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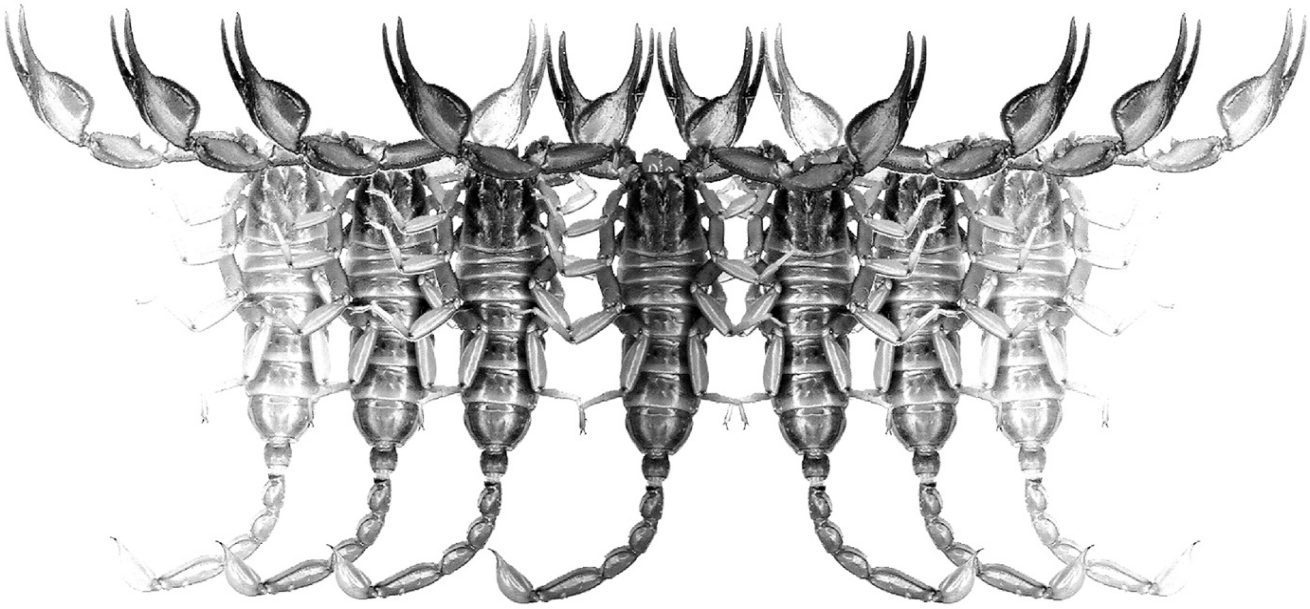

Integrative species delimitation and taxonomic status of the scorpion genus *Vaejovis* Koch, 1836 (Vaejovidae) in the Santa Catalina Mountains, Arizona

Emma E. Jochim, Lillian-Lee M. Broussard & Brent E. Hendrixson

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Integrative species delimitation and taxonomic status of the scorpion genus *Vaejovis* Koch, 1836 (Vaejovidae) in the Santa Catalina Mountains, Arizona

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Summary

Scorpions belonging to the *Vaejovis vorhiesi* species complex are widely distributed throughout the southwestern United States and northern Mexico. Most species are endemic to single mountain ranges but two species, *Vaejovis deboerae* Ayrey, 2009 and *V. brysoni* Ayrey & Webber, 2013, have been documented from the Santa Catalina Mountains in Arizona. We reevaluated the taxonomic diversity of these scorpions by integrating data from several different sources. Phylogenetic analyses indicate that scorpions in the Santa Catalina Mountains are monophyletic but comprise two divergent mitochondrial lineages that overlap at the type locality of *V. deboerae*. We failed to detect congruence between these lineages and the remaining datasets which suggests that there is a single species that we refer to as *V. deboerae* (=*V. brysoni* **syn. nov.**). Our inability to gather molecular data from the female holotype of *V. deboerae* could be the basis for future nomenclatural volatility if future studies find that the mitochon-drial lineages are validated by other forms of data (e.g., male morphology). Results from this study underscore the importance of integrative methods for delimiting species in morphologically cryptic groups. Furthermore, we recommend generating DNA barcodes for holotypes as part of the description process to reduce future nomenclatural quagmires.

Introduction

Scorpions belonging to the *Vaejovis vorhiesi* species complex (hereafter referred to as the "vorhiesi group") are widely distributed throughout Arizona, southwestern New Mexico, and northwestern Mexico (see summary in Ayrey, 2020). Approximately half of the described species occur in the region colloquially referred to as the "Madrean Sky Islands" (MSI), a series of mountain ranges that form an archipelago of isolated woodlands and forests surrounded by seas of arid deserts and grasslands. These rather small (< 35 mm) and nondescript scorpions have limited dispersal capabilities and are restricted to the cool and moist conditions of the MSI. As a consequence, fragmentation of the MSI has facilitated diversification of isolated populations (Bryson et al., 2013), resulting in a distributional pattern whereby species are endemic to individual mountain ranges or complexes of ranges connected by woodlands (Stahnke, 1940; Graham, 2007; Ayrey, 2009; Hughes, 2011; Graham et al., 2012; Ayrey & Webber, 2013; Ayrey & Soleglad, 2014, 2015; Ayrey, 2018; Barrales-Alcalá et al., 2018; Ayrey & Myers, 2019; Ayrey, 2020). The general rule of thumb is that MSI ranges harbor only a single scorpion species in the vorhiesi group. However, two species have recently been reported from the Santa Catalina Mountains near Tucson, Arizona.

