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The Evolution of Ecomorphological Convergence in Dwarf Chameleons (*Bradypodion*)

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ABSTRACT: Environmental variation can drive patterns of directional selection, often leading to adaptive changes in shape and form. When selection pressures are strong, different lineages may converge on similar phenotypes, or phenotypes may be conserved for a particular habitat type. In the dwarf chameleons (*Bradypodion*) of southern Africa, it has been hypothesized that some lineages have converged on similar phenotypes (ecomorphs) when they occur in habitats with similar vegetation structure. However, the convergence of lineages into ecomorphs has not been explicitly tested across the genus and could be due to other factors such as morphological conservatism or shifts of entire clades to new trait states. To assess these alternatives, we measured functional morphological traits across 24 lineages of *Bradypodion* and tested for evidence of phenotypic convergence using time-calibrated phylogenies. Our results indicated that many lineages that do not share a common ancestor, but are from similar habitats, have converged on similar phenotypes (i.e., tail length, hand size, foot size, casque height), with ecology showing a stronger influence than evolutionary history. We interpret this as adaptive changes in morphology in response to the constraints imposed by different habitat types.

Keywords: Africa, adaptation, ecomorph, habitat, morphology, reptiles.

Introduction

Environmental influences are considered one of the major driving forces behind phenotypic diversification and adaptation (Huxley 1942; Simpson 1953; Schluter 2000, 2001). Species that occupy similar ecosystems and microhabitats frequently exhibit similar morphological and behavioral features owing to convergent evolution (Losos 2009; Kolbe et al. 2011; Vidal-García et al. 2015). Presumably, similar selective pressures within analogous habitats impose directional selection, resulting in convergent forms (Losos 2009; Esquerré and Keogh 2016; Moen et al. 2016). However, not all species occupying similar habitats will show convergence, as there are a number of factors that can constrain adaptation, including phylogenetic history, developmental constraints, genetic drift, and interspecific competition (Harvey and Pagel 1991; Losos and Miles 1994; Schluter 1996; McGuigan et al. 2005; Galis et al. 2018). Furthermore, similar phenotypes can also occur through stochastic evolutionary processes (Stayton 2015), through morphological conservatism of ancestral phenotypes (e.g., Branch et al. 2014; Pelegrin et al. 2021), or through entire clades evolving toward a new trait space (Garland et al. 1993). However, when morphological evidence is combined with environmental data and appropriate statistical tests, the presence of convergent evolution can be quantified and substantiated (Arbuckle et al. 2014; Stayton 2015).

Different ecomorphological forms are thought to occur within the dwarf chameleons (*Bradypodion*; Tolley et al. 2006; Hopkins and Tolley 2011; da Silva and Tolley 2013; da Silva et al. 2014). These chameleons are native to southern Africa, and the genus has undergone periods of rapid lineage diversification attributed to ecological speciation as a result of changes in natural environments since the Miocene epoch (e.g., Tolley et al. 2008, 2013, 2025). Previous work has suggested that there are two ecomorph types

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(Measey et al. 2009; Herrel et al. 2011; da Silva et al. 2014), classified as open habitat ecomorph and closed habitat ecomorph. Species of these ecomorphs occur in different habitats (open canopy heathlands/grassy savanna and closed canopy forest, respectively) and have morphological and performance differences that are adaptive in their respective habitats (Herrel et al. 2011, 2013; da Silva et al. 2014). For example, forest species have exaggerated ornamentation, vibrant coloration, and long tails. Tail length is likely to be beneficial for navigation of the complex array of branches, whereas the ornamentation and coloration traits are known to facilitate intraspecific communication (Stuart-Fox et al. 2007), which may be particularly important in the forest environment (Measey et al. 2009). In contrast, open canopy species have muted coloration, reduced ornamentation, and short tails (Hopkins and Tolley 2011; Tolley et al. 2025). These are features that may be adaptive in habitats that lack canopy cover, allowing for individuals to be more cryptic to predators (Hopkins and Tolley 2011; da Silva et al. 2014; Tolley et al. 2025). Furthermore, the open/closed ecomorphs types may be an oversimplification, as there is morphological evidence for an additional ecomorph type occurring in shrubby habitats (Tolley et al. 2025). Although shrub habitats also have a low open canopy similar to grasslands and fynbos habitats, they generally have thicker perches (Barends et al. 2024; Herrel et al. 2024). These shrub species tend to be heavily ornamented but with very short tails and muted, contrasting coloration (Tolley and Burger 2007; Tolley et al. 2025). The specific advantages of these features in shrub habitats have not been investigated but likely represent adaptations to the mechanical demands of thicker perches combined with selective pressures for reduced detectability and a habitat where bushes are sparsely placed, necessitating ground crossings and therefore a more terrestrial lifestyle.

The three ecomorphs occur throughout the phylogeny in different clades. In some cases, several species of a single ecomorph share a recent common ancestor, but in many instances, species of the same ecomorph have evolved independently in different clades, some separated by millions of years of divergence (Tolley et al. 2008, 2013). The ecomorph types are generally not disputed (e.g., da Silva and Tolley 2017; Tolley et al. 2019), but competing hypotheses regarding the evolutionary origin of the ecomorphs have not been tested. For example, the phylogenetic pattern may be due to repeated instances of convergence because of directional selection associated with habitat type. The habitats differ widely in the substrates available to chameleons (Barends et al. 2024), and these structural characteristics may exert different selective pressures, driving the evolution of the ecomorph types. Alternatively, given that forest is the ancestral habitat for *Bradypodion* (Tolley et al. 2008, 2013), retention of the forest ecomorph in divergent lineages could be the result of morphological conservatism, with an optimal forest phenotype retained for species that occur in forest habitats. Finally, some species of the open habitat ecomorph are clustered in the phylogeny, suggesting that entire clades may be evolving toward a new trait space as a whole. We therefore assessed whether lineages of *Bradypodion* that occupy similar environmental niches evolved similar morphological traits and can be classified into three ecomorphs. We also test whether there is significant evidence of convergent evolution among species within each of the three putative ecomorphs.

Material and Methods

Study Group

Bradypodion are small- to medium-sized chameleons (i.e., adult body length from 45 to 95 mm) that are near endemic to South Africa (Tolley and Burger 2007). There are 20 described species and a number of additional lineages that are thought to represent either undescribed species or morphologically divergent populations of described species (Tolley and Burger 2007). Although they occur across different habitat types (see below), they are absent from the arid interior of the country. All species are viviparous, with clutch sizes often up to 15 or more babies, each about 20–25 mm in body length (Tolley and Burger 2007; Anderson and Tolley 2011). Because of the fairly mild climate, most species are active throughout the year, breeding is asynchronous, multiple clutches can be produced annually, and individual clutches can have multiple paternity (Tolley and Burger 2007; Tolley et al. 2014). Population abundance is variable, survival is positively correlated with body size (Tolley et al. 2010; Katz et al. 2013), and dispersal distances and rates are low (Tolley et al. 2010; Rebelo et al. 2022). For most species, there is female-biased sexual dimorphism (Barends et al. 2024), and females are typically aggressive toward males (Stuart-Fox et al. 2006), with males exhibiting perching behaviors consistent with mate guarding rather than showing territoriality (Rebelo et al. 2022).

Data Collection

Morphological and ecological data were collected from 986 adult chameleons (499 females; 487 males) between 2008 and 2012 for 24 different lineages of *Bradypodion* (table 1), each representing a distinct evolutionary unit. In most cases, lineages represent described species, although we include a few morphologically or genetically divergent lineages that may represent candidate species that are not yet described (table 1; see Tolley and Burger 2007; Hopkins and Tolley 2011; da Silva and Tolley 2013; Tolley et al. 2019, 2025). Adults were classified as having a snout-vent

Table 1: Lineage name, ecomorph type, sample size (*N*), and general locality of the 24 *Bradypodion* lineages sampled from South Africa during this study

