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Research Article

Phylogeographic Investigations of the Widespread, Arid-Adapted Antlion *Brachynemurus sackeni* Hagen (Neuroptera: Myrmeleontidae)

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Several recent studies investigating patterns of diversification in widespread desert-adapted vertebrates have associated major periods of genetic differentiation to late Neogene mountain-building events; yet few projects have addressed these patterns in widespread invertebrates. We examine phylogeographic patterns in the widespread antlion species *Brachynemurus sackeni* Hagen (Neuroptera: Myrmeleontidae) using a region of the mitochondrial gene cytochrome oxidase I (COI). We then use a molecular clock to estimate divergence dates for the major lineages. Our analyses resulted in a phylogeny that shows two distinct lineages, both of which are likely distinct species. This reveals the first cryptic species-complex in Myrmeleontidae. The genetic split between lineages dates to about 3.8–4.7 million years ago and may be associated with Neogene mountain building. The phylogeographic pattern does not match patterns found in other taxa. Future analyses within this species-complex may uncover a unique evolutionary history in this group.

1. Introduction

Phylogeographic analyses investigate the relationship between genealogies and their geographic distribution [1]. Many recent studies have investigated the historical biogeography of the Nearctic arid lands through the phylogeographic analyses of wide-ranging, desert-adapted taxa [2–7]. These studies often associated major genetic divergences with mountain-building events that took place in the late Neogene. As a result of these late Neogene events, deeply divergent clades were found to be restricted to the eastern (Chihuahuan) and western (Mojave and Sonoran) deserts. While the various hypotheses detailing the causes of the diversification of the Nearctic's arid-adapted biota approach a generalized model [8], little work has been done on wide-ranging, arid-adapted arthropods [7]. Phylogeographic analyses of these organisms will aid in the development of a generalized model detailing diversification in the deserts.

In addition to the importance of phylogeographic analyses to historical biogeography, these analyses often uncover the existence of cryptic species [9–13]. Recognition of these complexes is an essential aspect of documenting biodiversity and can be beneficial in the development of conservation strategies [14, 15].

While some phylogeographic investigations have been done on arid-adapted arthropods, like beetles [16, 17], velvet ants [7, 18], and spiders [2, 19], several diverse arthropod groups remain unexplored. One such group are the antlions (Neuroptera: Myrmeleontidae). Antlions in the tribe Brachynemurini are ideal candidates for phylogeographic analyses investigating the history of the Nearctic deserts because they are most abundant in the arid or semiarid regions of the southwestern United States and northern Mexico [20]. Furthermore, because antlions are economically important as predators, adults commonly feed on caterpillars and aphids [21], and as potential pollinators—several species are associated with flowers and potentially transfer pollen [20]—understanding the genetic diversity and species limits of antlions may be helpful to biologists and land managers.

Table 1: Descriptive information for all of the taxa used in this study.

Species	Voucher ID	Lineage	Collection location	COI accession no.
Brachynemurus sackeni	MY02	1	NV: White Pine Co., near Cherry Creek	HQ386913
Brachynemurus sackeni	MY14	1	UT: Washington Co., near St. George	HQ386914
Brachynemurus sackeni	MY13	1	NV: Clark Co., Toquop Wash, W. Mesquite	HQ386915
Brachynemurus sackeni	MY15	1	TX: Dimmit Co., Chaparral Wildlife Management Area	HQ386916
Brachynemurus sackeni	MY03	1	CA: San Diego Co., Ocotillo Wells	HQ386917
Brachynemurus sackeni	MY19	1	CA: Imperial Co., Algodones Sand Dunes	HQ386918
Brachynemurus sackeni	MY09	1	CA: San Diego Co., Anza Borrego State Park	HQ386919
Brachynemurus sackeni	MY18	1	UT: San Juan Co., Valley of the Gods	HQ386920
Brachynemurus sackeni	MY16	1	UT: Emery Co., Green River	HQ386921
Brachynemurus sackeni	MY21	2	CA: San Bernardino Co., 8 mi N Big Bear City	HQ386922
Brachynemurus sackeni	MY08	2	CA: San Bernardino Co., 5 mi S Barstow	HQ386923
Brachynemurus sackeni	MY06	2	UT: Washington Co., Beaver Dam Slope	HQ386924
Brachynemurus sackeni	MY11	2	NV: Nye Co., Pahrump	HQ386925
Brachynemurus sackeni	MY12	2	CA: Riverside Co., Deep Canyon Reserve	HQ386926
Brachynemurus sackeni	MY05	2	NM: Harding Co., Kiowa National Grasslands	HQ386927
Brachynemurus sackeni	MY17	2	TX: Jeff Davis Co., Davis Mtns. State Park	HQ386928
Brachynemurus sackeni	MY04	2	TX: Brewster Co., Big Bend Ranch State Park	HQ386929
Brachynemurus hubbardi	MY23	2	AZ: Yavapai Co., near Camp Verde	HQ386930
Scotoleon yavapai	MY22	2	AZ: Cochise Co., happy camp road	HQ386931

