Genetic and Ecogeographic Controls on Species Cohesion in Australia's Most Diverse

Lizard Radiation

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Short Title: Controls on Species Cohesion.

Dryad data: https://doi.org/10.5061/dryad.jm63xsjbq

Title of Journal: The American Naturalist.

Abstract

Species vary extensively in geographic range size and climatic niche breadth. If range limits are primarily determined by climatic factors, species with broad climatic tolerances and those that track geographically widespread climates should have large ranges. However, large ranges might increase the probability of population fragmentation and adaptive divergence, potentially decoupling climatic niche breadth and range size. Conversely, ecological generalism in large-ranged species might lead to higher gene flow across climatic transitions, increasing species' cohesion and thus decreasing genetic isolation-by-distance (IBD). Focusing on Australia's iconic *Ctenotus* lizard radiation, we ask whether species range size scales with climatic niche breadth and the degree of population isolation. To this end, we infer independently evolving operational taxonomic units (OTUs), their geographic and climatic ranges, and the strength of IBD within OTUs based on genome-wide loci from 722 individuals spanning 75 taxa. Large-ranged OTUs were common and had broader climatic niches than small-ranged OTUs; thus, large ranges do not simply result from passive tracking of widespread climatic zones. OTUs with larger ranges and broader climatic niches showed relatively weaker IBD, suggesting that large-ranged species might possess intrinsic attributes that facilitate genetic cohesion across large distances and varied climates. By influencing population divergence and persistence, traits that affect species cohesion may play a central role in large-scale patterns of diversification and species richness.

Introduction

Biologists have long recognized that species distributions vary along two interrelated axes: geographic range size, the extent of the world's area that a species occupies, and climatic niche breadth, the range of climatic conditions a species tolerates (Darwin 1859; Willis 1926; Hutchinson 1957). Range sizes can differ by orders of magnitude, as seen, for instance, in Amazonian frogs, African primates, and North American plants (Gaston 2003; Mittermeier et al. 2013; Morueta- Holme et al. 2013; Guillory et al. 2020). Likewise, climatic niche breadths vary extensively. Some species are found in a limited set of climatic conditions, as is the case of tropical mountaintop endemics (e.g., Strangas et al. 2019). Others can thrive across a range of climates, as illustrated by human-mediated invasives (e.g., Harper and Bunbury 2015). If tolerance to climatic factors is a primary limit on species ranges (Andrewartha and Birch 1960; Lee-Yaw et al. 2016), we might expect species with broader climatic tolerances to occupy larger geographic areas, all else being equal (Slatyer et al. 2013). In addition, large geographic ranges are presumably more likely to span broader climatic gradients than small ranges, regardless of the factors behind range size (Sexton et al. 2009; Sizling et al. 2009). Therefore, differences in range size might both stem from and contribute to among-species variation in climatic niche breadth.

Empirical data are consistent with the expectation of a positive relationship between geographic range size and climatic niche breadth. As an illustrative example, figure 1A shows the significant relationship between these two variables across 900 terrestrial Australian lizard and snake taxa (linear regression: $F_{1,\,898} = 1{,}064$; $R^2 = 0.54$; $p < 0.001$; see Material and Methods for details on estimation). On one end of this relationship are taxa with large ranges and broad climatic niches, such as the scincid lizard *Ctenotus pantherinus*, whose distribution spans biomes

that range from arid temperate deserts to tropical grasslands and shrublands (figure 1B). On the

other end are taxa with small ranges and narrow climatic niches, such as *C. storri*, restricted to

tropical savannas in Australia's Top End (figure 1B). Even in closely related organisms within

the same geographic theater – as illustrated by scincid lizards on the Australian continent – we

find taxa that occur on opposite ends of the continuum defined by range size and climatic niche

breadth, underlying the pervasiveness of this pattern.

This positive relationship between range size and climatic niche breadth (figure 1) might

be viewed as expected. For instance, it is consistent with the hypothesis that climatic tolerances

are a primary driver of species range limits (Sexton et al. 2009; Slatyer et al. 2013; Lee-Yaw et

al. 2016). On the other hand, the apparent commonness of large-ranged taxa (figure 1) may be

unexpected, because large ranges spanning heterogeneous climates should be prone to

fragmentation (Pigot et al. 2012). For instance, such ranges are susceptible to dissection by

emerging geographic barriers (Rosenzweig 1995), favoring population divergence by genetic

drift (Barraclough 2019). Even in continuously distributed species, dispersal limitation can lead

to population differentiation via isolation-by-distance (Wright 1943; Irwin 2002). Additionally,

spatial climatic gradients can promote genetic divergence through selection and local adaptation

(Endler 1977; Bridle and Vines 2007; Schluter and Conte 2009; Hohenlohe et al. 2010), leading

to isolation-by-environment (Wang and Bradburd 2014). Given that these drivers of population

divergence are more likely to affect large-ranged species, the empirical pattern depicted in figure

1A highlights a major issue: how do species, particularly those that span large areas or varied

climates, remain cohesive in both genotype and phenotype?

One possibility is that species vary in their capacity for population connectivity, with

large-ranged species being more resistant to the differentiating effects of geographic separation

and spatial climatic gradients. Species cohesion across space has long been attributed to the homogenizing effects of population gene flow (Mayr 1963; Barker 2007; Barker and Wilson 2010). In turn, levels of gene flow may vary across species, as supported by studies reporting extensive variation in the degree of genetic isolation-by-distance (IBD) (Sexton et al. 2014; Singhal et al. 2018). We might expect large-ranged and ecologically generalized species to show lower IBD relative to small-ranged species because – all else being equal – a higher capacity to sustain population gene flow should decrease the likelihood of range splitting (Ackerly 2003). This capacity may stem, for instance, from intrinsic attributes that mediate dispersal (Ehrlich and Raven 1969) or population persistence and connectivity across climatic transitions (Ackerly 2003; Seebacher et al. 2012), leading to among-species variation in IBD. Under this model, the empirical pattern of a strong association between range size and climatic niche breadth (figure 1A) might ultimately reflect species' differential capacity to oppose the divergence of spatially separated populations in distinct climatic zones.

Comparative studies of distribution patterns and their potential climatic drivers have typically assumed that the underlying taxa are coherent and comparable units (Brown et al. 2014; González-Orozco et al. 2014; Zamborlini Saiter et al. 2016; Batista et al. 2020). However, many taxa once thought to be large-ranged are now known to consist of divergent phylogenetic lineages. This pattern has been observed repeatedly in Australian squamates (Smith and Adams 2007; Rabosky et al. 2017; Potter et al. 2019), potentially complicating our interpretation of the relationship between range size and climatic niche breadth and our ability to compare these two variables across taxa (figure 1). Traditionally, species delimitation has relied on organisms' phenotypic attributes, yet the traits that diverge across species can be cryptic to humans (Zozaya et al. 2019). Conversely, high phenotypic variation among populations can complicate species

delimitation, such that populations initially identified as multiple small-ranged taxa actually correspond to a single large-ranged polytypic species (Rabosky et al. 2014*b*). To properly understand the relationships between range size, climatic niche breadth, and IBD, the taxa must themselves be uniformly delimited. Studies of diversification dynamics have often recognized the need to delineate comparable evolutionary units (Smith et al. 2013; Ruane et al. 2014; Rabosky 2016; Singhal et al. 2018), yet macroecological analyses have rarely met this standard.

Here, we dissect the relationships between geographic range size, climatic niche breadth, and IBD in *Ctenotus*, a clade of lizards that occupies nearly all of Australia's major biomes. *Ctenotus* includes about 100 species-level taxa that are broadly similar in morphological and life-history traits (Cogger 2014). Therefore, we might expect them to have similar patterns of geographic, ecological, and genetic variation (Papadopoulou and Knowles 2016). However, whereas some of these taxa inhabit a relatively narrow set of climatic conditions within a single habitat type, others span climatically disparate forests, shrublands, grasslands, and deserts (Pianka 1969*a*, 1986). Australian *Ctenotus* occur in regions with little topographic variation, particularly in the extensive central arid zone (Pianka 1972; Pepper and Keogh 2021), which has likely minimized population isolation driven by geographic barriers (Potter et al. 2019). As a result, these lizards are a promising system to investigate the relationships between range size, climatic variability, and genetic isolation on a continental scale.

Recent genetic assessments of *Ctenotus* have found deeply divergent phylogenetic lineages within morphology-defined taxa and rampant taxon paraphyly and synonymy (Rabosky et al. 2014*b*, 2017; Singhal et al. 2018). To ensure our study compared equivalent units, we first performed a detailed analysis to delimit operational taxonomic units (OTUs) based on genome-wide loci, which allowed us to assess the commonness (or rarity) of genetically cohesive units

that span large areas and climatically heterogeneous regions. Based on the delimited OTUs, we

estimate geographic range sizes, climatic niche breadths, and within-OTU IBD to address three

questions. First, we ask whether the relationship between range size and climatic niche breadth is

robust to OTU delimitation and taxonomic practice, thus assessing the extent to which previously

reported patterns might have been influenced by limited comparability among the taxa

considered. Second, we ask whether OTUs exhibit different levels of genetic isolation per unit of

geographic distance, potentially reflecting variation in population gene flow and connectivity.

