XXX

ORDER TBD: Héctor Tejero-Cicuéndez[1,*], Iris Menéndez[2,3], Salvador Carranza[1], and Dean C. Adams[4]

22 September, 2022

[1]Institute of Evolutionary Biology (CSIC-Universitat Pompeu Fabra), Passeig Marítim de la Barceloneta 37-49, Barcelona 08002, Spain

[2]Departamento de Geodinámica, Estratigrafía y Paleontología, Facultad de Ciencias Geológicas, Universidad Complutense de Madrid, C/José Antonio Novais 12, Madrid 28040, Spain

[3]Departamento de Cambio Medioambiental, Instituto de Geociencias (UCM, CSIC), C/Severo Ochoa 7, Madrid 28040, Spain

[4]Department of Ecology, Evolution, and Organismal Biology, Iowa State University, Ames, Iowa, 50010 USA

[*]Correspondence: Héctor Tejero-Cicuéndez cicuendez93@gmail.com

Keywords: Phenotypic Evolution, Morphospace, Allometry, *Pristurus* geckos

Short Title: XXX

Author Contributions: All authors collaboratively developed the concept and contributed to all portions of this manuscript. HT-C, IM, and DCA performed the analyses. All authors approve of the final product and are willingly accountable for any portion of the content.

Conflicts of Interests: The authors declare no conflicts of interest.

Data Archiving: Data are available on DRYAD (doi:10.5061/dryad.xwdbrv1f6 (Tejero-Cicuéndez et al. 2021b)). R-scripts are available at **XXX**.

Acknowledgments: We thank XYZPDQ... This work was sponsored in part by XXX (to SC) DCA was funded in part by National Science Foundation Grant DEB-2140720, and a Fulbright Senior Scholar Grant.

Abstract

asdf

Introduction

The mechanisms through which phenotypic diversity emerges and evolves are an essential topic of study in evolutionary biology. Such diversity is the result of a combination of genetic, developmental, and environmental factors that determine the life history of organisms and the evolutionary trajectory of species. These factors might impose certain constraints or offer opportunities for morphological evolution, generating the diversity upon which natural selection acts culminating in the adaptation of species to their surrounding environments. Consequently, the differences in species' ecological preferences (e.g., the exploitation of different habitats) have the potential to drive morphological changes via distinct selective pressures.

Ecological specialization is one of the main sources of phenotypic diversity. When organisms colonize new and unique habitats, they are subjected to novel ecological dynamics that may impose different functional requirements. This ecomorphological relationship may result in the repeated evolution of certain phenotypes (i.e., convergence) and the appearance of the so-called ecomorphs, when the general morphological features of the species within a clade can be tightly related to specific ecological contexts. This includes emblematic examples of adaptive radiations such as the differential body size and shape of Anolis species exploiting different microhabitats (Losos 2009), the disparity in beak morphology in Darwin's finches (REFS) and Hawaiian honeycreepers (REFS), or the differences in jaw morphology among cichlid fishes (REFS).

However, while the patterns of morphological differences among distinct ecological contexts have been well documented in a variety of vertebrate taxa, the specific trajectories of morphological evolution are in many cases less known. A particularly interesting question is perhaps the extent to which evolutionary allometry can describe this phenotypic differentiation. ALLOMETRY BLABLABLA.

The Afro-Arabian geckos in the genus *Pristurus* afford the opportunity to elucidate the interdigitating effects of allometry and habitat specialization on clade-level patterns of phenotypic diversity. Prior work on this system (Tejero-Cicuéndez et al. 2021a) revealed that the colonization of ground habitats has been a trigger of morphological change, specifically reflected in an increase in body size and shape disparity. Interestingly, some ground-dwelling species are among the largest of the genus and also show increased relative head sizes and limb proportions, while some other species with this ecological specialization have evolved to be among the smallest of the group. Additionally, among the species exploiting rocky habitats (the most common ecological feature in *Pristurus*), there are also species with both considerably large and small body sizes (Tejero-Cicuéndez et al. 2021a). What remains unexplored, however, is how the evolution of body shape is related to differences in body size and whether habitat specialization has an impact in this relationship shape-size. (how this relationship shape-size differs among habitats.)

last paragraph In this study, we used a combination of multivariate morphometric and phylogenetic comparative analysis to interrogate macroevolutionary patterns of evolutionary allometry in *Pristurus* geckos of Afro-Arabia. Using a combination of phenotypic, phylogenetic, and ecological data, we characterized allometric trends in body form to discern the extent to which those patterns differed across species occupying distinct ecological habitats, and to explore how allometric differences related to overall patterns of phenotypic diversification in the group.

