



Morphological and molecular diversification of slender salamanders (Caudata: Plethodontidae: *Batrachoseps*) in the southern Sierra Nevada of California with descriptions of two new species

ELIZABETH L. JOCKUSCH¹, IÑIGO MARTÍNEZ-SOLANO^{1,2}, ROBERT W. HANSEN³, & DAVID B. WAKE⁴

¹Department of Ecology and Evolutionary Biology, 75 N. Eagleville Rd., U-3043, University of Connecticut, Storrs, CT 06269, USA. E-mail: elizabeth.jockusch@uconn.edu

²Instituto de Investigación en Recursos Cinegéticos (IREC) (CSIC-UCLM-JCCM), Ronda de Toledo, s/n 13005 Ciudad Real, Spain. E-mail: inigomsolano@irec.csic.es

³16333 Deer Path Lane, Clovis, CA 93619-9735, USA. E-mail: HerpReview@gmail.com

⁴Museum of Vertebrate Zoology, 3101 Valley Life Sciences Building, University of California, Berkeley, CA 94720-3160, USA. E-mail: wakelab@berkeley.edu

Abstract

Slender salamanders of the genus *Batrachoseps* achieve relatively high diversity in the Kern Canyon region at the southern end of the Sierra Nevada of California through high turnover of species with small geographic ranges. The status of several populations of *Batrachoseps* in this region is enigmatic, and both morphological and molecular data have suggested that some populations do not belong to any of the currently recognized species. Identification of species in this region is complicated by the apparent extinction of *Batrachoseps relictus* in the vicinity of its type locality in the Lower Kern River Canyon. Here we analyze a comprehensive morphological dataset to evaluate diversity in the Kern River Canyon region. We conclude that populations from Breckenridge Mountain are conspecific with *B. relictus*, while populations from north of the Kern River previously treated as *B. relictus* belong to an undescribed species. The morphological data also show the distinctiveness of populations from the Upper Kern River Canyon. Thus, we describe two new species, *Batrachoseps bramei* **sp. nov.** for populations from the Upper Kern River Canyon and *Batrachoseps altasierrae* **sp. nov.** for populations from the southern Sierra Nevada previously referred to *B. relictus*. *B. bramei* **sp. nov.** and *B. relictus* are members of the *nigriventris* group; *B. altasierrae* **sp. nov.** belongs to the group formerly called the *relictus* group, which we rename the *diabolicus* group. We conclude by presenting allozyme and mitochondrial DNA sequence data that support the distinctiveness of these newly described species and provide a hypothesis of relationships within the *nigriventris* group.

Key words: *Batrachoseps altasierrae* **sp. nov.**, *Batrachoseps bramei* **sp. nov.**, *Batrachoseps relictus*, Kern River Canyon, morphometrics, allozymes, cytochrome *b*

Introduction

Slender salamanders of the genus *Batrachoseps* (Caudata: Plethodontidae: Batrachosepini) are the most diverse clade of salamanders in western North America, with 19 currently recognized species (Stebbins & McGinnis in press). *Batrachoseps* is thought to be the sister taxon of the Neotropical salamander clade (Bolitoglossini, Vieites *et al.* 2011; Pyron & Wiens 2011) which contains about 45% of extant salamander diversity. *Batrachoseps* is widespread along the Pacific Coast of North America, with a nearly continuous range from the California/Oregon border south into Baja California, and some disjunct interior ranges from the Cascade Mountains of northern Oregon through the Sierra Nevada and Inyo mountain systems in California to the Sierra San Pedro Mártir near the southern end of its range in Baja California. This distribution is filled by five species groups (subgenus *Plethopsis* and the four species groups of the subgenus *Batrachoseps*: the *attenuatus* and *nigriventris* groups of Jockusch and Wake (2002), a group renamed herein but previously referred to as the *relictus* group (Jockusch & Wake 2002), and the *pacificus* group). Although sympatry is relatively limited, with at most two species reported from any site, high diversity is achieved in some regions through turnover of species over short geographic distances. The two areas

with the highest species diversity are the central Coast Ranges of California, where five species representing two species groups are found within 50 km of each other, and the Kern River Canyon region at the southern end of the Sierra Nevada, California (Fig. 1), where both subgenera and two species groups of subgenus *Batrachoseps* are found.

