The evolution and age of populations of *Scaphinotus petersi* Roeschke on Arizona Sky Islands (Coleoptera, Carabidae, Cychrini)

Karen Ober, Brian Matthews, Abigail Ferrieri, Sonia Kuhn

*Department of Biology, College of the Holy Cross, Worcester, MA 01610*

**Corresponding author:** Karen Ober (kober@holycross.edu)

**Academic editor:** T. Erwin | Received 7 September 2011 | Accepted 20 September 2011 | Published 16 November 2011

**Citation:** Ober K, Matthews B, Ferrieri A, Kuhn S (2011) The evolution and age of populations of *Scaphinotus petersi* Roeschke on Arizona Sky Islands (Coleoptera, Carabidae, Cychrini). In: Erwin T (Ed) Proceedings of a symposium honoring the careers of Ross and Joyce Bell and their contributions to scientific work. Burlington, Vermont, 12–15 June 2010. ZooKeys 147: 183–197. doi: 10.3897/zookeys.147.2024

**Abstract**

Populations of the ground beetle *Scaphinotus petersi* are isolated in subalpine conifer forest habitats on mountain ranges or Sky Islands in southeastern Arizona. Previous work on this species has suggested these populations have been isolated since the last post-glacial maximum times as warming caused this cool adapted species to retreat to high elevations. To test this hypothesis, we inferred the phylogeny from mitochondrial DNA sequence data from several Arizona Sky Island populations of *S. petersi* and estimated the divergence time of the currently isolated populations. We found two major clades of *S. petersi*, an eastern clade and a western group. Our results indicated most mountain ranges form clades except the Huachucas, which are polyphyletic and the Santa Catalinas, which are paraphyletic. We estimated the Pinaleño population is much older than the last glacial maximum, but the Huachuca and Pinal populations may have been fragmented from the Santa Catalina population since the post-glacial maximum times.

**Keywords**
carabid ground beetles, divergence dates, phylogeography

**Introduction**

Carabidae (ground beetle family) is one of the larger families of insects with approximately 40,000 described species (Lorenz 2005). The snail-eating beetles of the genus
Scaphinotus belong to the carabid tribe Cychrini. Cychrines consist of about 150 species in four genera and are restricted to the Northern Hemisphere; the Cychrini genus Scaphinotus, found only in North America, began its initial radiation about 35 million years ago (Osawa et al. 2004, Scudder 1900) into 55 species (Lorenz, 2005). Scaphinotus petersi is a large ground beetle confined exclusively to moist coniferous forests that occur in southern Arizona at elevations > 1800 m. Scaphinotus petersi is a specialist predator of land snails, using elongated and narrow mouthparts to penetrate and extract the soft parts of terrestrial snails (Digweed 1993, LaRochelle 1972). Scaphinotus petersi, like other Scaphinotus, is flightless, with reduced or absent flight wings under fused elytra, and thus a poor disperser. Six subspecies of S. petersi have been described (Ball 1966), and geographical variation among subspecies includes differences in size, head and neck characteristics, leg differences and color variation. All six S. petersi subspecies live only on mountains in the sub-Mogollon area of Arizona, a region known as the Sky Islands.

The Sky Islands (Heald 1951), also called the Madrean Archipelago, are a unique complex of mountain ranges and ecosystems in southeastern Arizona. At present, hot, dry, desert grasslands and desert scrub in the valleys (the sea between the Sky Islands) act as barriers to the movement of upland forest species such as S. petersi much as saltwater seas isolate flora and fauna on oceanic islands. As with oceanic islands, this separation of habitat limits genetic interchange between populations and creates environments with high evolutionary potential. The resulting Sky Island ecosystems, renowned for their biodiversity (Lomolino et al. 1989), support a high number of endemic species, including many threatened and endangered species, and are considered a biodiversity hot spot (Spector 2002). The Sky Islands are a natural laboratory in which to examine genetic differentiation and the evolutionary dynamics of vicariance. Mesic refuges, such as those in southwest mountains, may have been important centers of diversification during periods of dry climate for carabid beetles (Noonan 1992). Today, several Sky Island mountain ranges each contain a unique subspecies of S. petersi.

The goal of this study was to infer the biogeographic history of S. petersi in southeastern Arizona and investigate how the paleoclimatic oscillations of Quaternary affected the distribution of populations in the Sky Islands. We present a preliminary genealogy of mitochondrial DNA (mtDNA) sequences and use these data to address questions about population structure of this species and examine the potential role of the Pleistocene climate changes in the differentiation some of the Sky Island populations of S. petersi.