Lineage	Morph	Total sample size	Males (*N*)	Females (*N*)	General locality
B. atromontanum	LBC	16	7	9	Swartberg Mountains, Western Cape
B. barbatulum	LBC	75	42	33	Tsitsikamma Mountains, Eastern Cape
B. baviaanense	LBC	18	11	7	Baviaanskloof Mountains, Eastern Cape
B. caeruleogula	Forest	22	11	11	Dlinza Forest, KwaZulu-Natal
B. caffrum (forest)	Forest	25	15	10	Port St. Johns, Eastern Cape
B. caffrum (grass)	LBC	27	13	14	Mkambati, KwaZulu-Natal
B. damaranum	Forest	60	20	40	Knysna, Western Cape
B. dracomontanum	Forest	9	3	6	Drakensberg Mountains, KwaZulu-Natal
B. gutturale (lowland)	Shrub	4	3	1	Worcester, Western Cape
B. gutturale (montane)	LBC	14	6	8	Grootwinterhoek Mountains, Western Cape
B. kentanicum	Forest	19	7	12	Dwesa Nature Reserve, KwaZulu-Natal
B. melanocephalum	LBC	59	22	37	Durban, KwaZulu-Natal
B. nemorale	Forest	13	10	3	Nkandla and Kranskop Forest, KwaZulu-Natal
B. occidentale	Shrub	68	42	26	Port Nolloth, Northern Cape
B. pumilum (forest)	Forest	106	50	56	Stellenbosch, Western Cape
B. pumilum (fynbos)	LBC	124	57	67	Hottentot-Hollands Mountains, Western Cape
B. setaroi	Forest	31	21	10	St. Lucia, KwaZulu-Natal
Bradypodion sp. ("emerald")	Forest	49	18	31	Drakensberg Mountains, KwaZulu-Natal
Bradypodion sp. ("groendal")	LBC	4	3	1	Grootwinterhoek Mountains, Eastern Cape
Bradypodion sp. ("karkloof")	Forest	24	12	12	Karkloof Forest KwaZulu-Natal
B. taeniabronchum	LBC	42	22	20	Elandsberg Mountains, Eastern Cape
B. thamnobates	Forest	102	61	41	Dargle Valley, KwaZulu-Natal
B. transvaalense	Forest	45	22	23	Soutpansberg Mountains and Woodbush, Limpopo
B. ventrale	Shrub	30	21	9	Gqeberha, Eastern Cape

Note: LBC = little brown chameleon.

length (SVL) larger than 45 mm. Samples sizes ranged from four (*Bradypodion* sp. "groendal," *B. gutturale* [lowland]) to 124 (*B. pumilum* [fynbos]), with a mean and median sample size of 41.2 and 28.5, respectively (table 1). Of the 986 chameleons, 79.8% were measured on live individuals during field surveys, with 20.2% of the data collected from museum specimens to supplement the dataset. All museum specimens measured were from the Port Elizabeth Museum, Gqeberha, South Africa.

At each field site, chameleons were located at night using strong LED torches with focused beams, with two to four researchers walking transects for 1–3 h per session. Chameleons were captured by hand, their GPS coordinates were recorded, and the vegetation they were found on (tree, shrub, or grass) was marked with barricade tape. Chameleons were kept in cloth bags overnight and were measured the following day. Chameleons were subsequently released at their exact points of capture, as indicated by the barricade tape. To avoid recapturing chameleons if a site was revisited, each chameleon was marked with a visible black dot (3–4 mm in diameter) on the ventral surface using a nontoxic surgical skin marker (Tondaus). In addition, tail clips (~2–3 mm) of each individual were taken to collect DNA samples for a separate study. The black dot acted as a short-term batch mark (weeks to months), whereas the tail tip was used as a long-term batch mark, as chameleons do not have tail autotomy; therefore, any individual with a blunted tail tip was presumed to be a recapture. All necessary ethics and permits to conduct fieldwork were acquired (see the acknowledgements for details).

The 24 lineages were classified a priori into one of three habitat types (figs. 1, 2) based on the structural characteristics of their environment: little brown chameleons (LBCs), previously termed "open" ecomorph from grassy savanna or fynbos habitats ($N = 9$); forest chameleons, previously

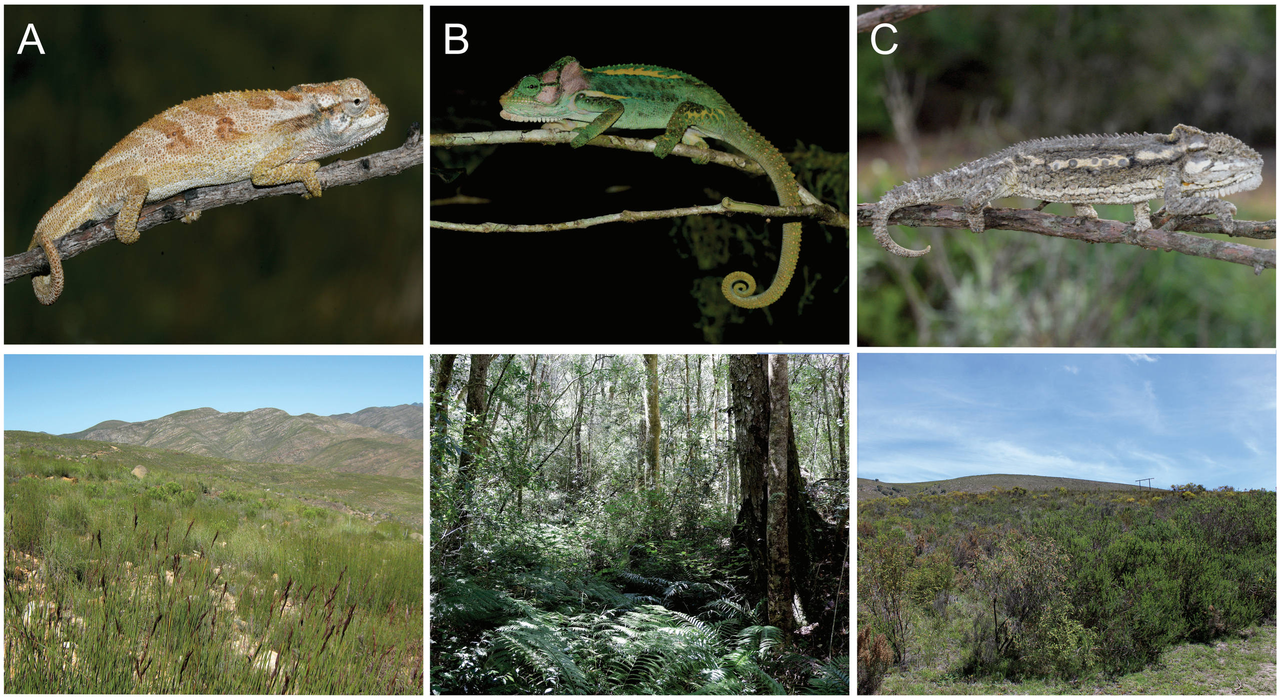

Figure 1: Examples of each ecomorph and its respective habitat for *Bradypodion*. *A*, *Bradypodion barbatulum*, little brown chameleon. *B*, *Bradypodion damaranum*, forest. *C*, *Bradypodion ventrale*, shrub. Photographs by K. A. Tolley.

termed "closed" ecomorph from closed forest habitats ($N = 12$); and a new category, shrub chameleons, from open shrubby, semiarid habitats ($N = 3$; figs. 1, 2). Specifically, LBCs occur in grassy savanna or fynbos habitats, with low vegetation cover (0.3–1.5 m in height) characterized by clusters of dense vertical perches (Barends et al. 2024) and a partly herbaceous understory. Lineages of LBCs have previously been shown to have a small body size with reduced ornamentation, including small casques, small gular scales, and muted brown to green coloration (Tolley and Burger 2007; Hopkins and Tolley 2011; da Silva and Tolley 2013). Forest chameleons occur in Afrotemperate, scarp, or coastal forests, which have a closed and dense high canopy (30 m or more) with a shrubby and herbaceous understory. Structurally, forests are diverse, with a mixture of vertical and horizontal perches (Herrel et al. 2011; da Silva and Tolley 2013; Barends et al. 2024). Forest species are generally large in body size, with long tails, and have conspicuous ornamentation, such as prominent casques, gular scales, tubercles, and dorsal crests (Tolley and Burger 2007; Hopkins and Tolley 2011; da Silva and Tolley 2013). They are brightly colored, with a range of colors such as green, purple, blue, orange, pink, and red. Shrub species occur in open shrubby, semiarid habitats with a low karroid or thicket vegetation (0.5–1.5 m in height) that has little herbaceous understory, with open ground between bushes (Barends et al. 2024). Shrub species are large in body size and sometimes have strongly contrasting, but not vibrant, coloration, often with gray, brown, green, and orange tones. While the fynbos/grass habitats are also characterized by low open canopy, shrub habitat has thicker perches (Barends et al. 2024; Herrel et al. 2024) and open ground between bushes, necessitating chameleons to cross bare ground between bushes.