The independent diversification of both Socotran and continental taxa, the ecological and behavioural diversity and the unique phenotypic dataset compiled in this study, make this group of geckos an attractive model system to investigate keystone dynamics in evolutionary biology such as the island effect and ecological adaptation, and their impact on morphological evolution.

Our findings

Overall our work has ... (some implication).

Our results demonstrate that differing tracectories of allometric growth can result in similar adult phenotypes... (Don't like it! We've got adults, yes?)

Materials and Methods

Data

We used a combination of phenotypic, phylogenetic, and ecological data to characterize and evaluate interspectific allometric trends. The data utilized here were obtained from our prior work on this system (Tejero-Cicuéndez et al. 2021a, 2022), and are briefly described here. First we used a time-dated, molecular phylogeny that included all members of the genus *Pristurus*, including several currently undescribed taxa. The tree was estimated in a Bayesian framework, using five mitochondrial markers, six nuclear markers, and 21 calibration points (for details see Tejero-Cicuéndez et al. 2022). Next we categorized each species as belonging to one of three ecological groups (ground, rock, or tree), based on descriptions of habitat use found in the literature (see Tejero-Cicuéndez et al. 2021a). Finally, we obtained a phenotypic data set containing body size (snout-vent length: SVL) and eight linear measurements (Figure 1) that described overall body form: trunk length (TrL), head length (HL), head width (HW), head height (HH), humerus length (Lhu), ulna length (Lun), femur length (Lfe), and tibia length (Ltb) (Tejero-Cicuéndez et al. 2021a). We restricted our study to those species represented by five or more individuals; resulting in a dataset of 687 individuals from 25 species (invidivuals per species: $\mu = 27$; min = 9, max = 56). Species in the phenotypic dataset were then matched to the phylogeny, which was subsequently pruned to arrive at the final topology. All measurements were log-transformed prior to statistical analyses. Additional details regarding data collection and formal descriptions of each linear measurement may be found in the original sources (see Tejero-Cicuéndez et al. 2021a, 2022). The data are found on DRYAD: https://doi.org/10.5061/dryad.xwdbrv1f6 (Tejero-Cicuéndez et al. 2021b).

Statistical and Comparative Analyses

EVOLUTIONARY ALLOMETRY Do just shape ~ size & shape ~size for species | phylogeny

THen mancova. . . .

- compare habitat slopes we found with individuals to those from species means (the 'usual' way of looking at evolutionary allometry)

We conducted a series of analyses to interrogate allometric trends and macroevolutionary changes in allometry, relative to diversification in body form. First, to determine whether allometric trends in body form differed across habitat groups, we performed a multivariate analysis of covariance, with body size (SVL), *habitat*, and $SVL \times habitat$ as model effects. Significance was evaluated using 999 iterations of a permutation procedure, where residuals from a reduced model were randomly permuted in each permutation (RRPP), model statistics were recalculated, and used to generate empirical null sampling distributions to evaluate the observed test statistics (following Freedman and Lane 1983; Collyer and Adams 2007; Collyer et al. 2015). Next we compared the multivariate allometric vectors for each habitat group by calculating pairwise differences in their angular direction in morphospace, and evaluating these relative to empirical sampling distributions obtained through RRPP (Collyer and Adams 2007; Adams and Collyer 2009; Collyer and Adams 2013). We then visualized patterns of multivariate allometry relative to body size via regression scores (Drake and Klingenberg 2008) and predicted lines (Adams and Nistri 2010), based on the coefficients and fitted values from the linear model described above.