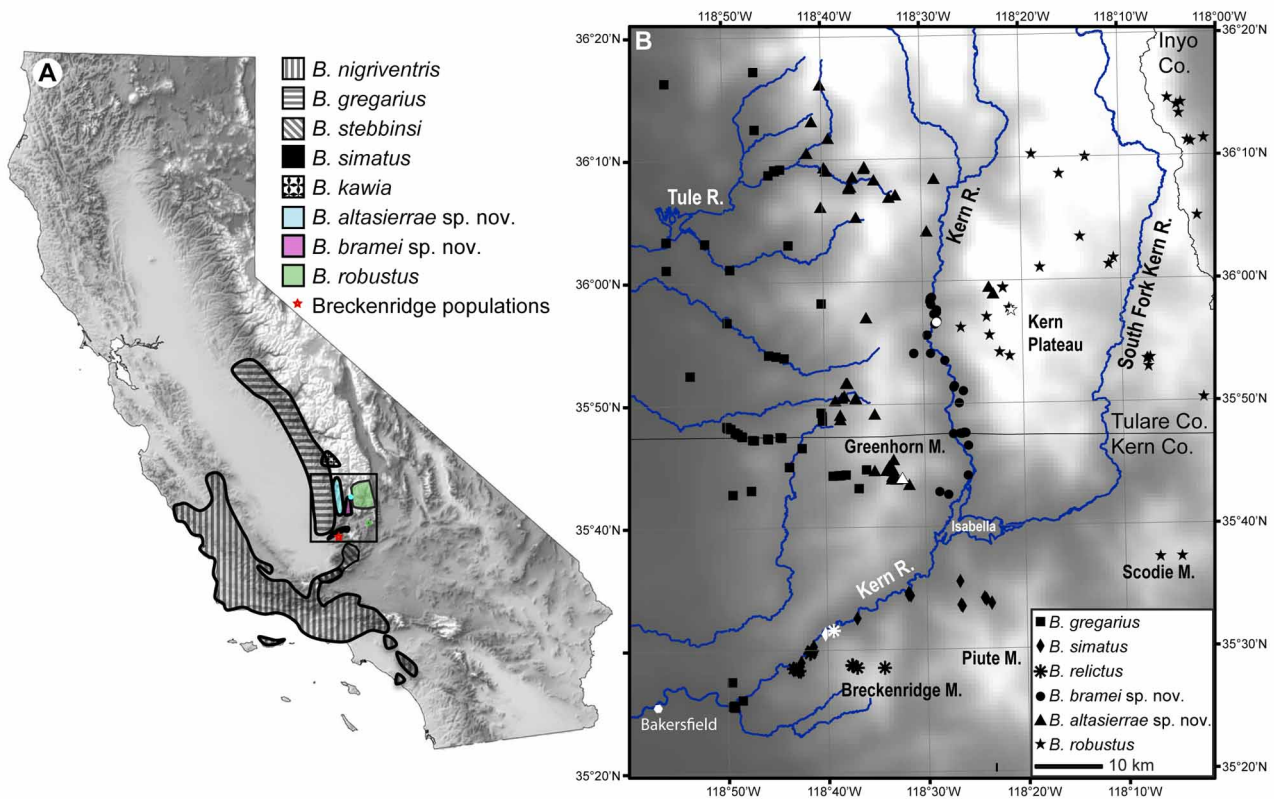


FIGURE 1. A. Map of California, USA, showing the ranges of *Batrachoseps nigriventris* group species and other species of *Batrachoseps* from the vicinity of the Kern River Canyon. The range of *B. relictus* in the Lower Kern River Canyon is contained within the range of *B. simatus*. Individual dots indicate isolated populations of *B. altasierrae* sp. nov. from the Kern Plateau (blue) and of *B. robustus* from the Scodie Mountains (green). B. Point localities for *Batrachoseps* from the Kern River Canyon and vicinity. Localities include all identified specimens from the collection of the Museum of Vertebrate Zoology, University of California, Berkeley, as well as additional specimens from the California Academy of Sciences and, for *B. relictus*, from the Los Angeles County (California) Museum. Lighter shading indicates higher elevation; blue indicates major streams or rivers. Type localities are indicated by open symbols for all species except *B. gregarius* (whose type locality is outside of the region shown). Refer to the web version of this article for color figure.

Two species, *B. relictus* Brame and Murray 1968 and *B. simatus* Brame and Murray 1968, have type localities in the Lower Kern River Canyon, and *B. gregarius* Jockusch, Wake and Yanev 1998 and *B. robustus* Wake, Yanev and Hansen 2002 are known from just outside the canyon (Fig. 1). The taxonomic identity of other populations from the area, including those in the Upper Kern River Canyon (Brame & Murray 1968; Hansen & Wake 2005a) and on Breckenridge Mountain (Jennings & Hayes 1994; Jockusch & Wake 2002), has been uncertain. Complicating identification of populations from the region was the apparent extinction of topotypic *B. relictus*, which has not been seen in the Lower Kern River Canyon since 1970 (this sighting was reported as 1971 in Jennings & Hayes 1994; Jockusch *et al.* 1998; Hansen & Wake 2005b) and is not represented in tissue collections or molecular datasets. Here we use multivariate morphometrics to show that populations from Breckenridge Mountain are most similar in morphology to topotypic *B. relictus*, and we refer these populations to that species. We also show that populations from the Upper Kern River Canyon are distinct in morphology and biochemical traits from their close relatives *B. gregarius* and *B. simatus*. We then describe two new species, one from the Upper Kern River Canyon and one for populations formerly treated as *B. relictus* from north of the Kern River, and present molecular data to confirm the distinctiveness of species in the southern Sierra Nevada.

Taxonomic history of *B. relictus*

Batrachoseps relictus was described by Brame and Murray (1968), who diagnosed the species primarily on the basis of a slightly more robust morphology compared to the widespread attenuate morph of *Batrachoseps*. The name *relictus* was derived from what Brame and Murray interpreted as a relict distribution, the result of extinctions that broke a once continuously distributed and widespread species into four geographically disjunct fragments: primarily higher elevations of the Sierra Nevada of California, USA; the central Coast Ranges, California, USA; Santa Cruz Island, California, USA; and the Sierra San Pedro Mártir, Baja California Norte, Mexico. The type locality for *B. relictus* is at the southern end of the Sierra Nevada in the Lower Kern River Canyon, 150 yards above the junction of State Hwy. 178 and the road turnoff to Democrat Hot Springs Resort (Brame & Murray 1968); additional populations extended along the river for about 16 km, and sympatry with *B. simatus* was found at several of these sites. Topotypic *B. relictus* have very short trunks and low vertebral counts, even in comparison to other robust populations referred to *B. relictus* (Brame & Murray 1968). With the advent of molecular data, Yanév (1980) showed that populations from separate geographic regions were not closely related to each other, a conclusion supported by subsequent research (Jockusch *et al.* 1998, 2001; Jockusch & Wake 2002; Fig. 2). Populations from central coastal California are now assigned to *B. luciae* Jockusch, Yanév and Wake 2001, *B. incognitus* Jockusch, Yanév and Wake 2001 and *B. minor* Jockusch, Yanév and Wake 2001; Santa Cruz Island populations are included in *B. nigriventris* Cope 1869 (Yanév 1978, 1980) and Mexican populations are included in *B. major* Camp 1915 (Wake & Jockusch 2000; Martínez-Solano *et al.* 2012). The fourth geographic unit, which ranges along the western Sierra Nevada from the Merced River to the Kern River, is currently called the *relictus* group (Yanév 1980; Jockusch & Wake 2002). High genetic divergence within this group led to the recognition of four species distributed without sympatry along the Sierra Nevada (Jockusch *et al.* 1998); the southernmost of these retained the name *B. relictus*, based on geographic proximity to the type locality. Thus, as treated most recently (Jockusch *et al.* 1998; Jockusch & Wake 2002), *B. relictus* included populations extending from the Tule River drainage to the Lower Kern River Canyon in the southern Sierra Nevada.