Figure 1: Male *Brachynemurus sackeni* from Deep Canyon, California.

In this study, we investigate the phylogeographic patterns among populations of a widespread antlion, *Brachynemurus sackeni* Hagen (Figure 1), in order to gain insight into the diversification of the Nearctic desert biota. We apply a molecular clock, in order to estimate the dates associated with the major divergences within this species and compare those dates to published records of diversification in other desert-adapted animals. This study represents the first phylogeography and one of the first molecular phylogenetic analyses conducted on antlions.

2. Materials and Methods

2.1. Taxon Sampling. Specimens were collected from sites across western North America (Figure 2) from 2002 to 2009 using black light traps and fluorescent lantern traps. All specimens were placed directly into 95% ethanol, and those used for molecular examination have been labeled as voucher specimens and deposited in the Department of Biology Insect Collection, Utah State University, Logan, UT (EMUS). Desert boundaries to discuss species distributions and historical biogeography are altered from Omernik [22].

2.2. Molecular Methods. DNA was extracted from a middle and hind leg of each specimen using the High Pure PCR Template Preparation Kit (Roche Pharmaceuticals, Indianapolis, IN). A portion of the mitochondrial gene cytochrome oxidase I (COI) was amplified using the primer pair LepF1 (ATTCAACCAATCATAAAGATATTGG) and LepR1 (TAAACTTCTGGATGTCCAAAAAATCA) [23], which amplified an approximately 700 bp DNA fragment of the mitochondrial COI gene. PCR took place in a 20 μL volume with the following conditions: 3 mM $MgCl_2$, 200 pM dNTPs, 2 units of *Taq* polymerase, 1 mM of each primer, and standard PCR buffer concentration. For each PCR, approximately 20 ng of template DNA was added to the reaction. The PCR program included an initial step of 94°C for 150 sec, followed by 35 cycles of 94°C for 30 sec, 47°C for 60 sec, and 72°C for 60 sec, with a final step of 72°C for 10 min. Amplified products were visualized on