Morphological Measurements

Chameleons were measured using Mitutoyo digital calipers to the nearest 0.01 mm for 19 measurements: SVL, tail length (TL), head length (from posterior-dorsal point of casque to snout; HL), head width behind the eyes (HW), head height including lower jaw (HH), lower jaw length (LJL), cantilever to anterior tip of jaw (CT), quadrate to anterior tip of jaw (QT), casque height from lower corner of the temporal fenestra to tip of the casque (CH), femur as measured from the thigh (FM), tibia as measured from the shin (TB), tarsal measured as the width across the fused lateral aspect of the metatarsals (TARS), length of medial hindfoot (i.e., tarsals, metatarsals, and phalanges excluding the distal phalange; MF), length of lateral hindfoot (i.e., tarsals, metatarsals, and phalanges excluding the distal phalange; LF), humerus as measured from the upper arm (HM), radius as measured from the forearm (RD), carpal

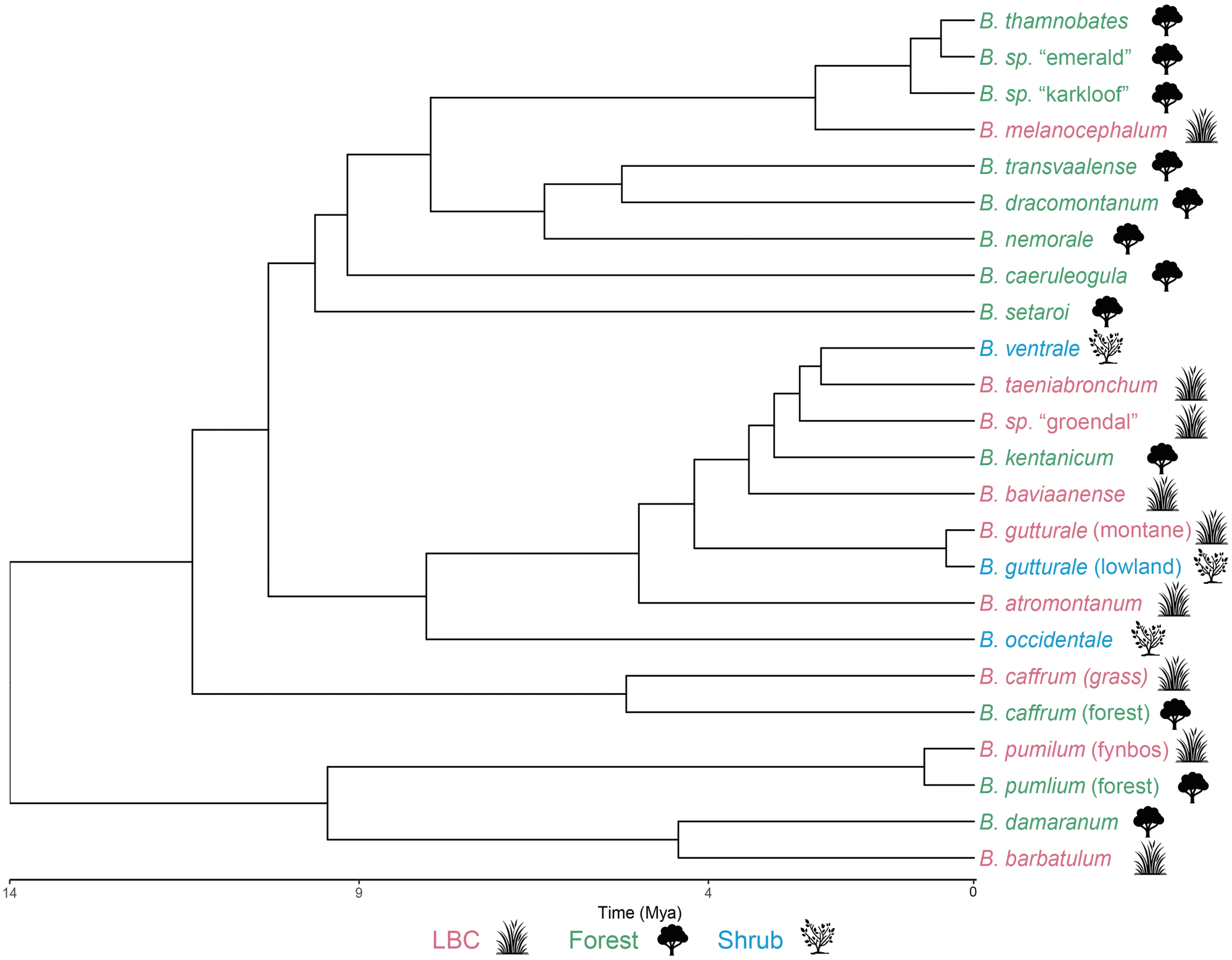

Figure 2: Phylogenetic tree of *Bradypodion* pruned to the lineages used to assess convergence of species/lineages into ecomorph types. Color and icon of the terminal tips represent ecomorph type: pink and grass indicate little brown chameleon (LBC), green and tree indicate forest, and blue and shrub indicate shrub. Described species are labeled accordingly, undescribed species are indicated as *Bradypodion* sp. (with nicknames in quotations), and lineages of described species that occur in two different habitats are indicated by that habitat type (i.e., grass, forest, fynbos), per Tolley and Burger (2007).

measured as the width across the fused lateral aspect of the metacarpals (CARP), length of the medial hand (i.e., carpals, metacarpals, and phalanges excluding the distal phalange; MH), and length of the lateral hand (i.e., carpals, metacarpals, and phalanges excluding the distal phalange; LH). All variables were $\log_{10}$ transformed before analyses to meet assumptions of homoscedasticity and normality. Raw morphological variables for all individuals can be found in table S1 (tables S1–S14 are available online).

Analyses of Ecomorphs

Phylogenetic Reconstruction. To control for the effect of phylogeny in assessing whether lineages are convergent for ecomorph type, a phylogenetic comparative approach was applied by constructing a phylogeny that incorporated one to three individuals for each of the 24 lineages, totaling 64 in-group taxa. The DNA sequence data for a total of 2,516 bp from two mitochondrial genes (*16S* = 381 bp, *ND2* = 747 bp) and two nuclear genes (*RAG-1* = 842 bp, *PRLR* = 528 bp) were either downloaded from GenBank or, if unavailable, generated for this study (table S2). New DNA sequences were generated using primers vMet2 and vTrp for *ND2* (Cunningham and Cherry 2004), 16Sa and 16Sb (Palumbi 1996) for *16S*, PRLR-ChamF1 and PRLR-ChamR1 for *PRLR* (Tolley et al. 2013), and RAG-ChamF1 and RAG1-ChamR1 for *RAG-1* (Tolley et al. 2013). The polymerase chain reactions (PCRs) were carried out in volumes of 25 μL containing approximately 25–50 ng/μL of genomic DNA, 1× PCR reaction buffer, 2.5 mM $MgCl_2$, 0.33 μM of each primer, 0.2 mM deoxynucleoside triphosphate solution, and 0.5 U/μL of SuperTherm Taq polymerase. Thermal cycling included an initial denaturation for 4 min at 94°C followed by 35 cycles with denaturation for 30 s at 94°C, annealing for 30–40 s at 52°C–55°C, extension for 40 s at 72°C, and final extension for 5 min at 72°C. PCR products were run on a 1% agarose gel and visualized under an ultraviolet light to verify amplification. Sanger sequencing was carried out at Macrogen (Amsterdam) using the forward primers for each gene. Sequences were edited and aligned using Geneious (ver. 11.1.5; https://www.geneious.com) using the MUSCLE alignment algorithm with default settings. Heterozygous sites for nuclear genes were coded as ambiguities.

For use in the phylogenetic comparative analysis, a time-calibrated ultrametric phylogeny was generated for a combined dataset of four genes and included representatives of all other genera of Chamaeleonidae (except *Palleon*) downloaded from GenBank (table S2). In addition, to provide fossil calibrations to allow for dating of the phylogeny, an additional 27 squamate taxa were included from different families plus Sphenodontidae for a total of 205 taxa. The software jModelTest (ver. 1.1; Posada 2008) was used to assess the evolutionary model that best fitted each of the genes using the Akaike information criterion (AIC) test. BEAST (Bayesian evolutionary analysis sampling trees) was run with XML files created in BEAUTi (ver. 2). Gene partitions were unlinked, with the GTR+I+G for *ND2* and *16S* and HYK+I+G for *RAG-1* and *PRLR*. Substitution rates, invariable sites, and gamma distribution (α) were input based on the values estimated by jModelTest. A lognormal relaxed clock (unlinked) was used, and the Yule speciation model was applied with linked trees. Following Tolley et al. (2013), divergence times were estimated by constraining nine nodes and placing a calibration at each, corresponding to the oldest known fossil(s) for that clade (tables S3, S4). One fossil node was at the lepidosaurian root, and seven were within the squamate clade. One additional node constrained the family Chamaeleonidae, with a secondary calibration point based on the age of that node in Tolley et al. (2013). Each calibration constraint followed the same lognormal prior distribution and zero offset (i.e., age of the fossil) used in Tolley et al. (2013), except for the node constraining Chamaeleonidae, at which a normal prior distribution was placed. As in Tolley et al. (2013), the mean and standard deviation for each prior distribution covering the 95% highest posterior density were approximately 1% more recent than the estimated fossil age, and the bulk of the probability was placed near the estimated fossil age, but with long probability tails providing a soft maximum bound for the node age.