We then proceed to test a core prediction from our hypothesis that species have differential

capacities for cohesiveness in the presence of geographic and climatic heterogeneity: do large-

ranged and ecologically generalized OTUs have weaker IBD relative to OTUs with small ranges

and narrow climatic niches?

Material and Methods

A note on terminology

Throughout this manuscript, we used *taxa* and *taxon* to refer to the nominal taxa currently

recognized in *Ctenotus* taxonomy, most of which were defined based on morphological

attributes. These taxa are taxonomic entities that may or may not correspond to *species* in an

evolutionary sense. By *species*, we specifically refer to a conceptual category corresponding to

separately evolving metapopulation lineages (De Queiroz 1998, 2007). Applying this concept to

taxonomic practice is often not trivial. Our approach employs *Operational Taxonomic Units*

(OTUs), which we delimited based on attributes expected to be present in true species (*species*

criteria; see below). As such, these OTUs are our best approximation of species, and can be understood as *candidate species*.

Climatic niche estimates should be interpreted as a measure of the *realized climatic niche*, i.e., the climatic envelope occupied by a taxon or OTU under constraints imposed by barriers to dispersal and biotic interactions (Jiménez et al. 2019). By contrast, the *fundamental niche* is typically measured at taxonomic scales smaller than ours through ecophysiological experimentation (Lee-Yaw et al. 2016; but see Angilletta et al. (2019) for a contestation of the biological reality of the fundamental niche concept).

Geographic range size and climatic niche breadth estimation

To provide context for this investigation, we started by examining the relationship between range size and climatic niche breadth across the terrestrial Australian lizards and snakes, as currently recognized in taxonomy (figure 1). For this analysis, we used the expert-derived taxon distribution polygons by Roll et al. (2017). We first generated 2,000 random points for each taxon, a number that evenly spanned the distribution of even the most widespread taxa while being computationally tractable. To obtain a similar spatial density of points across taxa with different range sizes, we used the *speciesThin* R package (Aiello-Lammens et al. 2015) to rarify these points ensuring a minimum distance of 5 km between them. We then used the *raster* R package (Hijmans et al. 2015) to extract, from each resulting point, values of four bioclimatic variables (at a 30 arc-second resolution): annual mean temperature, temperature seasonality, annual precipitation, and precipitation seasonality, obtained from the *Chelsa* database (Karger et al. 2017). By focusing on only four variables that capture Australia's major climatic regimes, we reduced niche dimensionality and the number of occurrence records needed for climatic niche

estimation. Based on these data, we estimated a climatic hypervolume for each taxon. For this

purpose, we used the multivariate kernel density estimation method of Blonder et al. (2014) as

implemented in the *hypervolume* R package (Blonder et al. 2018). Following Blonder et al.

(2014), we standardized the climatic variables across all points across taxa before hypervolume

estimation by scaling values by their quadratic mean. With that, the resulting volumes are

expressed in powers of quadratic means (i.e., a composite unit corresponding to the product of

the climatic variable units; Blonder et al. 2014). We used these volumes as an estimate of

climatic niche breadth. As a metric of range size for the Australian lizards and snakes, we used

the area of each taxon polygon from Roll et al. (2017), expressed in km^2. Lastly, we extracted the

number of biomes occupied by each taxon using the geospatial layer derived by Olson et al.

(2001). We did not include taxa with ranges smaller than 20 km^2 (n = 17). These taxa, which

generally occur in spatially restricted settings (e.g., islands) or are known solely from their type

locality, often had a single point for climatic values, precluding hypervolume estimation.

     We used the approach described above also to estimate climatic niche breadths for each

delimited *Ctenotus* OTU (see below). To calculate each OTU's hypervolume, we extracted

climatic information from the collection sites of individual samples (assigned to OTUs based on

the delimitation results; see below). We used the *sp* R package (Pebesma and Bivand 2015) to

estimate each OTU's range size (expressed in km^2) based on the area of a convex hull defined by

the outermost collecting sites of individual samples, clipping hulls at the coastal outline of the

Australian continent when needed.

Specimen sampling

To delineate comparable evolutionary units and estimate isolation-by-distance in *Ctenotus*, we used a double-digest restriction site-associated DNA dataset (ddRAD) (Peterson et al. 2012). Data for most specimens (ca. 75 %) were generated by previous investigations of Australian scincid lizards (Singhal et al. 2017, 2018). For the newly sampled individuals, we obtained tissue samples through our fieldwork and loans from the Cornell University Museum of Vertebrates (CUMV), Northern Territory Museum (NTMR), Queensland Museum (QM), South Australian Museum (SAM) and its associated Australian Biological Tissue Collection (ATBC), University of Michigan Museum of Zoology (UMMZ), and Western Australian Museum (WAM). The taxonomic identification of individuals was performed by the original collectors at the field or museum based on diagnostic morphological attributes.

Reduced-representation genomic datasets composed of divergent species can have high levels of missing data (Eaton et al. 2017), which might impair OTU delimitation. To minimize this issue, we assembled eight datasets corresponding to major *Ctenotus* clades, which mostly align with the traditional morphology-defined species groups in this genus (Storr et al. 1999): the *atlas*, *colletti*, *essingtonii*, *inornatus*, *leonhardii*, *pantherinus*, *schomburgkii*, and *taeniolatus* clades. A ninth major clade, the *labillardieri* clade, was represented by four taxa with less than two samples each and was thus not included in OTU delimitation analyses. To inform the taxon composition of clades, we followed comprehensive molecular phylogenetic studies of scincids (Rabosky et al. 2014*a*; Singhal et al. 2017, 2018).

Sampling imbalance in genetic structure analyses can result in the artificial merging of undersampled groups and spurious grouping of intensely sampled localities (Puechmaille 2016; Lawson et al. 2018) and biased estimates of population genetic parameters (Battey et al. 2020). To minimize these issues, we limited the maximum number of samples per taxon per collecting

site to five. Moreover, we did not include 18 taxa represented by just one or two samples after

genetic filtering (see below). After these steps and downstream filtering of single nucleotide

polymorphisms (SNPs) (see below), 505 individuals representing 49 nominal *Ctenotus* taxa were

included in the OTU delimitation analyses. However, to improve the inference of tree topology,

phylogenetic analyses included samples from all nine *Ctenotus* major clades, including samples

not used in delimitation analyses, totaling 722 samples from 75 species-level *Ctenotus* taxa.

Generation of genetic data

We extracted genomic DNA and generated ddRAD libraries following Singhal et al.

(2017). Briefly, extractions were digested with the restriction enzymes EcoRI and MspI, and the

resulting fragments were tagged with individual barcodes, PCR-amplified, multiplexed, and

sequenced on an Illumina HiSeq or NovaSeq platform (with pooling adjusted for platform read

output). We used the *ipyrad* v. 0.9.57 pipeline (Eaton and Overcast 2020) to de-multiplex and

assign reads to individuals based on sequence barcodes, allowing no nucleotide mismatches from

individual barcodes. The number of paired-end reads ranged from ~100 thousand to ~27 million

per sample, with a read length of 100 base pairs. Due to computational time constraints, only the

forward reads (R1) were used in downstream analyses. We used *ipyrad* to perform *de novo* read

assembly (minimum clustering similarity threshold = 0.90), align reads into loci, and call SNPs.

A minimum Phred quality score (= 33), sequence coverage (= 6x), read length (= 35 bp), and

maximum proportion of heterozygous sites per locus (= 0.5) were enforced while ensuring that

variable sites had no more than two alleles within an individual (i.e., a diploid genome). Newly

generated demultiplexed raw sequence data were deposited in the Sequence Read Archive

(BioProject PRJNA755251).

We used *ipyrad* to assemble an initial dataset for each clade where each ddRAD locus was present in at least 60% of the sampled individuals. We extracted SNPs from these loci and removed those with a minimum allele frequency lower than 0.05 to improve inferences of population genetic structure and history (Linck and Battey 2019) and minimize spurious SNPs that result from sequencing errors (Ahrens et al. 2018). To ensure independence of SNPs, we then extracted a single SNP per locus. After these filtering steps, individuals with data for less than 50% of the final SNPs were excluded from downstream OTU delimitation analyses. We performed filtering using VCFtools v. 0.1.16 (Danecek et al. 2011) and custom R scripts. Data used in all analyses are available through Dryad (https://doi.org/10.5061/dryad.jm63xsjbq) (Prates et al. 2021). Computer scripts used to prepare and filter the data and perform all analyses are available through GitHub (https://github.com/ivanprates/Ctenotus_species_cohesion) and Zenodo (https://doi.org/10.5281/zenodo.5258926).