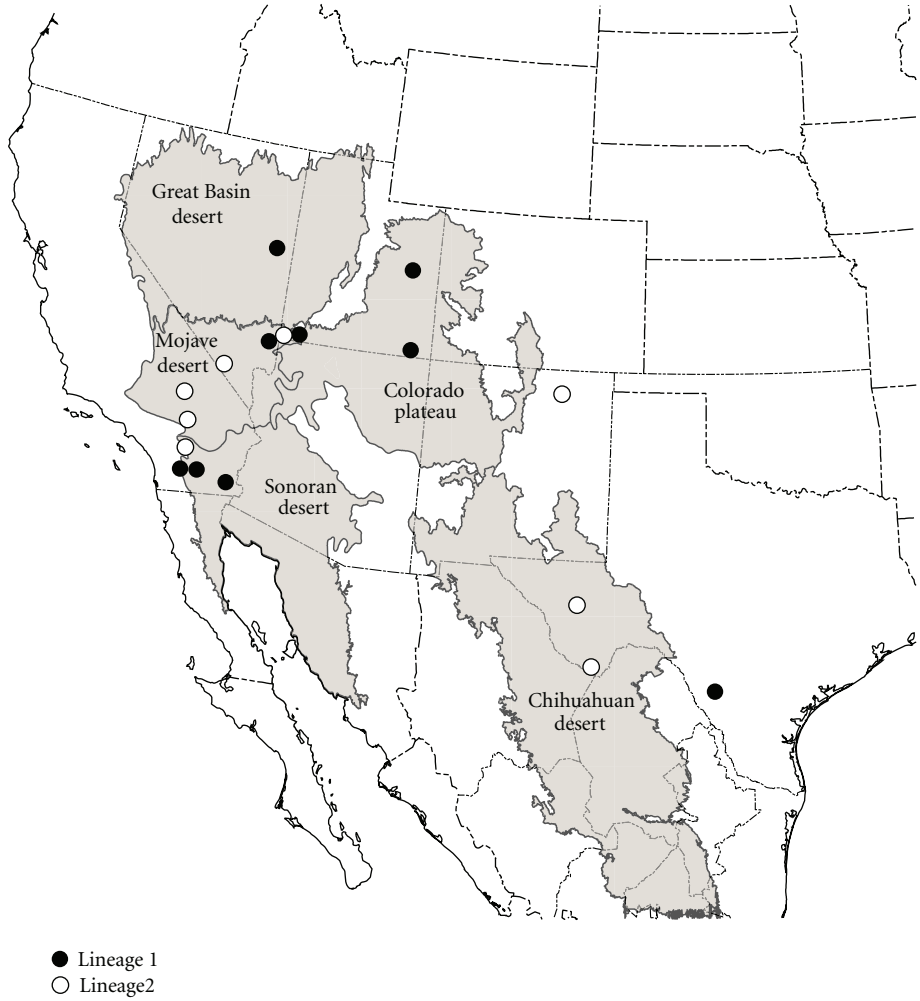

Figure 2: Map of Western North America showing the major deserts and arid lands altered from Omernik [22]. Closed circles (●) indicate collection locations of specimens from lineage 1, and open circles (○) indicate collection locations of specimens from lineage 2.

agarose gels, stained with ethidium bromide. Successful PCR products were cleaned using isopropanol purification. Sequences were analyzed with an ABI Prism 3730 Genetic Analyzer. PCR products were sequenced in both directions and sequence contigs assembled using Sequencher 4.0 (Gene Code Corp., Ann Arbor, MI). DNA sequences were aligned using Clustal W [24] and alignments were visually inspected and corrected in MacClade 4.07 [25]. All COI sequences were deposited in GenBank (Accession numbers HQ386913-HQ386931: Table 1). Genetic distances between major clades were calculated as pairwise percentages by determining the number of differences (point mutations and insertions or deletions) divided by the number of base pairs of the longer of the two sequences.

Two additional DNA regions, the internal transcribed spacer regions 1 and 2 (ITS1 and ITS2), were amplified for a limited number of specimens to investigate the possibility of these intergenic regions being useful for phylogeographic analysis. The primers 5′-GATTACGTCCCTGCCCTTTG-3′ (forward-18S) and 5′-CGATGATCAAGTGTCCTGCA-3′ (reverse-5.8S) [26] were used for the ITS1 locus and 5′-GGCTCGTGGAATCGATGAAGAACG-3′ (forward 5.8S) modified from Weekers et al. [27] and 5′-GCTTATTAATATGCTTAAATTCAGCGG-3′ [27] were used for ITS2. The PCR programs included an initial step of 94°C for 150 sec, followed by 35 cycles of 94°C for 30 sec, 52°C (ITS1) or 56°C (ITS2) for 60 sec, and 72°C for 60 sec, with a final step of 72°C for 10 min.

2.3. Phylogenetic and Network Analyses. The genetic locus COI was subjected to Bayesian analysis using MrBayes v3.1.2 [28]. Sequences were analyzed according to the general time-reversible model of sequence evolution [29] with invariant sites and gamma-distributed rate variation across sites (GTR + I + Γ) and with all parameters unlinked across loci. Bayesian analyses included four independent runs with three heated chains and one cold chain in each run. The MCMC (Markov Chain Monte Carlo) chains were set for 3,000,000 generations and sampled every 100 generations; chains were run until the average standard deviation of the split frequencies dropped below 0.01. The burn-in period