Genetic analyses confirm that samples currently treated as *B. relictus* from north of the Kern River are closely related to each other and are members of a single species; this species is also found on the Kern Plateau, east of the main branch of the Kern River (Jockusch & Wake 2002). However, in the absence of molecular data from *B. relictus* from the type locality or elsewhere in the Lower Kern River Canyon, it is unknown whether these more northern populations are conspecific with *B. relictus*. Populations of *B. relictus* from the Lower Kern River Canyon are morphologically distinct from more northern populations, differing in traits including vertebral number, number of maxillary teeth and some body proportions (Brame & Murray 1968; Jockusch *et al.* 1998). These populations are also differentiated in habitat, with populations north of the river occurring at mid- to high elevations in coniferous forests, while populations in the Lower Kern River Canyon were all found below 750 m in oak woodlands, though intimately associated with seepages and creeks (Brame & Murray 1968). Additionally, the Kern River serves as a biogeographic break in some species (including *B. simatus*, which is known only from the south side of the river). In essence, populations from north of the Kern River were retained in *B. relictus* by default.

Additional populations that are relevant to the identity of *B. relictus* have been discovered since the work of Brame and Murray (1968). These were found on Breckenridge Mountain (Fig. 1), a large, geographically isolated peak on the south side of the Lower Kern River. The first of these populations was discovered in 1979, high on the mountain. After allozyme analyses (see below) revealed its distinctiveness from all other genetically characterized populations, repeated searches of the area were undertaken on an almost annual basis; however, animals were not observed again until 2001. A second population was discovered in 2001, lower down on the north-facing slope of Breckenridge Mountain, along the upper reaches of Lucas Creek (which flows into the Kern River). This site is separated from the type locality of *B. relictus* by less than 12 km (although there is a substantial elevational difference of more than 900 m) and old samples of *B. relictus* from the Lower Kern River Canyon were reported from the major streams that enter the Kern River just upstream and downstream of Lucas Creek (Brame & Murray 1968). At both sites on Breckenridge Mountain, specimens were found only in close association with water, reminiscent of the “semiaquatic” habitat reported for *B. relictus* from the Lower Kern River Canyon (Brame & Murray 1968).

Molecular data (see below) show that the two Breckenridge Mountain populations are closely related to each other, but distantly related to all other populations, and allow us to confidently exclude them as members of any molecularly characterized species, including populations from north of the Kern River treated as *B. relictus* (e.g.,

Brame & Murray 1968; Jockusch *et al.* 1998), as well as nearby *B. simatus*. The Breckenridge populations belong to the *nigriventris* group, while populations from north of the Kern River are most closely related to *B. kawia* Jockusch, Wake and Yanev 1998, a member of what is currently called the *relictus* group (Jockusch & Wake 2002; Fig. 2). Attempts to amplify a fragment of *cob* from formalin-preserved *Batrachoseps* have been unsuccessful to date (B. Stuart, pers. comm.). Thus, while the high-elevation populations from opposite sides of the Kern River belong to distinct species, at least one of which is undescribed, molecular data do not resolve which, if any, is appropriately referred to *B. relictus*. To address this question, we used multivariate analyses of morphometric data.