Estimating comparable evolutionary units

To compare geographic range sizes, climatic niche breadths, and genetic isolation-by-distance in *Ctenotus*, we first defined comparable operational taxonomic units (OTUs). We refrain from implementing coalescent-based delimitation approaches because they are computationally intractable for datasets as large as ours. Instead, we considered three sources of evidence widely used to support species delimitation (Mayr 1963; Dobzhansky 1971; Cracraft 1987; De Queiroz 1998; Mallet 2013, 2020): 1) the composition of genotypic groups from a genetic clustering approach, 2) the historical relationships between samples from a phylogenetic analysis, and 3) geographic distribution patterns (see below). We then compared the resulting

OTUs to morphology-defined *Ctenotus* taxa based on the original identification of sampled individuals.

To perform genotypic clustering, we used sparse Nonnegative Matrix Factorization (sNMF), a method that is robust to departures from traditional population genetic model assumptions (Frichot et al. 2014). We ran sNMF based on the unlinked SNP data for each major *Ctenotus* clade separately using the R package *LEA* (Frichot and François 2015). To infer the best-fit number of clusters (K), we compared the fit of schemes under K = 1–20, with 50 replicates for each K. The K value that led to the lowest cross-entropy value across replicates was considered the best-fit K. We then ensured that samples assigned to the same cluster grouped in genotypic space. For that purpose, we performed a principal component analysis (PCA) on the unlinked SNP data using the *LEA* R package and inspected biplots of the first four principal components.

To evaluate whether the inferred genotypic clusters correspond to phylogenetic lineages, we examined their correspondence with clades within *Ctenotus*. For that purpose, we performed phylogenetic inference under maximum likelihood for the entire *Ctenotus* genus using RaxML-HPC v. 8.2.12 (Stamatakis 2014) through the CIPRES Science Gateway (Miller et al. 2010). We used the GTRCAT model of nucleotide evolution and estimated node support based on 100 bootstrap replicates. As outgroups, we included six representatives from other scincid clades, namely *Eremiascincus fasciolatus*, *Lerista bipes*, and *Lerista ips*. The final phylogenetic dataset was composed of 83,083 SNPs, each present in at least 50% of the samples. We used the resulting phylogeny (figure S1) also to account for the historical relationships between OTUs when testing for associations between range size, climatic niche breadth, and population genetic isolation (see below). For that, we made the tree ultrametric based on penalized likelihood using

the *ape* R package (Paradis et al. 2004) and randomly sampled one individual per OTU. To

visualize phylogenetic trees, we used the *ape* (Paradis et al. 2004), *ggtree* (Yu et al. 2017), and

phytools (Revell 2012) R packages.

Quantifying population isolation and testing for relationships with range size and climatic niche breadth

We tested whether OTUs with larger distributions and broader climatic niches show

lower genetic isolation levels across their distribution relative to OTUs with narrow ranges and

niches. To do so, we compared patterns of genetic isolation-by-distance (IBD) based on F_{ST}, a

metric of genetic differentiation (Weir and Cockerham 1984; Weir and Hill 2002). Within each

delimited OTU, we estimated pairwise F_{ST} between individuals based on the unlinked SNP data

using the *BEDASSLE* R package (Bradburd et al. 2013). To calculate a matrix of geographic

distances, we used the *fossil* R package (Vavrek 2011). We then estimated the slope of the

relationship between genetic distances ($F_{ST}/1$-F_{ST}) and geographic distances for each OTU

(hereafter, IBD slopes). We used these IBD slopes as an estimate of spatial genetic isolation

within each OTU, and, thus, as a proxy of cohesion across geographic space. Specifically, we

considered a less pronounced IBD slope to indicate lower genetic isolation, and thus higher

cohesion, over an OTU's range. To determine whether the relationship between genetic and

geographic distances was statistically significant for each OTU, we used multiple matrix

regression with randomization in R (Wang 2013) employing 1,000 permutations. We then tested

whether IBD slope varies as a function of range size and climatic niche breadth across the

Ctenotus clade by implementing linear regressions in R. We also accounted for the historical

relationships between OTUs in these analyses by implementing phylogenetic generalized least

squares under Brownian motion (lambda = 1) using the *caper* R package (Orme et al. 2013).

The number of samples available varied across OTUs (mean = 10.3, range = 3–37). For

instance, out of 48 OTUs used for IBD slope estimation, six were represented by 3–4 samples, a

number that emerged automatically from our delimitation process (and thus could not be

anticipated). Because our sampling strategy aimed to span a broad spatial area and include taxa

collected only infrequently, we did not exclude OTUs represented by fewer samples from our

analyses. Instead, we directly assessed whether the relationship between IBD slope and range

size might have been affected by sample size. For that purpose, we performed an analysis of

covariance (ANCOVA) with IBD slope as a dependent variable, range size as an independent

variable, and the number of samples per OTU as a covariate.

Another potential confounding factor in these analyses is that species with large (or

small) ranges might be concentrated in biomes that lead to lesser (or greater) IBD. To examine

whether the relationship between IBD slope and range size is the same across biomes (as per

Olson et al. 2001), we implemented another linear model in R with IBD slope as a dependent

variable, range size as an independent variable, and the most frequent biome where a species

occurred as a factor.

Estimates of population genetic differentiation (such as F_{ST}) might be affected by levels

of within-population genetic diversity (Charlesworth 1998; Cruickshank and Hahn 2014).

Additionally, genetic diversity is expected to be higher in populations with larger effective sizes

(Charlesworth 2009; Lanfear et al. 2014), which might be the case of our large-ranged delimited

OTUs. Therefore, we examined the potential associations between IBD slope, range size, and

expected heterozygosity (a standard metric of genetic diversity) across the *Ctenotus* clade using

linear regressions. To estimate expected heterozygosity based on the SNP data, we used the *adegenet* package in R (Jombart 2008).

Lastly, to assess how delimitation schemes may affect estimates of IBD, range size, and climatic niche breadth, we compared OTU-based estimates to estimates for the corresponding taxa to which individuals within each OTU were originally assigned based on morphological attributes.

Results

Delimitation of comparable evolutionary units

The overwhelming majority of the currently recognized *Ctenotus* taxa were described based on morphological attributes. Our analyses indicate that 24 out of 49 of the taxa included in our OTU delimitation analyses correspond to distinct genetic pools and are thus likely to represent separately evolving units. However, we also found evidence of both unrecognized diversity and taxonomic over-splitting within *Ctenotus*. As an illustration of the former, figure 2 presents OTU delimitation results for the *schomburgkii* clade (see figure S2 and S3 for results for the other seven major *Ctenotus* clades). In this group, samples originally assigned to the taxon *C. schomburgkii* formed two major genotypic clusters (light and dark blue in figure 2 respectively) that are reciprocally monophyletic, have adjacent geographic distributions in central versus southwestern Australia, and show no evidence of admixture – a pattern consistent with evolutionary independence. Similarly, we inferred *C. strauchii* samples to correspond to two non-sister clades, each corresponding to a geographically coherent genotypic cluster in central and eastern Australia (yellow and light green in figure 2, respectively). We found multiple OTUs

within morphologically defined taxa in most of the major *Ctenotus* clades (figure S2). As

expected, the number of OTUs inferred within taxa increased with taxon range size ($F_{1, 37} = 6.93$;

$R^2 = 0.14$; $p = 0.01$; figure S4).

In other cases, OTU delimitation analyses suggested that multiple nominal taxa may

correspond to the same genetic pool. For instance, in the *schomburgkii* clade, samples assigned

to *C. eutaenius* and *C. euclae* composed a single major genetic group (pink in figure 2; see also

figure S2). In some instances, this pattern results from taxon misidentification, particularly

among taxa with a subtle or unclear morphological diagnosis. For example, several individuals

originally identified as *C. brooksi* clustered with samples attributed to *C. eutaenius* and *C.

euclae*. Misidentification appeared particularly common in the *inornatus* clade, previously found

to show rampant phenotypic parallelism and high levels of intraspecific trait variability (figure

S2) (Rabosky et al. 2014*b*).

In total, delimitation analyses inferred 53 genetically and geographically coherent OTUs

across the 49 nominal taxa included in delimitation analyses. Of these OTUs, 48 were

represented by three or more climatically unique collecting sites, allowing climatic niche

estimation. These uniformly delimited putative species were used in downstream analyses to

quantify climatic niche breadths, range sizes, and genetic isolation over geographic space.

Relationship between geographic range size and climatic niche breadth

The range sizes and climatic niche breadths seen in the delimited *Ctenotus* OTUs

spanned nearly the entire range seen in the Australian squamates as a whole (figure 3). Even

after accounting for potentially cryptic species within *Ctenotus*, widely distributed OTUs were

common. This result supports that species that span large areas and varied climates are not

artifacts from unrecognized diversity under the current taxonomy but a real biological pattern.