**Methods**

**DNA sequence data**

We collected DNA sequence data from 45 specimens of four of the six subspecies of S. petersi in five localities in four mountain ranges (Table 1, Fig. 1). We included three outgroup species from the tribe Cychrini. One species of a related genus Sphaeroderus, and two other distantly related Scaphinotus species. Outgroup choices
**Table 1.** Specimens, collection localities, and GenBank numbers included in this study.

| Specimen                  | Collection locality                                                                 | Specimen number | COI GenBank  | ND1 GenBank |
|---------------------------|--------------------------------------------------------------------------------------|-----------------|-------------|-------------|
| *Sphaeroderus lecontei*   | MA: Worcester Co. Wachusett Reservoir 71.6849°W, 42.4048°N 120m elev.               | 001             | JN639333    | JN641890    |
| *Scaphinotus crenatus*    | CA: Kern Co., Silvia Rd. 37°29.789′N, 119°53.369′W                                  | 002             | JN639334    | JN641891    |
| *Scaphinotus sp.*         | CA: Kern Co. Hwy 49A 37°22.806′N, 119°43.879′W                                      | 030             | JN639335    | JN641892    |
| *Scaphinotus petersi*     | AZ: Graham Co., Pinaleño Mts., Columbine Corral Camp/Ash Creek 32.7065°N, 109.9131°W elev. 2904m | 040             | JN639336    | JN641893    |
| *Scaphinotus petersi*     | AZ: Graham Co., Pinaleño Mts., Ladybug Trail 32.6589°N, 109.8540°W elev. 2716m      | 041             | JN639337    | JN641894    |
| *Scaphinotus petersi*     | AZ: Graham Co., Pinaleño Mts., Columbine Corral Camp/Ash Creek 32.7065°N, 109.9131°W elev. 2904m | 075             | JN639369    | JN641926    |
| *Scaphinotus petersi*     | AZ: Graham Co., Pinaleño Mts., Columbine Corral Camp/Ash Creek 32.7065°N, 109.9131°W elev. 2904m | 076             | JN639370    | JN641927    |
| *Scaphinotus petersi*     | AZ: Graham Co., Pinaleño Mts., Columbine Corral Camp/Ash Creek 32.7065°N, 109.9131°W elev. 2904m | 077             | JN639371    | JN641928    |
| *Scaphinotus petersi*     | AZ: Graham Co., Pinaleño Mts., Columbine Corral Camp/Ash Creek 32.7065°N, 109.9131°W elev. 2904m | 078             | JN639372    | JN641929    |
| *Scaphinotus petersi*     | AZ: Graham Co., Pinaleño Mts., Columbine Corral Camp/Ash Creek 32.7065°N, 109.9131°W elev. 2904m | 079             | JN639373    | JN641930    |
| *Scaphinotus petersi*     | AZ: Cochise Co., Huachuca Mts., Carr Canyon Trail 31.4272°N, 110.3069°W elev. 2186m   | 044             | JN639340    | JN641897    |
| *Scaphinotus petersi*     | AZ: Cochise Co., Huachuca Mts., Carr Canyon Trail 31.4272°N, 110.3069°W elev. 2186m   | 045             | JN639341    | JN641898    |
| *Scaphinotus petersi*     | AZ: Cochise Co., Huachuca Mts., Carr Canyon Trail 31.4272°N, 110.3069°W elev. 2186m   | 046             | JN639342    | JN641899    |
| *Scaphinotus petersi*     | AZ: Cochise Co., Huachuca Mts., Carr Canyon Trail 31.4272°N, 110.3069°W elev. 2186m   | 047             | JN639343    | JN641900    |
| *Scaphinotus petersi*     | AZ: Cochise Co., Huachuca Mts., Carr Canyon Trail 31.4272°N, 110.3069°W elev. 2186m   | 048             | JN639344    | JN641901    |
| *Scaphinotus petersi*     | AZ: Cochise Co., Huachuca Mts., Carr Canyon Trail 31.4272°N, 110.3069°W elev. 2186m   | 049             | JN639345    | JN641902    |
| Specimen                  | Collection locality                      | Specimen number | COI GenBank | ND1 GenBank |
|---------------------------|------------------------------------------|-----------------|-------------|-------------|
| Scaphinotus petersi biedermanni | AZ: Cochise Co., Huachuca Mts., Carr Canyon Trail 31.4272°N, 110.3069°W elev. 2186m | 050             | JN639346    | JN641903    |
| Scaphinotus petersi biedermanni | AZ: Cochise Co., Huachuca Mts., Carr Canyon Trail 31.4272°N, 110.3069°W elev. 2186m | 051             | JN639347    | JN641904    |
| Scaphinotus petersi biedermanni | AZ: Cochise Co., Huachuca Mts., Carr Canyon Trail 31.4272°N, 110.3069°W elev. 2186m | 052             | JN639348    | JN641947    |
| Scaphinotus petersi biedermanni | AZ: Cochise Co., Huachuca Mts., Carr Canyon Trail 31.4272°N, 110.3069°W elev. 2186m | 073             | JN639367    | JN641924    |
| Scaphinotus petersi biedermanni | AZ: Cochise Co., Huachuca Mts., Carr Canyon Trail 31.4272°N, 110.3069°W elev. 2186m | 074             | JN639368    | JN641925    |
| Scaphinotus petersi catalinae | AZ: Pima Co., Santa Catalina Mts., Marshall Gulch 32.4279°N, 110.7052°W elev. 2432m | 042             | JN639338    | JN641895    |
| Scaphinotus petersi catalinae | AZ: Pima Co., Santa Catalina Mts., Marshall Gulch 32.4279°N, 110.7052°W elev. 2432m | 043             | JN639339    | JN641896    |