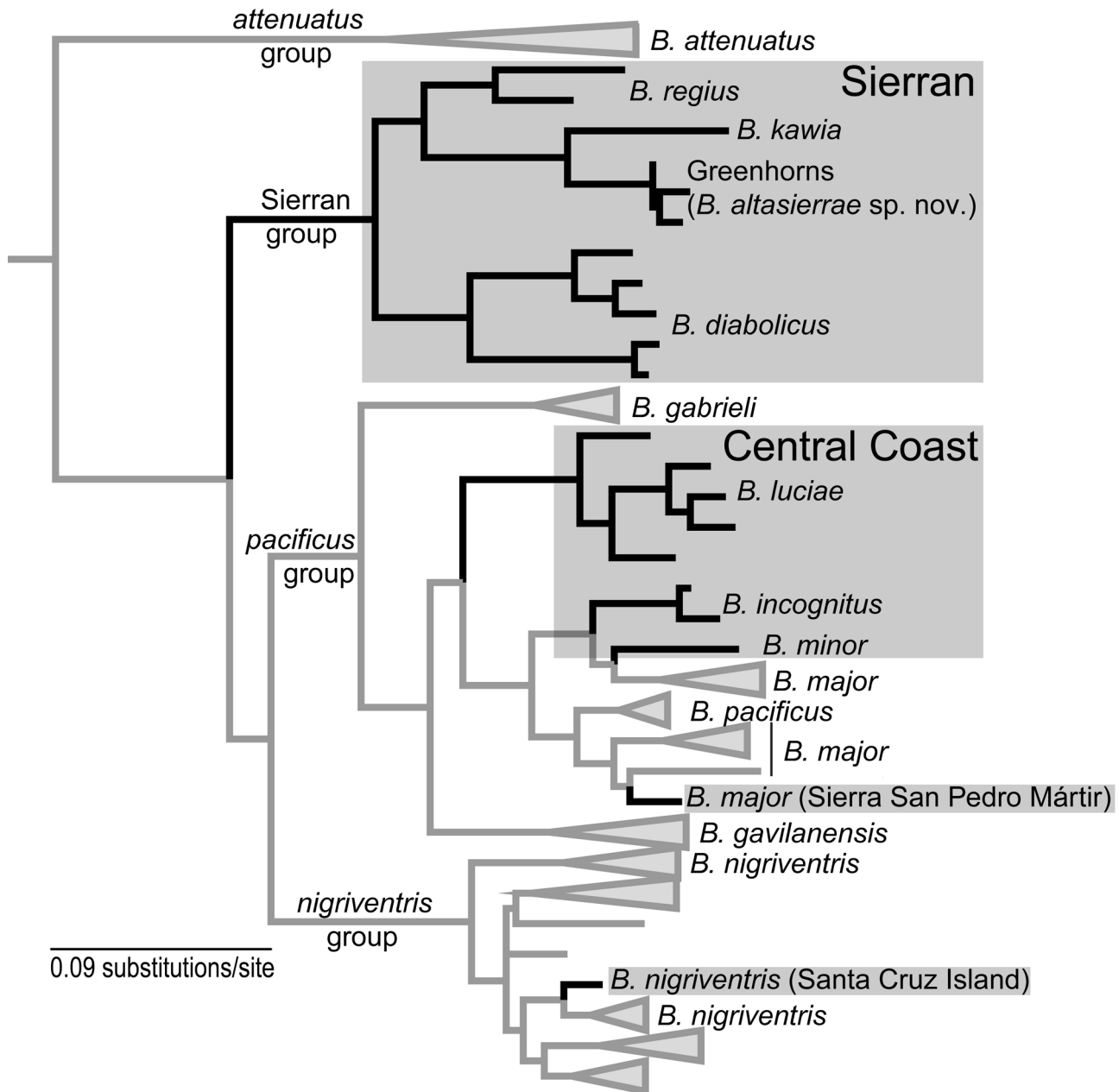


FIGURE 2. Overview of relationships in the subgenus *Batrachoseps*, based on *cob* dataset analyzed by Jockusch and Wake (2002). The four major clades are named (*attenuatus*, Sierran, *pacificus* and *nigriventris* groups) and terminals are labeled in all groups except the *nigriventris* group. Shaded boxes indicate the four geographic units of *B. relictus*, as recognized by Brame and Murray (1968). Terminal taxa that do not contain populations previously placed in *B. relictus* are collapsed. Note that topotypic *B. relictus* are not included in this tree. The clade labeled ‘Greenhorns’ has been retained in *B. relictus* until the present, but is described here as *B. altasierrae* sp. nov.

Analyses of morphology

Nine linear measurements (standard length, SL, which is the length from the tip of the snout to the posterior angle of the vent, head width, snout–gular fold length, axilla–groin length, chest width behind the forelimbs, forelimb length, hind limb length, maximum foot width and head depth behind the eyes) were taken from eight specimens of *B. relictus* from Cowflat Creek in the Lower Kern River Canyon (from the vicinity of the type locality; referred to below as topotypic *B. relictus*) and 527 additional specimens. These specimens represent all species in the subgenus *Batrachoseps* known to occur in and around the Kern River Canyon, and *B. kawia*, the closest relative of populations from the southern Sierra Nevada currently treated as belonging to *B. relictus* (Table 1). All measurements were taken by the same individual (IMS). To study variation in shape, residuals of the regression of each variable against SL were analyzed. Correlations between residuals were calculated, and the forelimb-length residual was excluded because of its high correlation ($R^2 = 0.90$) with the hind limb-length residual. Principal Component Analysis (PCA) of the correlation matrix of the remaining seven residuals, as implemented by the `prcomp` command in R (R Development Core Team 2008), was used to capture the main axes of morphological variation. Each PC was subjected to ANOVA, with species as fixed factors. For PCs showing significant differences, post-hoc Tukey HSD tests were then used to determine the probability that each pair of species differed. All statistical analyses were implemented in R v. 2.7.0–2.7.2 (R Development Core Team 2008). The 1st principal component (PC1), explaining 65.3% of the variance, is a measure of elongation, with axilla–groin length residuals loading positively and all other residuals loading negatively (Table 2). The 2nd and 3rd PCs accounted for 13.8% and 7.4% of the variance, respectively. ANOVA showed significant species effects for all PCs (Table 2).

These analyses were used to address the following questions. First, is *B. relictus* from vicinity of the type locality in the Lower Kern River Canyon more similar morphologically to Breckenridge Mountain *Batrachoseps* or to populations from north of the Kern River Canyon currently assigned to *B. relictus*? For this purpose, each of these three units was treated as a species. Second, how do populations from the Upper Kern River Canyon differ from their close relatives *B. simatus* and *B. gregarius*? Additionally, because samples from the Upper Kern River Canyon do not form a monophyletic group in the mtDNA tree (Jockusch & Wake 2002, and see below), we examined morphological variation between populations in this region by conducting ANOVA followed by Tukey HSD tests, treating populations as fixed factors. For these analyses, each of the four mitochondrial clades in the Upper Kern River Canyon was treated as a population.