The positive relationship between range size and climatic niche breadth was statistically

significant across OTUs ($F_{1, 46} = 59.4$; $R^2 = 0.55$; $p < 0.001$), and remained so after accounting

for phylogenetic autocorrelation ($F_{1, 46} = 32.5$; $R^2 = 0.4$; $p < 0.001$). OTUs with large ranges

invariably had broader climatic niches. The number of biomes occupied by an OTU generally

increased with range size and climatic niche breadth (figure 3). However, certain OTUs with

broader niches were restricted to a single biome, reflecting the apparent climatic diversity within

some biomes (Pepper and Keogh 2021). These findings seem to contradict the possibility that

large ranges merely result from tracking narrow sets of climatic conditions that span large

geographic areas.

Patterns of isolation-by-distance

Pairwise F_{ST} estimates reveal a pattern of increasing genetic distance with geographic

distance in 46 out of 48 *Ctenotus* OTUs. This positive relationship was statistically significant in

30 OTUs ($p < 0.05$; multiple matrix regression with randomization). Despite this consistent

pattern of genetic isolation-by-distance (IBD), the slope of this relationship varied substantially

across OTUs (figure 4, figure S5).

Across *Ctenotus* OTUs, levels of genetic isolation by geographic distance – as described

by the slope of IBD – decreased with increasing range sizes ($F_{1, 46} = 32.5$; $R^2 = 0.4$; $p < 0.001$;

figure 5A) and niche breadths ($F_{1, 46} = 12.8$; $R^2 = 0.2$; $p < 0.001$; figure 5B). After accounting for

phylogenetic autocorrelation, these negative relationships remained statistically significant for

range size ($F_{1, 46} = 30.7$; $R^2 = 0.39$; $p < 0.001$) but not climatic niche breadth ($F_{1, 46} = 3.4$; $R^2 =$

0.05; p = 0.07). These results suggest generally lower genetic isolation over the landscape in

OTUs that span large areas and varied climates relative to small-ranged and climatically less

diverse OTUs.

When accounting for the effect of biome in the relationship between IBD slope and

range size or climatic niche breadth (figure S6), we found that the interactions between biome

and both range size ($F_{2, 41}$ = 2.3; p = 0.12) and niche breadth ($F_{2, 41}$ = 0.7; p = 0.51) were not

significant. After removing the non-significant interaction terms, there was a significant effect of

both range size ($F_{1, 43}$ = 31; p < 0.001) and niche breadth ($F_{1, 43}$ = 13.2; p < 0.001) on IBD slope,

but no effect of biome ($F_{3, 43}$ = 1.5; p = 0.22). These results support that the relationships

between IBD slope and range size or climatic niche breadth are not driven by consistent

differences in IBD among biomes.

We found no association between expected heterozygosity and range size ($F_{1, 46}$ = 3.88; p

= 0.06; R^2 = 0.06), suggesting no consistent differences in genetic diversity levels across small-

and large-ranged OTUs (figure S7). Additionally, we found no association between IBD slope

and expected heterozygosity ($F_{1, 46}$ = 3; p = 0.09; R^2 = 0.04), supporting that variation in the

strength of IBD across OTUs does not simply reflect variation in genetic diversity levels (figure

S7).

An analysis of the relationship between IBD slope and range size including the number of

samples per OTU as a covariate found no significant effect of sample size ($F_{2, 45}$ = 15.89; p =

0.94). By contrast, range size continued to have a significant effect on the slope of IBD (p <

0.001), confirming that the negative association between those two variables cannot be explained

by variation in the number of samples among OTUs.

Effects of species delimitation on macroecological estimates

Species delimitation scheme affected estimates of range size, climatic niche breadth, and IBD (figure 6). For all three metrics, estimates based on the morphologically defined taxa to which individuals were originally assigned resulted in both higher and lower values relative to estimates based on the delimited OTUs. Taxon-based range sizes and climatic niche breadths (figure 6A,B) spanned the same range of values as OTU-based estimates. By contrast, taxon-based IBD slopes were up to ten times as high as OTU-based estimates (figure 6C).

Discussion

Few studies have applied a standardized scheme to address the impacts of inconsistent species delimitation on macroecological patterns. Therefore, reported patterns of geographic range variation have typically relied on unstated assumptions about the comparability of the underlying units (e.g., Letcher and Harvey 1994; Blackburn and Gaston 1996; Waldron 2007; Slatyer et al. 2013; Zagmajster et al. 2014; Cardillo 2015; Pie and Meyer 2017). Using a uniform approach to delimit operational taxonomic units (OTUs) in *Ctenotus* lizards, we confirmed that range variation does not simply reflect heterogeneous species delimitation. The OTUs showed extensive variation in range size and climatic niche breadth, which spanned the same range of values we estimated for the (largely morphology-defined) Australian lizard and snake taxa as a whole. Even under this uniform delimitation scheme, large-ranged and ecologically generalized OTUs were common.

Additionally, our results provide little support for the hypothesis that large ranges result from narrow climatic envelopes that spread over extensive geographic areas (James and Shine 2000; Ackerly 2003). Instead, OTUs with larger ranges generally had broader climatic niches

and often occurred in multiple biomes. These large-ranged and ecologically diverse OTUs typically had relatively weaker genetic isolation-by-distance (IBD), which suggests a possible mechanism for the observed relationship between range size and climatic niche breadth. In particular, species with attributes that facilitate population connectivity should be able to maintain coherent ranges across large areas. In contrast, species that cannot maintain such connectivity will tend to fragment into smaller units. Under this model, the pattern of a strong coupling between range size and climatic niche breadth (figure 1, 3) might ultimately originate from species traits that oppose the genetic differentiation of spatially separated populations in distinct climatic zones.

Effects of species delimitation on macroecological patterns

To assess the relationship between range sizes, climatic niche breadths, and IBD, we first delimited comparable OTUs – a step that is rarely part of macroecological analyses. To this goal, we relied on three species criteria widely employed in delimitation studies: that 1) conspecific individuals tend to share derived genetic variants and thus form a monophyletic group; that 2) conspecifics comprise a cohesive genetic pool, sharing strongly correlated genome-wide allele frequency patterns; and that 3) conspecifics span a coherent and mostly continuous geographic area, allowing population gene flow (Mayr 1963; Dobzhansky 1971; Cracraft 1987; De Queiroz 1998; Mallet 2013, 2020). Many of the OTUs we delimited under these expectations correspond to long-recognized *Ctenotus* taxa, primarily described based on morphological attributes (e.g., Storr 1973, 1975, 1988; Ingram 1979; Horner 2009). However, our results also highlight cases that may require additional investigation of taxon limits and composition. In some instances, genotypic clustering analyses grouped samples that composed distinct clades and were initially

assigned to different taxa based on morphology, as was the case of *C. euclae and C. taeniatus*. In

other cases, OTU pairs corresponded to a single taxon, as seen in *C. schomburgkii* (figure 2).

These intra-taxon OTUs were geographically coherent and formed distinct clades and genotypic

clusters. They may also correspond to known phenotypic breaks within taxa, as is the case of *C.

schomburgkii*, where the OTUs that emerged from our analyses may correspond to known color

morphs (Storr et al. 1999; Wilson and Swan 2017).

Employing a uniform framework to outline OTUs affected some downstream inferences

of macroecological variables. OTU-based estimates of range size and climatic niche breadth

were both greater and less than estimates based on the taxa to which individuals were originally

assigned (figure 6). Taxon-based estimates of IBD were up to twice as high as OTU-based

estimates, a pattern consistent with lumping of different species under the same taxon name.

These findings support that incomplete delimitation and variation in taxonomic practice can

influence estimates of species richness and turnover, ecological niches, and population genetic

differentiation. We note, however, that these effects are likely clade-specific, reflecting the status

of the associated taxonomic knowledge (Melville et al. 2021; Moura and Jetz 2021).

Extrinsic and intrinsic drivers of range size and climatic niche breadth variation

Both extrinsic and intrinsic factors could generate the range size variation seen in

Ctenotus and other taxa (Gaston 2003; Sexton et al. 2009; Slatyer et al. 2013). One potential

extrinsic factor is geographic barriers to dispersal (Sheth et al. 2020), long recognized to limit

species distributions (Wallace 1854; Darwin 1859). However, macroecological studies have

noted that taxon range limits in *Ctenotus* cannot be readily attributed to landscape features that

might limit dispersal (Pianka 1969*b*), in agreement with an apparent pattern of idiosyncratic

range limits among our delimited OTUs (figure S2). Accordingly, Australia generally lacks

major physiographic features that might limit dispersal by terrestrial vertebrates in obvious ways,

particularly in its vast and largely featureless central arid zone (Pianka 1972; James and Shine

2000; Pepper and Keogh 2021). In addition to such barriers, current species ranges may have

been influenced by historical shifts in habitat distributions (Graham et al. 2006; Carnaval and

Moritz 2008; Prates et al. 2016). However, while information on *Ctenotus* is lacking, studies of

many Australian taxa have found idiosyncratic responses to Plio-Pleistocene climate change

(Byrne et al. 2011; Potter et al. 2018; Pepper and Keogh 2021). This situation contrasts with

other world regions where major transitions in the composition of regional species pools (or

phylogenetic lineages) overlap with landscape features such as mountains and rivers or the

presumed distributions of past habitats (e.g., Hewitt 2000; Graham et al. 2009; Oliveira et al.

