(b) Extinction, ghost lineages and the fossil record

One important caveat of this study is that it does not take extinct forms into account. It remains possible that non-random extinction patterns that resulted in substantial loss of morphological diversity within the *Gabunia* group, but not in the *Cryptus* group, could mislead interpretations by generating a false impression of morphological constraint. Phylomorphospace construction uses ancestral state estimations generated by squared-change parsimony [69], which assumes that the inferred ancestor was somewhat intermediary to its descendant taxa. Hence, the method is sensitive to non-random sampling bias, such as the extinction of taxa with morphologies not represented in the extant members of a group. Such a limitation is hard to address empirically, as the fossil record for Ichneumonidae as a whole is still very scarce, and most of the few described taxa need to be re-examined and re-interpreted [70]. Hence, the current state of understanding of fossil diversity precludes any definitive conclusion about the existence of such ghost lineages, and our results should be interpreted as contingent on the existing evidence for the group. An increasing knowledge of the ichneumonid fossil record [70–73] will serve as a fitting test to the patterns observed herein, allowing more powerful interpretations of morphological evolution through time.

(c) Constrained walks in the morphospace

Our results reinforce the idea that clades with high morphological diversity are not necessarily the ones that have undergone higher rates of phenotypic change, but sometimes are instead the clades that distributed change most widely, exploring novel regions of the available morphospace [29]. Likewise, low morphological diversity in a clade may be owing to constraints in morphological innovation. Still, most

recent work on morphological diversification has been concentrated on the magnitude of change [54,74,75]. Many of these studies have investigated morphological diversification under an *a priori* assumption of Brownian motion [12,71,76], which can lead to misleading conclusions if morphological variance does not increase over time as a diffusive process [77,78]. Our approach, following Sidlauskas [29], allows us to explicitly test the observed patterns against a null model of Brownian motion and to accurately quantify deviations from the model. Furthermore, while molecular branch lengths were relatively good predictors for morphometric variation, the relatively low coefficient of determination ($R^2 = 0.570$), leaves room for considerable decoupling between morphospace occupation and overall evolution rates.

Conceptually, evolutionary constraint has often been conflated with phylogenetic inertia [7], a term that has often been used to explain apparently nonadaptive or maladaptive traits, or to describe phenotypic stasis (e.g. [79–81]). Our results help to highlight a distinction between evolutionary stasis—that is, reduced morphological change or lack thereof— and constraint in a broader sense. As shown by our evolutionary rate analyses, members of the *Gabunia* group have undergone a substantial amount of morphological change through the course of their evolution; in fact, probably as much as the remaining Cryptini. Hence, constraints imposed by a particular ecological trait or life strategy should not necessarily be expected to prevent morphological change altogether, but to confine it to a specific, limited set of morphotypes. Our results are in agreement with such an idea: one of our studied clades roams free in developing new morphologies, while the other one seems not to be stuck, but rather running in circles in phylomorphospace.

Ethics. This study adheres to local and national guidelines and regulations and is based upon material collected and obtained under the appropriate licences and permits.

Data accessibility. Data used in this study is available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.41r1q12 [48].

Authors' contributions. B.F.S. and A.P. conceived and designed the study, and analysed the data. B.F.S. collected the data, B.F.S., S.G.B. and A.P. contributed specimens/materials/analysis tools. B.F.S., A.P. and S.G.B. wrote the paper.

Competing interests. The authors have identified no conflict of interest to disclose.