| Scaphinotus petersi catalinae | AZ: Pima Co., Santa Catalina Mts., Ski Valley 32.4507°N, 110.7789°W elev. 2499m | 053             | JN639348    | JN641905    |
| Scaphinotus petersi catalinae | AZ: Pima Co., Santa Catalina Mts., Ski Valley 32.4507°N, 110.7789°W elev. 2499m | 054             | JN639349    | JN641906    |
| Scaphinotus petersi catalinae | AZ: Pima Co., Santa Catalina Mts., Ski Valley 32.4507°N, 110.7789°W elev. 2499m | 055             | JN639350    | JN641907    |
| Scaphinotus petersi catalinae | AZ: Pima Co., Santa Catalina Mts., Ski Valley 32.4507°N, 110.7789°W elev. 2499m | 056             | JN639351    | JN641908    |
| Scaphinotus petersi catalinae | AZ: Pima Co., Santa Catalina Mts., Ski Valley 32.4507°N, 110.7789°W elev. 2499m | 058             | JN639352    | JN641909    |
| Scaphinotus petersi catalinae | AZ: Pima Co., Santa Catalina Mts., Ski Valley 32.4507°N, 110.7789°W elev. 2499m | 059             | JN639353    | JN641910    |
| Scaphinotus petersi catalinae | AZ: Pima Co., Santa Catalina Mts., Ski Valley 32.4507°N, 110.7789°W elev. 2499m | 060             | JN639354    | JN641911    |
| Scaphinotus petersi catalinae | AZ: Pima Co., Santa Catalina Mts., Ski Valley 32.4507°N, 110.7789°W elev. 2499m | 061             | JN639355    | JN641912    |
| Scaphinotus petersi catalinae | AZ: Pima Co., Santa Catalina Mts., Ski Valley 32.4507°N, 110.7789°W elev. 2499m | 062             | JN639356    | JN641913    |
| Specimen                               | Collection locality                              | Specimen number | COI GenBank   | ND1 GenBank   |
|----------------------------------------|--------------------------------------------------|-----------------|---------------|---------------|
| Scaphinotus petersi catalinae          | AZ: Pima Co., Santa Catalina Mts., Ski Valley 32.4507°N, 110.7789°W elev. 2499m | 063             | JN639357      | JN641914      |
| Scaphinotus petersi catalinae          | AZ: Pima Co., Santa Catalina Mts., Ski Valley 32.4507°N, 110.7789°W elev. 2499m | 064             | JN639358      | JN641915      |
| Scaphinotus petersi catalinae          | AZ: Pima Co., Santa Catalina Mts., Ski Valley 32.4507°N, 110.7789°W elev. 2499m | 065             | JN639359      | JN641916      |
| Scaphinotus petersi catalinae          | AZ: Pima Co., Santa Catalina Mts., Ski Valley 32.4507°N, 110.7789°W elev. 2499m | 066             | JN639360      | JN641917      |
| Scaphinotus petersi catalinae          | AZ: Pima Co., Santa Catalina Mts., Ski Valley 32.4507°N, 110.7789°W elev. 2499m | 067             | JN639361      | JN641918      |
| Scaphinotus petersi catalinae          | AZ: Pima Co., Santa Catalina Mts., Ski Valley 32.4507°N, 110.7789°W elev. 2499m | 068             | JN639362      | JN641919      |
| Scaphinotus petersi catalinae          | AZ: Pima Co., Santa Catalina Mts., Ski Valley 32.4507°N, 110.7789°W elev. 2499m | 069             | JN639363      | JN641920      |
| Scaphinotus petersi catalinae          | AZ: Pima Co., Santa Catalina Mts., Ski Valley 32.4507°N, 110.7789°W elev. 2499m | 070             | JN639364      | JN641921      |
| Scaphinotus petersi catalinae          | AZ: Pima Co., Santa Catalina Mts., Ski Valley 32.4507°N, 110.7789°W elev. 2499m | 071             | JN639365      | JN641922      |
| Scaphinotus petersi catalinae          | AZ: Pima Co., Santa Catalina Mts., Ski Valley 32.4507°N, 110.7789°W elev. 2499m | 072             | JN639366      | JN641923      |
| Scaphinotus petersi petersi           | AZ: Gila Co., Pinal Mts., Icehouse Canyon F1rail 198 33. 2925°N, 110.8311°W elev. 2302.5m | 081             | JN639375      | JN641932      |
| Scaphinotus petersi petersi           | AZ: Gila Co., Pinal Mts., Icehouse Canyon F1rail 198 33. 2925°N, 110.8311°W elev. 2302.5m | 082             | JN639376      | JN641933      |
| Scaphinotus petersi petersi           | AZ: Gila Co., Pinal Mts., Icehouse Canyon F1rail 198 33. 2925°N, 110.8311°W elev. 2302.5m | 083             | JN639377      | JN641934      |
| Scaphinotus petersi petersi           | AZ: Gila Co., Pinal Mts., Icehouse Canyon F1rail 198 33. 2925°N, 110.8311°W elev. 2302.5m | 084             | JN639378      | JN641935      |
| Scaphinotus petersi petersi           | AZ: Gila Co., Pinal Mts., Icehouse Canyon F1rail 198 33. 2925°N, 110.8311°W elev. 2302.5m | 085             | JN639379      | JN641936      |
| Scaphinotus petersi petersi           | AZ: Gila Co., Pinal Mts., Icehouse Canyon F1rail 198 33. 2925°N, 110.8311°W elev. 2302.5m | 086             | JN639333      | JN641890      |
Figure 1. Study location A. *S. petersi* distribution is circled area. Habitat above 1830m is shown in black and between 1500 and 1830m is shown in grey B. Shaded relief map of study area. Black dots denote sampling localities of *S. petersi* used in this study (see Table 1) abbreviated as follows: P, Pinal Mountains; SC, Santa Catalina Mountains; PN, Pinaleño Mountains; and H, Huachuca Mountains. Figure courtesy of Sara Mitchell.