Second, we examined changes in allometric trends across the phylogeny, treating the head dimensions and limb dimensions separately. Because both the head and limb data were multivariate, we accomplished this by first performing a partial least squares analysis (Rohlf and Corti 2000) of the head traits versus SVL, and the limb traits versus SVL, and retaining the PLS scores for each individual from the first dimension of this analysis. Species-specific slopes describing the extent of head and limb allometry within each species were then obtained from an analysis of covariance modeled as: $PLS1_{head} \sim SVL * species$ and $PLS1_{limb} \sim SVL * species$ respectively. Species' slopes were then mapped on the phylogeny of *Pristurus* using a Brownian motion model of evolution, to qualitatively evaluate shifts in allometry across species (for a similar approach see Adams and Nistri 2010).

Finally, to relate within-species allometric trends with patterns of phenotypic diversification in the group we generated a phylomorphospace, based on the size-standardized species means obtained from a phylogenetic regression (see Tejero-Cicuéndez et al. 2021a). Here, phenotypic similarities among species, relative to their phylogenetic relationships and habitat affiliations, were observed. All analyses were conducted in R 4.2.1 (R Core Team 2022), using `RRPP` version 1.3.1 (Collyer and Adams 2018; Collyer and Adams 2022), and scripts written by the authors (available at **XXX**).

Results

Our analyses revealed significant differences in the allometry of body form among *Pristurus* that utilized distinct habitats (Table 1). Further, comparisons of multivariate allometric vectors identified that ground-dwelling *Pristurus* displayed a distinct allometric trend as compared with *Pristurus* occupying both the rock and tree habitats (Table 2). A visualization of multivariate allometric trends (Fig. 2) confirmed these statistical findings, and indicated that the allometric trajectory in rock-dwelling animals was more extreme as compared with either ground or tree-dwelling *Pristurus*. Inspection of individual regression coefficients for each trait (Supplemental Information) further corroborated this, revealing steeper allometric coefficients for all head and limb traits in ground-dwelling *Pristurus* as compared with rock and tree-dwelling taxa. Overall, these findings revealed that larger individuals of ground-dwelling *Pristurus* species displayed proportionately larger heads and limbs, as compared with large individuals in taxa utilizing other habitat types.

Ground: head and body dimensions are more positively allometric (relative to SVL) than in rock/tree groups, and whereas allometric coefficients more similar in rock & tree.

Formally evaluated using PLS: confirming .... WHen mapped on the phylogeny (....) Here traitgrams (by SVL) elucidated that heads more strongly allometric in XXX, implying that larger individuals of these species display proportionately larger heads relative to the 'typical' trend in the genus. By contrast, ... less strong (negative?) allometry

steeper/shallower slopes? resulting in ...

**Careful! use steeper slope, not positive/negative.

When viewed in light of phylomorphospace....

Discussion

References

Adams, D. C., and M. L. Collyer. 2009. A general framework for the analysis of phenotypic trajectories in evolutionary studies. Evolution 63:1143–1154.

Adams, D. C., and A. Nistri. 2010. Ontogenetic convergence and evolution of foot morphology in european cave salamanders (family: plethodontidae). BMC Evolutionary Biology 10:1–10. BioMed Central.

Collyer, M. L., and D. C. Adams. 2007. Analysis of two-state multivariate phenotypic change in ecological studies. Ecology 88:683–692.

Collyer, M. L., and D. C. Adams. 2013. Phenotypic trajectory analysis: Comparison of shape change patterns in evolution and ecology. Hystrix 24:75–83.

Collyer, M. L., and D. C. Adams. 2022. R: RRPP: Linear model evaluation with randomized residuals in a permutation procedure. Vsn. 1.3.1. R Foundation for Statistical Computing, Vienna, Austria.

Collyer, M. L., and D. C. Adams. 2018. RRPP: An r package for fitting linear models to high-dimensional data using residual randomization. Methods in Ecology and Evolution 9:1772–1779.

Collyer, M. L., D. J. Sekora, and D. C. Adams. 2015. A method for analysis of phenotypic change for phenotypes described by high-dimensional data. Heredity 115:357–365.

Drake, A. G., and C. P. Klingenberg. 2008. The pace of morphological change: Historical transformation of skull shape in st bernard dogs. Proceedings of the Royal Society B: Biological Sciences 275:71–76.

Freedman, D., and D. Lane. 1983. A nonstochastic interpretation of reported significance levels. Journal of Business & Economic Statistics 1:292–298.