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References

1. Erwin DH. 2007 Disparity: morphological pattern and developmental context. *Palaeontology* **50**, 57–73. (doi:10.1111/j.1475-4983.2006.00614.x)
2. Haber A. 2016 Phenotypic covariation and morphological diversification in the ruminant skull. *Am. Nat.* **187**, 576–591. (doi:10.1086/685811)
3. Losos JB. 2011 Convergence, adaptation, and constraint. *Evolution* **65**, 1827–1840. (doi: 10.1111/j.1558-5646.2011.01289.x)
4. McGhee GR. 2011 *Convergent evolution: limited forms most beautiful*, Cambridge, MA: MIT Press.
5. Bale R, Neveln ID, Bhalla APS, MacIver MA, Patankar NA. 2015 Convergent evolution of mechanically optimal locomotion in aquatic invertebrates and vertebrates. *PLoS Biol.* **13**, e1002123. (doi:10.1371/journal.pbio.1002123)
6. Diniz-Filho, JAF, Sant'Ana CER, Bini, LM. 1998 An eigenvector method for estimating phylogenetic inertia. *Evolution* **52**, 1247–1262. (doi:10.1111/j.1558-5646.1998.tb02006.x)
7. Blomberg SP, Garland T. 2002 Tempo and mode in evolution: phylogenetic inertia, adaptation and comparative methods. *J. Evol. Biol.* **15**, 899–910. (doi:10.1046/j.1420-9101.2002.00472.x)
8. Pienaar J, Ilany A, Geffen E, Yom-Tov Y. 2013 Macroevolution of life–history traits in passerine birds: adaptation and phylogenetic inertia. *Ecol. Lett.* **16**, 571–576. (doi:10.1111/ele.12077)
9. Foote M. 1996 Models of morphological diversification. In *Evolutionary paleobiology* (eds Jablonski D, Erwin DH, Lipps JH), pp. 62–86. Chicago, MI: University of Chicago Press.
10. Foote M. 1997 The evolution of morphological diversity. *Annu. Rev. Ecol. Syst.* **28**, 129–152. (doi:10.1146/annurev.ecolsys.28.1.129)
11. Cooper W, Steppan SJ. 2010 Developmental constraint on the evolution of marsupial forelimb morphology. *Aust. J. Zool.* **58**, 1–15. (doi:10.1071/ZO09102)
12. Collar DC, Near TJ, Wainwright PC. 2005 Comparative analysis of morphological diversity: does disparity accumulate at the same rate in two lineages of centrarchid fishes? *Evolution* **59**, 1783–1794. (doi:10.1111/j.0014-3820.2005.tb01826.x)
13. Hughes M, Gerber S, Wills MA. 2013 Clades reach highest morphological disparity early in their evolution. *Proc. Natl Acad. Sci. USA* **110**, 13 875–13 879. (doi:10.1073/pnas.1302642110)
14. Eastman JM, Alfaro ME, Joyce P, Hipp AL, Harmon LJ. 2011 A novel comparative method for identifying shifts in the rate of character evolution on trees. *Evolution* **65**, 3578–3589. (doi:10.1111/j.1558-5646.2011.01401.x)
15. Venditti C, Meade A, Pagel M. 2011 Multiple routes to mammalian diversity. *Nature* **479**, 393–396. (doi:10.1038/nature10516)
16. Lovette IJ, Bermingham E, Ricklefs RE. 2002 Clade-specific morphological diversification and adaptive radiation in Hawaiian songbirds. *Proc. R. Soc. Lond. B* **269**, 37–42. (doi:10.1098/rspb.2001.1789)
17. Dumont ER, Dávalos LM, Goldberg A, Santana SE, Rex K, Voight CC. 2012 Morphological innovation, diversification and invasion of a new adaptive zone. *Proc. R. Soc. B* **279**, 1797–1805. (doi:10.1098/rspb.2011.2005)
18. Maia R, Rubenstein DR, Shawkney MD. 2013 Key ornamental innovations facilitate diversification in an avian radiation. *Proc. Natl Acad. Sci. USA* **110**, 10 687–10 692. (doi: 10.1073/pnas.1220784110)
19. Derrickson EM, Ricklefs RE. 1988 Taxon-dependent diversification of life-history traits and the perception of phylogenetic constraints. *Funct. Ecol.* **2**, 417–423. (doi:10.2307/2389415)
20. Reginato M, Michelangeli FA. 2016 Diversity and constraints in the floral morphological evolution of *Leandra* s.str. (Melastomataceae). *Ann. Bot.* **118**, 445–458. (doi:10.1093/aob/mcw116)
21. Westneat MW, Alfaro ME, Wainwright PC, Bellwood DR, Grubich JR, Fessler JL, Clements KD, Smith LL. 2005 Local phylogenetic divergence and global evolutionary convergence of skull function in reef fishes of the family Labridae. *Proc. R. Soc. B* **272**, 993–1000. (doi:10.1098/rspb.2004.3013)
22. Roelants K, Haas A, Bossuyt F. 2011 Anuran radiations and the evolution of tadpole

morphospace. *Proc. Natl Acad. Sci. USA* **108**, 8731–8736. (doi:10.1073/pnas.1100633108)