The analysis was run twice in parallel for 200 million generations, with one run saving trees every 1,000 generations and the other run saving trees every 5,000 generations, to assess the effect of sampling density. The analyses produced sets of 100,000 and 40,000 trees, respectively, each followed by a 50% burn-in. The log files were checked in Tracer (Rambaut et al. 2018) to examine tree likelihood and parameter estimates for evidence of mixing and convergence, evaluated by effective sample size greater than 200 (after burn-in). TreeAnnotator (ver. 2.1.2; Bouckaert et al. 2014) was used to produce an ultrametric consensus maximum clade credibility tree for the set of post-burn-in trees, setting the posterior probability limit to 0.5. Both analyses produced the same topology and node support, so the runs were combined using LogCombiner (part of the BEAST package), and a single consensus tree was generated for downstream analyses. The out-group taxa were removed, and the in-group tips were pruned to remove multiple individuals, retaining one tip per lineage (table S2; fig. S2; figs. S1–S3 are available online). The final phylogenetic tree was subsequently trimmed to include only the lineages in our analyses using the drop.tip() function from the ape package (ver. 5.8; Paradis et al. 2004) in R (ver. 4.4.0; R Core Team 2024; fig. 2).

Phylogenetic Analyses of Variance. To remove the effects of body size in the analyses, all variables were regressed against $\log_{10}$ SVL with a phylogenetic regression, using the phyl.resid function of the phytools package (ver. 2.3-0; Revell 2012; tables S5, S6). Size correction was applied to quantify shape variation, as it has been previously shown that body size itself varies among ecomorphs (Barends et al. 2024). For this purpose, the mean value of each morphological variable was estimated for each lineage.

To identify whether the putative ecomorphs differ significantly in shape, phylogenetic multivariate analyses of variance (MANOVAs) were conducted on lineage means of each size-corrected variable separately by sex, with ecomorph as the fixed factor. The MANOVAs were conducted by first running a generalized least squares model in the mvgls function of the mvMORPH package (ver. 1.2.1; Clavel et al. 2015) in R, using the Brownian motion (BM) evolutionary model and the penalized log-likelihood cross validation. The mvgls object was then passed to the manova.gls function of the same package. For each MANOVA, we ran 1,000 simulations and Wilks's λ was used as the test statistic. When significant differences were highlighted among ecomorphs ($P \leq .05$), subsequent phylogenetic ANOVAs were conducted for all traits using the phylANOVA function in the phytools package (ver. 2.3-0) with a Holm's post hoc correction and 1,000 simulations to identify which ecomorph pairs differed.

Phylomorphospace Plot. To visualize morphological patterns among ecomorphs while taking into account phylogeny, a phylomorphospace plot was created. To achieve this, phylogenetic principal component analyses (pPCAs) were conducted on the size-corrected morphological variables for each sex separately. The pPCAs were performed using the gm.prcomp function of the geomorph package (ver. 4.0.6; Adams and Otárola-Castillo 2013). The proportion of total variance explained by each of the pPCA vectors was calculated, and the loadings of the original morphological variables were evaluated to identify the contribution of each trait on each vector. The phylogenetic tree was projected into morphospace for each sex, using the phylomorphospace function of the phytools package (ver. 2.3-0; Revell 2012).

Lineage Convergence for Ecomorph Types. The presence of convergent evolution among lineages of the same ecomorph was investigated using the search.conv function of the RRPhylo package (ver. 2.8.1; Castiglione et al. 2018). The search.conv function uses the time-calibrated phylogeny to identify convergent evolutionary changes by evaluating whether branches associated with similar phenotypic states exhibit greater similarity than expected under a BM model of evolution. By applying a time-calibrated phylogeny, the timing of convergence across lineages could also be assessed. We employed the "state" version rather than the "clade" version because we were testing for convergence among lineages sharing similar ecomorph characteristics that may be distributed across different phylogenetic clades. Occurrence of convergence among lineages is assessed through mean angles, which are the phenotypic distances between species, with large mean angles indicating greater phylogenetic distance. Two angle metrics are given: ang.state and ang.state.time. The former is based on the angular distance between evolutionary trajectories in the phenotypic space, while ang.state.time incorporates a temporal dimension to test whether convergence occurred in the same time frame. Significance of these mean angles is then calculated, with a statistically significant value indicating convergence.

The strength of convergent evolution for individual traits was estimated by the Wheatsheaf index. This statistic estimates the average pairwise distance between lineages of the same ecomorph group (i.e., the focal group) compared with those in the other ecomorph groups (i.e., nonfocal group) while considering phylogenetic relatedness. If lineages within the same ecomorph group are morphologically more similar than expected by chance, they are considered to have stronger convergence and thus have a higher Wheatsheaf index. For this analysis, the size-corrected morphological variables were grouped into functionally related groups to highlight evidence of biologically relevant convergence and to reduce the number of variables used for the analyses. These functional sets were head (HL, HW, HH, LJL, CT, QT, and CH), tail length (TL), forelimb (HM and RD), hindlimb (FM and TB), forefoot (CARP, MF, and LF), and hindfoot (TARS, MH, and LH). Strength of convergence was tested using lineage means of each functional set for each sex and for each ecomorph group. Statistical significance ($P \leq .05$) of the Wheatsheaf index was estimated using 1,000 bootstrap replications. Significant P values indicate that convergence is significantly stronger than expected compared with a random distribution. The analysis was run using the test.windex function of the windex package (ver. 2.0.8; Arbuckle et al. 2014; Arbuckle and Minter 2015).

The convergence among lineages was also quantified using Ct (convergence at time t) metrics, which evaluate how closely the focal lineages (i.e., those of a given ecomorph) resemble each other relative to other lineages in the phylogeny. Four Ct metrics were calculated for each ecomorph group: Ct1 is the ratio of the observed phenotypic similarity at the tips of the tree to the maximum ancestral distance between lineages, providing a relative measure of convergence; Ct2 is the absolute difference between the tip distance and the maximum ancestral distance; Ct3 scales Ct2 by the total amount of evolution that has occurred along the branches of the two focal lineages; and Ct4 scales Ct2 by the total amount of evolution that has occurred across the entire phylogeny (Stayton 2015). The mean Ct values of all pairwise comparisons incorporating all morphological traits for each ecomorph group were extracted, with values closer to 1 indicating strong convergence and values closer to 0 indicating weak convergence. All Ct analyses were performed using the calcConvCt function of the convevol package (ver. 2.2.1; Brightly and Stayton 2024).

Phylogenetic Signal. We estimated whether phylogenetic signal is present among lineages of each ecomorph using the K statistic of Blomberg et al. (2003) via the multiPhylosignal function of the picante package in R (ver. 1.8.2; Kembel et al. 2010). This analysis can be used to identify whether related lineages are more similar morphologically to each other than they are to other lineages in the clade drawn at random given a phylogeny (Blomberg et al. 2003). Phylogenetic signal was tested using lineages means of each size-corrected variable for each sex. Values of K close to 0 imply low phylogenetic signal (morphological similarity is not likely due to shared ancestry), whereas K values close to 1 indicate phylogenetic signal as expected under a model of BM (random evolution of traits), and $K > 1$ suggests strong phylogenetic signal (thus evolution of traits are constrained by evolutionary history). Significance of the test statistic was evaluated by permutating the trait values across the phylogeny 999 times.

Phylogenetic Uncertainty. To ensure that our results were not due to phylogenetic or divergence time uncertainty, we extracted a random sample of 100 trees from the posterior distribution of our Bayesian phylogenetic analysis and reran all of our analyses across these trees (expect for the polymorphospace analysis). Methods for each of these analyses are the same as those described above. We then summarized the results to assess the consistency of our results across the different trees.

Evolutionary Model Comparison. To test which evolutionary model best explains our data, and to test for evidence of adaptive evolution, we compared four evolutionary models using the mvMORPH package separately for each sex (Clavel et al. 2015). For this, we analyzed the morphological traits that showed significant differences between ecomorphs in the phylogenetic ANOVA tests. The four models compared were Brownian motion (BM1), Brownian motion multiple rates (BMM), single-optimum Ornstein-Uhlenbeck (OU1), and multiple-optima Ornstein-Uhlenbeck (OUM). For the BM models, the mvBM function was used, and for the OU models, mvOU was used. For the models requiring selective regime mapping (BMM and OUM), we used mapped ecomorph states onto the phylogenetic tree using the make.simmap function in phytools (Revell 2012). Models were fitted using the optimizer parameter Nelder-Mead for all analyses. Model selection was based on AIC, with lower values indicating better model fit.

Results

Phylogenetic Reconstruction

The ultrametric phylogenetic tree was consistent with previous *Bradypodion* phylogenies (Tolley et al. 2004, 2006, 2008) as well as with a higher-level phylogeny for the Chamaeleonidae (Tolley et al. 2013). Previous phylogenies, however, did not include all possible *Bradypodion* lineages, so the current phylogeny can be considered the most up to date for the genus. Most nodes that distinguished the lineages were supported by posterior probabilities > 0.98 (fig. 2). Nodes that were not well supported were those uniting closely related lineages with short branches.

Phylogenetic Analyses of Variance

Size-corrected phylogenetic MANOVAs comparing traits among ecomorphs highlighted significant differences for both sexes (males: Wilks's $\lambda = 0.331$, $P = .015$; females: Wilks's $\lambda = 0.438$, $P = .002$). Size-corrected post hoc phylogenetic ANOVAs showed that tail length differed significantly across all ecomorphs, regardless of sex, with the shrub ecomorph lineages having the shortest tails and forest ecomorph lineages having the longest (table 2; fig. 3). Size-corrected distal limbs (forefoot and hindfoot) also differed overall for both sexes, with shrub and LBC lineages having smaller feet than forest lineages (table 2; fig. 3). However, differences were significant only among females. Additionally, size-corrected casque height was significantly lower in female LBC ecomorph lineages compared with shrub ecomorphs (fig. 3).