R Core Team. 2022. R: A language and environment for statistical computing. Version 4.2.1. R Foundation for Statistical Computing, Vienna, Austria.

Rohlf, F. J., and M. Corti. 2000. Use of two-block partial least-squares to study covariation in shape. Systematic Biology 49:740–753.

Tejero-Cicuéndez, H., A. H. Patton, D. S. Caetano, J. Šmíd, L. J. Harmon, and S. Carranza. 2022. Reconstructing squamate biogeography in afro-arabia reveals the influence of a complex and dynamic geologic past. Systematic Biology 71:261–272.

Tejero-Cicuéndez, H., M. Simó-Riudalbas, I. Menéndez, and S. Carranza. 2021a. Ecological specialization, rather than the island effect, explains morphological diversification in an ancient radiation of geckos. Proceedings of the Royal Society B: Biological Sciences 288:20211821.

Tejero-Cicuéndez, H., M. Simó-Riudalbas, I. Menéndez, and S. Carranza. 2021b. Ecological specialization, rather than the island effect, explains morphological diversification in an ancient radiation of geckos. Dryad digital repository. (Doi:10.5061/dryad.xwdbrv1f6).

Table 1: Multivariate analysis of covariance describing variation in body form in extitPristurus.

	Df	SS	MS	Rsq	F	Z	Pr(>F)
svl	1	516.036559	516.0365588	0.9203096	10188.69842	9.490057	0.001
habitat	2	6.218510	3.1092552	0.0110902	61.38957	9.322480	0.001
svl:habitat	2	3.974307	1.9871536	0.0070879	39.23464	7.077264	0.001
Residuals	681	34.491245	0.0506479	0.0615124			
Total	686	560.720622					

Table 2: Pairwise comparisons of multivariate allometry vectors. Effect sizes ($Z_{\theta_{12}}$) based on pairwise differences in angular direction are below the diagonal, and their corresponding significance levels are above diagonal. Significant values in bold.

	Ground	Rock	Tree
Ground	0	**0.001**	**0.001**
Rock	**6.872**	0	0.261
Tree	**3.657**	0.649	0

Figures

Figure 1. Linear Measurements used in this study. SVL = snout-vent length, TL = trunk length, HL = head length, HW = head width, HH = head height, Lhu = humerus length, Lun = ulna length, Lfe = femur length, Ltb = tibia length (for details see Tejero-Cicuéndez et al. 2021a).

Figure 2. Plot of regression scores and predicted lines representing the relationship between linear body measurements and size (SVL). Individuals re colored by habitat use: rock (beige), ground (dark purple), and tree (magenta).

Figure 3. Traitgrams showing the evolution of body size (SVL) through time based on the phylogenetic tree of *Pristurus*. Colors represent an evolutionary mapping of regression slopes describing the relationship of (A) head morphology versus body size, and (B) limb proportions versus body size (see text for descriptions). Species names are colored by habitat use: rock (beige), ground (dark purple), and tree (magenta).

Figure 4. Phylomorphospace of *Pristurus*, based on residuals from a phylogenetic regression of body measurements on size (SVL). Species means are colored by habitat use: rock (beige), ground (dark purple), and tree (magenta). Large and small rock-dwelling and ground-dwelling are highlighted with darker colors to highlight their differentiation and relative positions in morphospace.