Vaejovis deboerae Ayrey, 2009, the largest member of the vorhiesi group, was described from a high-elevation locality in the Santa Catalina Mountains (Willow Canyon, 2142 m elevation) that is characterized by ponderosa pine forest and scattered oaks. Four years later, *V. brysoni* Ayrey & Webber, 2013 was described from a lower-elevation site (Seven Cataracts Vista, 1626 m) in open oak woodland. The authors differentiated the two species based on a handful of quantitative (e.g., measurements, morphometric ratios, pectinal tooth counts) and qualitative (e.g., subaculear tubercle development) morphological features but examined only a limited number of specimens from each type locality despite acknowledging that "their ranges may overlap, perhaps along the mid-elevation pine-oak woodlands between 1800–1900 m" (Ayrey & Webber, 2013).

Based on this information, we had numerous questions concerning the validity of the two species in the Santa Catalina Mountains. First, this is the only example where two species are documented from the same mountain range. This is rather unusual, and the authors did not propose a biological explanation for this observation. For example, did *V. deboerae* and *V. brysoni* colonize the Santa Catalina Mountains independently or did they diverge *in situ* (see Weaver et al., 2010 for an example of *in situ* diversification in a MSI range)? If the latter, how might have this occurred and how does each species maintain cohesion in zones of syntopy? Second, the vorhiesi group is morphologically homogeneous which leads

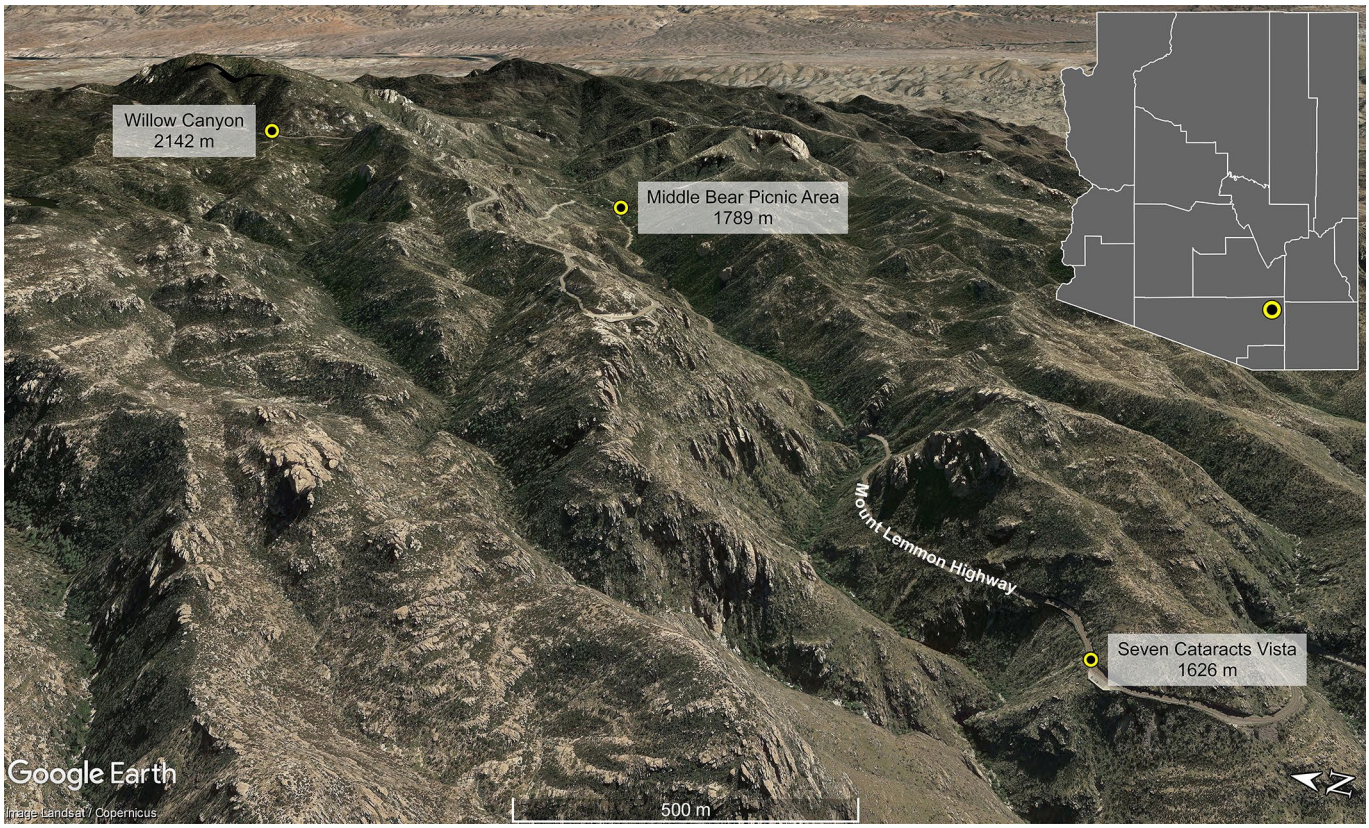

Figure 1. Google Earth (Image Landsat/Copernicus) terrain map of the Santa Catalina Mountains showing the sampling sites for vorhiesi group scorpions examined in this study (refer to Table 1 for GPS coordinates, vegetation zones, and the number of individuals sampled for each site). Willow Canyon and Seven Cataracts Vista are the type localities for *Vaejovis deboerae* and *V. brysoni*, respectively; these sites are separated by only 11.0 km along the Mount Lemmon Highway. Inset map of the state of Arizona depicting the approximate location of the Santa Catalina Mountains.