were limited by material available for DNA analysis. Genomic DNA was extracted following the protocol outlined in Maddison et al. 1999. PCR reactions were performed using a modification of the procedure described in Maddison et al. 1999. Reactions used a 53–56°C annealing temperature. This procedure was used to amplify approximately 1200bp of ND1 and adjacent RNA genes, and either a 500 bp portion or 1400 bps of COI. Macrogen Inc. (Korea) carried out DNA sequencing using an Applied Biosystems ABI 3730 48-capillary DNA analyzer with Big Dye Terminator Technology according to the manufacturer’s protocols (Applied Biosystems). The primers used for PCR amplification and DNA sequencing is given in Table 2. DNA sequence data was visualized using the SEQUENCHER 3.0 software (Gene Codes Corp.). Sequences were easily aligned by eye using MACCLADE 4.06 (Maddison and Maddison 2005). Data matrices are available from the corresponding author. Voucher specimens are in KAO insect collection at the College of the Holy Cross, Worcester, MA.

Phylogenetic reconstruction

Phylogeographic patterns were examined by inferring phylogenetic relationships from mitochondrial sequence data from all specimens collected. The combined COI and ND1 data set (2678 characters) was partitioned in five unlinked subsets (COI pos 1 and 2, COI pos 3, ND1 pos 1 and 2, ND1 pos 3, mtRNA). Maximum likelihood
Table 2. Primers used for DNA amplification (PCR) and sequencing for the ND1 and COI mitochondrial genes.

| Gene                        | Primer        | Direction | Sequence 5′ to 3′                                      |
|-----------------------------|---------------|-----------|-------------------------------------------------------|
| Cytochrome Oxidase I (COI)  | SK            | Forward   | CGCTCTAGAACTAGTGGATCAANAAYCAYAARGAYATYG               |
|                             | Pat (L2-N-3014 (Simon et al. 1994)) | Reverse   | TCCAATGCACTAATCTGCCATATTA                             |
|                             | Ron (C1-J-1751 (Simon et al. 1994)) | Forward   | GGATCACCTGATAGCATAGCATTCCC                            |
|                             | Nancy (C1-N-2191 (Simon et al. 1994)) | Reverse   | CCCGGTAAATATAAATATAAACCCTTC                           |
| NADH1 dehydrogenase (ND1)   | ND1F          | Forward   | ACATGAATTGGAGCTCGACCAGT                              |
|                             | 16sR (LR-N-12866 (Simon et al. 1994)) | Reverse   | ACATGATCTGAGTTCAACCAGG                               |

models were selected using MODELTEST 3.7 (Posada 2005) and likelihood searches were completed using GARLI-PART 0.97 (Zwickl 2010) using a GTR+I+Γ model of evolution for each subset. Other search settings were default. The searches employed a heuristic search strategy and were repeated 20 times starting from random trees keeping only the tree with the best likelihood score. Support for the relationships found in these searches was evaluated by 200 replicate bootstrap analyses with two addition sequences per replicate.

Bayesian analyses were completed in MRBAYES 3.12 (Ronquist and Huelsenbeck 2003) using four runs of 10 million generations each. The same partition strategy and model of evolution as above was used. Each run used four separate chains, sampling every 1,000 generations. Independent runs were combined using LOGCOMBINER1.5.4 (Rambaut and Drummond 2010). For each analysis, the trees in a burn-in period were excluded (the first 25% of the runs), and the majority-rule consensus tree of the remaining trees was calculated by PAUP* (Swofford 2002) to determine Bayesian Posterior Probabilities of clades. The average standard deviation of split frequencies was below 0.01 and all parameters appeared to have reached stationarity.