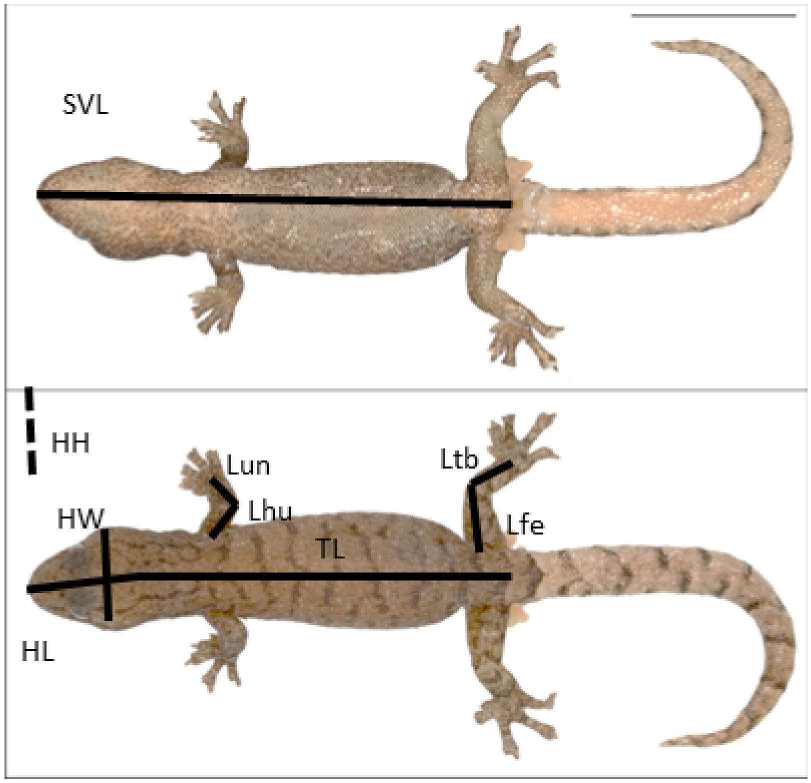

Figure 1: Linear Measurements used in this study. SVL = snout-vent length, TL = trunk length, HL = head length, HW = head width, HH = head height, Lhu = humerus length, Lun = ulna length, Lfe = femur length, Ltb = tibia length (for details see Tejero-Cicu'endez et al. 2021a).