Sampling Location	Coordinates	Elevation	Vegetation Zone	*n*
Seven Cataracts Vista	32.35796°N 110.72538°W	1626 m	oak woodland	8
Middle Bear Picnic Area	32.37358°N 110.69223°W	1789 m	pine-oak woodland	10
Willow Canyon	32.38695°N 110.69583°W	2142 m	pine forest	14

Table 1. Characteristics of sample sites for vorhiesi group scorpions from the Santa Catalina Mountains.

to some questions regarding the efficacy of morphological features used for distinguishing species in the first place (although see Hughes, 2011 and Graham et al., 2012). In addition, the diagnostic features used for separating *V. deboerae* from *V. brysoni* are based on relatively small sample sizes (i.e., individuals *and* locations) so limits of variation remain poorly understood. How would these features hold up after examining more individuals from more localities? Could the observed differences be due to clinal variation along an elevational gradient? Third, despite occurring in notably different vegetation zones, the type localities for *V. deboerae* and *V. brysoni* are geographically proximate (i.e., only 4.2 km "as the crow flies" or 11.0 km along the Mount Lemmon Highway) and there are no obvious barriers to dispersal or gene flow. The authors did not sample scorpions from mid-elevation pine-oak woodlands that link the respective type localities so the story of vorhiesi group scorpions in the Santa Catalina Mountains remains incomplete.

Based on these observations, the primary objective of this study is to reevaluate species boundaries in vorhiesi group scorpions from the Santa Catalina Mountains. The approach we adopt herein essentially "starts from scratch" and makes no a priori assumptions regarding the nomenclatural identity of individual scorpions. We integrate data from several sources (mtDNA, nDNA, morphology, and geography/ecology) to generate species hypotheses and then compare these delimited entities to the two nominal species to assess their taxonomic status.

Materials and Methods

Specimen Sampling

Scorpions were collected from three sites along an elevation gradient in the Santa Catalina Mountains (Table 1, Fig. 1), including the type localities for *V. deboerae*

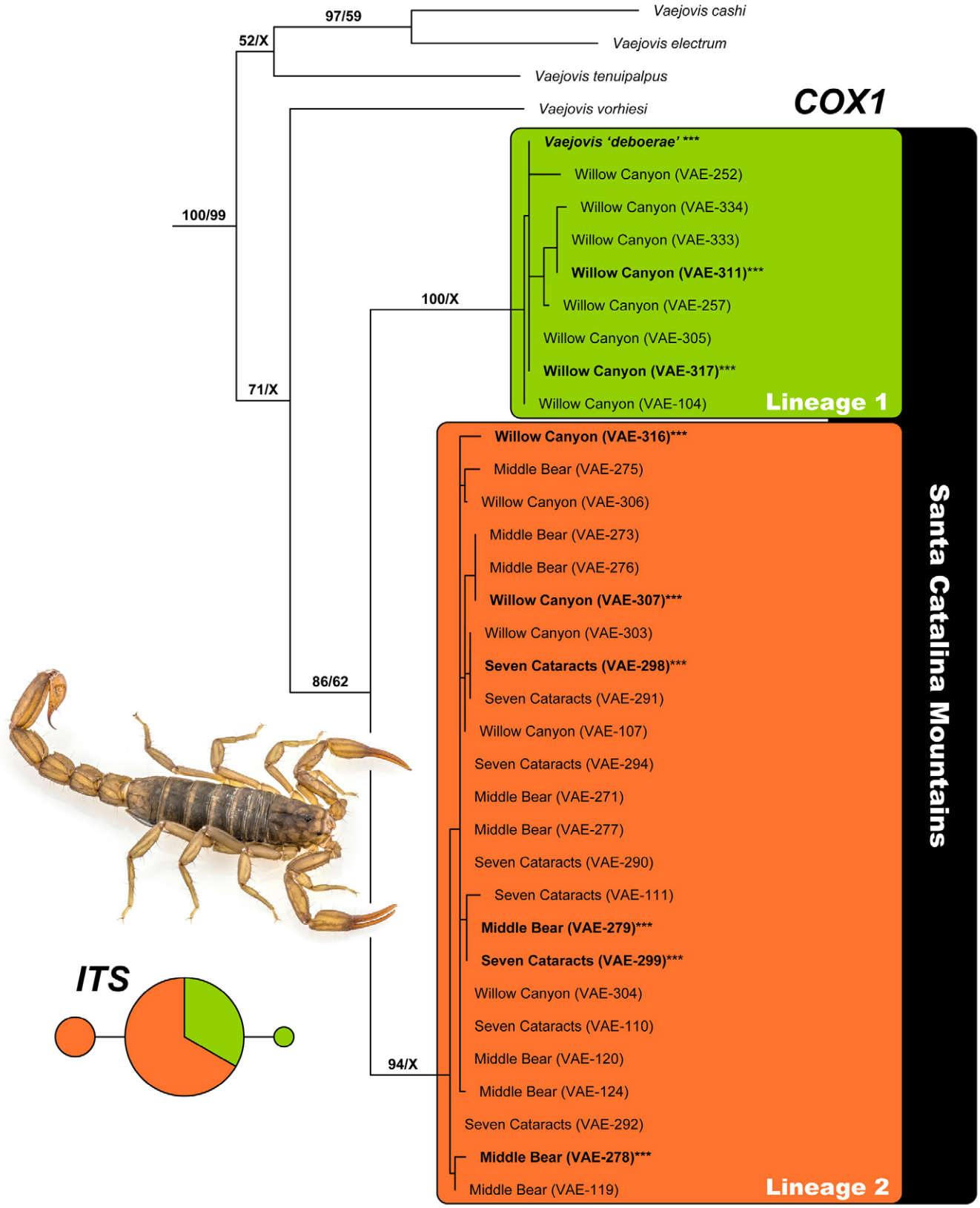

Figure 2. Maximum likelihood phylogeny of vorhiesi group scorpions based on *COX1* (distant outgroups removed) and a median-joining network for samples from the Santa Catalina Mountains based on *ITS*. Specimens in bold-faced type with triple asterisks (***) specify individuals that were sampled for *ITS*. Colors depict the two divergent *COX1* lineages identified by the ABGD analysis (green = Lineage 1, orange = Lineage 2). Bootstrap support values along nodes are listed as *COX1*/*ITS* ("X" indicates clades that were not recovered in the *ITS* analysis). The sample named *Vaejovis 'deboerae'* is the individual included in Bryson et al. (2013). Different sized circles in the *ITS* network indicate the relative proportion of individuals possessing a particular genotype; branches represent a single mutational step.