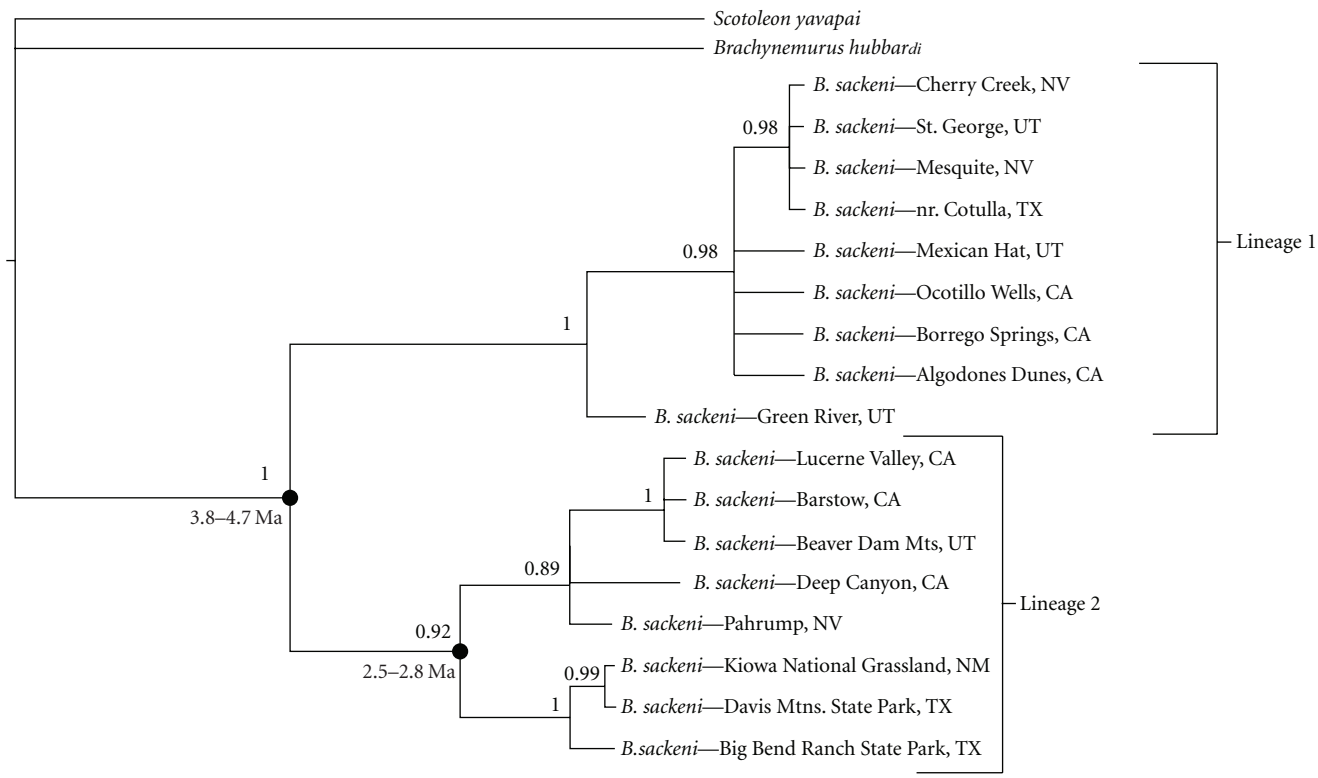

Figure 3: Consensus tree of the Bayesian analysis done on the aligned COI data set. Numbers at each node represent posterior probabilities. Collection locations for each *B. sackeni* specimen are given. Estimated divergence dates are given for two nodes marked with black circles.

of 3,000 samples was removed for each analysis after graphical determination of stationarity. *Brachynemurus hubbardi* Currie and *Scotoleon yavapai* (Currie) were included as outgroups, because they are closely related to *B. sackeni* [20].

We constructed a parsimony-based haplotype network using the aligned CO1 sequences for all *B. sackeni* specimens using TCS version 1.21 [30]. The program estimated the 95% reconnection limit between haplotypes with gaps treated as missing data.

3. Results

3.1. Phylogenetic, Haplotype Network Results. Sequences from the two intergenic regions (ITS1 and ITS2) were A/T rich (ITS1: A = 42.7%, T = 42%, C = 6%, G = 9.3%. ITS2: A = 42.1%, T = 42%, C = 8.1%, G = 7.8%). Because both of these regions were A/T rich, which can be problematic phylogenetic analyses, we did not use these sequences in the subsequent analyses.

