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Comparative Population Genomic Diversity and Differentiation in Trapdoor Spiders and Relatives (Araneae, Mygalomorphae)

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ABSTRACT

Although patterns of population genomic variation are well-studied in animals, there remains room for studies that focus on non-model taxa with unique biologies. Here we characterise and attempt to explain such patterns in mygalomorph spiders, which are generally sedentary, often occur as spatially clustered demes and show remarkable longevity. Genome-wide single nucleotide polymorphism (SNP) data were collected for 500 individuals across a phylogenetically representative sample of taxa. We inferred genetic populations within focal taxa using a phylogenetically informed clustering approach, and characterised patterns of diversity and differentiation within- and among these genetic populations, respectively. Using phylogenetic comparative methods we asked whether geographical range sizes and ecomorphological variables (behavioural niche and body size) significantly explain patterns of diversity and differentiation. Specifically, we predicted higher genetic diversity in genetic populations with larger geographical ranges, and in small-bodied taxa. We also predicted greater genetic differentiation in small-bodied taxa, and in burrowing taxa. We recovered several significant predictors of genetic diversity, but not genetic differentiation. However, we found generally high differentiation across genetic populations for all focal taxa, and a consistent signal for isolation-by-distance irrespective of behavioural niche or body size. We hypothesise that high population genetic structuring, likely reflecting combined dispersal limitation and microhabitat specificity, is a shared trait for all mygalomorphs. Few studies have found ubiquitous genetic structuring for an entire ancient and species-rich animal clade.

1 | Introduction

Understanding the many factors that determine how genetic variation is distributed within and among populations, and how this partitioning varies across species and more inclusive clades, is a central question in evolutionary biology. Broadly speaking, patterns of genetic variation reflect a combination of genomic architectures, demographies, landscapes, ecologies and lineage histories. Although generally well-studied at broad phylogenetic scales (Romiguier et al. 2014; Ellegren and Galtier 2016; Chen, Glémin, and Lascoux 2017; Buffalo 2021), studies focused on non-model taxa are still relevant, as the unique biologies of such taxa might allow for the isolation of particular explanatory variables (Vachon, Whitehead, and

Frasier 2018; De Kort et al. 2021; Larkin et al. 2023; Segovia-Ramírez et al. 2023). Also, explanatory variables at deep phylogenetic scales often do not capture patterns of genetic variation within individual focal clades (Singhal et al. 2017). Understanding specific determinants of genetic variation and differentiation are also important in informing taxon-specific conservation strategies (De Kort et al. 2021; Segovia-Ramírez et al. 2023).

The relationship between ecological and life history variables, and measures of diversity and differentiation within and among populations, has been the focus of hundreds of studies. Only recently have studies begun to account for phylogenetic non-independence across lineages and taxa. At broad phylogenetic scales across all animals, short-lived or highly fecund species are more genetically diverse (as measured by nucleotide diversity, π) than long-lived or low-fecundity species (Romiguier et al. 2014). Arthropod examples include parasitic feather lice, where *host* body size positively predicts genetic estimates of louse effective population size (Doña and Johnson 2023), and butterflies, where small-bodied species have higher genetic diversity (Mackintosh et al. 2019). Determinants of among-population genomic differentiation (e.g., as measured by F_{st}) have also been studied in arthropods from an explicitly phylogenetic comparative perspective. Bees that are more social and with larger body sizes have lower population differentiation (López-Uribe, Jha, and Soro 2019). In true spiders (Infrorder Araneomorphae), species living higher in the vegetation are less genetically structured than ground-dwelling species (Domènech et al. 2022).

This study involves a comparative analysis of population genomic patterns and potential explanatory variables in mygalomorph spiders, a group including the trapdoor spiders, tarantulas and relatives. Mygalomorphs include about 3500 described species (World Spider Catalog 2024), with a primary phylogenetic division separating the species-poor atypoids (purseweb spiders and kin, $n = 104$ species) from the more diverse avicularioids (tarantulas and kin, remaining taxa). Most mygalomorphs live in silk-lined burrows constructed underground, although a minority of species live in tree burrows, or opportunistically live in cracks or beneath ground shelter (Coyle 1986; Pérez-Miles and Perafán 2017; Wilson et al. 2023). Ground-dwelling mygalomorphs tend to build their burrows in specific soils or microhabitats (Coyle and Icenogle 1994; Řezáč, Řezáčová, and Pekár 2007; Rix et al. 2023). Many mygalomorphs are remarkably long-lived for terrestrial invertebrates, with females living 5–25 years, including individual females documented to have lived for over 40 years in the wild (Mason, Wardell-Johnson, and Main 2018).

Burrowing behaviour and burrow entrance silken constructs in mygalomorphs are easily measured, and can be used to characterise a burrowing 'behavioural niche' across taxa (Coyle 1986). Wilson et al. (2023) recently conducted an ecomorphological analysis across mygalomorphs, showing that behavioural niche space is comparatively simple in these spiders, with relatively few discrete alternative states. Niche categories in a majority of taxa included opportunistic web-builders, burrowing taxa with simple burrow entrances and burrowing taxa with modified burrow entrances (e.g., trapdoors). Phylogenetic analyses show that these distinct burrowing behaviours have evolved repeatedly and convergently, with multiple independent clades evolving similar syndromes (Wilson et al. 2023).

Mygalomorph dispersal typically occurs in one of three ways. Spiderlings leaving maternal burrows can disperse, but because of microhabitat specificity, small physical size and non-cursorial morphologies, are often clustered near maternal burrows (Decae, Caranhac, and Thomas 1982; Coyle and Icenogle 1994; Ferretti et al. 2014). The exception to this limited spiderling vagility includes the small number of taxa that disperse via ballooning, aerial dispersal on silken threads (reviewed in Buzatto, Haeusler, and Tamang 2021). As immatures mature, either as males or females, spiders might abandon old burrows and relocate, but again, detailed life history studies indicate that burrow fidelity is strong (Main 1987; Vincent 1993; Rix et al. 2019, 2023). Finally, after reaching sexual maturity males leave thier burrows to find females, in the vagrant phase of the mygalomorph life history. For example, relatively large bodied adult male tarantulas disperse up to 1300 m (Janowski-Bell and Horner 1999). Although exceptions exist, the generalised life history of mygalomorph species includes conspicuously sedentary lives, remarkable longevity, in spatially clustered demes found in specific microhabitats.

Given the life history traits summarised above, genetic studies of these spiders might be expected to reveal genetic evidence for low diversity within- but high differentiation among populations. To the extent that this has been measured, this expectation generally holds true. Strong genetic structuring has been measured in taxa from several continents (e.g., Bond et al. 2001; Arnedo and Ferrández 2007; Stockman and Bond 2007; Hamilton, Formanowicz, and Bond 2011; Hedin, Starrett, and Hayashi 2012; Opatova and Arnedo 2014; Opatova, Bond, and Arnedo 2016; Castalanelli et al. 2014; Harvey et al. 2015; Montes de Oca, D'Elía, and Pérez-Miles 2016; Starrett et al. 2018). Despite this extensive history of study, knowledge gaps remain. For example, most studies have primarily used mitochondrial evidence, which because of sex-biased dispersal and smaller effective sizes, tend to show more population structure. More generally, mitochondrial diversity in animals weakly predicts variation in nuclear π (see Ellegren and Galtier 2016; Singhal et al. 2017; Vachon, Whitehead, and Frasier 2018). More recent mygalomorph studies using phylogenomic nuclear evidence have emphasised phylogeographic divergence among populations (e.g., Newton et al. 2020; Marsh, Bradford, and Cooper 2023; Monjaraz-Ruedas, Mendez, and Hedin 2023; Starrett et al. 2024; Opatova, Bourguignon, and Bond 2024), rather than measuring both nuclear genomic diversity and differentiation. Aside from allozyme studies (Ramirez and Chi 2004; Ramirez et al. 2013), we are unaware of sequence-based studies that have estimated nuclear population genomic diversity values in mygalomorph spiders, despite their interesting biologies.

Here we used comparable nuclear single nucleotide polymorphism (SNP) data to assess patterns of genomic diversity and differentiation in an ecologically and phylogenetically representative sample of mygalomorphs. We used phylogenetically informed genetic clustering methods to define genetic populations within sampled focal taxa, then calculated within-cluster genomic diversity metrics including π, observed

TABLE 1 | Summary statistics for focal taxa, including geographical area covered, sample sizes, SNPs, π, D_{xy}, F_{st} and carapace lengths (CL).