Age estimates of populations

We inferred divergence dates of *S. petersi* populations using a Bayesian relaxed clock uncorrelated lognormal method in BEAST (Drummond and Rambaut 2007) for all data combined. We partitioned the combined data into the same five subsets as used in the phylogenetic analyses. We chose unlinked GTR+I+Γ models with four gamma
categories, a coalescent extended Bayesian skyline plot for the tree prior, and an uncorrelated lognormal relaxed clock model of rate variation estimated for each partition with a normal distribution and a mean for each gene based on the rates for each gene from Pons et al. (2010). We constrained all *S. petersi* to be monophyletic because it was clearly monophyletic in the maximum likelihood analyses and to simplify the BEAST analyses. After an initial period of fine-tuning the operators, two separate MCMC analyses were run for 20 million generations with parameters sampled every 1000 generations. Independent runs were combined using LOGCOMBINER 1.5.4 (Rambaut and Drummond 2010), and the first 30% of the generations from each run was discarded as burnin. Convergence of the chains was checked using TRACER 1.4 (Rambaut and Drummond 2007). The searches achieved adequate mixing as assessed by the high effective sampling size (ESS) values for all parameters of 100 or greater. Node ages and upper and lower bounds of the 95% highest posterior density interval for divergence times was calculated using TreeAnnotator 1.5.4 and visualized using FIGTREE 1.3.1 (Rambaut 2010).

**Results**

**Phylogenetic analyses**

Both maximum likelihood and Bayesian analyses of mtDNA found similar topologies. The best maximum likelihood tree (Fig. 2) had a log-likelihood score of -6033.6277, and the Bayesian analysis converged on a set of trees with a mean log-likelihood score of -5797.5. Within a monophyletic *S. petersi*, two well-supported major clades were identified, corresponding to geographic relationships between collection localities (Fig. 2) and spatially structured genetic variation at deep and shallow scales. A clade of *S. p. grahami* from the Pinaleño Mountains was clearly phylogenetically distinct from a western clade of *S. petersi* from the Santa Catalina, Huachuca, and Pinal Mountains. The Santa Catalina population (*S. p. catalinae*) was paraphyletic with respect to a clade of *S. p. petersi* from the Pinal Mountains and *S. p. biedermani* from the Huachuca Mountains. The *S. p. biedermani* population did not appear to be monophyletic with one specimen grouping with members of *S. p. catalinae* from the Santa Catalina Mountains (Fig. 2). The overall phylogenetic tree topology estimate from GARLI and MRBAYES was similar to the BEAST analyses (Fig. 3).

**Estimates of divergence times**

Divergence time estimates for mtDNA lineages from BEAST reveal a deep and complex history of diversification (Fig. 3 and Table 3). The *S. petersi grahami* population in the Pinaleño Mountains diverged from the western populations in this study approximately 95,200 years ago. The *S. p. petersi* population in the Pinal Mountains diverged from the Santa Catalina Mountain population approximately 11,000 years ago. More
Figure 2. Maximum likelihood tree of *S. petersi* populations from combined COI and ND1 data. Outgroups are removed to show greater detail. Specimen numbers are removed, but the mountain range from which they were collected is indicated. Support for branches is indicated by Bayesian Posterior Probability/Maximum Likelihood bootstrap values. Scale bar units are substitutions per site.

than one dispersal event from the Santa Catalinas to the Huachucas may have occurred about 8,900 years ago and also 7,400 years ago (Fig. 3 and Table 3).

Discussion

Phylogeography and genetic structure of *Scaphinotus petersi*

Our phylogenetic analyses indicated geographic and genetic structure within the *S. petersi*, and most clades corresponded to isolated mountain ranges. There was strong support for two major clades in this species; an eastern clade of *S. p. grahami* from the Pinaleño Mountains and a western clade of *S. p. petersi, S. p. catalinae*, and *S. p. biedermani* from the Pinal Mountains, Santa Catalina Mountains, and Huachuca Mountains, respectively. While it appears the Pinaleño clade is reproductively isolated from the rest of *S. petersi*, caution must be taken in interpreting genealogy patterns from mitochondrial data only, as it is a single locus and represents the maternal lineage only. The phylogenetic analyses suggested the Santa Catalina population is paraphyletic with respect
Figure 3. Phylogeny of *S. petersi* dated using a Bayesian relaxed molecular clock in BEAST. Outgroups are removed to show greater detail. Specimen numbers are removed, but the mountain range from which they were collected is indicated. Branches are proportional to time in thousands of years. Shading indicates the two most recent glacial maxima. 95% confidence intervals for the ages of major clades in the tree are indicated with blue bars. The capital letters indicate population fragmentation between mountain ranges (see Table 3).

to the Pinal and Huachuca populations that were derived from independent dispersal events from the Santa Catalinas. The history of the Huachuca population shows two relatively recent dispersal events from the Santa Catalinas to the Huachucas indicating there may have been suitable habitat in the past for low elevation Santa Catalina populations to migrate to the Huachucas. Based on morphological data, Ball (1966) suggested the Pinaleño population is fairly derived and experienced the earliest relative divergence from other *S. petersi*, and that later, lower elevation Santa Catalina populations may have given rise to *S. p. petersi* and *S. p. biedermani* based on the pronotum and body size. Trees inferred from molecular data were in agreement with this early hypothesis.