Usable COI sequences were obtained from a total of 17 *B. sackeni* specimens that were collected from sites across the Nearctic deserts (Figure 2). A total of 679 bp of COI were sequenced. Bayesian analysis of the molecular data produced a tree that clearly shows two divergent *B. sackeni* lineages (Figure 3). The genetic distances between these two lineages are relatively high (8.7–10.9%). There is no clear biogeographic pattern between the two major lineages, but there is some geographic structuring within Lineage 2 (Figure 3). Lineage 2 is split into two subclades, one made up of populations from the Mojave and western Sonoran desert and the other from the Chihuahuan Desert and nearby areas. While some phylogenetic structuring is found within Lineage 1, there is no clear biogeographic pattern to these clades.

Haplotype Network analysis also shows that a large amount of genetic variation exists among populations of *B. sackeni*. A total of seven networks were formed based on the aligned COI dataset (Figure 4). Four of these networks are composed of single individuals. The populations associated with Lineage 1 formed a single network except for one individual from Green River, Utah, and one individual from Mexican Hat, UT, which were each placed in their own network. Lineage 2 was split into two monotypic networks and two networks composed of three populations each (Figure 4).

4. Discussion

Stange [20] suggested that *B. sackeni* is highly variable morphologically, and more detailed studies may lead to division of the species. Our analysis shows that this species is also highly variable genetically. Genetic distances, phylogenetic estimation, and haplotype networks suggest that *B. sackeni* should be split into two lineages (our Lineage 1 and Lineage 2: Figure 3) and is likely two different species. *Brachynemurus sackeni* has two synonyms, which were originally distinguished by color and size. Based on subsequent