Species	Area km²	# Included samples after SNP + Phylogeny filtering	No biallelic SNPs	Ave π	Ave D_{xy}	Ave F_{st}	CL	CL reference
Hexurella apachea	14,657	15	41,886	0.012	0.027	0.488	1.1	Gertsch and Platnick (1979)
Megahexura fulva	70,313	79	8492	0.007	0.025	0.524	5.6	Gertsch and Platnick (1979)
Aliatypus thompsoni	25,081	64	8382	0.008	0.016	0.373	6.35	Coyle (1974)
Atypoides riversi	12,124	20	20,603	0.006	0.013	0.444	6.4	Coyle (1968)
Microhexura montivaga	3603	32	2349	0.003	0.005	0.399	1.32	Coyle (1981)
Bothriocyrtum californicum	51,732	86	23,163	0.013	0.025	0.421	7.75	Pers obs.
Calisoga longitarsis	72,447	140	15,733	0.010	0.022	0.466	8.26	Monjaraz-Ruedas et al. (2024)
Aptostichus icenoglei	18,948	40	16,872	0.013	0.015	0.129	7.68	Bond (2012)
Apomastus kristenae	2308	26	17,422	0.006	0.010	0.376	5.95	Bond (2004)

heterozygosity, expected heterozygosity and F_{IS}. We used D_{xy}, F_{st} and isolation-by-distance (IBD) metrics to characterise patterns of among-population genomic differentiation. Using phylogenetic comparative methods we tested whether geographical range sizes and ecomorphological variables (behavioural niche and body size) might explain observed genetic patterns. We predicted more genetic diversity in genetic populations with larger geographical distributions, as larger distributions imply larger census sizes (N_c), which under neutral theory correlate positively (but imperfectly) with population effective sizes (N_e) (Wright 1931; Kimura 1969). We also predicted more genetic diversity in small-bodied taxa, as also found in butterflies (Mackintosh et al. 2019), reflecting either higher population densities (Buffalo 2021) and/or shorter lifespans (Romiguier et al. 2014). Regarding population differentiation, we predicted that miniature taxa would be more dispersal-limited than larger-bodied and longer-legged taxa, reflected in higher differentiation among populations (López-Uribe, Jha, and Soro 2019). We also predicted that spiders closely tied to underground burrows, such as obligate burrowing taxa, would disperse over the landscape less than 'opportunistic, web entrance' taxa, again reflected in higher genetic differentiation among populations.

2 | Materials and Methods

2.1 | Focal Taxon Sampling

We characterised genomic variation within and among inferred genetic populations for nine focal taxa. Each taxon is either currently classified as a single species, or represents a single clade from a complex of previously documented cryptic species. Sampling included the following Atypoidea: *Hexurella apachea* Gertsch & Platnick (1979), *Megahexura fulva* (Chamberlin, 1919), *Aliatypus thompsoni* Coyle, 1975 and the North lineage of *Atypoides riversi* O. Pickard-Cambridge, 1883. Prior research has shown that *A. riversi* is likely a cryptic species complex (Hedin, Starrett, and Hayashi 2012), so we included members of one geographical lineage (=putative species). Avicularioids included *Microhexura montivaga* Crosby & Bishop, 1925, *Bothriocyrtum californicum* (O. Pickard-Cambridge, 1874), *Aptostichus icenoglei* Bond, 2012, *Apomastus kristenae* Bond, 2004 and the "ring complex" of *Calisoga longitarsis* (Simon, 1891). Similar to *Atypoides*, *Calisoga* likely includes cryptic species (Leavitt et al. 2015; Monjaraz-Ruedas et al. 2024), so we included only a monophyletic sublineage of this complex. We acknowledge that some of our focal taxa might still include multiple cryptic species (see Section 4), but to be concise refer to these as single 'species' in all text below.

For each focal species we included a similar geographical sample of specimens, so that estimated genetic measures would be comparable; final sample sizes included 502 individuals, with a median number of 40 spiders per focal taxon (see Section 3). Seven species were sampled from the California Floristic Province (CAFP), with *Hexurella* and *Microhexura* sampled from outside of the CAFP. Total geographical areas sampled for each species are included in Table 1, calculated from a polygon obtained with Google Maps.

Included species represented the 'opportunistic, web entrance' (*Hexurella*, *Megahexura*, *Microhexura*), 'burrowing, open entrance' (*Atypoides*, *Calisoga*, *Apomastus*) and 'burrowing, trapdoor entrance' (*Aliatypus*, *Bothriocyrtum*, *Aptostichus*) behavioural niche categories of Wilson et al. (2023). We grouped *Atypoides*, which technically builds a turret entrance, into the similar 'burrowing, open entrance' category. Included species also varied in body size. We used adult female mean carapace lengths as a proxy for body size (see Table 1 for references), and placed taxa into three discrete categories (below 2 mm, intermediate, above 7 mm).

2.2 | UCE Data Collection, Processing, Calling SNPs

Voucher specimens are deposited in the San Diego State University Terrestrial Arthropods Collection (SDSU_TAC) and the UC Davis Bohart Museum of Entomology (BME; Table S1). Genomic DNA was extracted from leg tissues using the DNeasy Kit (Qiagen GmbH, Hilden, Germany), quantified using a Qubit Flex Fluorometer (Thermo Fisher Scientific) and quality checked using agarose gels. Ultraconserved element (UCE) library preparation was performed at SDSU or UC Davis using previously standardised methods for arachnids (Starrett et al. 2017), or at RAPID Genomics. Target enrichment was performed using the myBaits UCE Spider 2Kv1 kit (Arbor Biosciences; Kulkarni et al. 2020). Libraries were sequenced using 150 bp, paired-end sequencing on an Illumina HiSeq 4000 at the UC Davis DNA Technologies Core or at RAPID Genomics.

Data processing was performed on the HPC Mesxuuyan at SDSU, or the UC Davis Farm Bioinformatics Cluster. Raw demultiplexed UCE reads were quality-filtered and cleaned of adapter contamination using Trimmomatic (Bolger, Lohse, and Usadel 2014) using parameters: `PE ILLUMINACLIP:$adaptersfasta:2:30:10:2:keepBothReads LEADING:5 TRAILING:15 SLIDINGWINDOW:4:15 MINLEN:40`. Cleaned reads were assembled into contigs using SPADES v3.13.0 (Prjibelski et al. 2020). For each species, we extracted UCE alignments using PHYLUCE (Faircloth 2016) to map contigs and identify UCE loci using the merged arachnid and spider probeset of Maddison et al. (2020), using default (80, 80) matching values. UCE loci were aligned and trimmed using MAFFT (Katoh, Asimenos, and Toh 2009) and Gblocks (Castresana 2000) respectively, using parameters: `b1: 0.50, b2: 0.70, b3: 10, b4: 4`. We filtered alignments by loci and sample following Monjaraz-Ruedas et al. (2024).

SNP calling and data filtering was conducted for each focal taxon independently. For each, we created a consensus reference of all UCE alignments using the function `--make_consensus` in CIAlign (Tumescheit, Firth, and Brown 2022). We mapped cleaned fastq files against this pseudo-reference using the `bwa-mem` function (Li 2013), followed by sorting, fixing mates and marking duplicates with SAMtools (Danecek et al. 2021). We merged samples and called variants using Bcftools v1.11 (Danecek et al. 2021) functions `mpilup` and `call`. To retain invariant sites needed for computing some diversity metrics (see below), we retained all positions using command: `bcftools call -m -Oz -f GQ` (resulting in an '*all sites*' VCF file). This VCF file was filtered using VCFtools v0.1.16 (Danecek et al. 2011), by site and genotype retaining variants with a minimum quality and depth of 30 and 10 respectively, a maximum of 80% missing data, sites with a mean max depth of 200 and indels removed (`--remove-indels --max-missing 0.8 --minQ 30 --min-meanDP 10 --max-meanDP 200`). We also filtered the '*all sites*' VCF file in order to get biallelic SNPs with a minimum allele count of 1, a minimum allele frequency of 0.05 and removed samples with more than 80% missing data (=*biallelic SNPs* file). Finally, we subsampled sites keeping one random SNP per locus (=*unlinked SNPs* file).