2017).

Range sizes might also be influenced by extrinsic environmental factors. For instance,

species with narrow climatic niches might acquire large distributions by tracking geographically

widespread climates (Ackerly 2003). It has been suggested that such tracking might account for

the high richness of *Ctenotus* assemblages in the arid zone, given the larger size of this biome

relative to others (James and Shine 2000). Contrary to this hypothesis, we found that large-

ranged OTUs mainly have broad climatic niches (figure 3). While the range limits of certain

OTUs roughly align with the climatic transitions that correspond to Australian biome boundaries

(González-Orozco et al. 2014), several OTUs span multiple biomes, and both widely and

narrowly distributed OTUs occur within any given biome (figure S2). These patterns suggest that

spatial climatic transitions are insufficient to explain the extensive range size and climatic niche

breadth variation seen in *Ctenotus* lizards.

Another extrinsic environmental factor that might limit species distributions is biotic interactions. Several studies of *Ctenotus* have focused on the contribution of interspecific competition to ecological assemblage structure. For instance, regional co-occurrence of 14 or more *Ctenotus* taxa in Australia's arid zone has been attributed to divergence in diet, time of activity, habitat, and microhabitat use (Pianka 1969*a*; Rabosky et al. 2011). Competition-driven character displacement was invoked to explain assemblage-wide overdispersion in ecologically relevant traits in *Ctenotus* (and other lizard clades) at a local spatial scale (Rabosky et al. 2007, 2011). However, assemblage-wide trait diversity is nearly constant at a broader regional scale (Rabosky et al. 2007), and possible links between competition and taxon distributions remain unclear. How other types of biotic interactions – such as predation, parasitism, and hybridization with closely related species (Ricklefs 2010) – affect distribution patterns in *Ctenotus* is yet to be determined.

Among-species variation in range size and niche breadth might also be influenced by intrinsic organismal factors (Sheth et al. 2020). One example is traits that affect dispersal, invoked to explain range size variation in birds, bats, and insects, for instance (Böhning-Gaese et al. 2006; McCulloch et al. 2017; Luo et al. 2019). In *Lerista*, the clade sister to *Ctenotus*, taxa show varying degrees of limb reduction, which, in turn, predict some of the variance in range size (Lee et al. 2013). Limb reduction across *Lerista*, *Ctenotus*, and closely related clades is also associated with higher IBD (Singhal et al. 2018), suggesting that traits that mediate dispersal capacity may impose limits to range size by constraining gene flow across regions. Organismal traits might also contribute to among-species variation in climatic niche breadth. For instance, physiological studies of ectothermic organisms have reported among-population variation in thermal performance curves matching local climates (Wilson 2001; Gaitán-Espitia et al. 2014;

Llewelyn et al. 2016; Kosmala et al. 2018). Lastly, the range of conditions where a species can

occur is influenced by behavioral traits. For instance, lizards can adjust basking frequency and

microhabitat use to local temperatures (Adolph 1990; Navas 2002), thus bypassing the need for

adaptation (Buckley et al. 2015). Variation in thermal behavior has been reported among closely

related taxa, which can range from thermoregulators to thermoconformers (Huey et al. 2009;

Ibargüengoytía et al. 2010). Unfortunately, these types of data are largely lacking for *Ctenotus*,

preventing a test of which phenotypic attributes may contribute to range size and climatic niche

breadth variation. To fill these gaps, future studies will benefit from gathering information on

morphophysiological and behavioral variation across this diverse clade.

Evolutionary causes and consequences of species cohesion

Our finding of many large-ranged OTUs poses the question of what factors maintain

species cohesion over large geographic distances. We found that large-ranged OTUs have

shallower IBD slopes relative to OTUs with smaller ranges, consistent with the hypothesis of a

higher capacity (or propensity) for cohesion in large-ranged species. This pattern might indicate

that range size is primarily determined by dispersal, under the premise that IBD variation tracks

differences in dispersal capacity across species (Wright 1943; Sexton et al. 2014). However, it is

also possible that both range size and IBD are independently determined by species' climatic

tolerances, such that species with broad tolerances might be able to persist across a range of

environmental conditions without the ensuant disruption of gene flow. This combination of

broad tolerances with sustained gene flow might be achieved through phenotypic plasticity and

even local adaptation, as long as the behavioral and morphophysiological traits under selection

(and their underlying genes) are unrelated to reproductive isolation (Ackerly 2003; Seebacher et

al. 2012). By allowing populations to persist in varied conditions while remaining interconnected, factors that broaden species-level environmental tolerances may play a central role in species cohesion over large areas.

The potentially central role of gene flow in species cohesion has long been recognized (Mayr 1963; Barker and Wilson 2010). Nevertheless, other factors may buffer species from genetic (and phenotypic) change (Ehrlich and Raven 1969; Barker 2007), some of which may disproportionately affect species with large ranges. For instance, these species might have larger effective population sizes, resulting in less genetic divergence from drift (Excoffier and Ray 2008). However, we found no relationship between range size and heterozygosity, which is expected to scale with effective population size (Lande and Barrowclough 1987). Conversely, larger population sizes also increase the effectiveness of selection and thus the potential for adaptation (Charlesworth 2009; Lanfear et al. 2014). It is currently unknown whether populations of large-ranged *Ctenotus* species show genetically determined adaptations to local climates. Nevertheless, our finding of relatively lower IBD in OTUs with broad climatic niches suggests that spatial climatic gradients do not constrain population genetic connectivity. Accordingly, whereas local adaptation can sometimes contribute to reproductive isolation (Nosil 2012; Sobel et al. 2010), there is increasing evidence that this process rarely leads to speciation between parapatric populations (Seehausen et al. 2014). Instead, locally adapted populations can sustain high gene flow levels even when the genomic regions that underpin adaptive phenotypes become markedly differentiated (Feder et al. 2012; Harrison 2012).

Our finding of extensive variation in IBD across OTUs has implications for our understanding of speciation and extinction over macroevolutionary timescales. If certain species are more prone to range fragmentation, they may have a greater probability of diversifying into

multiple new species (Rabosky 2016). By affecting speciation probability, variation in species

cohesion might influence evolutionary diversification and regional species richness. In this view,

relative cohesiveness would be similar to other emergent species-level traits that lead to

differential species proliferation (or "species selection") through its impact on speciation and

extinction rates (Arnold and Fristrup 1982; Jablonski 2008). For instance, levels of genetic

structure correlate with speciation rates in birds, although much variation in speciation remains

unexplained (Harvey et al. 2017). By contrast, the strength of IBD did not predict speciation rate

variation across the sphenomorphine lizard clade (in which *Ctenotus* is nested), though this

association might be hard to identify at this narrow phylogenetic scale (Singhal et al. 2018).

Establishing direct links between species cohesion and evolutionary diversification can also be

complicated by the expected higher extinction rate of isolated populations, for instance due to

small population sizes (Harvey et al. 2019; Prates and Singhal 2020). Whether isolated

populations will complete speciation or go extinct might be context-dependent, as determined,

for instance, by long-term habitat persistence tied to climatic stability (e.g., Carnaval et al. 2014;

Dynesius and Jansson 2014). A synthetic understanding of the population-level controls on

macroevolutionary dynamics is likely to require further investigations of the intrinsic and

extrinsic factors that influence species cohesion through evolutionary time.

Statement of Authorship

IP, SS, and DLR conceived and designed the study. SS and DLR supervised the study. SS, CCM, SD, and DLR acquired funding. CCM, SD, and DLR provided resources. SS, MRMR, MRG, and DLR designed data collection. SS, MRMR, and MRG collected data. IP wrote computer scripts and prepared figures. IP, SS, and DLR analyzed data and drafted the manuscript. IP, SS, MRMR, MRG, CCM, SD, and DLR edited the manuscript.