In this study we sampled only four of the six subspecies of *S. petersi*, and only a few of the known populations of *S. p. petersi*, *S. p. biedermani*, and *S. p. grahami*. Future work will include the additional subspecies and populations for a fuller picture of *S. petersi* evolution and biogeography. We predict, with the inclusion of these samples, the phylogeography of *S. petersi* subspecies will follow, in large part, Ball’s (1966) hypotheses of relationships based on morphological characteristics. Ball (1966) suggested the *S. p.*
Sky Island Scaphinotus petersi

Table 3. Ages of selected nodes estimated from molecular data in *Scaphinotus petersi* phylogeny from BEAST analysis. Letters correspond to nodes in Fig. 3.

| Node | Split between populations          | Age in years | 95% C.I. age in years |
|------|-----------------------------------|--------------|-----------------------|
| A    | Pinaleño vs western populations   | 95,200       | 8,000–225,000         |
| B    | Huachuca vs Catalina 1            | 7,400        | 1,200–18,500          |
| C    | Huachuca vs Catalina 2            | 8,900        | 1,500–21,300          |
| D    | Catalina vs Pinal                 | 11,200       | 1,800–28,200          |

grahami from the Pinaleño Mountains shared traits with *S. p. kathleenae* from the Santa Rita Mountains and *S. p. corvus* from the Chiricahua Mountains. Thus we would predict these three subspecies form a clade even though the Santa Rita Mountains are more geographically close to the Huachuca Mountains where *S. p. biedermani* are found. Based on morphological similarity, Ball (1966) hypothesized *S. petersi* in the Rincon Mountains are closely related to those in the Huachuca Mountains, however, based on the amount of dispersal from the Santa Catalina Mountains to neighboring mountain ranges and the amount of morphological variation Ball (1966) found there, we predict the population in the Rincon Mountains may be more closely related to a lineage of *S. p. catalinae* instead of other *S. p. biedermani* found in the Huachuca Mountains.

The distribution of genetic diversity in *S. petersi* is structured across southeastern Arizona, indicating extrinsic barriers to gene flow are probably responsible for phylogeographic structure. It appears that a historical corridor of shared, linked habitat existed along a north – south ridge in the Western clade of *S. petersi* enabling dispersal from the Santa Catalinas to the Huachuca and Pinal Mountains. This north – south ridge of connectivity pattern in biogeography has been seen in other Sky Island arthropods (Maddison and McMahon 2000, Smith and Farrell 2005a). Future phylogeographic studies will include additional populations of *S. petersi* from Eastern and Western clades as well as closely related species in Arizona and New Mexico to further investigate the role geographic barriers have played in population isolation.

**Divergence time of isolated populations**

The divergence time estimates suggested the Pinaleño population (*S. p. grahami*) is considerably older than the end of the last glacial period, perhaps indicating that this population was isolated during previous interglacial events in the Pliocene and persisted during Pleistocene glaciations. The western populations of *S. p. petersi* from the Pinals and *S. p. biedermani* from the Huachucas have more recent divergence times, indicating that these areas were more recently isolated, perhaps since the end of the last glacial maximum (LGM). It is important to note that the error bars for the time estimates of nodes are large, making it difficult to pinpoint with certainty divergence dates and the impact particular changes in climate have had on population isolation. Additional loci could reduce variation in estimated time to coalescence.
Ball (1966) suggested that all subspecies of *S. petersi* could have evolved within the time span of the classical Wisconsin stage and Holocene. He hypothesized that during the pluvial stages of the Pleistocene, the montane coniferous forests occurred in the lowlands, probably along watercourses, and *S. petersi* dispersal took place. In subsequent pluvial stages, range expansion of populations could have led to contact between previously isolated lineages. The results from our current molecular study are in concordance with this original hypothesis. During interglacial periods, contact between neighboring lineages of *S. petersi* probably occurred in low elevation populations. These same populations were also probably the first to be extirpated during elevational shifts in habitat caused by post-glacial climate warming, leaving no signature of gene flow after the loss of these contact populations. Thus lineage boundaries like those between *S. p. grahami* in the Pinaleños and *S. p. catalinae* in the Santa Catalinas were maintained during glacial age population expansion and interglacial range retraction.