2.3 | Inferring Genetic Populations within Species

Because of population structuring (see Section 1), focal mygalomorph taxa are suspected to represent metapopulations, with varying numbers of Wright–Fisher (~panmictic) 'genetic populations' (Battey, Ralph, and Kern 2020). We attempted to infer comparable genetic populations within each focal taxon using a combination of population clustering analyses and phylogenetic information. Using unlinked SNPs (Table 1), we estimated ancestry proportions and intraspecific genetic clusters ('populations') using Sparse Non-Negative Matrix Factorization (sNMF) implemented in the R package LEA (Frichot et al. 2014; Frichot and François 2015). Ten runs with 1×10^5 iterations and alpha = 10 were performed for K values ranging from 1 to 20. We used a cross-entropy validation approach to select optimal K values; results which minimised the cross-entropy value of each K run were selected as the best run for data visualisation. When the cross-validation method was not decisive for K, we selected the first value to reach the asymptote, without exploring further population structuring. Thirty-three samples returned too few SNPs and were excluded from sNMF clustering (samples in red Table S1).

For each focal taxon we also reconstructed phylogenomic relationships among all sampled individuals using a concatenated maximum likelihood (ML) search of UCE alignments. We used IQ–TREE 2 (Minh et al. 2020) with 1000 replicates of ultrafast bootstrapping and chose optimal models using ModelFinder (Kalyaanamoorthy et al. 2017). Using these phylogenies as a reference, we found that most inferred sNMF clusters were also recovered as monophyletic. However, we discovered that some sNMF clusters included a paraphyletic grouping of samples; in these cases, we excluded some samples and focused on a monophyletic subsample from these paraphyletic clusters (see Figures S1–S9). Finally, we sometimes recovered either individual samples, or groups of samples, as highly admixed at best K. If this admixed group also formed a clade, we treated this as a separate genetic population (Figures S1–S9). If instead admixture was confined to a single individual, we removed this sample from analysis. Again, the overarching objective here was to apply a consistent set of criteria in defining comparable 'genetic populations' for downstream summary statistics and comparative analyses. Our approach was similar to that of Singhal et al. (2017), who used coalescent-based population discovery methods to define consistent 'lineages' for comparative analyses.

2.4 | Measuring Genetic Diversity and Differentiation

Using the *all sites* VCF files, measures of nucleotide diversity (π) within inferred genetic populations were estimated using the program *pixy* (Korunes and Samuk 2021). To calculate a genome wide estimate of π, we used a window size of 1000 bp, followed by a post hoc aggregation of all windows for each genetic population (see https://pixy.readthedocs.io/en/latest/output.html#post-hoc-aggregating). We also estimated observed heterozygosity, expected heterozygosity and F_{IS} using *biallelic SNP*s in VCFtools v0.1.16 (Danecek et al. 2011), using the `--het` function, averaged over individuals within genetic populations.

We measured differentiation among genetic populations using D_{xy}, F_{st} and IBD. D_{xy} values (=average number of pairwise differences between populations, excluding comparisons within populations, Nei and Li 1979) were preferred over F_{st} because these values represent absolute measures of genetic differentiation, while F_{st} values are relative measures, correlated with nucleotide diversity within genetic populations (Cruickshank and Hahn 2014). D_{xy} values were estimated by averaging values for each population across all pairwise specimen comparisons, using *pixy* (Korunes and Samuk 2021). Genome wide estimates of F_{st} were calculated in *pixy* using the *all sites* VCF with a window size of 1000 bp and averaged across windows.

We note here that IBD technically can be used to measure differentiation both *within* and among genetic populations, which was our approach. IBD plots were calculated using individual pairwise Euclidean genetic and geographical distances from *biallelic SNPs*, grouping individuals into either genetic populations or more inclusive species. When grouping by genetic population, only those populations including two or more different geographical locations were included. We tested for IBD statistical significance using a Mantel Test with 1000 bootstrap replicates, using the R packages Adegenet, DartR and vcfR (Jombart 2008; Knaus and Grunwald 2017; Gruber et al. 2018).

2.5 | Phylogenomics and Phylogenetic Comparative Methods

Phylogenetic trees for phylogenetic generalised least squares (PGLS) analyses were reconstructed by randomly selecting one specimen per genetic population, for all populations and focal species. UCE alignments were filtered by completeness using an 80% occupancy threshold, resulting in 654 alignments for 61 terminals. Trees were estimated using the weighted hybrid version of ASTRAL-III, wASTRAL-hybrid v1.15.2.3 (Zhang et al. 2018; Zhang and Mirarab 2022), which improves estimation by considering branch lengths and support for individual gene trees. Gene trees for individual UCE alignments were estimated using ML in IQ–TREE 2 (Minh et al. 2020) with 1000 replicates of ultrafast bootstrapping and treated as unrooted. ASTRAL internode branch lengths were estimated in coalescent units, with branch support measured as local posterior probabilities. ASTRAL trees were time-calibrated using the least square dating (LSD2) method in IQ-TREE (To et al. 2016). We used three calibration points from Hedin et al. (2019) as follows: the root of the tree was set to a mean age of 322 mya, the MRCA for Atypoidea was set to a mean age of 254 mya and finally the MRCA for Avicularoidea was set to a mean age of 211 mya. Hedin et al. (2019) included Hexurellidae, sister to the remaining Atypoidea, which pulls the crown age of Atypoidea back in time.

PGLS analyses were carried out using the function *pgls* in the R package *caper* (Paradis, Claude and Strimmer 2004; Orme et al. 2023) to test the phylogenetic correlation and non-independence of selected traits (Freckleton, Harvey, and Pagel 2002; Orme et al. 2023). For the covariance matrix we used the time calibrated LSD2 tree. PGLS analyses were conducted using both genetic populations ($n = 61$) and species ($n = 9$) as tree terminals. Phylogenetic signal (lambda; Freckleton, Harvey, and Pagel 2002) was assessed using ML with default bounds.

Several predictive variables for measures of nucleotide diversity (π) were investigated. For genetic populations we used estimated range sizes for individual genetic populations as predictive variables. For those genetic populations known only from single locations we arbitrarily set the estimated range size as 1 km^2. For species we used body size (three categories), behavioural niche ('opportunistic', 'open entrance' and 'trapdoor' categories) and estimated range sizes (an average of genetic population range sizes) as predictive variables. We fit PGLS models to explore the contribution of each predictive variable independently, and all three in combination. We checked for residuals normality using a Shapiro–Wilk test as implemented in the R package *stats*, and to meet the condition of normally distributed residuals transformed data using the square root of range size (for both species and genetic populations).

We also investigated several predictive variables for measures of population differentiation (D_{xy}). For species we used body size, behavioural niche and range sizes (an average of genetic population range sizes) as predictive variables. We fit PGLS models to explore the contribution of each predictive variable independently and all three in combination. We did not conduct population differentiation analyses at the level of genetic populations because predictive variables either did not vary at this level (body size, behavioural niche), or a priori predictions were unclear (relationship between range size and D_{xy}).

For IBD we fit a simple linear regression using the function *lm* from the R package *stats*, using the natural logarithm of individual pairwise genetic and geographical distances within genetic populations and species. We extracted the values of each independent slope and used these as response variables in PGLS. For genetic populations, we used estimated range sizes as predictive variables, expecting that larger distributions would encompass greater landscape heterogeneity, resulting in stronger IBD (see Pelletier and Carstens 2018). Trees used here included fewer genetic populations ($n = 41$, Figure S10), excluding genetic populations sampled from two or fewer geographical locations. For species we used body size, behavioural niche and range sizes as predictive variables for IBD. We fit PGLS models to explore the contribution of each predictive variable independently and all three in combination.

3 | Results

3.1 | Data

We characterised population genomic variation for nine focal taxa, with filtered VCF files including approximately 2.3–42K biallelic SNPs per species (Table 1). After excluding samples with too few SNPs, or excluded based on phylogenetic filtering, we included 502 of 586 samples, with a median number of 40 spiders per species (Table 1, Tables S1 and S2). All scripts, analysis files (input, log, output) and a ReadMe file can be found at the Dryad repository (https://doi.org/10.5061/dryad.mw6m9064s). Raw UCE data newly generated for this project is deposited under BioProject PRJNA1157860 on the GenBank SRA repository, please refer to Table S1 for detailed information for sample accession numbers.

3.2 | Defining Genetic Populations

The number of phylogenetically informed sNMF genetic populations per species ranged from 4 to 12, with an average value of 6.8 (Table S2, Figures S1–S9). Geographical range sizes of genetic populations are in general more comparable than overall range sizes of sampled species (Figure 1, Table 1, Table S2). The geographical distributions of inferred genetic populations are shown in Figures S1–S9.