23. Eberle J, Myburgh R, Ahrens D. 2014 The evolution of morphospace in phytophagous scarab chafers: no competition – no divergence? *PLoS ONE* **9**, 1–16. (doi:10.1371/journal.pone.0098536)
24. Raup DM. 1961 The geometry of coiling in gastropods. *Proc. Natl Acad. Sci. USA* **47**, 602–609. (doi:10.1073/pnas.47.4.602)
25. Raup DM. 1966 Geometric analysis of shell coiling: general problems. *J. Paleontol.* **40**, 1178–1190.
26. Gerber S. 2017 The geometry of morphospaces: lessons from the classic Raup shell coiling model. *Biol. Rev.* **92**, 1142–1155. (doi:10.1111/brv.12276)
27. Winemiller KO, Fitzgerald DB, Bower L, Pianka ER. 2015 Functional traits, convergent evolution, and periodic tables of niches. *Ecol. Lett.* **18**, 737–751. (doi:10.1111/ele.12462)
28. Pianka ER, Vitt LJ, Pelegrin N, Fitzgerald DB, Winemiller KO. 2017 Toward a periodic table of niches, or exploring the lizard niche hypervolume. *Am. Nat.* **190**, 601–616. (doi:10.1086/693781)
29. Sidlauskas B. 2008 Continuous and arrested morphological diversification in sister clades of characiform fishes: a phylomorphospace approach. *Evolution* **62**, 3135–3156. (doi:10.1111/j.1558-5646.2008.00519.x)
30. Maddison WP, FitzJohn RG. 2014 The unsolved challenge to phylogenetic correlation teste for categorical characters. *Syst. Biol.* **64**, 127–136. (doi:10.1093/sysbio/syu070)
31. Yu DS, van Achterberg C, Horstmann K. 2012 World Ichenumonoidea 2011. Internet version available at Home of Ichneumonoidea. See http://www.taxapad.com/ (updated 28 April 2012).
32. Townes HK. 1970 The genera of Ichneumonidae, Part 2. *Mem. Am. Entom. Inst.* **12**, 1–537.
33. Quicke DLJ. 2015 *The braconid and ichneumonid parasitoid wasps: biology, systematics, evolution and ecology*. Hoboken, NJ: Wiley Blackwell.
34. Santos BF. 2017 Phylogeny and reclassification of Cryptini (Hymenoptera, Ichneumonidae, Cryptinae), with implications for ichneumonid higher-level classification. *Syst. Entom.* **42**, 650–676. (doi:10.1111/syen.12238)
35. Quicke DLJ, Wyeth P, Fawke JD, Babisuyuk HH, Vicent JFV. 1998 Manganese and zinc in the ovipositors and mandibles of hymenopterous insects. *Zool. J. Linn. Soc.* **124**, 387–397. (doi:10.1006/zjls.1997.0146)
36. Gauld ID. 2006 Family Ichneumonidae. In *Hymenoptera de La región neotropical* (eds PE Hanson, ID Gauld). *Mem. Am. Entom. Inst.* **77**, 446–487.
37. Faircloth BC, McCormack JE, Crawford NG, Harvey MG, Brumfield RT, Glenn TC. 2012 Ultraconserved elements anchor thousands of genetic markers spanning multiple evolutionary timescales. *Syst. Biol.* **61**, 717–726. (doi:10.1093/sysbio/sys004).
38. Faircloth BC, Branstetter MG, White ND, Brady SG. 2015 Target enrichment of ultraconserved elements from arthropods provides a genomic perspective on relationships among Hymenoptera. *Mol. Ecol. Resour.* **15**, 489–501. (doi:10.1111/1755–0998.12328).
39. Branstetter MG, Longino JT, Ward PS, Faircloth BC. 2017 Enriching the ant tree of life: enhanced UCE bait set for genome-scale phylogenetics of ants and other Hymenoptera. *Methods Ecol. Evol.* **8**, 768–776. (doi:10.1111/2041-210X.12742)
40. Faircloth BC. 2016 PHYLUCE is a software package for the analysis of conserved genomic loci. *Bioinformatics* **32**, 786–788. (doi:10.1093/bioinformatics/btv646)
41. Faircloth BC. 2013 Illumiprocessor: a trimmomatic wrapper for parallel adapter and quality trimming. (doi:10.6079/J9ILL). See https://github.com/faircloth-lab/illumiprocessor.
42. Grabherr MG *et al.* 2011 Full–length transcriptome assembly from RNA–Seq data without a reference genome. *Nat. Biotechnol.* **29**, 644–652. (doi:10.1038/nbt.1883).
43. Katoh K, Misawa K, Kuma K, Miyata T. 2002 MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Res.* **30**, 3059–3066. (doi:10.1093/nar/ gkf436).
44. Castresana J. 2000 Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Mol. Biol. Evol.* **17**, 540–552. (doi:10.1093/oxfordjournals.molbev.a026334)
45. Talavera G, Castresana J. 2007 Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. *Syst. Biol.* **56**, 564–577. (doi:10.1080/ 10635150701472164)
46. Stamatakis A. 2014 RAxML version 8: a tool for phylogenetic analysis and post–analysis of large phylogenies. *Bioinformatics* **30**, 1312–1313. (doi:10.1093/bioinformatics/btu033)