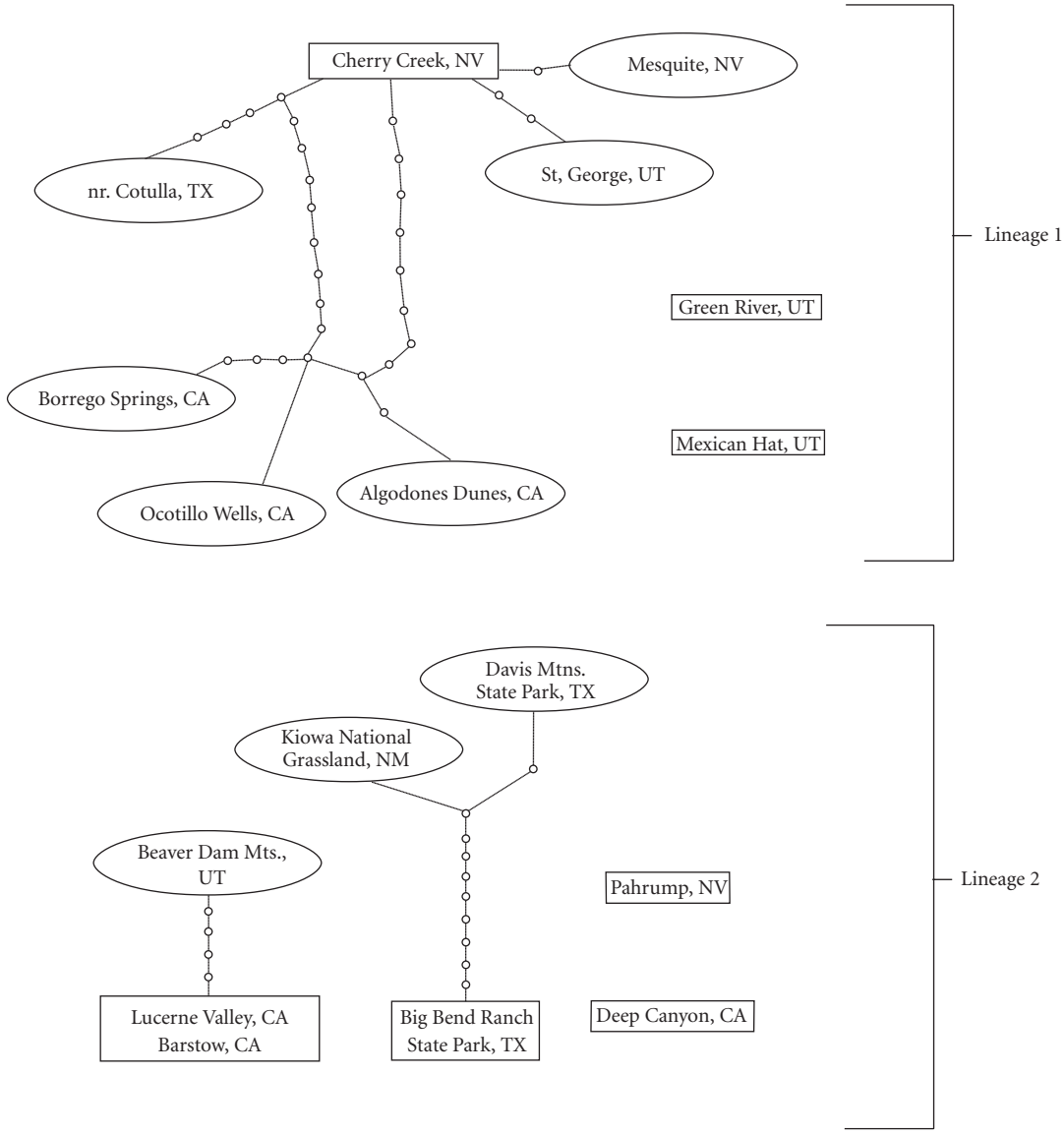

Figure 4: Haplotype networks based on the aligned COI data set. Populations are named based on their collection locality, and populations corresponding to Lineage 1 and Lineage 2 from Figure 3 are marked. Haplotypes surrounded by a rectangle were estimated to be the ancestral haplotype for network.

analysis of the holotypes, Stange [20] suggested that the coloration and size differences fit within the variation seen in *B. sackeni*. While our analyses suggest that *B. sackeni* is composed of multiple species, we agree with Stange [20] that color and size differences are variable and do not represent distinct species in this case because multiple color forms and sizes are present throughout both major lineages of our phylogeny. Detailed analysis of morphological characters, especially genetalia, may uncover morphological differences between the two *B. sackeni* species. Because these species are genetically distinct, yet cannot easily be distinguished at present based on the morphological characters presented in the literature, *B. sackeni* represents the first known cryptic species-complex in Myrmeleontidae.

While several of the analyses investigating the historical biogeography of the Nearctic deserts have described an east/west split in closely related species with sister species often being restricted to eastern (Chihuahuan) and western (Mojave and Sonoran) deserts [2–5, 7], our analysis did not uncover this pattern between the species in the *B. sackeni* cryptic species-complex. Instead we collected species 1 in the western Sonoran, Mojave, and Great Basin deserts as well as the Colorado Plateau and in southern Texas near the Chihuahuan Desert (Figure 2). Similarly, we collected species 2 in the western Sonoran, Mojave, and Chihuahuan deserts, as well as the high plains of northern New Mexico (Figure 2). While we do not see any biogeographical pattern among species in the *B. sackeni* cryptic species-complex, we do see

an east/west split among populations in species 2, with one lineage being found in the Mojave and western Sonoran Desert, and the other lineage being found in the Chihuahuan Desert and nearby areas (Figure 3). Patterns like this have been linked to both late Neogene mountain-building events [3–5, 7] and Pleistocene climate change [2, 18]. Divergence dates are needed in order to understand what processes led to diversification within the *B. sackeni* cryptic species-complex.

Using our COI sequence data and a global arthropod molecular clock estimate of 2.3% sequence divergence between lineages per million years [2, 31], we roughly dated the divergence time between species, and between major lineages in species 2. Divergence time estimates for the split between species suggest that the speciation event occurred around 3.8–4.7 million years ago. The split between major lineages in species 2 was estimated to be from 2.5–2.8 million years ago. Because of the wide range of dates that have been proposed for the uplift of the mountain ranges in western North America, divergence dates ranging from 2–15 million years could likely be associated with mountain-building events [32]. Therefore, even though no clear biogeographic pattern exists between species, the major divergences within this species-complex, and those divergences within species 2, may be linked to mountain building events in the late Neogene that caused the formation of many of the western deserts and drove diversification in numerous arid-adapted species.

Because no biogeographic pattern can be seen between species in the *B. sackeni* cryptic species-complex, it is likely that both vicariant events and dispersal events shaped the history of this group. Given that this analysis is based on a limited number of specimens, it must be viewed as preliminary. Future analyses examining more detailed phylogeographic patterns within this species-complex may uncover additional patterns of diversity that could aid in the understanding of the processes that led to diversification in the Nearctic deserts and arid lands.

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