Data and Code Availability

Data used in all analyses are available through the Dryad Digital Repository database: https://doi.org/10.5061/dryad.jm63xsjbq (Prates et al. 2021). Newly generated de-multiplexed raw DNA sequence data were deposited in the Sequence Read Archive (BioProject PRJNA755251). R and UNIX shell scripts used to prepare and filter the data and perform all

analyses are available through GitHub

(https://github.com/ivanprates/Ctenotus_species_cohesion) and Zenodo

(https://doi.org/10.5281/zenodo.5258926). A copy of the Supplementary Material is available at

Zenodo (https://doi.org/10.5281/zenodo.5258928).

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Figure Legends

Figure 1. Variation in the spatial and climatic structure of taxon distributions. (A) The

relationship between geographic range size and climatic niche breadth in terrestrial Australian

lizards and snakes (n = 900) (R^2 = 0.54; p < 0.001) for currently recognized taxa. Range size

corresponds to the spatial area occupied by a taxon as per Roll et al. (2017). Climatic niche

breadth corresponds to a hypervolume defined by four climatic variables that describe

temperature and precipitation annual means and seasonality (see Material and Methods). The

color of circles indicates the number of biomes occupied by each taxon. Roman numerals

indicate representative *Ctenotus* lizard taxa that show different combinations of range size and

climatic niche breadth, as follows: (I) large range size despite a relatively narrow climatic niche

breadth, (II) large range and broad climatic niche, (III) small range and narrow climatic niche,

and (IV) relatively small range given a broad climatic niche. Dashed lines indicate the mean

value of each axis. (B) Australia's biomes as per Olson et al. (2001). (C) Geographic distribution

of the four representative *Ctenotus* taxa (I–IV) highlighted in A (as per Roll et al. (2017)). Taxon

ranges indicated in blue. Biomes (indicated in grayscale) as in B.

Figure 2. Operational Taxonomic Unit (OTU) delimitation in this study, as illustrated by

Ctenotus schomburgkii and allied taxa. (A) Phylogenetic relationships inferred under maximum

likelihood and all ddRAD SNPs. (B) Ancestry proportions and assignment of sampled

individuals to major genetic groups based on the unlinked SNP data. (C) Geographic

distributions of inferred genetic groups. Labels above maps indicate species-level taxa assigned

to the majority of each OTU's samples. (D) Sample clustering in genotypic space based on a

principal component analysis on the SNP data. Plot shows PC1 and PC2; groups further separate

along PC3 and PC4 (see figure S3). Results for the other seven major *Ctenotus* clades included

in the OTU delimitation analyses are presented as supplementary information (figure S2).

Figure 3. The relationship between geographic range size and climatic niche breadth in *Ctenotus*

lizards based on the delimited OTUs (n = 48). Blue line and colored circles correspond to

estimates for *Ctenotus* OTUs, with circle color indicating the number of biomes where an OTU

occurs. The gray line and circles indicate estimates for 900 Australian lizard and snake taxa

(from Roll et al. (2017), the same data presented in figure 1A). Density plots show the

corresponding range of climatic niche breadth (top) and range size (right) values. Even after

accounting for cryptic diversity and variation in taxonomic practice, there is a positive

relationship between range size and climatic niche breadth ($R^2 = 0.55$; $p < 0.001$).

Figure 4. The relationship between genetic isolation and geographic distance, illustrated by

OTUs in the *Ctenotus schomburgkii* clade (figure 2). Pairwise F_{ST} based on the SNP data was

used as an estimate of within-OTU genetic isolation. To facilitate comparison, axes span the

same range of values across plots. More pronounced slopes indicate higher isolation-by-distance

between individuals within an OTU. Results for other seven major clades of *Ctenotus* are

presented as supplementary information (figure S5).

Figure 5. Predictors of genetic isolation-by-distance (IBD) levels across all *Ctenotus* OTUs (n =

48). Lower IBD slopes indicate lower genetic isolation-by-distance within a given OTU. (A)

Within-OTU IBD slopes are negatively correlated with range size ($R^2 = 0.4$; $p < 0.001$). (B)

These IBD slopes are also negatively correlated with climatic niche breadth ($R^2 = 0.2$; p <

0.001).

Figure 6. The effect of the delimitation scheme on estimates of (A) range size, (B) climatic niche

breadth, and (C) IBD slope. Each plot compares estimates based on the delimited OTUs

(horizontal axes) versus the corresponding morphology-based taxa to which individuals were

originally assigned (vertical axes). The blue lines correspond to identical values on both axes

(i.e., x = y). Taxon-based estimates were both overestimated (points above the lines) and

underestimated (points below the lines) relative to OTU-based estimates.
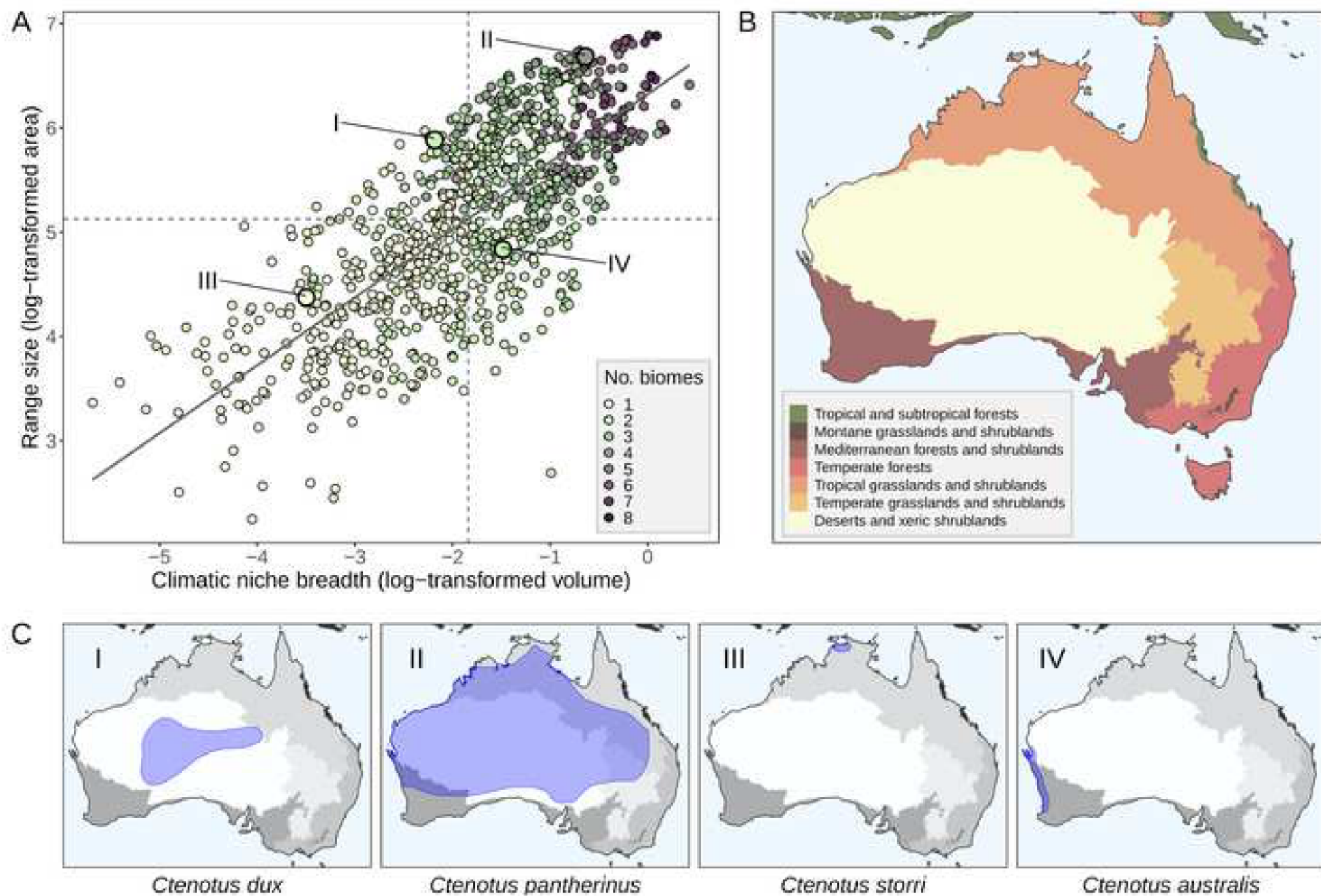
Figure 1

A — Range size (log-transformed area) vs Climatic niche breadth (log-transformed volume)

No. biomes
1
2
3
4
5
6
7
8

B — Layer

Tropical and subtropical forests
Montane grasslands and shrublands
Mediterranean forests and shrublands
Temperate forests
Tropical grasslands and shrublands
Temperate grasslands and shrublands
Deserts and xeric shrublands