47. Paradis E, Claude J, Strimmer K. 2004 APE: analyses of phylogenetics and evolution in R language. *Bioinformatics* **20** 289–290. (doi:10.1093/bioinformatics/btg412)
48. Santos BF, Perrard A, Brady SG. 2019 Data from: Running in circles in phylomorphospace: host environment constrains morphological diversification in parasitic wasps. Dryad Digital Repository. (http://dx.doi.org/10.5061/dryad.41r1q12)
49. Adams DC, Otarola-Castillo E, Sheratt E. 2014 geomorph: software for geometric morphometric analyses. R package version 2.0. See http://cran.r–project.org/web/packages/geomorph/index.html.
50. Revell LJ. 2012 phytools: an R package for phylogenetic comparative biology (and other things). *Methods Ecol. Evol.* **3** 217–223. (doi:10.1111/j.2041-210X.2011.00169.x)
51. Warton DI, Duursma RA, Falster DS, Taskinen S. 2012 Smatr 3–an R package for estimation and inference about allometric lines. *Methods Ecol. Evol.* **3**, 257–259. (doi:10.1111/j.2041-210X.2011.00153.x)
52. Dryden IL, Mardia KV. 1993 Multivariate shape analysis. *Sankhya* **55**, 460–480.
53. Rohlf FJ. 1999 Shape statistics: procrustes superimpositions and tangent spaces. *J. Class.* **16**, 197–223. (doi:10.1007/s003579900054)
54. Adams DC. 2014 Quantifying and comparing phylogenetic evolutionary rates for shape and other high–dimensional phenotypic data. *Syst. Biol.* **63**, 166–177. (doi:10.1093/sysbio/syt105)
55. Stayton TC. 2015 The definition, recognition, and interpretation of convergent evolution, and two new measures for quantifying and assessing the significance of convergence. *Evolution* **69**, 2140–2153. (doi:10.1111/evo.12729)
56. Gavrilets S, Losos JB. 2009 Adaptive radiation: contrasting theory with data. *Science* **323**, 732–737. (doi:10.1126/science.1157966)
57. Yoder JB *et al.* 2010 Ecological opportunity and the origin of adaptive radiations. *J. Evol. Biol.* **23**, 1581–1596. (doi:10.1111/j.1420-9101.2010.02029.x)
58. Slater, GJ, Price SA, Santini F, Alfaro ME. 2010 Diversity versus disparity and the radiation of modern cetaceans. *Proc. R. Soc. B* **277**, 3097. (doi:10.1098/rspb.2010.0408)
59. Dornburg A, Sidlauskas B, Santini F, Sorenson L, Near TJ, Alfaro ME. 2011 The influence of an innovative locomotor strategy on the phenotypic diversification of triggerfish (Family: Balistidae). *Evolution* **65**, 1912–1926. (doi:10.1111/j.1558-5646.2011.01275.x)
60. Laurenne NM, Karatolos N, Quicke DLJ. 2009 Hammering homoplasy: multiple gains and losses of vibrational sounding in cryptine wasps (Insecta: Hymenoptera: Ichneumonidae). *Biol. J. Linn. Soc.* **96**, 82–102. (doi:10.1111/j.1095-8312.2008.01114.x)
61. Henaut A. 1990 Study of the sound produced by *Pimpla instigator* (Hymenoptera: Ichneumonidae) during host selection. *Entomophaga* **35**, 127–139. (doi:10.1007/BF02374309)
62. Otten H, Wäckers FL, Isidoro N, Romani R, Dorn S. 2002 The subgenual organ in *Pimpla turrionelae* L. (Hymenoptera Ichneumonidae): ultrastructure and behavioral evidence for its involvement in vibrational sounding. *Redia* **85**, 61–76.
63. Vilhelmsen L, Isidoro N, Romani R, Basibuyuk HH, Quicke DLJ. 2001 Host location and oviposition in a basal group of parasitic wasps: the subgenual organ, ovipositor apparatus and associated structures in the Orussidae (Hymenoptera, Insecta). *Zoomorphology* **121**, 63–84. (doi:10.1007/s004350100046)
64. Broad GR, Quicke DLJ. 2000 The adaptive significance of host location by vibrational sounding in parasitoid wasps. *Proc. R. Soc. Lond. B* **267**, 2304–2409. (doi:10.1098/rspb.2000.1298)
65. Quicke DLJ, Palmer-Wilson J, Burrough A, Broad GR. 2004 Discovery of calcium enrichment in cutting teeth of parasitic wasp ovipositors (Hymenoptera: Ichneumonoidea). *Afr. Entomol.* **12**, 259–264.
66. Santos BF, Perrard A. 2013 Testing the dutilleul syndrome: host use drives the convergent evolution of multiple traits in parasitic wasps. *J. Evol. Biol.* **31**, 1430–1439. (doi:10.1111/jeb.13343)
67. Godfray HCJ. 1994. *Parasitoids: behavioral and evolutionary ecology*. Princeton, NJ: Princeton University Press.
68. Forbes AA, Bagley RK, Beer MA, Hippee AC, Widmayer HA. 2018 Quantifying the unquantifiable: why Hymenoptera, not Coleoptera, is the most speciose animal order. *BMC Ecol.* **18**, 21. (doi:10.1186/s12898-018-0176-x)
69. Rholf FJ. 2002 Geometric morphometrics and phylogeny. In *Morphology, shape and phylogeny*