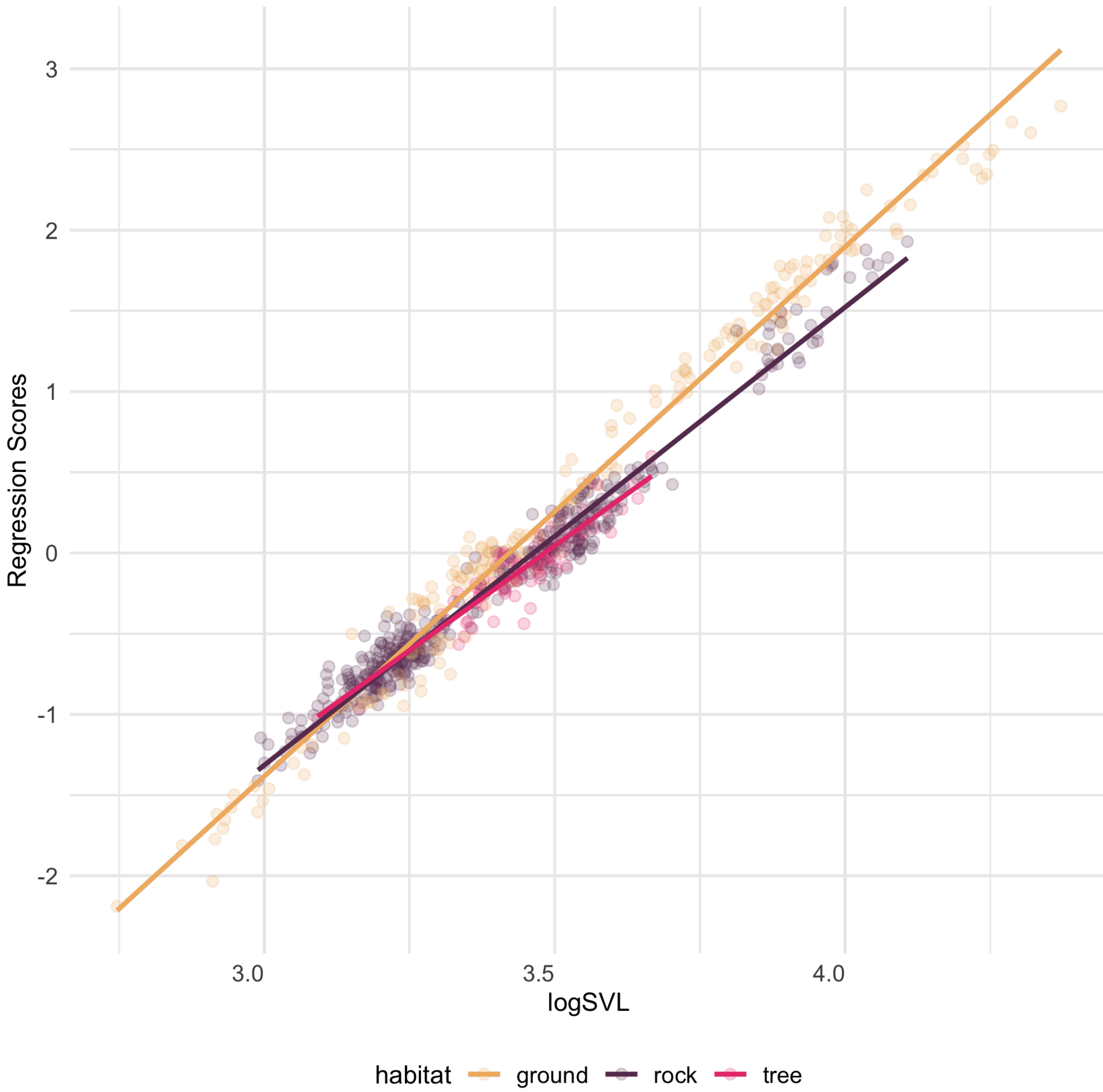

Figure 2: Plot of regression scores and predicted lines representing the relationship between linear body measurements and size (SVL). Individuals re colored by habitat use: rock (beige), ground (dark purple), and tree (magenta).

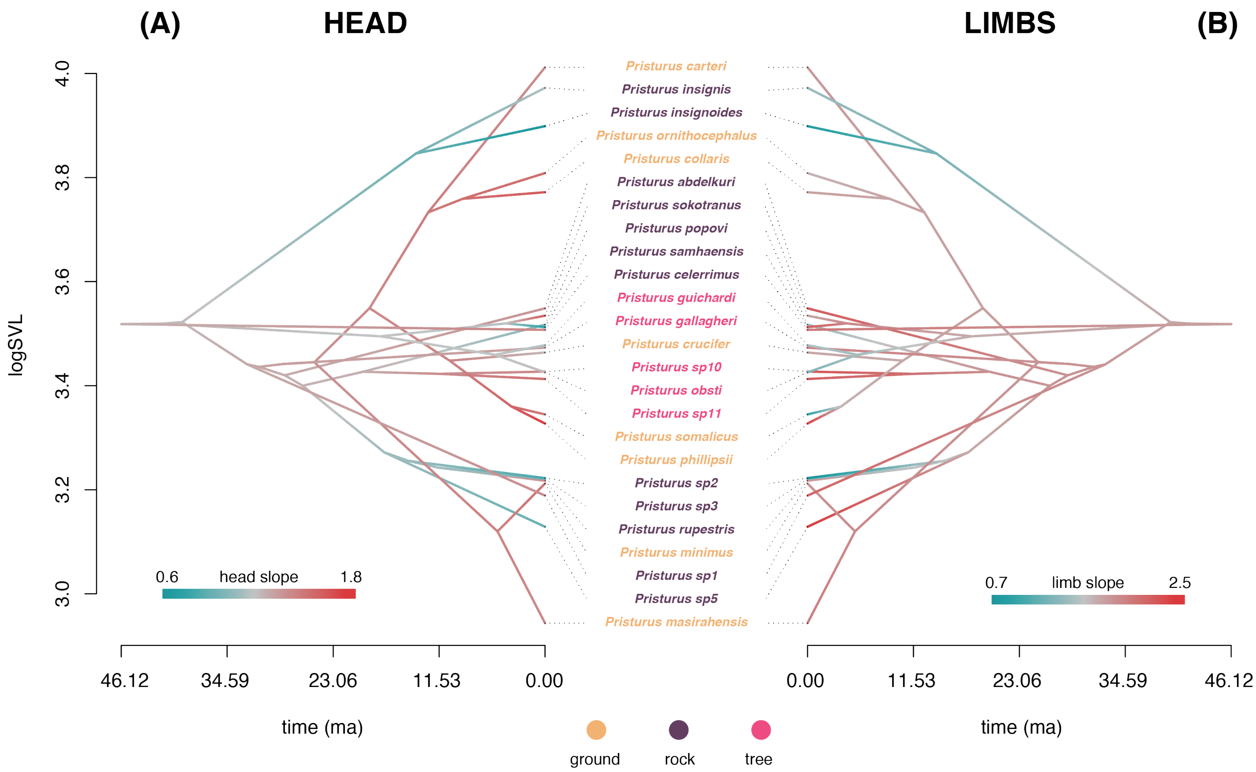

Figure 3: Traitgrams showing the evolution of body size (SVL) through time based on the phylogenetic tree of *Pristurus*. Colors represent an evolutionary mapping of regression slopes describing the relationship of (A) head morphology versus body size, and (B) limb proportions versus body size (see text for descriptions). Species names are colored by habitat use: rock (beige), ground (dark purple), and tree (magenta).