Morphometric data show that *B. relictus* from the Kern River Canyon (topotypic *B. relictus*) is more similar to *Batrachoseps* from Breckenridge Mountain than it is to populations from north of the Kern River (Fig. 3). Topotypic *B. relictus* was not significantly different from *Batrachoseps* from Breckenridge Mountain at PC1 or PC2; both differed significantly from all species other than the Upper Kern River Canyon populations at PC1 and from all other species at PC2 (Fig. 3). Breckenridge Mountain *Batrachoseps* and topotypic *B. relictus* differed significantly at PC4 ($p=0.022$), and there was a suggestion of a difference in PC3 ($p=0.069$) and PC7 ($p=0.075$). Populations from north of the Kern River differed significantly from topotypic *B. relictus* in PCs 1, 2 and 4; however, they could not be distinguished from their sister group *B. kawia* using morphometric data ($p > 0.48$ for all PCs except PC5, for which $p = 0.058$). Because the average SL of topotypic *B. relictus* (30.2 ± 4.3 mm) was closer to that of populations from north of the Kern River (34.7 ± 4.3 mm) than to the SL of populations on Breckenridge Mountain (39.3 ± 5.0 mm), differences in body size cannot explain these patterns (Fig. 3).

Other traits that vary within and between species of *Batrachoseps* include the number of trunk vertebrae, a trait that is sexually dimorphic (Jockusch 1997), and tooth counts and arrangements. Comparisons of these traits show that topotypic *B. relictus* is more similar to *Batrachoseps* from Breckenridge Mountain than from the Greenhorn Mountains, north of the Kern River. Breckenridge Mountain *Batrachoseps* and topotypic *B. relictus* have similar numbers of maxillary teeth in females ($p=0.90$; Tukey HSD test), while Greenhorn Mountains *Batrachoseps* have fewer ($p<0.0004$; 44.9 ± 6.3 in Breckenridge; 46.5 ± 0.7 in topotypic *B. relictus*, 21.9 ± 3.3 in Greenhorns); the number of maxillary teeth did not differ significantly in males, which had small sample sizes (ANOVA; $F=2.15$; $p=0.15$). Plethodontid salamanders typically have a row of teeth on each vomer, extending from the body of the vomer out toward the end of the preorbital process. Teeth are added posteriorly and migrate onto the bone where they are retained until shed (Lawson *et al.* 1971). *Batrachoseps* differs from most other plethodontids in having a rudimentary (subgenus *Plethopsis*) or no (subgenus *Batrachoseps*) preorbital process (Wake 1989). As a consequence, the vomerine tooth row is truncated. In members of the subgenus *Batrachoseps* these teeth can be retained

longer in the tooth replacement cycle than in outgroup taxa. This leads to a "pile-up" of teeth in two or even three rows, giving the impression of a patch. The degree to which this occurs varies among species, and thus the teeth may be in a row, a semi-patch, or a patch. In topotypic *B. relictus* and most individuals from Breckenridge Mountain, vomerine teeth are arranged in a series, whereas in individuals from the Greenhorn Mountains they are typically arranged in patches. Topotypic *B. relictus* has the lowest numbers of trunk vertebrae recorded in the subgenus *Batrachoseps*, with a modal number of 17 in both males and females (Brame & Murray 1968). Vertebral counts as low as 17 (or costal groove counts of 16) occur at low frequency in both populations on Breckenridge Mountain, while *Batrachoseps* from the Greenhorn Mountains has a minimum of 18 trunk vertebrae.

TABLE 1. Summary of populations included in morphometric analyses. In most cases, samples were from the same locality or a very small geographic region; when they were more widespread, population designations were based on mtDNA sequences and latitude and longitude are listed for the locality from which the largest number of specimens was measured. All localities are in California, USA. Shading indicates populations that are currently treated as conspecific with *B. relictus* from Cowflat Creek (southern Sierra Nevada) or that are candidates for inclusion in *B. relictus* (Breckenridge).

Taxon	Locality	Lat (°N)	Long (°W)	N
<i>B. relictus</i>	Cowflat Creek, Kern Co.	35.51	118.69	8
Breckenridge	vicinity Squirrel Meadow, Kern Co.	35.48	118.57	8
Breckenridge	Lucas Creek, Kern Co.	35.48	118.62	14
southern Sierra Nevada	Greenhorn Mountains, N side of Kern River, Kern and Tulare counties	35.82	118.61	42
southern Sierra Nevada	Tule River, Tulare Co.	36.18	118.69	65
<i>B. simatus</i>	Kern River Canyon, below Lake Isabella, Kern Co.	35.47	118.72	18
<i>B. simatus</i>	Erskine Creek Canyon, Kern Co.	35.57	118.40	12
<i>B. gregarius</i>	Mariposa Co.	37.46	119.65	43
<i>B. gregarius</i>	Fresno Co.	36.82	119.34	38
<i>B. gregarius</i>	Kaweah River, Tulare Co.	36.39	118.88	60
<i>B. gregarius</i>	Tule River, Tulare Co.	36.02	118.82	34
<i>B. gregarius</i>	Arrastre Creek, Tulare Co.	35.79	118.74	40
Upper Kern	Cannell Creek, Kern and Tulare counties	35.79	118.42	24
Upper Kern	Fairview, Kern River Canyon, Tulare Co.	35.93	118.49	64
Upper Kern	South Falls Creek at Kern River, Tulare Co.	35.97	118.49	6
Upper Kern	Wofford Heights, Kern Co.	35.71	118.46	14
<i>B. kawia</i>	South Fork Kaweah River, Tulare Co.	36.38	118.86	42

Based on the results of the PCA and comparisons of additional characters, we conclude that populations of *Batrachoseps* from higher elevations on Breckenridge Mountain are conspecific with *B. relictus* and that populations of *Batrachoseps* from north of the Kern River previously included in *B. relictus* were inappropriately referred to that taxon. Taxonomically, there are two options for these latter populations: they could be subsumed into *B. kawia*, the sister group and geographically neighboring taxon to the north, or they could be described as a new species. Based on high genetic divergence (Yanev 1980; Jockusch *et al.* 1998; and see below) and evidence for an abrupt genetic transition between *B. kawia* in the Kaweah River drainage and more southern populations from the Tule River drainage and Greenhorn Mountains, below we describe these southern Sierran populations as a new species, *Batrachoseps altasierrae* **sp. nov.**