(eds N MacLeod, PL Forey), pp. 175–193. Boca Ratón, FL: CRC Press.
70. Spasojevic T, Broad GR, Bennett AMR, Klopfstein S. 2017 Ichneumonid parasitoid wasps from the Early Eocene Green River Formation: five new species and a revision of the known fauna (Hymenoptera, Ichneumonidae). *PalZ* **92**, 35–63. (doi:10.1007/s12542-017-0365-5)
71. Khalaim AI. 2008. Fossil ichneumon wasps (Hymenoptera: Ichneumonidae) from Biamo (Russia), Oligocene. *Alavesia* **2**, 101–112
72. McKellar RC, Kopylov DS, Engel MS. 2013 Ichneumonidae (Insecta: Hymenoptera) in Canadian Late Cretaceous amber. *Fossil Record* **16**, 217–227. (doi:10.1002/mmng.201300011)
73. Harmon LJ, Schulte JA, Larson A, Losos JB. 2003 Tempo and mode of evolutionary radiation in iguanian lizards. *Science* **301**, 961–964. (doi:10.1126/science.1084786)
74. O'Meara BC, Aneé C, Sanderson MJ, Wainwright PC. 2006 Testing for different rates of continuous trait evolution using likelihood. *Evolution* **60**, 922–933. (doi:10.1111/j.0014-3820.2006.tb01171.x)
75. Ackerly DD, Nyffeler R. 2004 Evolutionary diversification of continuous traits: phylogenetic tests and application to seed size in the California flora. *Evol. Ecol.* **18**, 249–272. (doi:10.1023/B:EVEC.0000035031.50566.60)
76. Collar DC, Wainwright PC. 2006. Discordance between morphological and mechanical diversity in the feeding mechanism of centrarchid fishes. *Evolution* **60**, 2575–2584. (doi:10.1111/j.0014-3820.2006.tb01891.x)
77. Felsenstein J. 1985. Phylogenies and the comparative method. *Am. Nat.* **125**, 1–15. (doi:10.1086/284325)
78. Ricklefs RE. 2006 Global variation in the diversification rate of passerine birds. *Ecology* **87**, 2468–2478. (doi:10.1890/0012-9658(2006)87[2468:GVITDR]2.0.CO;2)
79. Wilson EO. 1975 *Sociobiology. The New synthesis*. Cambridge, MA: Harvard University Press.
80. Gould SJ, Lewontin RC. 1979. The spandrels of San Marco and the panglossian paradigm: a critique of the adaptationist programme. *Proc. R. Soc. Lond. B* **205**, 581–598. (doi:10.1098/rspb.1979.0086)
81. Bon R, Joachim J, Maublanc ML. 1995. Do lambs affect feeding habitat use by lactating female mouflons in spring in areas free of predators? *J. Zool.* **235**, 43–51. (doi:10.1111/j.1469-7998.1995.tb05126.x)