3.3 | Measures of Genetic Diversity and PGLS

Nucleotide diversity (π) values ranged from 0.002 to 0.018 across genetic population comparisons (Figure 1, Table 1, Table S2). π values are low for all genetic populations of the miniature *Microhexura*, generally high for all genetic populations of trapdoor spiders *Aptostichus* and *Bothriocyrtum* and notably variable in the miniature *Hexurella* (Figures 1 and 2). Observed heterozygosity values exceeded expected values for all genetic populations of *Apomastus* and *Microhexura*, resulting in negative F_{IS} values (Table S2). Conversely, observed heterozygosity values were lower than expected in all *Atypoides* and *Bothriocyrtum* populations, with positive F_{IS} values possibly indicating inbreeding (Table S2). Positive F_{IS} values were not consistently found in other taxa.

Phylogenomic relationships recovered for PGLS analyses were as expected (Figures 1 and 2), congruent with more comprehensive phylogenomic results for Atypoidea (Hedin et al. 2019) and Avicularioidea (Opatova et al. 2020). Phylogenetic signal for π ranged from 0.5 to 0.7 for genetic populations (Table S3) but increased in species analyses to match a Brownian motion model (lambda = 1, Table S3).

In PGLS analyses of genetic populations, estimated range sizes significantly predict π (larger ranges with higher π), albeit with a low R^2 value (Figure 1, Table S3). In PGLS analyses of species, significant individual predictive variables included body size (larger spiders with higher π than medium-sized spiders), behavioural niche (trapdoor spiders with higher π than open burrow spiders) and range sizes (Figure 2, Table S3). Combined variables did not significantly predict π at the species level (Table S3).

3.4 | Measures of Genetic Differentiation and PGLS

Pairwise F_{st} values are generally high (mostly above 0.3) in all species, except for *Aptostichus* with notably lower F_{st} values (Figures 1 and 3 upper, Table 1, Table S2). Average pairwise D_{xy} values ranged from 0.0037 to 0.0280 across genetic population comparisons (Figure 1, Table 1, Table S2). D_{xy} values are notably low among populations in the miniature *Microhexura* (Figures 1 and 2), which also shows low π values. In PGLS analyses of species, the three included variables do not significantly predict D_{xy} values, either individually or in combination (Figure 2, Table S3).

Positive and significant IBD values were estimated for most genetic populations, and for all species (Figure S10, Figure 3). As expected, IBD slopes are steeper (more genetic divergence per unit area) for species as compared to genetic populations (Figure 3, Figure S10), as species comparisons include individual pairwise comparisons across individuals from different genetic populations (see also Wacker and Winger 2024). Most species show similar slopes, with significant differences mainly for *Aptostichus* and *Microhexura* (Figure 3, Table 2).

In PGLS analyses of genetic populations, estimated range sizes do not predict IBD slopes (Table S3). In PGLS analyses of species, the three variables do not individually predict IBD slopes (Figures 3 and 4, Table S3), but the combination of medium body size and range size does significantly predict IBD slope (Figure 4, Table S3).

4 | Discussion

We predicted that geographical range sizes would influence genomic diversity and differentiation at the level of genetic populations, and that range sizes, body sizes and behavioural niche details would influence patterns of genomic diversity and differentiation at the level of species. These predictions followed from known mygalomorph biologies (e.g., demic clustering, dispersal limitation, etc.), and patterns reported in the literature (e.g., larger distributions correlated with more genetic diversity, etc.). Significant predictors of genetic diversity for both populations and species were recovered, although results did not always follow our a priori predictions.

We mostly failed to recover significant predictors of genetic differentiation for both populations and species. We did however find overwhelming evidence for genetic differentiation as a general feature of mygalomorph metapopulations. In particular, we discovered high and ubiquitous IBD, irrespective of body size and/or behavioural niche. Consistent with previous work, we confirm that highly structured metapopulations might represent a shared feature for all or most mygalomorphs, an ancient and relatively species-rich animal clade.

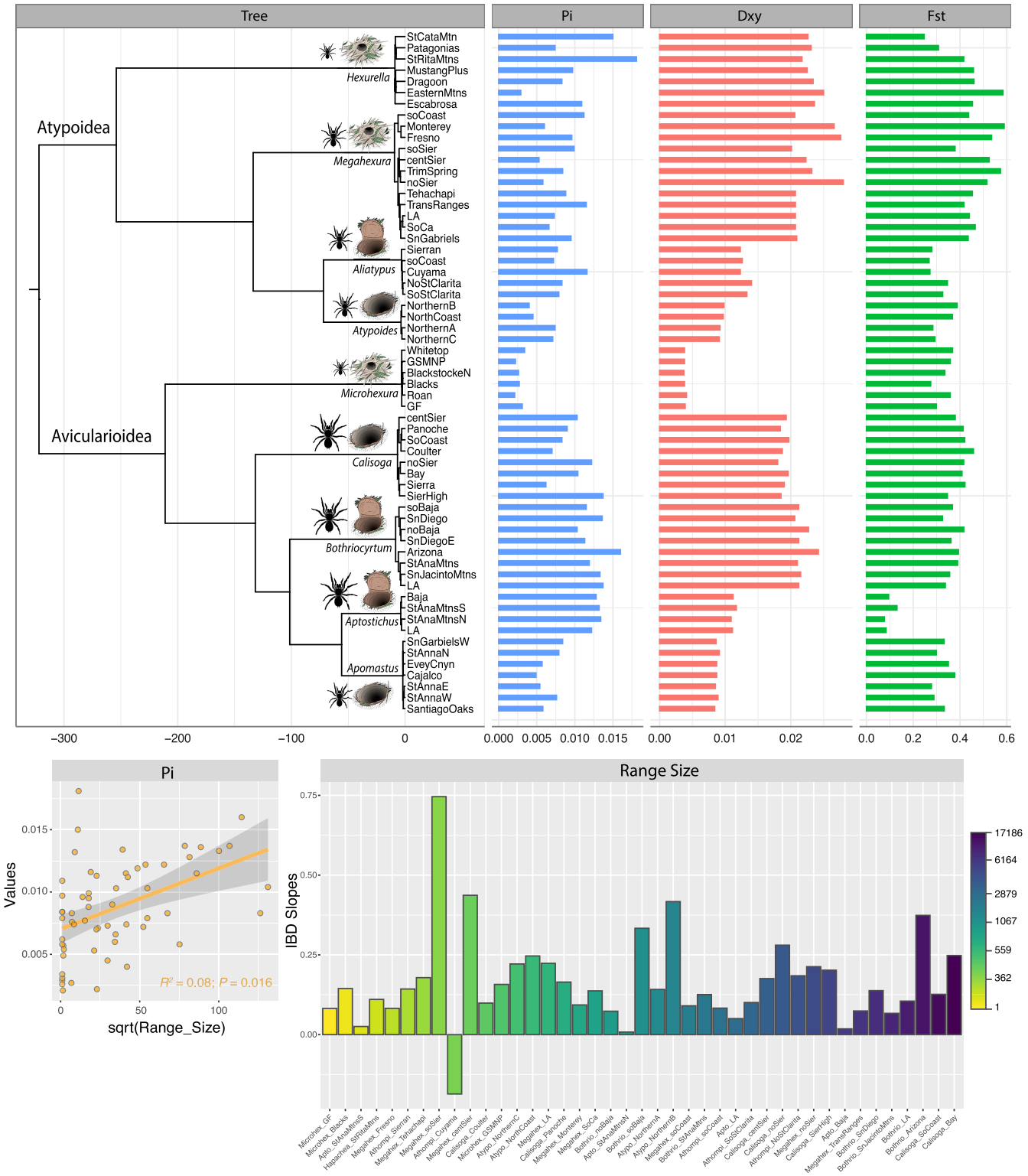

FIGURE 1 | Upper panels: Reconstructed ASTRAL phylogeny with one representative sample per genetic population as terminal taxa; π, D_{xy} and F_{st} values for each genetic population. Icons represent body size and behavioural niche ('opportunistic, web entrance', 'burrowing, open entrance' and 'burrowing, trapdoor entrance'). Informal names for genetic populations as in Table S2. Lower left panel: Correlation of genetic population π values with estimated range sizes. Lower right panel: IBD slopes per genetic population, sorted by range size, with darker colours denoting larger range sizes (in km^2).