The PCA also highlights the distinctiveness of samples from the Upper Kern River Canyon (Fig. 3). Upper Kern River Canyon *Batrachoseps* is significantly more robust than all species (PC1; $p < 10^{-7}$) other than the two groups identified as *B. relictus* above ($p > 0.89$), as indicated by its PC1 score; it differs significantly from all other species in PC2 ($p < 5 \times 10^{-7}$). PC1 ($p < 10^{-7}$) and PC2 ($p < 10^{-7}$) are the only axes along which Upper Kern River Canyon *Batrachoseps* differs significantly from *B. gregarius*. A significant difference from its close relative *B. simatus* was

also detected along PC3 ($p=8.9 \times 10^{-6}$; Fig. 3). PC2 and PC7 ($p<0.002$) are the only axes along which a significant difference between Upper Kern River Canyon and Breckenridge Mountain populations was detected (Fig. 3). ANOVA of just the Upper Kern River Canyon PCs showed that there is variation across populations in PC2 ($F=7.29$, $p<0.0002$), PC3 ($F=8.11$, $p<0.0001$) and PC5 ($F=2.83$, $p=0.042$), with the Fairview population differing significantly from the Cannell Creek and Wofford Heights populations in PC2 and PC3. The small sample size from the South Falls Creek population ($N=6$) reduces the power to detect morphological differentiation of this population.

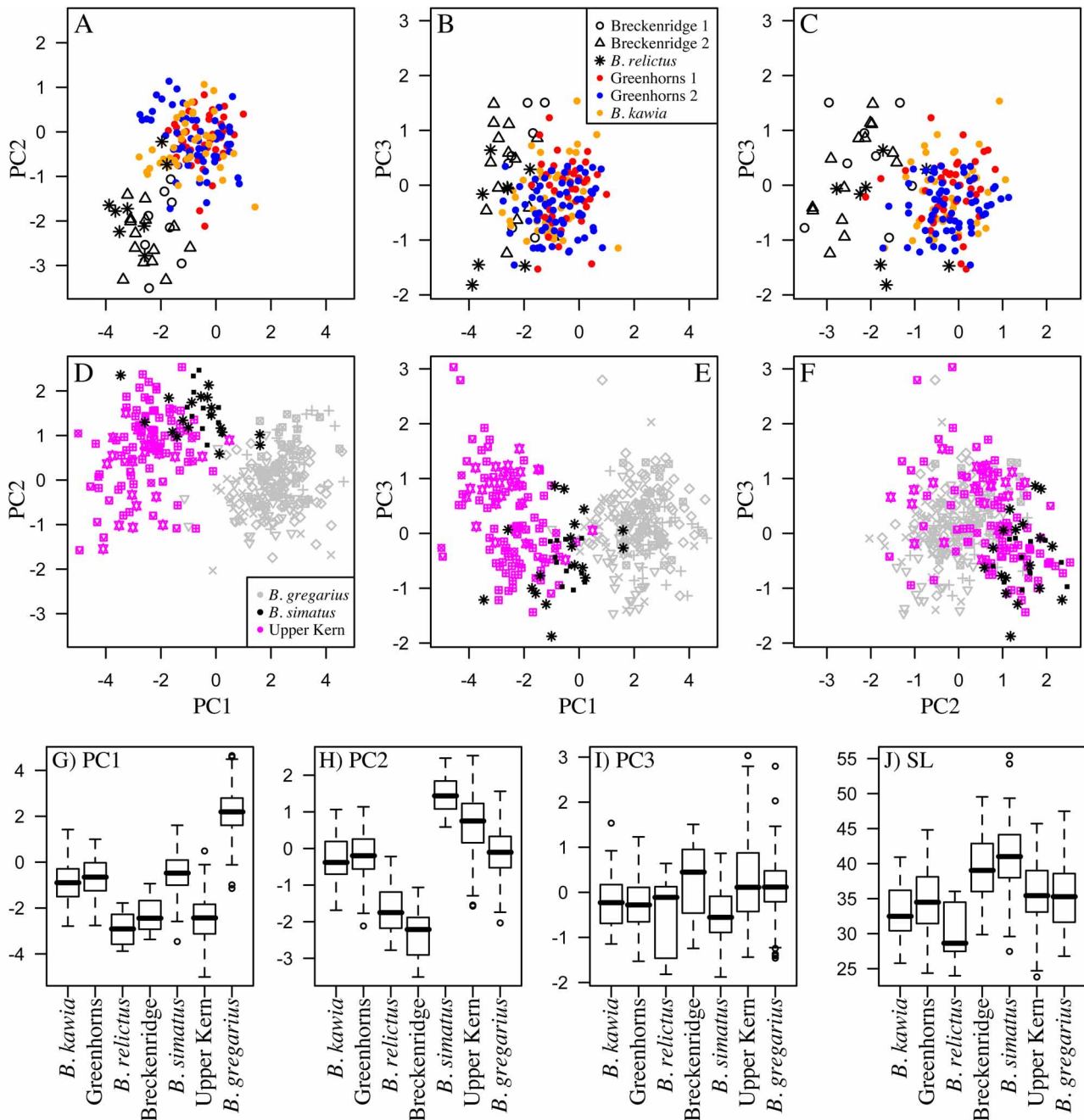


FIGURE 3. Morphological comparisons of *Batrachoseps* from the Kern River Canyon. A–F. Principal components analysis of correlation matrix of regression residuals of specimens from in and around the Kern River Canyon. Each color/symbol combination indicates a population. A–C compare *B. relictus* from the Lower Kern River Canyon, two candidates for inclusion in *B. relictus* (Breckenridge Mountain represented by the Lucas Creek and Squirrel Meadow populations and Greenhorn Mountains 1 and 2 from north of the Kern River), and *B. kawia*, which is the sister taxon to the Greenhorn populations. D–F show differentiation of the Upper Kern River Canyon populations (magenta), described here as *Batrachoseps bramei* sp. nov., from their close relatives *B. gregarius* (gray) and *B. simatus* (black). G–J. Boxplots of principal components (G–I) and SL (J) for species in and around Kern River Canyon. Refer to the web version of this article for color figure.