C

I — *Ctenotus dux*

II — *Ctenotus pantherinus*

III — *Ctenotus storri*

IV — *Ctenotus australis*

Figure 2

Fig2_schomburgkii_gr_mi0.5_ms0.4_a500_Australia_n88.png
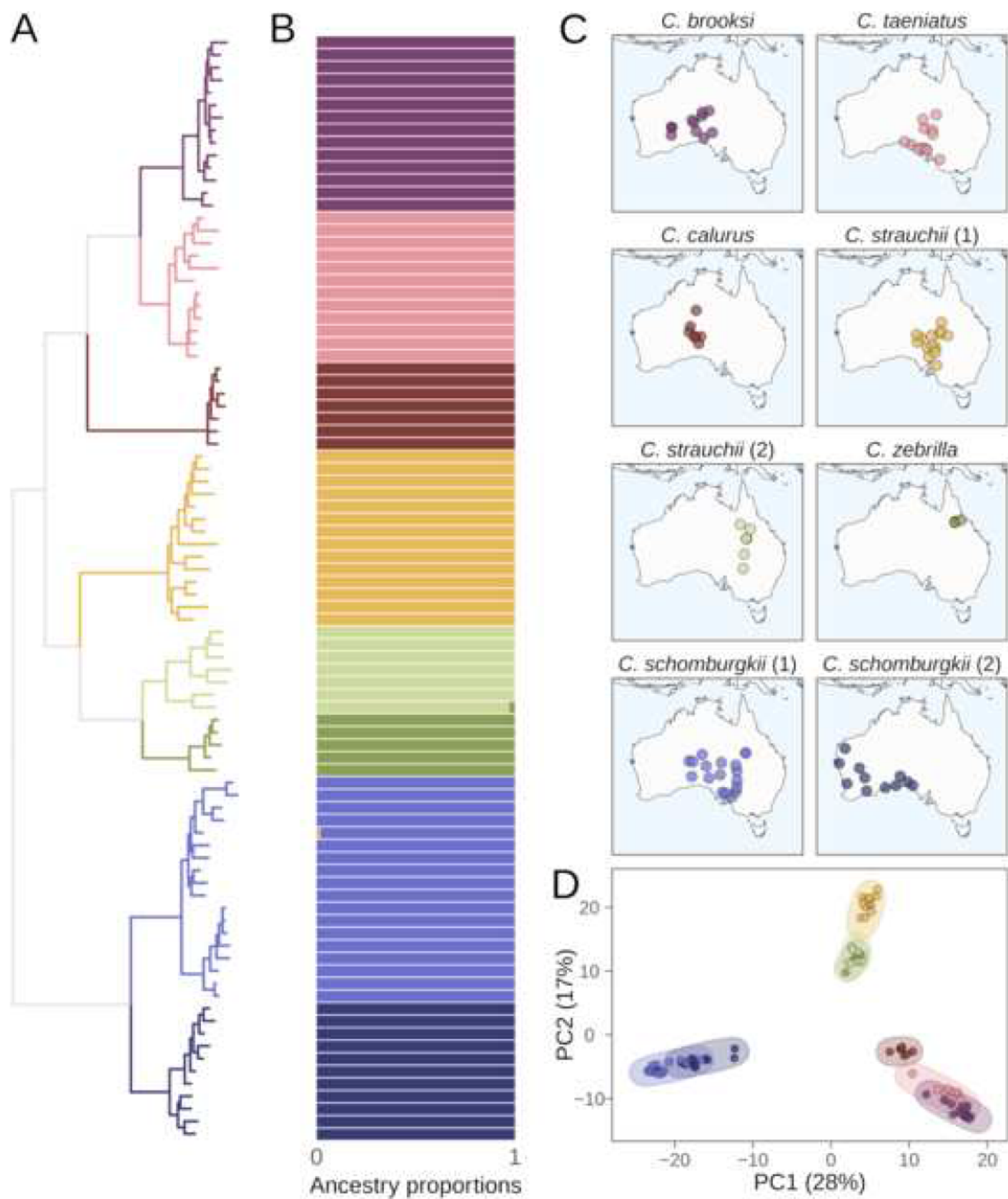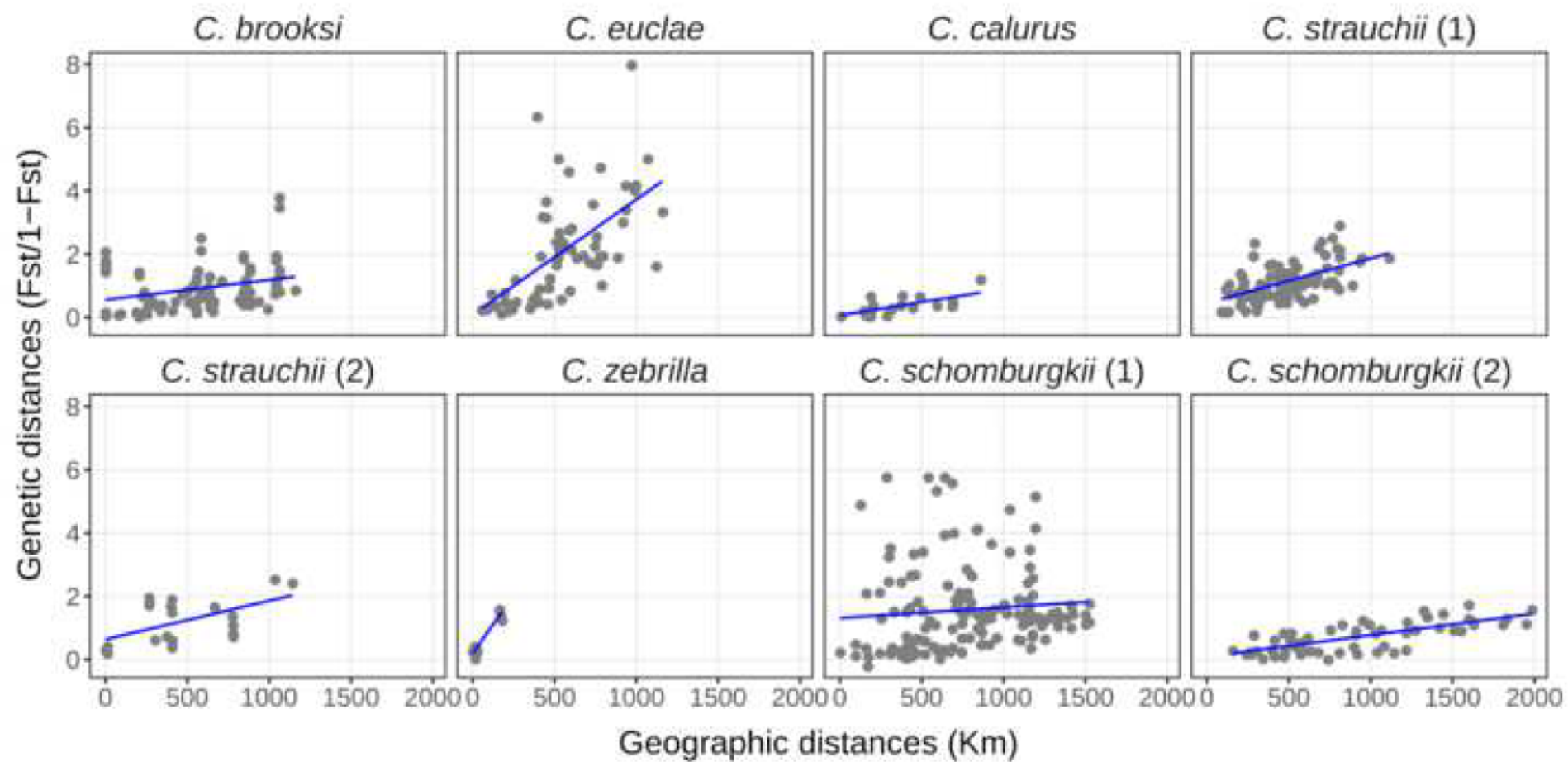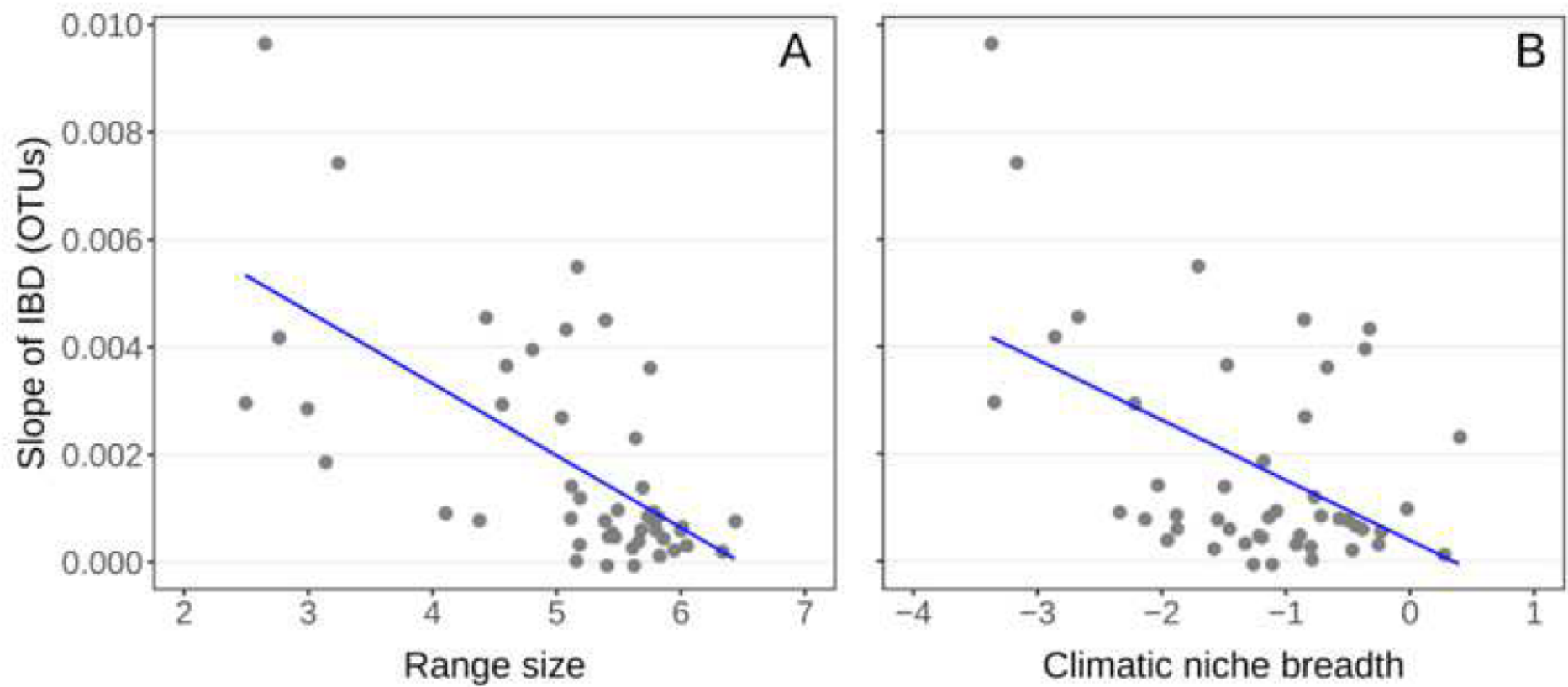
Figure 3
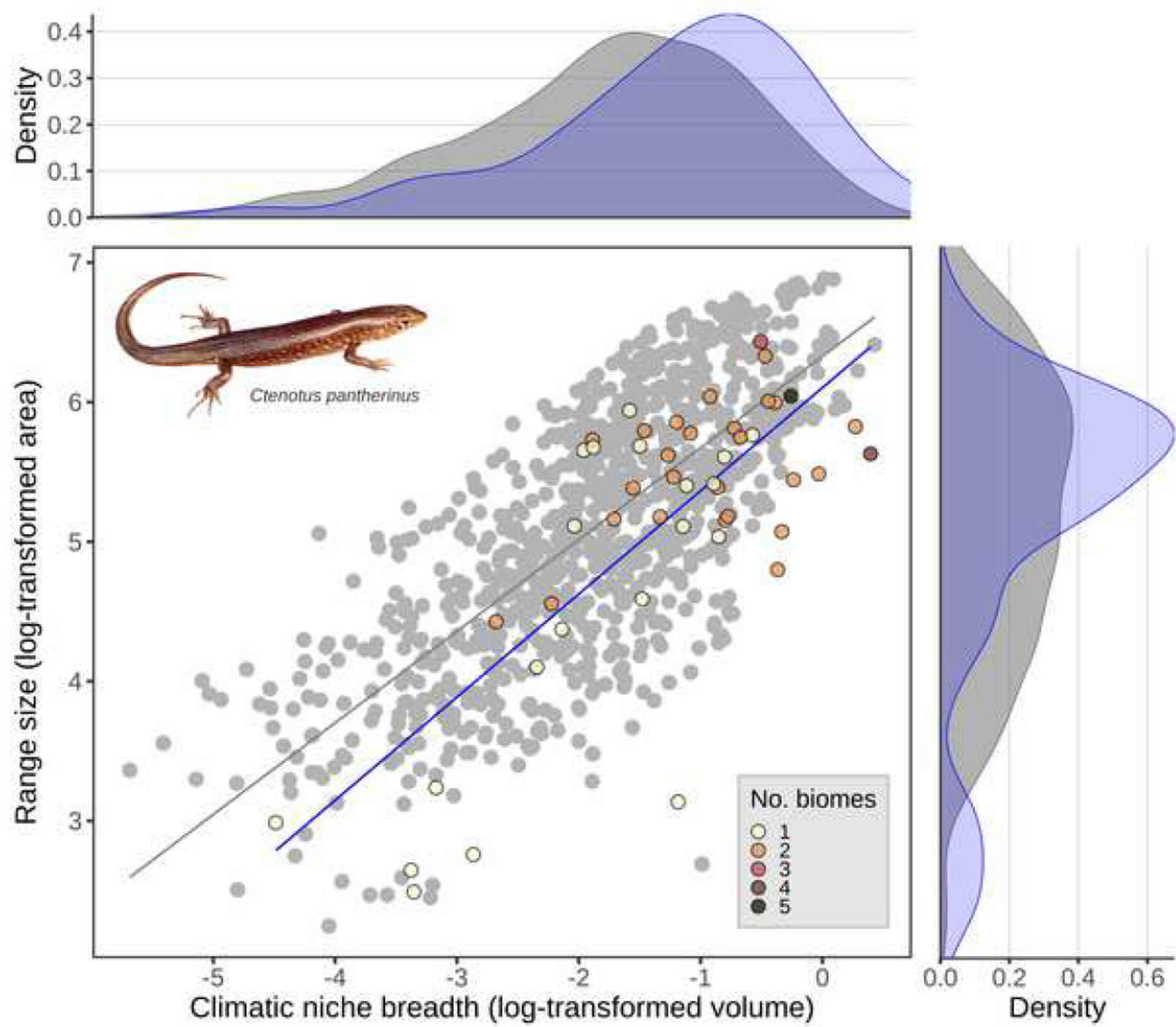
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Figure 4