4.1 | Genetic Diversity

Although spiders comprise a clade of over 52,000 described species (World Spider Catalog 2024), we are unaware of explicitly phylogenetic, predictive studies of genetic diversity within spider populations. We predicted more genetic diversity in populations with larger geographical ranges, a common surrogate for census sizes (N_c), as found in many other animal studies (figure 5 of

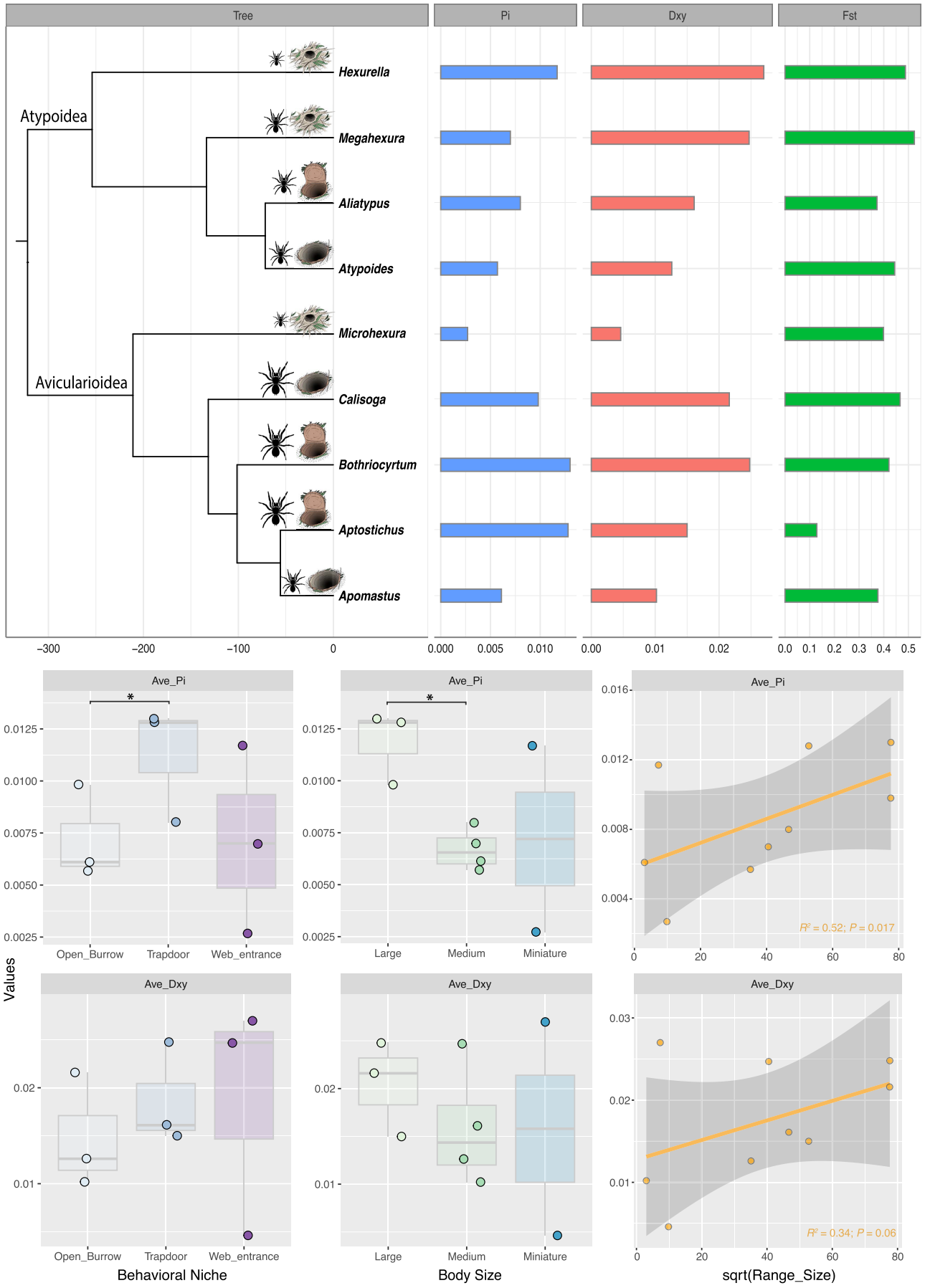

FIGURE 2 | Legend on next page.

FIGURE 2 | Upper panels: Reconstructed ASTRAL phylogeny with species as terminal taxa; π, D_{xy} and F_{st} values for each species. Icons represent body size and behavioural niche ('opportunistic, web entrance', 'burrowing, open entrance' and 'burrowing, trapdoor entrance'). Lower panels: Distribution of average π and D_{xy} values by predictive variable. Statistical significance denoted by lines connecting boxes marked with an asterisk.

Singhal et al. 2017; Larkin et al. 2023). An arthropod comparative phylogenetic example includes parasitic bird lice, where a positive correlation exists between measures of local genetic diversity (N_e) and host body size, reflecting larger louse deme sizes on larger hosts (Doña and Johnson 2023). Conversely, there is no relationship between range size and genetic diversity in butterflies (Mackintosh et al. 2019). In PGLS analyses of mygalomorph genetic populations and species we found that estimated range sizes positively predict genetic diversity values (Figures 1 and 2).

We also predicted more genetic diversity in small-bodied taxa, as found broadly in animals, including arthropods (Buffalo 2021), and in specific arthropod clades (Mackintosh et al. 2019). This relationship is hypothesised to reflect either an expected body size <> population density relationship based on macroecological principles (more smaller animals), and/or body size differences in longevity and fecundity (short-lived, small-bodied taxa producing many offspring) (Romiguier et al. 2014; Chen, Glémin, and Lascoux 2017). In PGLS analyses of mygalomorph species we found an opposite trend, larger spiders showing higher π values than medium-sized spiders (Figure 2). This opposite relationship might reflect the fact that fecundity and/or lifespan variation in mygalomorphs does not strictly follow general theory expectations. For example, miniature *Microhexura* egg sacs include fewer than 10 spiderlings (Coyle 1981), mean clutch sizes in medium-sized *A. thompsoni* include 124 eggs (Coyle and Icenogle 1994), while those in large *Aphonopelma* tarantulas include 588 eggs (Punzo and Henderson 1999). Regarding lifespans, miniature *Microhexura* are known to live for multiple years (Coyle 1981). Overall, we hypothesise that these reverse fecundity expectations, or other unmeasured variables, might explain higher π values in larger mygalomorphs. We also found that trapdoor spiders have higher π values than open burrow spiders (Figure 2), but cannot easily explain this pattern.

We anticipated genetic evidence for inbreeding in the sampled taxa, reflecting the often-seen microhabitat clustering of mygalomorph populations (Decae, Caranhac, and Thomas 1982; Main 1987; Vincent 1993; Coyle and Icenogle 1994; Ferretti et al. 2014; Rix et al. 2019, 2023). Such a potential signal was only observed in the open burrower *Atypoides* and the trapdoor spider *Bothriocyrtum*, where all genetic populations for each taxon show positive F_{IS} values (Table S2). In *Atypoides*, large female burrows are often surrounded by miniature burrows (Vincent 1993; personal observation), the likely offspring of these long-lived females. Evidence for inbreeding is not surprising in this taxon, although Ramirez and Chi (2004) found no such evidence using allozyme data. Similar allozyme data for *Bothriocyrtum* reveals mixed evidence for inbred genetic populations (Galindo-Ramirez and Beckwitt 1986; Ramirez et al. 2013). Both *Atypoides* and *Bothriocyrtum* do share genetic populations with relatively large geographical distributions, so it remains possible that reduced observed heterozygosity (over expected) might reflect the Wahlund Effect, where a merging of subpopulations gives a false signal of inbreeding. The fact that we found positive IBD slopes (Figure S10), as a measure of differentiation *within* genetic populations, supports this possibility.

Notably low π values were found in the miniature web-building species *M. montivaga* in comparison to other sampled mygalomorphs (Figures 1 and 2). This US federally endangered taxon is only known from imperilled microhabitats on isolated mountaintops in the southern Appalachian Mountains (Coyle 1981; Hedin, Carlson, and Coyle 2015). Data presented here show that these individual mountaintop populations are genetically depauperate. Combined with small geographical distributions and a predicted continuing loss of high-elevation forest habitats under future climate scenarios (Ulrey et al. 2016), our genomic diversity findings have important conservation implications.

4.2 | Genetic Differentiation

Many animal studies have examined how variation in ecological and life history variables influence patterns of genetic differentiation (e.g., wing shapes in birds, Harvey et al. 2017; pelagic larval duration in fishes, Donati et al. 2021). Such comparative studies have also been conducted in true spiders. In European cave spiders, more population structure and higher IBD is found in cave obligate species, in comparison to surface-dwelling taxa (Pavlek et al. 2022). Non-dispersive true spider lineages are more genetically structured than dispersive taxa, in some (but not all) of the Canary Islands (Suárez et al. 2022). Although the above spider studies are comparative, neither strictly used phylogenetic comparative methods to assess the relationship between predictive and response (genetic differentiation) variables.