From/To	Lineage 1	Lineage 2	Total	% Correct
Lineage 1	4	4	8	0.500
Lineage 2	8	16	24	0.667
Total	12	20	32	0.625

Table 2. Jackknifed cross-validation classification matrix for the discriminant function analysis of vorhiesi group scorpions from the Santa Catalina Mountains.

(Willow Canyon) and *V. brysoni* (Seven Cataracts Vista), and an intermediate site located within a pine-oak woodland (Middle Bear Picnic Area). Scorpions were found either by flipping rocks during daylight hours or by using ultraviolet lights at night. Specimens were placed into 95% ethanol, assigned unique voucher identification numbers (VAE-000), and subsequently stored at -20°C until processed for DNA extraction. All vouchers used in this study are stored in the Hendrixson Lab at Millsaps College (Jackson, Mississippi, USA).

Several dozen scorpions were found at each collection site but analyses were restricted to specimens that met two conditions: (1) they had to be adult females; and (2) they had to yield adequate molecular data. Females were chosen because males were infrequently encountered and the holotypes for *V. deboerae* and *V. brysoni* are both females; consequently, females provide the nomenclatural framework for evaluating the taxonomic diversity of these scorpions. Only specimens with molecular data were included so we could attribute morphological information to specific individuals based on their phylogenetic placement.

Mensuration

We measured 23 standard features (see Graham et al., 2012) on each specimen using the landmarks defined by Stahnke (1971) and Sissom et al. (1990). All measurements (reported in millimeters) were performed with a Leica MZ9.5 stereomicroscope equipped with a calibrated ocular micrometer. We did not measure body length due to variations in how this measurement is obtained (compare Sissom et al., 1990 and Graham et al., 2012); we consider carapace length a proxy for body length.

Molecular Protocols and Phylogenetic Analyses

Muscle tissues were collected by removing all of the legs on the right side of each scorpion and total genomic DNA was isolated using the DNeasy Blood & Tissue Kit (Qiagen). We amplified and sequenced a portion of cytochrome *c* oxidase subunit I (*COX1*)—the animal barcoding gene—from the mitochondrial genome following the procedures outlined in Bryson et al. (2016). Based on those results, eight individuals were sequenced spanning the breadth of *COX1* diversity for a fragment that includes internal transcribed spacer II and portions of 5.8S and 28S rRNA from the nuclear genome using protocols described by Hendrixson et al. (2013). This fragment will hereafter be referred to as *ITS*. Purified amplicons were sequenced in both directions using the same primers that were used for PCR and the ABI Big Dye Terminator ver. 3.1 Cycle Sequencing Ready Reaction Kit. All sequences have been deposited in Genbank under accession numbers MT863664–MT863695 for *COX1* and MT872179–MT872186 for *ITS*. We obtained previously published sequences (Bryson et al., 2013) from Genbank for the following outgroup taxa (*COX1/ITS*): *Paruroctonus boreus* (Girard, 1854) (JX909544/JX909361), *Vaejovis montanus* Graham & Bryson, 2010 (JX909603/JX909404), *V. cashi* Graham, 2007 (JX909587/JX909394), *V. electrum* Hughes, 2011 (JX909593/JX909398), *V. tenuipalpus* Sissom, Hughes, Byson & Prendini, 2012 (JX909610/JX909409), and *V. vorhiesi* Stahnke, 1940 (JX909611/JX909410). We also included sequences from a specimen previously identified as *V. deboerae* (JX909592/JX909397); it should be noted that this individual was collected from a site near—but not at—the type locality (R. Bryson, pers. comm., 2017).

Alignment of the *COX1* dataset was straightforward, and sequences were translated and checked for unexpected stop codons in Mesquite ver. 3.61 (Maddison & Maddison, 2019). We aligned the *ITS* dataset with MUSCLE ver. 3.8.425 (Edgar, 2004) in Geneious Prime ver. 2020.0.4 (Biomatters) using default parameters and truncated the sequences at both ends. We analyzed each dataset separately using maximum likelihood (ML) in IQ-TREE (Nguyen et al., 2015) through the W-IQ-TREE web server (Trifinopoulos et al., 2016). The substitution model was set to "Auto" which allows IQ-TREE to determine the best-fit substitution model for the data using ModelFinder (Kalyaanamoorthy et al., 2017); the FreeRate heterogeneity (+R) option was also selected. Clade support was estimated using 1,000 ultrafast bootstrap replicates (Hoang et al., 2018).

Due to limited variation in the *ITS* dataset for scorpions from the Santa Catalina Mountains, we also constructed a median-joining network in Network ver. 10.1.0.0 (Fluxus Technology, available at https://www.fluxus-engineering.com).

Species Delimitation

Integrative methods that incorporate data from a variety of sources have been useful for delimiting morphologically cryptic arachnid species in the Madrean Sky Islands (Hendrixson et al., 2015; Hamilton et al., 2016; Hendrixson, 2019). The general work protocol we follow here is the "integrative taxonomy by congruence" approach described by DeSalle et al. (2005) and Padial et al. (2010). This method is based on the idea that concordant patterns of differentiation among different types of taxonomic characters (e.g., geography, molecules, morphology, behavior, ecology, etc.)