Phylomorphospace Plot

The first two principal components of the pPCA explained 79.75% of the total variance for males and 79.57% for females. The first principal component (pPC1) for males (69.63% variation) had strong positive loadings for variables relating to feet (MH, MF, LH, LF). Examination of the phylomorphospace (fig. 4*A*) shows LBC and shrub lineages correlating negatively with pPC1, suggesting that they have short hands and feet, whereas forest lineages correlate positively with pPC1, indicating that they have long hands and feet. For pPC2 (10.12% variation), tail length showed a strong positive loading, and forefoot width (CARP) showed a strong negative loading. The scatterplot shows that the forest species correlate positively with pPC2, indicating that they have longer tails but thinner forefeet than the LBC and shrub lineages.

For females, pPC1 (58.25% variation) had positive loadings for variables related to hindfeet and forefeet length (CARP, MH, MF, LH, LF) and tail length. The scatterplot shows that lineages of the forest ecomorph correlate positively with pPC1 and, similar to males, suggests that they have longer feet/hands and tail. For pPC2 (17.54% variation), there were strong negative loadings for head measurements and hindfoot and forefoot width (CH, HH, LJL, TARS, CARP), with moderately strong negative loadings for hindlimb lengths. The scatterplot does not show a strong differentiation of ecomorphs on pPC2 (table 3).

Convergence

The tests for convergent evolution show an overall tendency for the lineages within each ecomorph to be convergent (ang.state). This was significant for females for all ecomorphs (table 4). For males, there was a notable signature for convergence of lineages for all ecomorphs, but the *P* values were slightly above the significance threshold for LCB and shrub lineages (table 4). In contrast, for the timing of convergence (ang.state.time), only females of the forest ecomorph showed significant timing for convergence of lineages. This suggests that, for most instances, evolution of the three ecomorphs was not coeval (i.e., not having the same date of origin), and lineages

Table 2: Phylogenetic ANOVA results for lineage means of *Bradypodion* ecomorphs using size-corrected morphological traits for body and head

	Male				Female			
	LBC vs. forest	LBC vs. shrub	Forest vs. shrub	Overall *P* value	LBC vs. forest	LBC vs. shrub	Forest vs. shrub	Overall *P* value
Tail:								
TL	.003*	.003*	.003*	.001*	.003*	.044*	.003*	.001*
Hindlimb:								
FM	1	1	1	.692	.574	.174	.21	.175
TB	1	1	1	.441	.758	.012*	.082	.081
Hindfoot:								
TARS	1	1	1	.599	1	1	1	.661
MF	.276	.955	.608	.178	.435	.435	.435	.151
LF	.114	.276	.114	.031*	.072	.408	.104	.031*
Forelimb:								
HM	.606	1	1	.383	.997	.042*	.15	.125
RD	.69	.982	.982	.433	.883	.222	.322	.289
Forefoot:								
CARP	1	1	1	.838	1	1	1	.86
MH	.141	.436	.172	.055	.063	.418	.098	.031*
LH	.072	.354	.084	.029*	.018*	.372	.030*	.004*
Head:								
HL	1	.888	1	.644	.424	.018*	.424	.134
HW	.904	.693	.904	.544	1	1	1	.997
HH	1	1	1	.947	.756	.114	.756	.288
LJL	.789	.789	.789	.432	.708	.018*	.226	.139
CT	.822	.822	.822	.538	.332	.027*	.332	.085
QT	.933	.933	.933	.540	.712	.045*	.316	.168
CH	.414	.219	.595	.265	.39	.006*	.39	.045*

Note: TL = tail length; FM = femur; TB = tibia; TARS = tarsal; MF = medial fore toes; LF = lateral fore toes; HM = humerus; RD = radius; CARP = carpal; MH = medial hind toes; LH = lateral hind toes; HL = head length (from posterior-dorsal point of casque to snout); HW = head width behind the eyes; HH = head height (including lower jaw); LJL = lower jaw length; CT = cantilever to anterior tip of jaw; QT = quadrate to anterior tip of jaw; CH = casque height (lower corner of temporal fenestra to tip of casque).

* Significant.

converged on their respective ecomorph form at different time points.

The strength of convergence assessed by the Wheatsheaf indices showed tail length (TL) to be strongly convergent among lineages for the forest ecomorph for both sexes (table 5). Other functional trait groupings did not show strong convergence. However, tail length in LBC females, forefoot in shrub males, and hindfoot in shrub females were near the threshold for statistical significance, possibly suggesting convergence ($P = .053$, .056, and .053, respectively).

The Ct convergence metrics indicated that strong convergence was present in female LBC and shrub lineages (Ct1 = −0.68 and −0.72, respectively; table S7), with moderate convergence in forest groups for both males and females (Ct1 = −0.60 and –0.53) and in LBC male lineages (Ct1 = −0.58). Male shrub lineages showed weaker convergence (Ct1 = 0.19). The Ct2–Ct4 values had generally smaller values than Ct1; however, these could not be reliably interpreted for forest groups because of infinite estimates (table S7), suggesting that there may be limited phenotypic divergence within those lineages.

Phylogenetic Signal

Phylogenetic signal was not present for most of the traits, with $K < 1$ (table 6). However, a few traits did show evidence of phylogenetic signal, with a statistically significant K close to or greater than 1, particularly for distal limbs (forefoot and hindfoot). Notably, all other analyses showed that these traits are characteristically different for the LBC/shrub ecomorphs (shorter) in contrast to the forest ecomorph (longer). Thus, the strong phylogenetic signal for these traits is consistent with the LBC/shrub lineages clustering mainly (but not fully) within one clade (fig. 2; *Bradypodion taeniabronchum*, *Bradypodion* sp. "groendal," *B. baviaanense*, *B. gutturale* [montane],