Mygalomorphs have an extensive history of genetic studies that have revealed high population genetic structuring (see Section 1). The few exceptions might include mygalomorph taxa that disperse using ballooning (aerial dispersal on silken threads). However, studies of some taxa known to balloon (summarised in Buzatto, Haeusler, and Tamang 2021), such as *Ummidia*, still reveal extensive multilocus population genetic structuring (Opatova, Bond, and Arnedo 2016). The same holds for *Atypus affinis*, which balloons, but is highly genetically structured based on nuclear allozymes (Pedersen and Loeschcke 2001). In the end, the only exceptions to the mygalomorph rule might include special cases where populations deviate from mutation-drift equilibrium, such as in recent range expansions (e.g., some *Aphonopelma hentzi*, Hamilton, Formanowicz, and Bond 2011).

We have extended prior studies in important ways, by including nuclear population genomic datasets (vs mitochondrial only), in taking an explicitly phylogenetic comparative approach and in measuring differentiation using multiple metrics. Although specific differentiation predictions (i.e., miniature taxa with more differentiation, obligate burrowing taxa with more differentiation) were not significant in PGLS, our results reinforce the

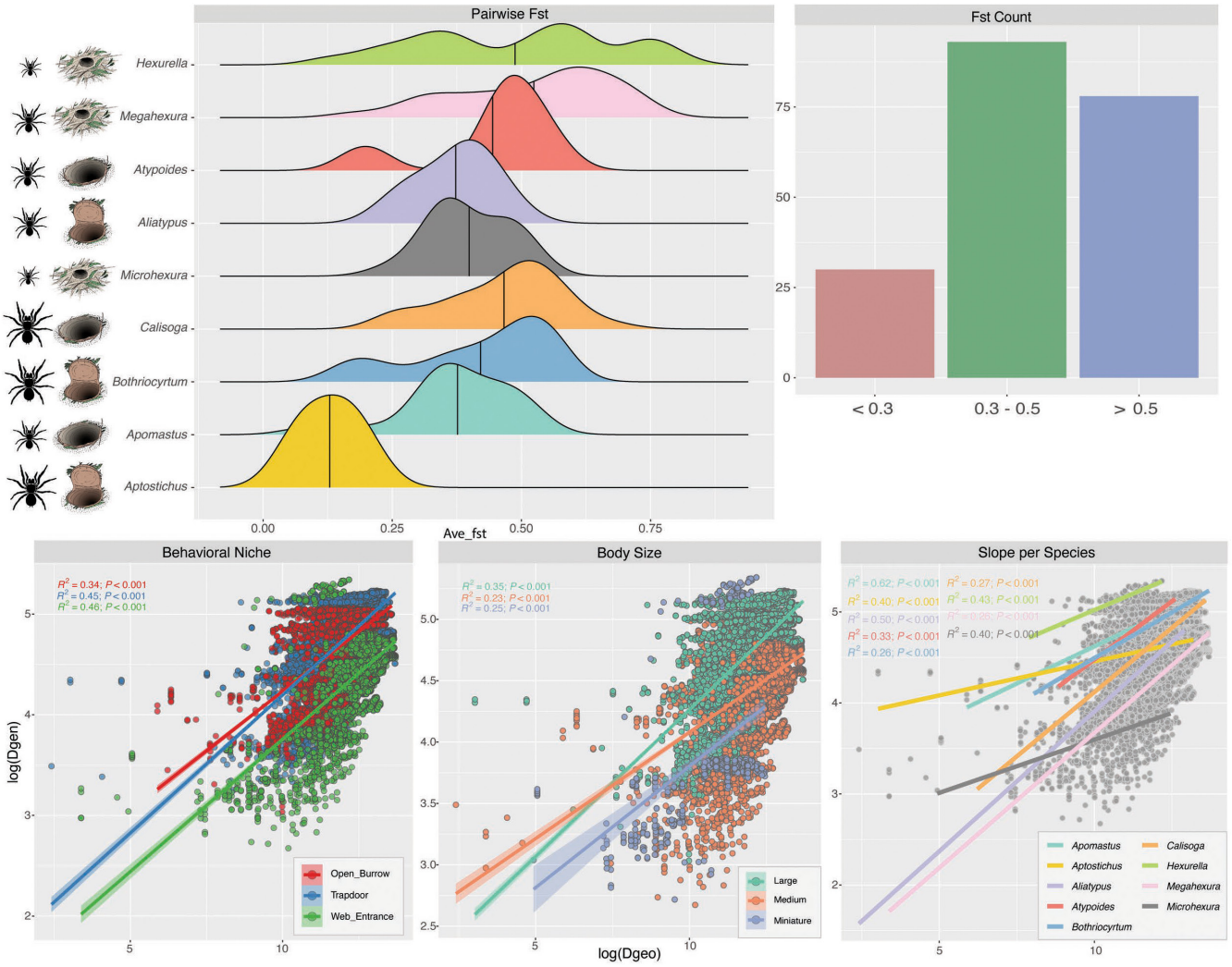

FIGURE 3 | Upper panel: Summary of F_{st} values. Vertical black lines on each species distribution correspond to mean values for respective taxon. Inset includes F_{st} values for all genetic populations (pairwise within species), tallied across all species, binned into three categories: Low < 0.3, mid 0.3–0.5, high > 0.5. Lower panels: IBD analyses for species and predictive variables (behavioural niche and body size).

notion that differentiation of genetic populations is a ubiquitous feature of mygalomorph taxa.

Roux et al. (2016) conducted a comparative analysis of 61 population pairs in a phylogenetically broad sample of animals, relating direct measures of genetic differentiation to estimates of gene flow inferred using approximate Bayesian computation (ABC). These authors demonstrated a 'grey zone' of intermediate differentiation and gene flow. Below this grey zone are conspecific populations with limited differentiation and high gene flow; above this are clearly distinct species with greater differentiation and reduced gene flow. Because of among-locus heterogeneity in gene flow and population effective size, this grey zone is not homogeneous across the genome. With respect to measures of differentiation, Roux et al. (2016) measured F_{st} and showed that under different ABC models, the grey zone fell from F_{st} of 0.1–0.3 (homogeneous migration models), to 0.2–0.6 (heterogeneous migration models; supplemental figure 6, Roux et al. 2016). Although we do not know whether homogeneous versus heterogeneous models best fit mygalomorphs, under homogeneous models, most mygalomorph F_{st} values across genetic populations *within focal taxa* (Figure 3 upper) are consistent with species level divergences, above the grey zone. Even under heterogeneous migration models, observed F_{st} values are high in comparison to other animals, either in or above the grey zone. The high genealogical divergence indexes (Jackson et al. 2017) reported for *Cyclocosmia* trapdoor spider populations (Opatova, Bourguignon, and Bond 2024) would also generally fall above this grey zone.

Similarly, Pelletier and Carstens (2018) conducted a global survey of IBD in over 8000 plant and animal species. This dataset included over 6000 arthropods, of which 15% showed evidence for IBD. Moreover, the majority of these arthropod datasets were mitochondrial, expected to show more genetic differentiation than most nuclear loci because of sex-biased dispersal and smaller effective sizes. In mygalomorphs, we found IBD to be a ubiquitous feature of *nuclear* metapopulation structure, with positive and significant IBD values estimated for 80% of genetic clusters (Figure S10), and for all focal species (Figure 3). Although not measured, we would expect this

TABLE 2 | Tukey HSD results for IBD mean slope differences among focal species.