TABLE 2. Results of the principal components analysis of the correlation matrix of residuals of measurements regressed against standard length. The top row shows the percent of the total variance explained by each principal component (PC). The second row shows the results of the ANOVA testing for species effects (***) indicates $p < 10^{-10}$; ** indicates $p < 0.0015$; * indicates $p < 0.05$). The remaining rows show loadings of residuals on each PC. AG=axilla–groin length; SG=snout to gular fold length; HW=maximum head width; HD=head depth behind the eyes; CHW=chest width behind the forelimbs; HL=hind limb length; FW=maximum foot width.

	PC1 (65.3%)	PC2 (13.8%)	PC3 (7.4%)	PC4 (4.8%)	PC5 (3.3%)	PC6 (3.0%)	PC7 (2.5%)
F	363.3***	88.23***	11.29***	3.98**	2.36*	3.69**	4.98**
AG	0.364	-0.136	-0.739	0.493	-0.144	0.165	0.105
SG	-0.398	0.350	-0.026	-0.052	-0.620	0.545	0.186
HW	-0.422	-0.193	-0.096	0.259	-0.071	0.040	-0.838
HD	-0.285	-0.699	-0.321	-0.531	-0.099	-0.001	0.186
CHW	-0.370	-0.388	0.347	0.611	0.179	0.116	0.415
HL	-0.406	0.261	-0.256	0.140	-0.214	-0.773	0.198
FW	-0.384	0.338	-0.393	-0.106	0.709	0.252	0.081

Molecular data (Jockusch & Wake 2002 and see below) confirm the distinctiveness of the Upper Kern River Canyon populations relative to all other *Batrachoseps*. Here, we describe these populations as a new species.

Description of new species

Batrachoseps bramei new species

Suggested common name: Fairview Slender Salamander

Figures 4–5

B. simatus (part) Brame and Murray 1968:16

Holotype. Museum of Vertebrate Zoology (MVZ) 217944, an adult female from Packsaddle Canyon, adjacent to Kern River, 1137 m elevation, Tulare Co., California, USA (35.945°N, 118.476°W), collected 9 March 1991 by Robert W. Hansen.

Paratypes. MVZ 168749–168755, same locality as holotype, collected 10 March 1979 by Robert W. Hansen; MVZ 217945, same data as holotype; MVZ 168756–168763 and MVZ 168950 from Brin Canyon at junction Kern River, Tulare Co., California, USA (35.952°N, 118.471°W), collected 11 March 1979 by Robert W. Hansen; MVZ 168737–168738 and MVZ 168740 from Fairview, Tulare Co., California, USA (35.927°N, 118.494°W), collected 10 March 1979 by Robert W. Hansen.

Referred specimens. MVZ 107168–107169 from 3.6 km S Fairview, E side of Kern River, Tulare Co., California, USA (35.892°N, 118.464°W), collected 18 February 1973 by Samuel Sweet and Marjorie Reaka; MVZ 107170 (collected 18 February 1973 by Samuel Sweet), MVZ 168739, MVZ 168741–168748 (collected 10 March 1979 by Robert W. Hansen) all from Fairview, E side of Kern River, Tulare Co., California, USA (35.927°N, 118.494°W); MVZ 217946–217954 from Cannell Creek, S side, W of Aqueduct, E of Kern River, Kern and Tulare counties, California, USA (35.794°N, 118.434°W), collected 10 March 1991 by Robert W. Hansen; MVZ 224858–224859 from Upper Kern River Canyon at Mountain Highway 99 bridge over Kern River at confluence with South Falls Creek, E side of Kern River, Tulare Co., California, USA (35.968°N, 118.486°W), collected 27 March 1994 by Elizabeth L. Jockusch, David B. Wake and others; MVZ 267129–267137 from trail along W side of Kern River, ca. 0.8–1.0 km N Mountain Highway 99 bridge over Kern River at confluence with South Falls Creek, Tulare Co., California, USA (35.973°N, 118.488°W to 35.977°N, 118.487°W), collected 28 January 2010 by Elizabeth L. Jockusch, Elizabeth K. Timpe and Chris Evelyn; MVZ 267140–267142 from Tobias Creek drainage, along trail in vicinity of its crossing of Tobias Creek, Tulare Co., California, USA (35.902°N, 118.516°W), collected 22 March 2007 by Elizabeth L. Jockusch and Iñigo Martínez-Solano; MVZ 267154 from Whiskey Flat Trail, ca. 1 km N of Bull Run Creek, W side of Kern River, Tulare Co., California, USA (35.793°N, 118.452°W), collected 20 March

2008 by Iñigo Martínez-Solano and Elizabeth L. Jockusch; MVZ 267118–267122 from Plater Rd., just N junction Burlando Rd., Kern Co., California, USA (35.736°N, 118.428°W), collected 21 March 2008 by Elizabeth L. Jockusch and Iñigo Martínez-Solano; MVZ 251741–251749 from WNW of Wofford Heights, Tillie Creek drainage, Kern Co., California, USA (35.714°N, 118.476°W), collected 27 March 2005 by Brad Alexander.