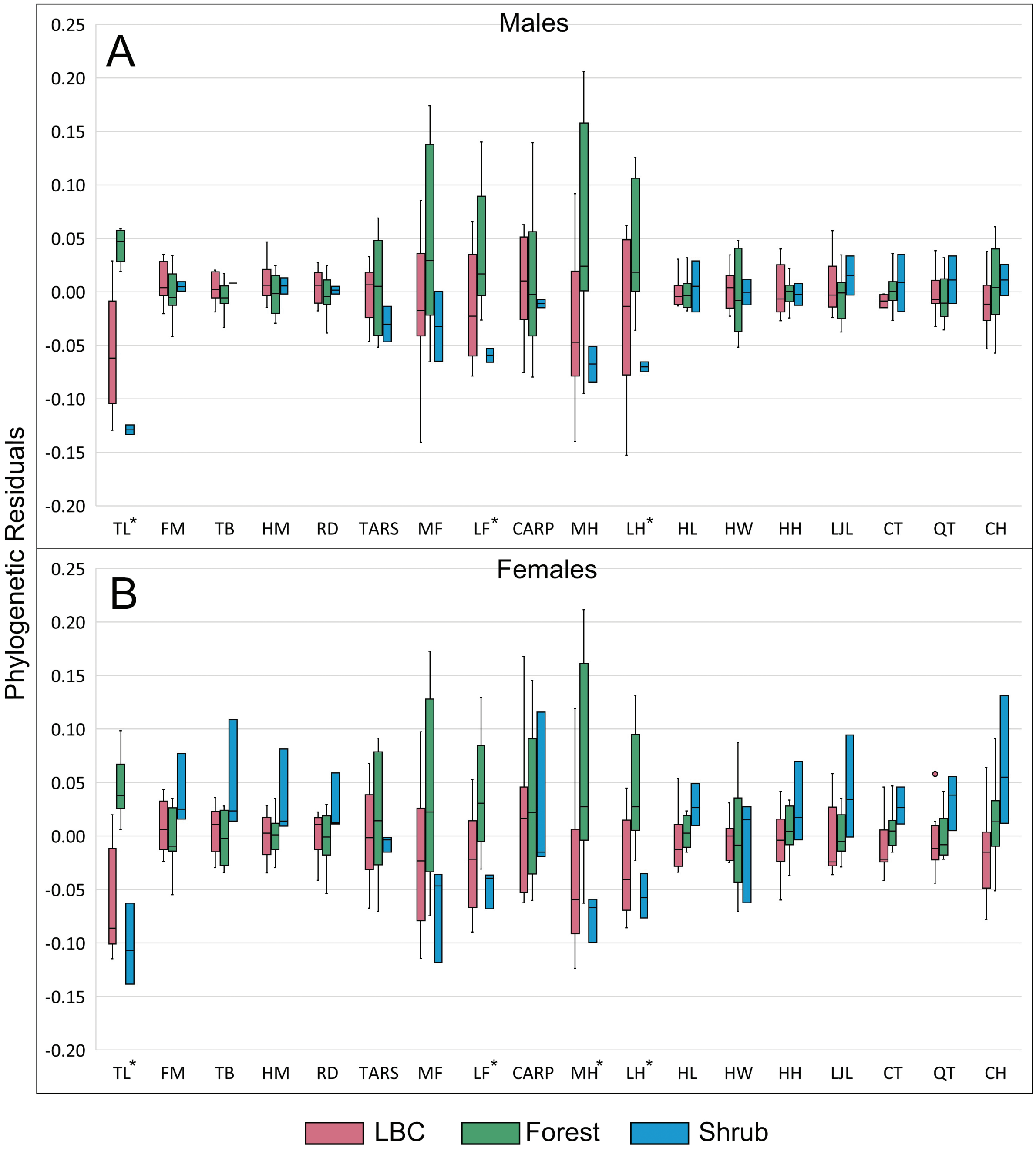

Figure 3: Box-and-whisker plots of the size-corrected morphological variables for all lineages of *Bradypodion* grouped into three ecomorphs: little brown chameleon (LBC; pink), forest (green), and shrub (blue). The boxes represent the interquartile range, with whiskers as the minimum and maximum points and the central line as the median. Outliers are shown as circles. *A*, Males. *B*, Females. Asterisks indicate the traits that were significantly different. TL = tail length; FM = femur; TB = tibia; HM = humerus; RD = radius; TARS = tarsal; MF = medial fore toes; LF = lateral fore toes; CARP = carpal; MH = medial hind toes; LH = lateral hind toes; HL = head length (from posterior-dorsal point of casque to snout); HW = head width behind the eyes; HH = head height (including lower jaw); LJL = lower jaw length; CT = cantilever to anterior tip of jaw; QT = quadrate to anterior tip of jaw; CH = casque height (lower corner of temporal fenestra to tip of casque).

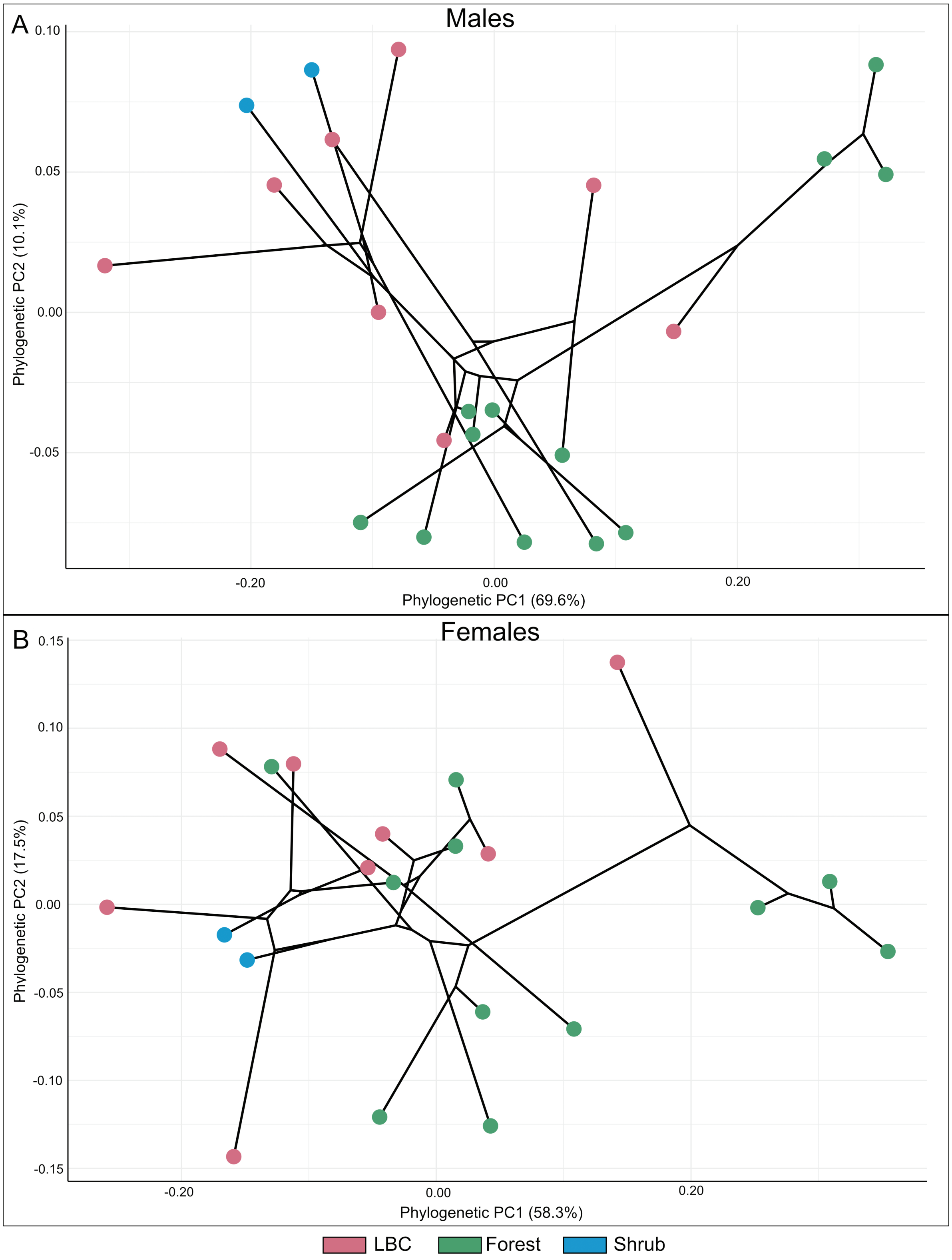

Figure 4: Phylogenetic morphospace in *Bradypodion* ecomorphs. The first two phylogenetic principal components are shown for males (*A*) and females (*B*). Points represent each of the 24 lineages, colored by ecomorph: little brown chameleon (LBC; pink), forest (green), and shrub (blue). Connecting branches show phylogenetic relationships projected into morphospace. The variances explained by each principal component are indicated as percentages on the respective axes.

Table 3: Loadings of original variables for the phylogenetic principal component analysis for *Bradypodion*

	Male		Female	
	PC1	PC2	PC1	PC2
Tail:				
TL	.32	.79[a]	.33[a]	<.01
Hindlimb:				
FM	−.04	.02	−.06	−.20
TB	−.06	−.04	−.07	−.26[a]
Hindfoot:				
TARS	.12	−.21	.16	−.30[a]
MF	.45[a]	−.21	.47[a]	.08
LF	.35[a]	.02	.35[a]	−.04
Forelimb:				
HM	−.02	−.04	−.01	−.18
RD	−.04	.05	−.05	−.16
Forefoot:				
CARP	.21	−.49[a]	.21	−.38[a]
MH	.56[a]	−.10	.57[a]	.03
LH	.42[a]	.03	.36[a]	−.06
Head:				
HL	−.04	−.01	−.02	−.21
HW	<.01	−.03	.01	−.07
HH	<.01	−.08	−.01	−.30[a]
LJL	−.09	−.07	−.08	−.29[a]
CT	−.03	−.02	−.03	−.23
QT	−.09	.01	−.07	−.23
CH	−.03	−.12	−.02	−.52*

Note: Traits are grouped according to function. TL = tail length; FM = femur; TB = tibia; TARS = tarsal; MF = medial fore toes; LF = lateral fore toes; HM = humerus; RD = radius; CARP = carpal; MH = medial hind toes; LH = lateral hind toes; HL = head length (from posterior-dorsal point of casque to snout); HW = head width behind the eyes; HH = head height (including lower jaw); LJL = lower jaw length; CT = cantilever to anterior tip of jaw; QT = quadrate to anterior tip of jaw; CH = casque height (lower corner of temporal fenestra to tip of casque).

[a] Highest loading.

B. atromontanum). This could provide support for the shift to a new trait space for that clade overall rather than convergence of the traits among lineages. Despite this exception, across the larger phylogeny, closely related species are not more likely to resemble one another morphologically.

Phylogenetic Uncertainty

The results from the multitree analyses were consistent with our original results using the maximum clade credibility tree. The phylogenetic MANOVAs were significant across all 100 trees for both sexes (males: mean $P = .01$, proportion significant = 1.00; females: mean $P = .01$, proportion significant = 1.00; table S8). The phylogenetic ANOVAs were also consistent with tail length, LF (hindfoot), MH, and LH (forefoot) measurements all showing significant results across all trees for both sexes (table S9).