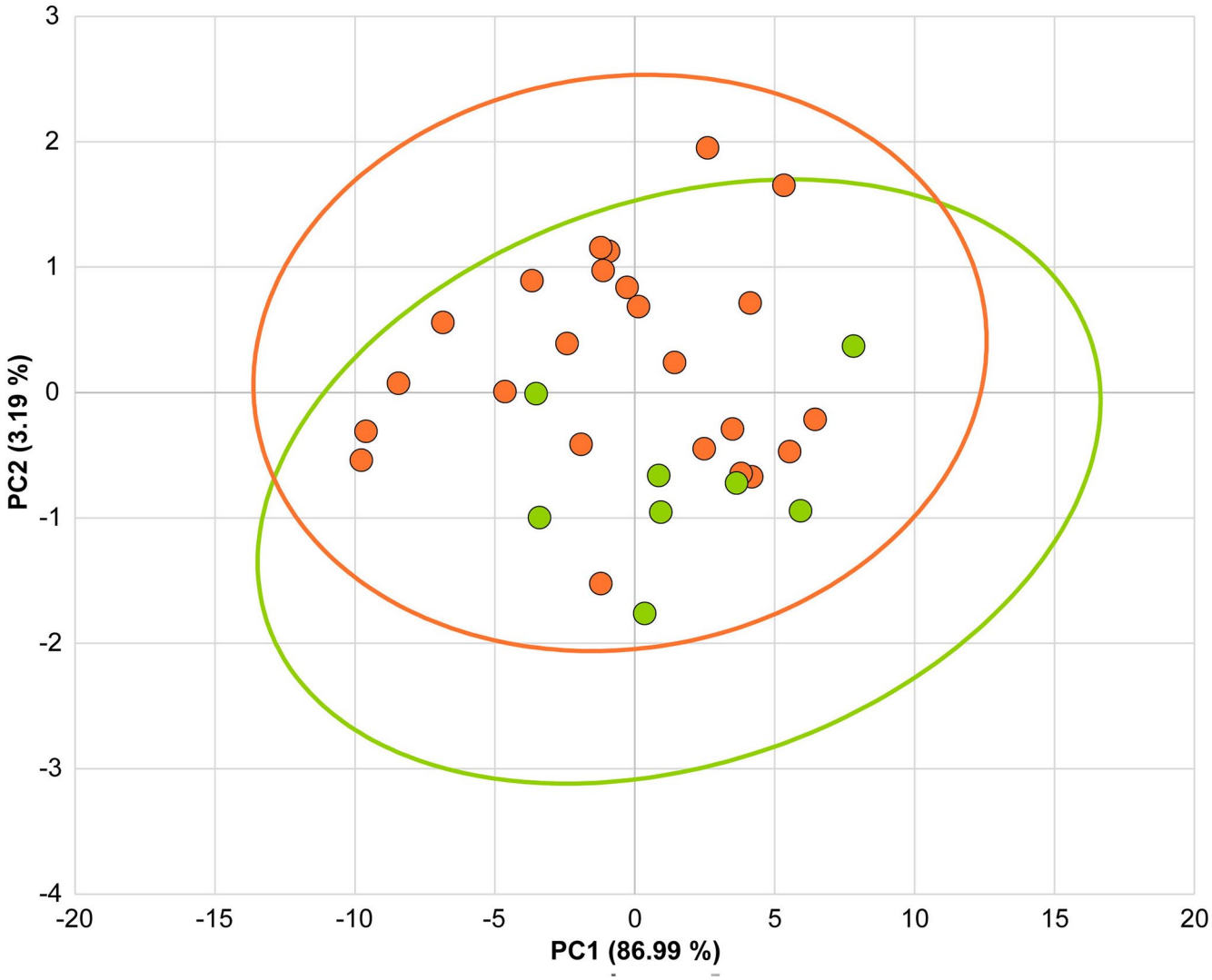

Figure 3. Scatterplot of principal components for measurements of vorhiesi group scorpions from the Santa Catalina Mountains. Colors depict the two divergent *COX1* lineages identified by the ABGD analysis (green = Lineage 1, orange = Lineage 2). Percentage values indicate the proportion of variation explained by a particular principal component (PC1 and PC2). Prediction ellipses are such that with a probability of 0.95, a new observation from the same group will fall inside the ellipse.

are indicative of complete lineage separation. A significant limitation to this approach is that it may underestimate species-level diversity, but a key advantage is that it promotes taxonomic stability (Padial et al., 2010). We first establish whether a mtDNA dataset comprises two or more distinct lineages and then determine if these entities are congruent with other types of data. We used the Automatic Barcode Gap Discovery (ABGD) method (Puillandre et al., 2012) to partition the *COX1* dataset into distinct clusters. A FASTA file was uploaded to the ABGD server (https://bioinfo.mnhn.fr/abi/public/abgd/abgdweb.html), converted to a distance matrix using the "Simple Distance" method, and analyzed using default parameters: *P*min = 0.001, *P*max = 0.1, Steps = 10, X = 1.5, Nb bins = 20.

The ABGD method identified two *COX1* clusters from the Santa Catalina Mountains (see Results) so we conducted a series of multivariate statistical analyses on the measurement data to determine if morphology corroborated the mtDNA data. We first performed a principal component analysis (PCA) of the raw measurements to explore patterns of morphological variation, using the clusters identified by ABGD as the grouping variable. Additionally, we conducted a discriminant function analysis (DFA) using the same grouping variable to determine which morphological features—if any—differentiated the two groups. Significance of the canonical functions was assessed through jackknifing and cross-validation (see Devitt et al., 2008; Hughes, 2011; Graham et al., 2012). We also explored the efficacy of the diagnostic characteristics used by Ayrey & Webber (2013) for distinguishing *V. brysoni* from *V. deboerae* by determining if these features could be used to differentiate the two clusters identified by ABGD; the means for quantitative features (i.e., measurements, morphometric ratios, pectinal tooth counts) were compared using Student's t-tests. Statistical analyses were performed in XLSTAT ver. 2020.2.3 (Addinsoft) and JMP ver. 15.1.0 (SAS Institute).