Figure 5
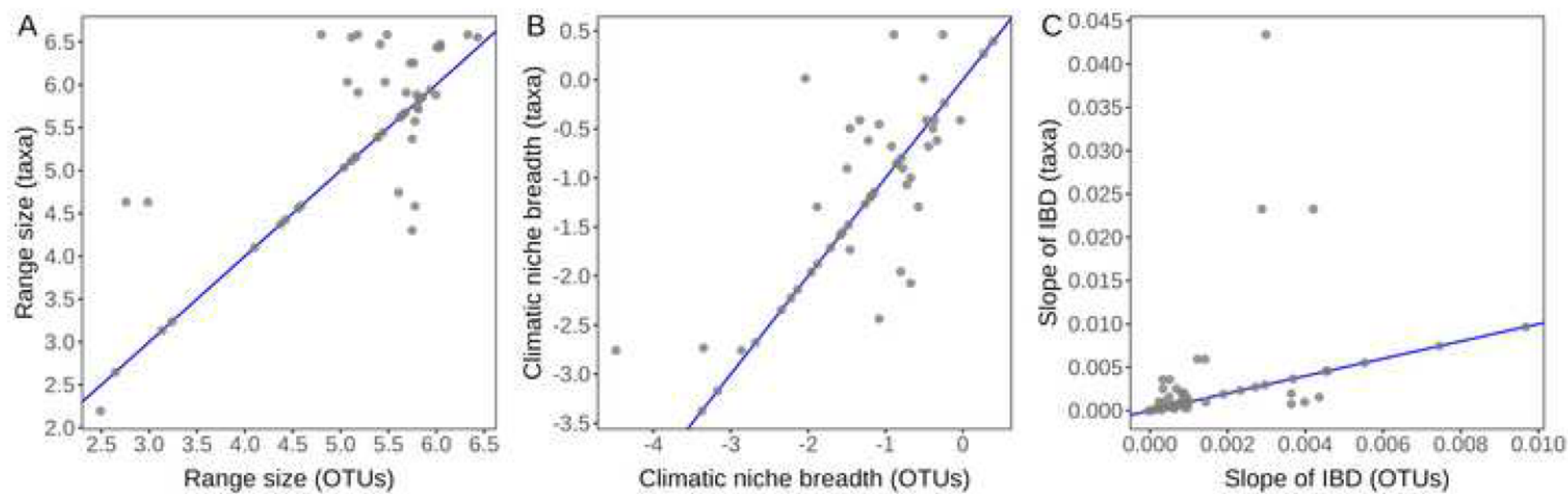

Fig6_slope_of_IBD_cluster_FstL_bysample.png

Figure 6

Fig3.jpeg