The convergence tests were also unaffected by phylogenetic uncertainty, with female lineages showing consistent convergence across ecomorphs, with more than 98% of the trees having statistically significant results (table S10). Male lineages showed more variance in convergence patterns for LBC and shrub ecomorphs (proportion significant = 0.56 and 0.54, respectively). Male forest ecomorphs were consistently significant, matching our original results, which highlighted only this ecomorph as convergent for males. The strength of convergence tests also supported our original findings, with tail length in forest ecomorphs showing strong convergence across all trees (proportion significant = 1.00 for both males and females; table S11). Variation in Ct measures were also generally low, with the only notable exception being high variation in Ct1 for female forest ecomorphs (SD = 1.98; table S12). Finally, phylogenetic signal patterns were also consistent with our original findings. Traits related to the hindfoot and forefoot all showed the strongest phylogenetic signal and consistent significance patterns across all of the 100 trees (table S13).

Evolutionary Model Comparison

Model comparison for males showed support for OUM as the best-fitting evolutionary model, with the lowest AIC value (AIC = −247.41). For females, the OU1 model was best supported with the lowest AIC value (AIC = −402.28) with moderate support over the OUM model (AIC = −397.20). For females, for successful convergence of the optimizer for both OU models, the maximum

Table 4: Results for tests of evidence of convergent evolution for *Bradypodion* ecomorphs, separated by sex

	Male				Female			
Ecomorph	State	*P*	Time	*P*	State	*P*	Time	*P*
LBC	83.93	.060	4.41	.097	81.90	.023*	4.87	.220
Forest	81.46	.002*	4.66	.093	82.32	.004*	4.57	.026*
Shrub	39.93	.065	2.55	.094	51.37	.021*	4.49	.423

Note: The mean angles of phenotypic distance are reported for the test of convergence using ang.state (state) and the test of convergence with timing of diversification using ang.state.time (time), with the corresponding *P* values. LBC = little brown chameleon.

* Significant.

Table 5: Wheatsheaf indices (*w*), with upper and lower 95% confidence bounds and *P* values, used to identify convergent evolution of morphological traits between ecomorphs

	Male				Female			
	w	Lower bound	Upper bound	*P*	*w*	Lower bound	Upper bound	*P*
LBC:								
TL	1.228	1.141	1.423	.266	1.524	1.439	1.840	.053
Forelimb	1.063	1.012	1.249	.506	1.084	1.002	1.269	.482
Hindlimb	.966	.891	1.113	.719	1.206	1.079	1.330	.340
Forefoot	1.176	1.100	1.354	.306	1.181	1.099	1.342	.290
Hindfoot	1.277	1.208	1.482	.167	1.180	1.101	1.307	.299
Head	1.024	.982	1.172	.616	1.112	1.046	1.272	.412
Forest:								
TL	3.695	3.434	5.087	.002*	2.754	2.642	3.315	≤.001*
Forelimb	.997	.945	1.035	.668	1.139	1.045	1.217	.291
Hindlimb	1.022	.960	1.106	.601	1.120	1.027	1.207	.313
Forefoot	1.070	1.027	1.115	.403	1.114	1.076	1.174	.287
Hindfoot	.978	.956	1.017	.755	.981	.958	1.029	.613
Head	1.102	1.057	1.164	.307	1.160	1.103	1.241	.188
Shrub:								
TL	24.165	22.594	NA	.117	3.501	3.332	6.584	.217
Forelimb	4.578	4.298	NA	.208	1.389	1.323	10.664	.827
Hindlimb	8.842	7.985	NA	.098	1.274	1.216	5.194	.847
Forefoot	12.447	11.627	NA	.056	2.961	2.755	10.373	.203
Hindfoot	4.248	4.021	NA	.229	4.825	4.501	9.138	.053
Head	1.619	1.552	NA	.865	1.559	1.496	2.807	.770

Note: Size-corrected traits were grouped into functional sets: head (HL, HW, HH, LJL, CT, QT, and CH), tail length (TL), forelimb (HM and RD), hindlimb (FM and TB), forefoot (CARP, MF, and LF), and hindfoot (TARS, MH, and LH). NA indicates instances where the sample sizes were insufficient to calculate the indices. HL = head length (from posterior-dorsal point of casque to snout); HW = head width behind the eyes; HH = head height (including lower jaw); LJL = lower jaw length; CT = cantilever to anterior tip of jaw; QT = quadrate to anterior tip of jaw; CH = casque height (lower corner of temporal fenestra to tip of casque); TL = tail length; FM = femur; TB = tibia; TARS = tarsal; MF = medial fore toes; LF = lateral fore toes; HM = humerus; RD = radius; CARP = carpal; MH = medial hind toes; LH = lateral hind toes.

* Significant.

limit iteration had to be increased to 100,000. Both sexes showed evidence for adaptive evolution over neutral drift, with OU models outperforming BM models (table S14).

Discussion

Comprehensive analyses of an extensive morphological dataset suggest that many *Bradypodion* lineages within each ecomorph have converged on a similar morphology independently. However, a shift to shorter forefeet/hindfeet within a single clade of closely related LBC lineages (*B. atromontanum* clade) does suggest that phylogeny has played a role in some cases, with a shift to a new trait space for that clade as a whole. Regardless, ecology appears to have a stronger effect on morphology than evolutionary history overall, with significant differences between ecomorphs when accounting for phylogeny, limited phylogenetic signal, and evidence of convergent evolution.

Several morphological traits appear to represent ecomorphological adaptations with a functional basis in *Bradypodion*, mainly tail length, forelimb and hindlimb sizes, and casque height (Measey et al. 2009; Herrel et al. 2011, 2013, 2024; da Silva et al. 2014; Higham et al. 2015). Tail length is a particularly important trait in relation to habitat type, with significant differences observed between all ecomorphs. Forest lineages have significantly longer tails and longer distal limb elements (forefoot and hindfoot lengths). Forest lineages occupy habitat types that are structurally complex, characterized by large gaps between suitable perches and increased dimensionality, with forests having significantly wider perches than other habitats (Barends et al. 2024). This requires adaptations that facilitate navigation in these environments (Herrel et al. 2011; da Silva and Tolley 2013; Barends et al. 2024). Longer distal limbs and longer tails in the forest likely provide enhanced gripping ability on wider perches to improve stability, which is consistent with previous findings of hand size and gripping ability on larger perches being positively correlated in chameleons (Herrel et al. 2013, 2024). Furthermore, long tails likely increase functional performance by allowing coiling of the tail around branches, which contributes to surface friction (Luger et al. 2020).

Table 6: Phylogenetic signal associated to morphological traits for *Bradypodion* ecomorphs by sex assessed by Blomberg's *K*

	Male		Female	
	K	*P*	*K*	*P*
Tail:				
TL	.550	.050*	.447	.037*
Hindlimb:				
FM	.431	.109	.217	.549
TB	.519	.054	.191	.700
Hindfoot:				
TARS	.594	.003*	.407	.056
MF	.741	.005*	.989	.001*
LF	1.041	.001*	.683	.004*
Forelimb:				
RD	.271	.612	.128	.856
HM	.361	.225	.224	.456
Forefoot:				
CARP	.566	.021*	.189	.668
MH	1.193	.001*	1.176	.001*
LH	.993	.001*	.706	.001*
Head:				
HL	.563	.045*	.242	.397
HW	.184	.824	.185	.660
HH	.295	.442	.172	.702
LJL	.532	.030*	.258	.381
CT	.594	.011*	.207	.482
QT	.562	.028*	.282	.236
CH	.370	.178	.147	.832

Note: $K > 1$ indicates stronger phylogenetic signal than expected under Brownian motion, $K = 1$ indicates signal consistent with Brownian motion, and $K < 1$ indicates lack of phylogenetic signal. Significant *P* values denoted by an asterisk indicate traits showing more phylogenetic signal than expected by random distribution across the phylogeny. HL = head length (from posterior-dorsal point of casque to snout); HW = head width behind the eyes; HH = head height (including lower jaw); LJL = lower jaw length; CT = cantilever to anterior tip of jaw; QT = quadrate to anterior tip of jaw; CH = casque height (lower corner of temporal fenestra to tip of casque); SVL = snout-vent length; TL = tail length; FM = femur; TB = tibia; TARS = tarsal; MF = medial fore toes; LF = lateral fore toes; HM = humerus; RD = radius; CARP = carpal; MH = medial hind toes; LH = lateral hind toes.

* Significant.

Notably, the forest lineages show evidence of strong convergent evolution for tail and forelimbs/hindlimbs and moderate convergence overall based on the Ct1 metrics. However, forest is the ancestral habitat for *Bradypodion* (Tolley et al. 2008, 2013), which could suggest the retention of the forest phenotype in lineages of that ecomorph rather than "convergence" of traits. Convergence would require the independent loss and regaining of forest traits for nearly half of all *Bradypodion* lineages. Given that previous ancestral habitat reconstructions did not contain all lineages assessed here, a post hoc analysis was run to accommodate all lineages of *Bradypodion* (see "Supplementary Methods and Results" in the supplemental PDF, fig. S3). This analysis supports earlier findings, suggesting that the most parsimonious explanation is that the forest lineages share an ancestral phenotype that is conservative and has been retained in those lineages over time. The exception is true convergence of the forest ecomorph for *B. kentanicum* (figs. 2, S3), which occurs in a clade with an ancestral habitat of open vegetation (fynbos/grassy) and contains primarily LBC and shrub ecomorph lineages. Given that forest habitat is ancestral for most *Bradypodion* lineages, we suggest that either convergence among most forest lineages occurred very early in the evolution of *Bradypodion* (e.g., mid-Miocene) and/or that the forest ecomorph traits are conservative.