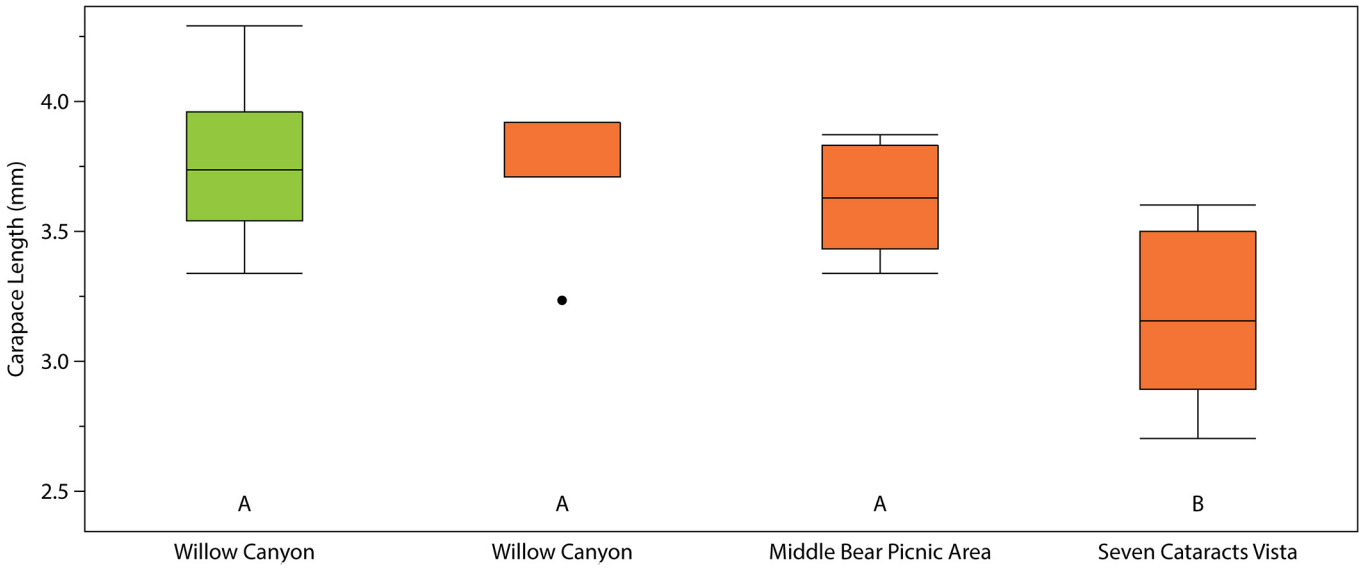

Figure 4. Boxplots showing variation in carapace length (mm) for vorhiesi group scorpions from the Santa Catalina Mountains at each sampling site. Colors depict the two divergent *COX1* lineages identified by the ABGD analysis (green = Lineage 1, orange = Lineage 2). Letters below the boxplots designate statistically similar/different groupings as identified by a Tukey HSD test.

Results

Molecular Characteristics and Phylogenetic Analyses

Useable *COX1* data for the newly sequenced individuals (n = 32) varied in length from 372–826 bp; the final *COX1* alignment comprised 854 bp (including the outgroups) and 155 parsimony-informative sites. ModelFinder determined that the best-fit substitution model for the data (based on the Bayesian Information Criterion) was TIM3+F+G4. The ML tree topology (log-likelihood score = –3084.470) (Fig. 2) shows that scorpions from the Santa Catalina Mountains are monophyletic (86 bs support) with respect to the outgroups and that the clade comprises two strongly supported subclades (100 and 94 bs support, respectively): (1) a group that includes 8 newly sequenced individuals from the Willow Canyon site plus the specimen identified as *V. deboerae* in Bryson et al. (2013) (hereafter referred to as "Lineage 1"); and (2) a group that includes all 8 samples from the Seven Cataracts Vista site, all 10 samples from the Middle Bear Picnic Area site, and the remaining 6 individuals from the Willow Canyon site (hereafter referred to as "Lineage 2"). The average pairwise genetic distance (uncorrected P) between lineages is approximately 5.4%.

For the *ITS* dataset, each newly sequenced individual (n = 8) contained 241 bp; the final *ITS* alignment comprised 272 bp (including the outgroups) and 26 parsimony-informative sites. ModelFinder determined that the best-fit substitution model for the data (based on the Bayesian Information Criterion) was HKY+F+G4. The ML tree topology (log-likelihood score = –743.9075) (only ultrafast bootstrap values included in Fig. 2) shows that scorpions from the Santa Catalina Mountains are monophyletic (62 bs support) but with no internal resolution. For scorpions from the Santa Catalina Mountains, the median-joining network constructed from the *ITS* data revealed limited structure: six individuals from Willow Canyon and Middle Bear Picnic Area shared the same genotype, two individuals sequenced from Seven Cataracts Vista shared a single unique mutation, and one individual from Willow Canyon possessed a single unique mutation (Fig. 2).

Species Delimitation

The ABGD analysis partitioned the *COX1* distance matrix into eight clusters which corresponded to each of the outgroup taxa (n = 6) and the two lineages from the Santa Catalina Mountains. Lineage 1 and Lineage 2 overlapped extensively in PCA morphospace (Fig. 3) where the majority of variance (86.99%) was explained by PC1 (i.e., attributes of body size); PC2 explained 3.19% of the variance in the data and was most correlated with metasomal segment I length. Canonical analysis of discriminance showed no mean differences between Lineage 1 and Lineage 2 (Wilks' λ = 0.092, P = 0.078), and the jackknife cross-validation procedure correctly classified only 62.5% of the samples (Table 2).