Contrast	Estimate	SE	df	*t* ratio	*p*
Apomastus–Aptostichus	**3.83E-07**	**6.85E-08**	**2.02E+04**	**5.60E+00**	**<0.0001**
Apomastus–Aliatypus	**−6.26E-07**	**6.48E-08**	**2.02E+04**	**−9.65E+00**	**<0.0001**
Apomastus–Atypoides	−4.04E-07	1.34E-07	2.02E+04	−3.02E+00	6.45E-02
Apomastus–Bothriocyrtum	−1.63E-07	6.13E-08	2.02E+04	−2.66E+00	1.61E-01
Apomastus–Calisoga	**−5.19E-07**	**5.91E-08**	**2.02E+04**	**−8.78E+00**	**<0.0001**
Apomastus–Hexurella	8.20E-08	1.65E-07	2.02E+04	4.97E-01	1.00E+00
Apomastus–Megahexura	**−5.80E-07**	**6.13E-08**	**2.02E+04**	**−9.47E+00**	**<0.0001**
Apomastus–Microhexura	2.00E-07	6.63E-08	2.02E+04	3.02E+00	6.35E-02
Aptostichus–Aliatypus	**−1.01E-06**	**4.85E-08**	**2.02E+04**	**−2.08E+01**	**<0.0001**
Aptostichus–Atypoides	**−7.87E-07**	**1.27E-07**	**2.02E+04**	**−6.21E+00**	**<0.0001**
Aptostichus–Bothriocyrtum	**−5.47E-07**	**4.37E-08**	**2.02E+04**	**−1.25E+01**	**<0.0001**
Aptostichus–Calisoga	**−9.03E-07**	**4.05E-08**	**2.02E+04**	**−2.23E+01**	**<0.0001**
Aptostichus–Hexurella	−3.01E-07	1.59E-07	2.02E+04	−1.89E+00	6.20E-01
Aptostichus–Megahexura	**−9.64E-07**	**4.37E-08**	**2.02E+04**	**−2.21E+01**	**<0.0001**
Aptostichus–Microhexura	**−1.83E-07**	**5.05E-08**	**2.02E+04**	**−3.63E+00**	**8.60E-03**
Aliatypus–Atypoides	2.22E-07	1.25E-07	2.02E+04	1.78E+00	6.97E-01
Aliatypus–Bothriocyrtum	**4.63E-07**	**3.78E-08**	**2.02E+04**	**1.22E+01**	**<0.0001**
Aliatypus–Calisoga	**1.07E-07**	**3.41E-08**	**2.02E+04**	**3.14E+00**	**4.51E-02**
Aliatypus–Hexurella	**7.08E-07**	**1.58E-07**	**2.02E+04**	**4.48E+00**	**3.00E-04**
Aliatypus–Megahexura	4.55E-08	3.77E-08	2.02E+04	1.21E+00	9.55E-01
Aliatypus–Microhexura	**8.26E-07**	**4.54E-08**	**2.02E+04**	**1.82E+01**	**<0.0001**
Atypoides–Bothriocyrtum	2.40E-07	1.23E-07	2.02E+04	1.95E+00	5.77E-01
Atypoides–Calisoga	−1.15E-07	1.22E-07	2.02E+04	−9.44E-01	9.90E-01
Atypoides–Hexurella	4.86E-07	1.97E-07	2.02E+04	2.47E+00	2.46E-01
Atypoides–Megahexura	−1.77E-07	1.23E-07	2.02E+04	−1.43E+00	8.85E-01
Atypoides–Microhexura	**6.04E-07**	**1.26E-07**	**2.02E+04**	**4.81E+00**	**1.00E-04**
Bothriocyrtum–Calisoga	**−3.56E-07**	**2.68E-08**	**2.02E+04**	**−1.33E+01**	**<0.0001**
Bothriocyrtum–Hexurella	2.45E-07	1.56E-07	2.02E+04	1.57E+00	8.23E-01
Bothriocyrtum–Megahexura	**−4.17E-07**	**3.13E-08**	**2.02E+04**	**−1.33E+01**	**<0.0001**
Bothriocyrtum–Microhexura	**3.64E-07**	**4.03E-08**	**2.02E+04**	**9.03E+00**	**<0.0001**
Calisoga–Hexurella	**6.01E-07**	**1.56E-07**	**2.02E+04**	**3.86E+00**	**3.60E-03**
Calisoga–Megahexura	−6.12E-08	2.67E-08	2.02E+04	−2.29E+00	3.46E-01
Calisoga–Microhexura	**7.19E-07**	**3.68E-08**	**2.02E+04**	**1.96E+01**	**<0.0001**
Hexurella–Megahexura	**−6.62E-07**	**1.56E-07**	**2.02E+04**	**−4.23E+00**	**8.00E-04**
Hexurella–Microhexura	1.18E-07	1.59E-07	2.02E+04	7.46E-01	9.98E-01
Megahex–Microhexura	**7.81E-07**	**4.02E-08**	**2.02E+04**	**1.94E+01**	**<0.0001**

Note: Significant *p* values marked in bold.
Abbreviations: df, degrees of freedom; SE, standard deviation.

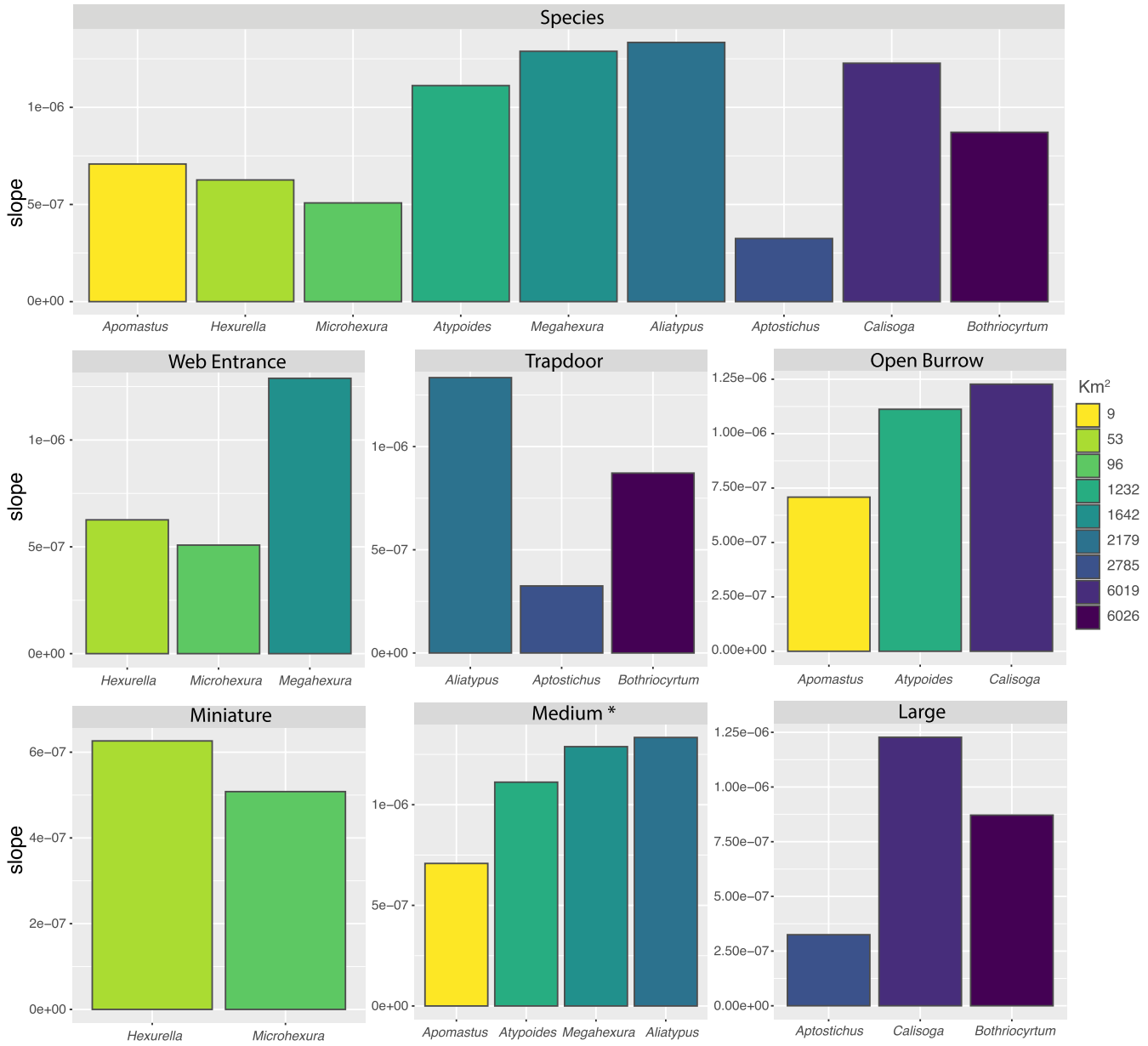

FIGURE 4 | Summary of IBD analyses per species by predictive variable in combination with range sizes. Plots sorted by range size, darker colours denote larger range sizes, measured in km^2. Asterisks denote statistical significance.

percentage to be higher for mitochondrial data. Unlike Pelletier and Carstens (2018), who showed range size to be an important predictive variable for IBD, estimated range sizes do not predict IBD slopes in mygalomorph genetic populations (Table S3). Also, at the species level, we found that taxa with body size and/or behavioural niche details differences show nearly identical patterns of IBD. This similarity suggests the possibility that disparate mygalomorph taxa share similar *neighbourhood sizes*, proportional to the average number of potential mates for an individual (Wright 1946), as often estimated from IBD slopes (Rousset 1997; Battey, Ralph, and Kern 2020).