Unlike the forest ecomorph, LBC and shrub lineages have shorter tails and short distal limbs (forefeet and hindfeet), which may reflect a reduced need to cross large gaps in the canopy, simultaneously facilitating ease of movement across the ground. These ecomorphs, particularly shrub lineages, occupy habitats with clumps of bushes that have densely clustered branches and open space between bushes. Short distal limbs and tails have been identified as characteristic of other chameleon species that regularly use the ground (Herrel et al. 2011, 2013), and it has been suggested that short tails also may be adaptive for crossing broad substrates (Boistel et al. 2010) such as the ground. Similar patterns have been identified in *Anolis* ecomorphs, where tail lengths align closely with habitat structure and locomotor demands (Beuttell and Losos 1999). In addition, shorter forefeet and hindfeet in LBC and shrub ecomorphs may be adaptive given that they use smaller-diameter perches (Barends et al. 2024). Their habitats are dominated by low bushy vegetation or sclerophyllous/grassy substrates, respectively, both with smaller-diameter perches than forest habitats, suggesting that the hand/foot size are optimized for these respective perch sizes. Thus, the convergence for short forefeet and hindfeet in LBC and shrub ecomorphs likely has functional advantages for the substrates they occupy. Furthermore, locomotor performance and grip strength across varying perch sizes and perch orientations between ecomorph types points to differences in biomechanical performance of the limbs (e.g. Higham et al. 2015; Herrel et al. 2024) and supports the hypothesis that substrate size and orientation are important drivers of ecomorphological convergence and conservatism.

Head traits did not show strong differences or signs of convergence among the three ecomorphs. This finding was surprising given that casque size has been shown to correlate with the outcome of aggressive encounters for male *Bradypodion* (Stuart-Fox et al. 2006) and appears to be linked to the ability to signal effectively (Petford et al. 2024). Furthermore, head width or head length explains bite force ability for both sexes of *Bradypodion* (Measey et al. 2009; da Silva et al. 2014, 2016; Dollion et al. 2017; Petford et al. 2024). For head traits, there was no significant differences among ecomorphs for males, but for females, head traits were smaller for the LBC ecomorph compared with the shrub ecomorph. Despite this, there was no easily discernible set of traits that could be attributed to the ecomorph lineages, with the LBC lineages spread widely across the region of morphospace attributed to the head traits. Thus, while there may be some indication of smaller head traits characterizing the LBC ecomorph for females, for males there was no indication that head traits can be used to define ecomorphs. The absence of this for males could suggest that sexual selection in males may be stronger than natural selection, with males of all ecomorphs retaining a prominent casque and large heads to improve their competitive advantage when signaling but also through biting ability when fighting rivals (e.g., Petford et al. 2024). A limitation of our study is that the casque height measurement was taken from the lower corner of the temporal fenestra to the tip of the bony casque spur, which includes areas of the skull to which mandibular musculature is attached and thus is a combination of musculature and ornamentation (Dollion et al. 2017). Thus, our findings that casque is not a trait that differentiates ecomorphs for males could be confounded by this measurement including aspects of the skull that also contribute to bite force.

Despite the limitations for interpreting the effect of casque on the ecomorph categories, we found that LBC females have a reduced casque height compared with other ecomorphs. We suggest that the reduced casque could facilitate crypsis in the open environment, as the more conspicuous individuals may be at higher risk of predation (Stuart-Fox and Moussalli 2007; Measey et al. 2009; da Silva et al. 2014; Petford et al. 2024). A trade-off, however, is that chameleons from open habitats are likely to be less effective in intraspecific signaling, resulting in more frequent direct and aggressive intraspecific encounters (e.g., Measey et al. 2009; Petford et al. 2024). Given the observed reduction in ornamentation, it appears that natural selection (crypsis and predation risk) is a more dominant force in driving the reduction in ornamentation than sexual selection in maintaining conspicuous ornamentation, at least for females. In contrast, the higher casque for shrub and forest ecomorphs may be adaptive in those habitats, where there is a greater complexity of branches in terms of orientation and widths (Barends et al. 2024). This may offer more opportunities for shelter and refugia from predators, allowing these ecomorphs to retain the large casque for signaling conspecifics.

Most traits did not show significant phylogenetic signal, with K values less than 1, suggesting that the overall convergence of traits within *Bradypodion* is not a consequence of evolutionary history. Consequently, most traits are assumed to have some degree of directional selection, driving lineages to converge on ecomorph types regardless of ancestry. However, forelimb and hindlimb traits showed phylogenetic dependence for both sexes, suggesting an interaction between evolutionary history and ecology driving the evolution of these traits. The presence of phylogenetic dependence for these traits may be dominated by specific subclades of closely related species within the phylogeny. In particular, the *B. atromontanum* clade contains five of the nine LBC lineages and all of the shrub lineages. Most lineages in this clade are young and closely related (Tolley et al. 2008, 2013; figs. S1–S3), resulting in lineages of these ecomorphs, in most cases, being sister taxa. Given that they are of the same ecomorph, they necessarily exhibit similar phenotypes and may represent a shift to a new trait space in general. This combination has the effect of inflating the overall phylogenetic signal while not necessarily representing a trend across all *Bradypodion* lineages. This phenomenon has been noted for subclades of plants from North America that exhibited greater phylogenetic signal than did the overall phylogeny, influencing the results of the full clade analysis (Ackerly 2009). These findings suggest that distal limb morphology and head lengths, particularly those of the LBC and shrub ecomorphs, are likely dependent on phylogeny but that the retention of these traits in these lineages is the result of morphological conservatism rather than convergence.

Evolutionary model comparisons showed support for OU models over BM models, which is consistent with adaptive morphological diversification toward phenotypic optima (Butler and King 2004). Males showed support for OUM, indicating convergence toward distinct ecomorphs, while females showed stronger support for OU1, which would suggest weaker ecomorph specialization. However, the difference in AIC between the OUM and OU1 model for females was minor. These findings have implications for our analytical approach, as our primary analyses assume BM evolution. The use of BM assumptions may be problematic when traits are actually under stabilizing selection toward adaptive optima, as they may overestimate evolutionary rates by interpreting variation around adaptive peaks as continued drift, ignore directional selection toward peaks, and misinterpret convergence as phylogenetic signal (Butler and King 2004; Beaulieu et al. 2012; Cooper et al. 2016). While the analyses we used in this study cannot account for OU models of evolution, they remain comparable to other comparative studies that use the same analytical approach. Support for OU models suggests that ecomorphological evolution in *Bradypodion* involves adaptive convergence toward distinct phenotypic optima rather than neutral drift.

In conclusion, we show morphological convergence among lineages of the three ecomorph types within the *Bradypodion* genus, but for some clades and traits, morphological conservatism is a strong factor in defining the ecomorphs. We suggest that habitat substrate is a strong driver of these patterns. The ecomorphs can be best characterized through tail length, with forest species having the longest tails, facilitating gripping and navigating though large gaps in the complex substrates of the canopy. In contrast, long tails may be disadvantageous when moving between bushes on the ground, explaining the short tails in the LBC and shrub lineages. Similarly, forefoot and hindfoot sizes also show significant differences between ecomorphs, with forest lineages having longer hands, likely as an adaptation to wider perches. Nevertheless, for some lineages the distal limb sizes may actually be the result of conservatism of those traits rather than convergence. Furthermore, the reduction in ornamentation (casque height) for LBC females suggests that natural selection is driving morphological adaptation in open habitats, where conspicuous ornamentation may increase predation risk. However, this pattern was not evident in males, indicating that sexual selection may be a stronger factor influencing adaption in males than natural selection, with ornamentation being retained for competitive signaling. Overall, we show that *Bradypodion* lineages can be characterized into three ecomorph types and that this strongly correlates to habitat structure as the driver of convergence or conservatism. Nevertheless, these inferences would be strengthened through an assessment of trade-offs between sexual selection and ecological adaptation in traits such as casque height. Given that the functionality of these traits has been previously shown to be strongly correlated to habitat (e.g., Measey et al. 2009; Herrel et al. 2011, 2013, 2024; da Silva et al. 2014; Higham et al. 2015), we propose that the lineages of these ecomorphs have adapted to their respective habitats.

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Statement of Authorship

M.A.P. contributed to conceptualization, data analysis, and writing (original draft, reviewing, and editing). A.H. contributed to conceptualization, data collection, data analysis, supervision, and writing (original draft, reviewing, and editing). K.A.T. contributed to conceptualization, data collection, data analysis, supervision, writing (original draft, reviewing, and editing), and funding acquisition.

Data and Code Availability

Supplementary data files and code have been deposited in the figshare repository (https://doi.org/10.25415/ujhb.30619118; Tolley 2025).

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