**Conclusions**

Several studies have focused on the biogeography of species on the Arizona Sky Island region including plants, arthropods, birds, lizards, and mammals (Downie 2004, Linhart and Permoli 1994, McCord 1994, Sullivan 1994, Slentz et al. 1999, Barber 1999a, b, Maddison and McMahon 2000, Masta 2000, Boyd 2002, Smith and Farrell 2005a, b, McCormack et al. 2008, Tennessen and Zamudio 2008). Most of these studies have shown significant morphological variation among populations and/or genetic structure in species on the Sky Islands. However, a biogeographic study of a galling insect (Downie 2004) and a study of squirrels (Lamb et al. 1997) failed to detect evidence for genetic divergence. Past climate changes have influenced the evolution of Sky Island species in very different ways. Phylogeographic studies in other arthropods such as spiders (Masta 2000), and beetles (Smith and Farrell 2005a, b) have tested hypotheses of divergence times among isolated populations. These studies suggest ancient divergence times among populations (more than one My), suggesting a much earlier vicariance event than the proposed post-LGM habitat fragmentation. Other studies of vertebrates (Sullivan 1994, Holycross and Douglas 2007, McCormack et al. 2008) suggest a more recent post-LGM effect on population genetic structure. In addition, concordant biogeographic patterns can be seen in populations of organisms distributed on the Sky Islands. Masta (2000), Boyd (2002), and McCormack et al. (2008) all reported a North-South mountain range relationship among populations with an East-West gap.

Both recent and more ancient global climate changes could be the causal mechanisms underlying the history of habitat fragmentation in *S. petersi*. Our results suggest *S. petersi* populations experienced a significant fragmentation into distinct eastern and western populations separated by the San Pedro River much earlier than the last glacial period. More recently, probably after the LGM, the western populations became more fragmented in the Pinal, Santa Catalina, and Huachuca Mountains. Future work will include more populations of *S. petersi* and closely related species from additional
mountain ranges, adding missing lineages. Additional nuclear genes will be included to provide a broader picture of genetic structure and a better estimate of divergence times. These efforts will help develop a general model for understanding the phylogeographic effects of climate change in Sky Island organisms.

Acknowledgements

The authors are deeply indebted to the work Ross and Joyce Bell have done on adephagan, rhysodine, and carabid systematics and natural history. It has formed the foundation of much of the work KAO has done on carabids, and it has truly inspired and facilitated her work in adephagan and carabid systematics. This paper is dedicated to the life and work of Ross and Joyce Bell. Some outgroup specimens were collected by Elizabeth Jockusch. The authors also thank the College of the Holy Cross, the Robert L. Ardizzone Faculty Excellence Fellowship, the Charles and Rosanna Batchelor Foundation Grant and the Richard B. Fisher Summer Research Fellowship for funding for this project. We thank Sean Devine and two anonymous reviewers for improvements to the manuscript.

References

Ball GE (1966) The taxonomy of the subgenus Scaphinotus Dejean with particular reference to the subspecies of Scaphinotus petersi Roeschke (Coleoptera: Carabidae: Cychrini). Transactions of the American Entomological Society 92: 687–722.
Barber PH (1999a) Patterns of gene flow and population genetic structure in the canyon treefrog, Hyla arenicolor (Cope). Molecular Ecology 8: 563–576. doi: 10.1046/j.1365-294x.1999.00594.x
Barber PH (1999b) Phylogeography of the canyon treefrog, Hyla arenicolor (Cope) based on mitochondrial DNA sequence data. Molecular Ecology 8:547–562. doi: 10.1046/j.1365-294x.1999.00593.x
Boyd AE (2002) Morphological analysis of Sky Island populations of Macromeria viridiflora (Boraginaceae). Systematic Botany 27: 116–126.
Cook JP, Youberg A, Pearthree PA, Onken JA, MacFarlane BJ, Haddad DE, Bigio ER, Kowler AL (2009) Mapping of Holocene river alluvium along the San Pedro River, Aravaipa Creek, and Babocomari River, Southeastern Arizona. A Report to the Adjudication and Technical Support Section Statewide Planning Division Arizona Department of Water Resources. Version 1.1 Arizona Geological Survey, Tucson, AZ 85701.
Digweed SC (1993) Selection of terrestrial gastropod prey by Cychrine and Pterostichine ground beetles (Coleoptera: Carabidae). Canadian Entomologist 125: 463–472. doi: 10.4039/Ent125463-3
Downie DA (2004) Phylogeography in a galling insect, grape phylloxera, Daktulosphaira vitifoliae (Phylloxeridae) in the fragmented habitat of the Southwest USA. Journal of Biogeography 31: 1759–1768. doi: 10.1111/j.1365-2699.2004.01075.x
Drummond AJ, Rambaut A (2007) BEAST: Bayesian evolutionary analysis by sampling trees. 
BMC Evolutionary Biology 7: 214. doi: 10.1186/1471-2148-7-214

Heald WF (1951) Sky Islands of Arizona. Natural History 60:56–63, 95–96.

Holycross AT, Douglas ME (2007) Geographic isolation, genetic divergence, and ecological 
on-exchangeability define conservation units in a threatened sky-island rattlesnake. Biological Conservation 134: 142–154. doi: 10.1016/j.biocon.2006.07.020

Lamb T, Jones TR, Wettstein PJ (1997) Evolutionary genetics and phylogeography of the tassel-
eared squirrels (Sciurus aberti). Journal of Mammalogy 78: 117–133. doi: 10.2307/1382645

LaRochelle A (1972) Notes on the food of Cychrini (Coleoptera: Carabidae). Great Lakes 
Entomologist 5: 81–83.