All morphological features previously used for distinguishing *V. deboerae* and *V. brysoni* (Ayrey & Webber, 2013) showed considerable overlap between Lineage 1 and Lineage 2 (Table 3). Subaculear tubercle shape varied both within and between lineages, pectinal tooth counts varied from 11–13 per comb (mode = 12) in both lineages, and only two morphometric ratios showed statistically significant differences between the lineages: metasomal segment I length/width and fixed finger length/chela length. When only the samples from the Willow Canyon site were analyzed, these two ratios showed no statistically significant differences between the lineages (P = 0.275 and P = 0.268, respectively).

Diagnostic Character	Lineage 1	Lineage 2
Carapace length (mm)	3.50–4.29 (3.757 ± 0.296)	2.70–3.92 (3.513 ± 0.367)
Telson vesicle length/width	1.74–1.96	1.70–2.04
Metasomal segment I length/width*	0.72–0.87 (0.800 ± 0.052)	0.76–0.97 (0.883 ± 0.061)
Chela fixed finger length/chela length*	0.45–0.54 (0.491 ± 0.025)	0.47–0.53 (0.507 ± 0.016)
Number of pectinal teeth	11–13 (11.875 ± 0.619)	11–13 (11.609 ± 0.537)

Table 3. Quantitative diagnostic features used by Ayrey & Webber (2013) to differentiate *Vaejovis deboerae* and *V. brysoni* as applied to *COX1* Lineages 1 and 2. Reported values show the range followed by the mean ± standard deviation in parentheses. Features designated by an asterisk (*) were statistically different ($P \leq 0.05$) between Lineage 1 and 2 but these values were no longer statistically significant when individuals from both lineages at Willow Canyon were compared to each other exclusive of samples from Seven Cataracts Vista and Middle Bear Picnic Area. Note: The values for telson vesicle length/width reported for *V. deboerae* in Ayrey & Webber (2013) do not agree with the measurements of the holotype reported in Ayrey (2009); our calculation of this ratio in the holotype (1.65) places the value much closer to what we observed in all vorhiesi group scorpions in the Santa Catalina Mountains.

Discussion

Diversity of Vorhiesi Group Scorpions in the Santa Catalina Mountains

Results from the phylogenetic analysis of *COX1* and *ITS* indicate that vorhiesi group scorpions in the Santa Catalina Mountains are monophyletic. This suggests that these scorpions colonized the mountain range on a single occasion and that *COX1* Lineages 1 and 2 diverged from each other *in situ*. The level of divergence detected between these lineages (5.4%) is impressive considering that it exceeds the largest *intraspecific* genetic distances observed for any other vorhiesi group species (3.0% for *V. cashi* from the Chiricahua, Peloncillo, and Animas Mountains) but more notably also exceeds the smallest *interspecific* genetic distances observed for any pair of described species in the group (2.7% between *V. vorhiesi* from the Huachuca Mountains and *V. islaserrano* from the Sierras Elenita and La Mariquita) (genetic distances calculated from *COX1* sequences reported in Bryson et al., 2013).

Despite these considerable differences in mtDNA, we were unable to detect concordant patterns of differentiation among any other taxonomic characters examined between Lineages 1 and 2. Hughes (2011) and Graham et al. (2012) demonstrated the efficacy of multivariate statistical analyses for differentiating female vorhiesi group scorpions (from different mountain ranges) based on external morphology, but our results indicate that the two lineages in the Santa Catalina Mountains have not diverged morphologically. They overlap extensively in PCA morphospace (Fig. 3), and the DFA failed to identify any mean morphological differences between them. The high percentage of incorrectly classified individuals from the jackknife cross-validation test similarly suggests that external morphology is a poor predictor of lineage identity. If two species were present, we might expect to observe divergent morphology between the two lineages where they occur in syntopy due to character displacement (e.g., Bond & Sierwald, 2002), but we have not observed this phenomenon in these scorpions. To the contrary, our data—albeit limited—point toward the opposite pattern. Specimens from both lineages are more similar morphologically (based on body size) when they occur in syntopy than when they occur in allopatry; the samples from Seven Cataracts Vista are significantly smaller than their higher-elevation counterparts (Fig. 4). This pattern of clinal variation suggests that scorpions in the Santa Catalina Mountains may follow Bergmann's Rule, an ecogeographical principle that states that organisms in cooler climates should be larger than those in warmer climates to conserve heat (see Shelomi, 2012 for a review of Bergmann's Rule in arthropods). This idea should be further explored.

Some integrative species delimitation studies have sought to detect habitat/ecological divergence between morphologically cryptic arachnid lineages statistically (e.g., Bond & Stockman, 2008; Hendrixson et al., 2013; Newton et al., 2020), but due to the limited number of sample sites and the unavailability of ultra-fine-scale bioclimatic data (the finest resolution data available at WorldClim are too coarse for our small study area), we were unable to perform these types of analyses. Based on our observations in the field, however, we would not consider these two lineages ecologically divergent even though Lineage 2 is found at the two lower-elevation sites where Lineage 1 has not been observed. Both lineages are syntopic at Willow Canyon and at a higher-elevation site (Palisades Visitor Center, 2440 m) that was not included in this study (samples from this location were not included because we were only able to collect molecular data from immature specimens). Specimens from both lineages have been observed hiding under the same rock during the day and foraging for prey in close proximity at night, suggesting that they have not partitioned their "niches" in syntopy (see Goodman & Esposito, 2020 for an example of niche partitioning in congeneric scorpions from southern

Mexico). Altogether, these observations suggest that there is considerable overlap in the bioclimatic and resource niches occupied by the two lineages and that they likely have not diverged ecologically from each other.

Finally, patterns of genetic variation in *ITS* are not concordant with *COX1*. The *ITS* dataset had limited variation and the majority of individuals (i.e., from both *COX1* lineages) shared identical *ITS* sequences (Fig. 2). This suggests that these two divergent mitochondrial lineages have experienced recent admixture and gene flow, but this hypothesis needs to be tested more formally using larger numbers of individuals and loci. If the lineages are indeed reproductively isolated, we have been unable to detect it with these molecular markers.