Overall, viewed in comparison to other animal taxa, both the F_{st} and IBD results emphasise the consistently high genetic differentiation seen in mygalomorph taxa, seemingly irrespective of species ecology or body sizes.

4.3 | Implications for Mygalomorph Speciation

The comparative population genomic data gathered here have implications for species delimitation and speciation in this group of spiders, and more generally.

Derkarabetian, Starrett, and Hedin (2022) showed that taxon-specific population genomic parameters (including folded-site frequency spectrum, pairwise difference ratio, F_{ST}, etc.) can be used to increase the reliability of supervised machine learning methods for species delimitation in non-model taxa. These authors used population genomic parameters derived from one dispersal-limited system to train a supervised model used in related taxa ('known informing the unknown'). This system-specific model resulted in more realistic species delimitations than an 'all taxa' (Pei et al. 2018) supervised model

parameterisation. Similarly, the comparative population genomic parameters presented here (in part) might profitably be used to develop a dispersal-limited mygalomorph supervised machine learning model. Again, species delimitation in these spiders is notoriously challenging, and applied algorithmic methods must accommodate population structure (e.g., Satler, Carstens, and Hedin 2013; Hedin, Carlson, and Coyle 2015; Starrett et al. 2024; Opatova, Bourguignon, and Bond 2024). A custom training model could be one step in this direction, particularly since our results demonstrate population genomic similarities across a broad swath of mygalomorph phylogeny, key to justifying a '*known informing the unknown*' approach.

Ultimately, and with more taxa, comparative population genomic data could be used to investigate the relationship between population structure and speciation rates in mygalomorphs. In birds, there is a positive relationship between levels of population structure and speciation rates (Harvey et al. 2017), while there is no relationship in squamates (Singhal et al. 2022; Burbrink et al. 2023). For spiders in particular, Suárez et al. (2022) found a positive relationship between differentiation and diversification in the Canary Island true spider (araneomorph) fauna. At first glance in mygalomorphs, these rates appear uncoupled at the level of large clade, as population structuring as measured by IBD is similar despite a large difference in described species diversity ($n = 104$ atypoids versus $n \sim 3400$ avicularioids). This might suggest that other drivers of speciation rate are relevant in mygalomorphs, including rates of extinction, or variable speciation completion rates (population lineage persistence with or without speciation).

4.4 | Caveats and Future Directions

As for any comparative study across natural populations, there are potentially confounding variables and possible areas for improvement. Our species sample sought to capture both phylogenetic and ecological variation, while maintaining comparable population genomic datasets across taxa. This study could be improved by increasing the number of mygalomorph species (and families) sampled, capturing more diversity in predictor and response variables over a more global fauna. UCE-based sequence capture data is a strength here, as future studies could use the same probeset to gather comparable SNP datasets. These future datasets would need to include broadly similar intraspecific sampling schemes, both in terms of number of sampled specimens and geographical scale, as these are expected to impact the inference of genetic populations and downstream genetic diversity measures.

Increased taxonomic sampling over broader geography does not come without trade-offs and additional confounding variables, as faced in the current study. Seven of our focal taxa are from the CAFP. Because these taxa are distributed in a similar climatic and topographic environment with a shared geologic history, we viewed this shared environment and history as a strength (one fewer confounding variable). This sampling scheme also minimised latitudinal variation, shown to be an important explanatory variable in other systems (e.g., Pelletier and Carstens 2018; Larkin et al. 2023). Conversely, this shared environmental regime could be viewed as a shared bias. The miniature species that we studied (*Hexurella* and *Microhexura*) occur outside of the CAFP, so here miniature size and environment are confounded variables. Future studies should increase sampling from different global areas, so that geographical region could be considered as a PGLS variable. The miniature species are also opportunistic, and our a priori predictions regarding ecology and genetic variation trend in opposite directions (opportunistic = more gene flow, small size = less gene flow). Including more large-bodied opportunistic taxa would be another important future goal.

Although we ostensibly sampled and compared intraspecific variation, it is possible (likely) that some of the included taxa comprise multiple morphologically cryptic species (see also Doña and Johnson 2023). This is a classic problem in mygalomorph spiders, and dozens of studies have shown morphological conservatism with deep genetic structuring. Here, we suspect that both *Megahexura* and *Bothriocyrtum* could house multiple cryptic taxa. However, our focus on the comparison of summary statistics for *inferred genetic populations* addresses this concern. So, for example, if *Megahexura* includes two or three species each with multiple genetic populations, our analyses at the level of genetic populations would not be impacted by this morphological crypsis.

We also emphasise that defining comparable genetic populations was challenging in this study. Our overarching objective was to maximise comparability across units (monophyletic genetic populations), but these may not be exactly equivalent across focal taxa. We could have used best K sNMF clusters alone, but these sometimes had clear problems, such as entire clades of highly admixed individuals, which are certain sNMF artefacts (see Lawson, Van Dorp, and Falush 2018). Our alternative approach was to combine phylogeny with sNMF, but here our treatment of monophyletic subsamples from paraphyletic sNMF clusters was ad hoc, although only involving 4 of 61 total inferred genetic populations (Figures S1–S9). Importantly, definitions of genetic populations did not include geographical criteria, which we measured a posteriori from defined units.

Lastly, the distribution and details of sequenced UCE loci within mygalomorph genomes is mostly unknown. Genetic diversity and differentiation measures are heterogeneous within and among genomes, reflecting genome sizes, rates of recombination, rates of differential gene flow, coding versus non-coding variation, and autosomal versus sex chromosomal variation, etc. (Ellegren and Galtier 2016; Roux et al. 2016; Chen, Glémin, and Lascoux 2017). Spider UCE loci have been shown to be mostly exonic (Hedin et al. 2019), implying that most SNP variation measured here should be synonymous, but UCEs also include flanking introns. Future genome re-sequencing efforts with annotated genomes and population-level sampling will be important to clarify the genetic architecture of genomic variation in these spiders.

4.5 | Conclusion

We tested several predictive variables previously shown to explain variation in genetic diversity and differentiation in other

spiders, other arthropods and other animals. Other than geographical range size (a surrogate for N_e), these were not recovered as consistently explanatory in mygalomorphs. Results revealed evidence for pervasive population genomic structuring in the form of IBD, regardless of species ecology or body size. We suggest that population genomic structuring is widespread in mygalomorph spiders, emphasising that this 'single clade' is very old, with a common ancestor estimated at 300–350 mya (Hedin et al. 2019; Opatova et al. 2020). Considering phylogenetic age only, mygalomorphs are akin to crown amniotes, for example. We also note that several spider clades in the phylogenetic neighbourhood of mygalomorphs show high population genetic structuring in available studies, although most are not phylogenomic. This includes Mesothelae (Xu et al. 2020), the Filistatid—Hypochilid Clade (Hedin and Wood 2002), Synspermiata (Magalhaes et al. 2014) and others. The implication is that high population genomic structuring might be a plesiomorphic condition for all spiders.

Author Contributions

Rodrigo Monjaraz-Ruedas and **Marshal Hedin:** conceptualisation. **Rodrigo Monjaraz-Ruedas, Marshal Hedin, James Starrett, Lacie Newton** and **Jason E. Bond:** sample collection and curation. **Rodrigo Monjaraz-Ruedas** and **Marshal Hedin:** methodology and data analysis. **Rodrigo Monjaraz-Ruedas** and **Marshal Hedin:** writing – original draft. **Rodrigo Monjaraz-Ruedas, Marshal Hedin, James Starrett, Jason E. Bond:** writing – review and editing. **Jason E. Bond** and **Marshal Hedin:** resources.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

All scripts, analysis files (input, log, output), and a ReadMe file can be found at the Dryad repository (https://doi.org/10.5061/dryad.mw6m9064s). Raw UCE data is deposited under BioProject PRJNA1157860 on the GenBank SRA repository, Table S1 details accession numbers for each sample.

Benefit-Sharing Statement

This research has conservation relevance in measuring genetic diversity in habitat-specialised and dispersal-limited animals in California, Arizona sky island and Appalachian sky island habitats. One focal taxon is a US Federally Endangered species. All data are archived in appropriate databases and are publicly available.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.