Linhart YB, Permoli AC (1994) Genetic variation in central and disjunct populations of Lilium parryi. Canadian Journal of Botany 72: 79–85. doi: 10.1139/b94-011

Lomolino MV, Brown JH, Davis R (1989) Island biogeography of montane forest mammals in 
the American Southwest. Ecology 70: 180–194. doi: 10.2307/1938425

Lorenz W (2005) Systematic list of extant ground beetles of the world (Insecta Coleoptera “Geadephaga”: Trachypachidae and Carabidae incl. Paussinae, Cicindelinae, Rhysodinae). 
Second edition. Published by the author, Hoermannstrasse 4, D-82327 Tutzing, Germany, 
i-iii, 1–530.

Maddison DR, Baker MD, Ober KA (1999) Phylogeny of carabid beetles as inferred from 
18S ribosomal DNA (Coleoptera: Carabidae). Systematic Entomology 24: 103–138. doi: 
10.1046/j.1365-3113.1999.00088.x

McCord RD (1994) Phylgoeny and biogeography of the land snail, Sonorella, in the Madrean 
Archipelago. In: DeBano LF, Ffolliott PF, Ortega-Rubio A et al. (Eds.) Biodiversity and 
Management of the Madrean Archipelago: The Sky Islands of Southwestern United States 
and Northwestern Mexico. US Department of Agriculture, Fort Collins, Colorado, 317– 
323.

McCormack JE, Bowen BS, Smith TB (2008) Integrating paleoecology and genetics of bird 
populations in two sky island archipelagos. BioMed Central Biology 6:28.

Noonan GR (1992) Biogeographic patterns of the montane Carabidae of North America north 
of Mexico (Coleoptera: Carabidae). In: Ball GB, Noonan GR, Stork NE (Eds.) The Biogeography of Ground Beetles of Mountains and Islands. Intercept, Andover UK, 1–41.

Osawa S, Su Z-H, Imura Y (2004) Molecular Phylogeny and Evolution of Carabid Ground 
Beetles. Springer-Verlag, New York, 191 pp. doi: 10.1007/978-4-431-53965-0

Pons J, Ribera I, Bertrandetit J, Balke M (2010) Nucleotide substitution rates for the full set 
of mitochondrial protein-coding genes in Coleoptera. Molecular Phylogenetics and Evolution. 56: 796–807. doi: 10.1016/j.ympev.2010.02.007
Posada D (2005) Modeltest: A tool to select the best-fit model of nucleotide substitution. Version 3.7. http://darwin.uvigo.es.

Rambaut A, Drummond AJ (2010) LogCombiner v1.5.4 MCMC Output Combiner. Institute of Evolutionary Biology, University of Edinburgh.

Rambaut A, Drummond AJ (2007) Tracer v1.4. http://beast.bio.ed.ac.uk/Tracer.

Rambaut A (2010) FigTree v1.3.1. Institute of Evolutionary Biology, University of Edinburgh.

Ronquist F, Huelsenbeck JP (2003) MRBAYES 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19: 1572–1574. doi: 10.1093/bioinformatics/btg180

Scudder SH (1900) Adephagous and clavicorn Coleoptera from Tertiary deposits at Florissant Colorado with descriptions of a few other forms and a systematic list of the non-rynchophorous Tertiary Coleoptera of North America. United States Geological Survey Professional Paper: 11–148.

Slentz S, Boyd AE, McDade LA (1999) Patterns of morphological differentiation among Madrean Sky Island populations of Castilleja austromontana (Scrophulariaceae). Madroño 46: 100–111.

Smith CI, Farrell BD (2005a) Phylogeography of the longhorn cactus beetle Moneilema appressum LeConte (Coleoptera: Cerambycidae): was the differentiation of the Madrean sky islands driven by Pleistocene climate changes? Molecular Ecology 14: 3049–3065. doi: 10.1111/j.1365-294X.2005.02647.x

Smith CI, Farrell BD (2005b) Range expansions in the flightless longhorn cactus beetles, Moneilema gigas and Moneilema armatum, in response to Pleistocene climate changes. Molecular Ecology 14: 1025–1044. doi: 10.1111/j.1365-294X.2005.02472.x

Spector S (2002) Biogeographic crossroads as priority areas for bio-diversity conservation. Conservation Biology 16: 1480–1487. doi: 10.1046/j.1523-1739.2002.00573.x

Sullivan RM (1994) Micro-evolutionary differentiation and biogeographic structure among coniferous forest populations of the Mexican woodrat (Neotoma mexicana) in the American Southwest: a test of the vicariance hypothesis. Journal of Biogeography 21: 369–389. doi: 10.2307/2845756

Swofford DL (2002) PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4.0b10. Sinauer Associates.

Tennessen JA, Zamudio KR (2008) Genetic differentiation among mountain island populations of the striped plateau lizard, Sceloporus virgatus (Squamata: Phrynosomatidae). Copeia 3: 558–564. doi: 10.1643/CG-06-038

Zwickl D (2010) GARLI-PART 0.97 Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion. https://www.nescent.org/wg_garli/Partition_testing_version#The_GARLI_partitioned_version.