Based on all the available data, then, we do not have sufficient evidence to recognize two species from the Santa Catalina Mountains (i.e., we cannot break out of the "taxonomic circle" *sensu* DeSalle et al., 2005). However, we acknowledge that excluding males (due to limited sample sizes and difficulties generating molecular data) may be considered a significant limitation for this study. Male characters involved in mating and courtship, such as hemispermatophore and pedipalp chela morphology, have been used for delimiting closely related scorpion taxa (e.g., Haradon, 1983; Sissom, 1991; Webber et al., 2012; Soleglad et al., 2016, 2017) and might be predicted to evolve rapidly and divergently due to sexual selection (Eberhard, 1985). Most species of vorhiesi group scorpions, however, show limited qualitative morphological differences and are primarily separated by various morphometric features (e.g., Ayrey, 2020). Nevertheless, this is a unique system and we must consider the possibility that these lineages can be validated by other forms of data. For example, behaviors that enhance prezygotic reproductive isolation (e.g., differences in courtship or breeding period) might be expected for closely related species that occur in syntopy (Coyne & Orr, 2004). Future studies (including karyotype analysis, see Šťáhlavský et al., 2018) should examine these types of characters further.

Nomenclatural Considerations

If we assume that we have sampled the described diversity of vorhiesi group scorpions in the Santa Catalina Mountains, recognition of only a single species necessitates a nomenclatural act. The name *Vaejovis deboerae* has priority over *V. brysoni* based on the date of publication (2009 and 2013, respectively), but nomenclatural considerations for these scorpions are complex. According to some species delimitation work protocols (e.g., the "candidate species approach" described in Vieites et al., 2009 and Padial et al., 2010), one of the divergent *COX1* lineages could be considered an "unconfirmed candidate species" (rather than a "deep conspecific lineage") because we lack data for males and cannot say that there are *not* any congruent differences in characters that mediate reproductive isolation. This is an important designation because it acknowledges the significant amount of molecular divergence observed between these two lineages and preserves the possibility that they are indeed different species. As a consequence, it places considerable weight on lineage affiliation for nomenclatural purposes. Even though we did not seek permission to sequence the holotype of *V. brysoni*, we are reasonably confident that it would belong to Lineage 2 because every individual sequenced from the type locality (Seven Cataracts Vista) grouped together in Lineage 2 (Fig. 2). Furthermore, the likelihood of encountering Lineage 1 individuals at Seven Cataracts Vista seems small given that every individual from the most geographic proximate site (Middle Bear Picnic Area) also belonged to Lineage 2. We have no confidence regarding lineage affiliation for *V. deboerae*, however, because we were denied permission to destructively sample and sequence the female holotype (L. Esposito, pers. comm., 2019), both lineages were sampled in comparable numbers at the type locality (Willow Canyon), and the two lineages are morphologically indistinguishable. Without molecular data or any other distinguishing features associated with the holotype, there is only a 50% chance of correctly assigning it to a lineage; this level of uncertainty could be the basis for future nomenclatural volatility.

Consider the following scenario: If future studies find that male scorpions exhibit morphological and/or behavioral differences that are concordant with lineage affiliation (i.e., both lineages are considered separate species), what names will be applied to the lineages? If we knew that the holotype for *V. deboerae* belonged to Lineage 1, then *V. deboerae* would be the name of Lineage 1, and *V. brysoni* would be the name of Lineage 2. If we knew that the holotype for *V. deboerae* belonged to Lineage 2, then *V. deboerae* would be the name of Lineage 2, *V. brysoni* would be considered a junior synonym of *V. deboerae*, and Lineage 1 would be considered a new species (i.e., "confirmed candidate species", see Vieites et al., 2009) in need of description. Since we do not know the lineage affiliation of the holotype for *V. deboerae*–and perhaps never will–neither nomenclatural act can be executed cleanly. The burden of proof, however, is on future researchers to establish that the lineages are *not* conspecific so we consider *V. deboerae* a senior synonym of *V. brysoni* (**syn. nov.**) at this time.

Conclusions

The objective of this study was to evaluate the diversity of vorhiesi group scorpions in the Santa Catalina Mountains, the only MSI range thought to harbor two distinct species. While we only recognize a single species (*V. deboerae*), the discovery of two deeply divergent but morphologically cryptic mtDNA lineages was unexpected and should be investigated in more detail. This observation suggests that these arachnids have experienced a complex evolutionary history *in situ* and that similar patterns of divergence may exist in other MSI mountain ranges (McCormack et al., 2009). This study also underscores the importance of increased sample sizes, increased geographic sampling, and the application of integrative methods for delimiting species in morphologically cryptic groups, especially in situations where sampling gaps preclude a full understanding of the distributional limits for the populations in

question. By modestly increasing sample sizes and including individuals from just a single new location, we found that the morphological features used for distinguishing *V. deboerae* and *V. brysoni* showed considerable overlap. These results are in agreement with Hughes (2011) who showed that many of the taxonomic features typically used for distinguishing vorhiesi group scorpions are highly variable when large sample sizes from numerous locations are examined. Unfortunately, the majority of species in the vorhiesi group are known only from their respective type localities so geographic limits of variation remain poorly understood. Finally, this study highlights the importance of linking morphological and molecular data to individual specimens for integrative taxonomic work. Not only were we able to show that the divergent mtDNA lineages were morphologically indistinguishable, but we also demonstrated that a lack of molecular data for the holotype of *V. deboerae* has the potential to disrupt nomenclatural stability in the group if future studies show that the lineages are indeed different species. When possible, we recommend generating *COX1* DNA barcodes for holotypes as part of the description process (e.g., Webber et al., 2012) to prevent or reduce future nomenclatural quagmires (see Webb et al., 2012; Mutanen et al., 2015).

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