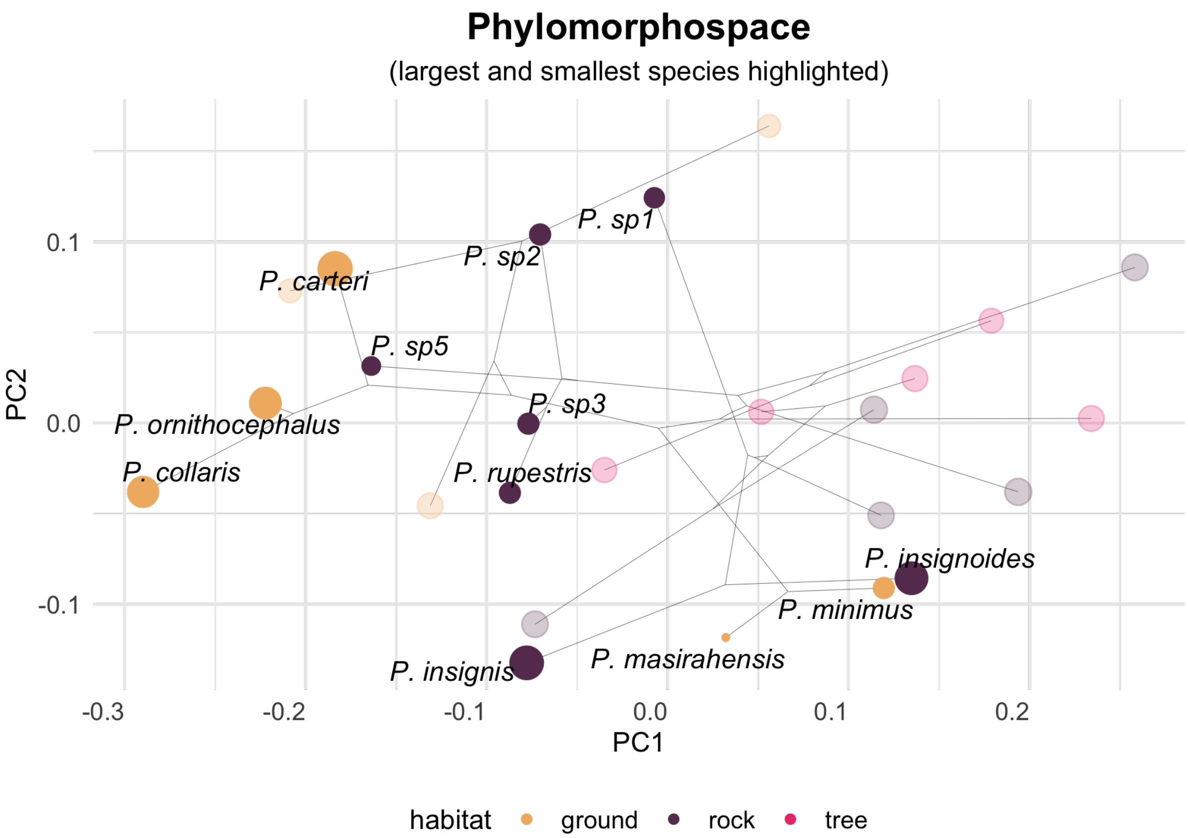

Figure 4: Phylomorphospace of *Pristurus*, based on residuals from a phylogenetic regression of body measurements on size (SVL). Species means are colored by habitat use: rock (beige), ground (dark purple), and tree (magenta). Large and small rock-dwelling and ground-dwelling are highlighted with darker colors to highlight their differentiation and relative positions in morphospace.