Diagnosis. A small, slender species (standard length, SL, of ten adult males $34.3 \text{ mm} \pm 2.4 \text{ mm}$; of eleven adult females $35.5 \text{ mm} \pm 3.8 \text{ mm}$) distinguished from other species of the *B. nigriventris* group as follows: from *B. simatus* by smaller adult size, somewhat more robust appearance (including a longer, broader head, longer limbs and larger feet) and fewer trunk vertebrae (mode 18–19 versus 20–21 in *B. simatus*); from *B. relictus* by its longer legs and wider feet; from *B. gregarius* and *B. nigriventris* by its substantially more robust appearance including a longer and broader head, longer limbs, and larger hands and feet; from *B. stebbinsi* Brame and Murray 1968 by its much smaller size and less robust morphology. Distinguished from other species of *Batrachoseps* in the southern Sierra Nevada as follows: from *B. campi* Marlow, Brode and Wake 1979 and *B. robustus*, both members of subgenus *Plethopsis*, by its unpaired premaxillary bones and smaller, less robust habitus and from *B. kawia* by its more robust, longer-limbed morphology.

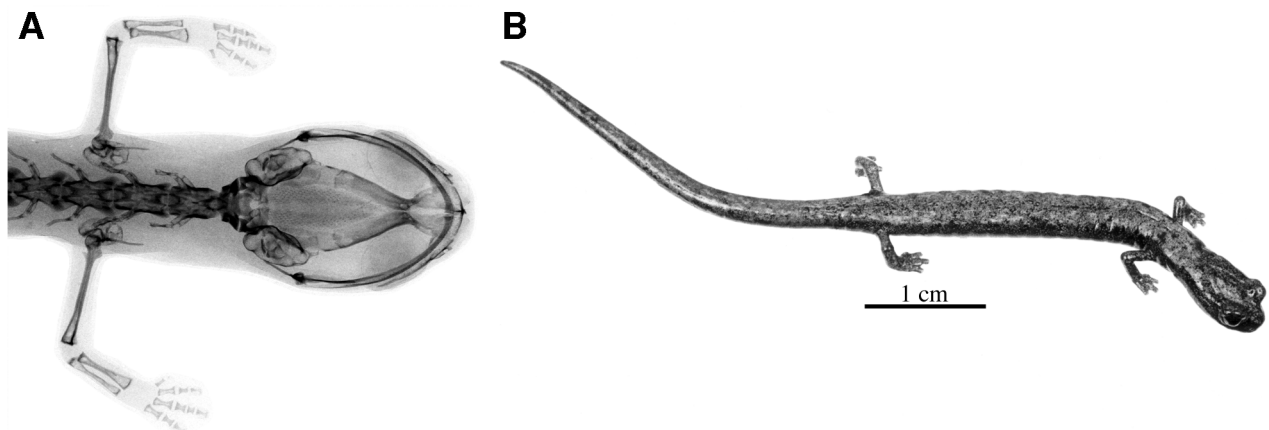


FIGURE 4. Holotype of *B. bramei* (MVZ 217944). A. Digital x-ray of skull and forelimbs. Digital x-rays were processed using the levels and curves functions in Adobe Photoshop. B. Photo of holotype in life.

Description. *Batrachoseps bramei* is a small (adults generally less than 45 mm standard length), slender species with a relatively broad, dorsoventrally flattened head and long limbs. The facial region (from the eyes to the snout) is relatively large and almost as broad as the posterior portion of the head; the eyes are moderately protuberant and visible projecting beyond the jaw line in ventral view. The nostrils are small and the nasolabial protuberances are slight to moderate. There is no evidence of a mental hedonic gland under the chins of males. Grooving patterns of the head, throat, and neck are typical of the genus. Standard length ranges from 7.2–8.1 (mean = 7.7) times head width in males and 7.5–9.9 (mean = 8.0) times head width in females. Teeth are relatively numerous: 6–11 (mean = 7.4) premaxillary teeth in males, 8–18 (mean = 13.7) in females; 31–49 (mean = 40.0) maxillary teeth in males, 32–68 (mean = 47.2) in females; 12–17 (mean = 14.8) vomerine teeth in males, 10–25 (mean = 17.9) in females. The vomerine teeth are arranged patchily to semi-patchily. Small maxillary teeth are borne in a long row extending about to the posterior end of the eyes in females. The premaxillary teeth are the same size as maxillary teeth in females; in males the premaxillary teeth are substantially enlarged. Both males and females have 17–18 costal grooves between the limb insertions. The tail is tapering and relatively short, exceeding body length only in the largest animals. The tail is 1.0–1.1 (mean = 1.0) times SL in males, and 0.9–1.1 (mean = 1.0) times in females in specimens lacking evidence of tail regeneration. There is no basal tail constriction. The postiliac gland is visible as an obscure pale spot. The limbs are relatively long; limb interval ranges from 4.5–6 (mean = 5.2) in males and 5–8 (mean = 5.9) in females. SL ranges from 4.5–6.0 (mean = 5.1) times hind limb length in males and 4.6–5.7 (mean = 5.0) times in females. The hands and feet are relatively large; foot width ranges from 1.7–2.2 mm (mean 2.0 mm) in both males and females. The digits are well formed and discrete with expanded tips that bear subterminal pads. Webbing occurs between the fingers and toes of some individuals, and extends to approximately the second phalanx. Fingers and toes in order of decreasing length are 3-2,4-1. Digit 1 is very reduced.



FIGURE 5. Color pattern variation in *B. bramei*. A. Specimen from the type locality; note the rust colored patches over the shoulders. The presence of these patches is typical of *B. bramei*. B. MVZ 267142, from Tobias Creek, Tulare Co., California. C–F. Four specimens from Cannell Creek, Kern and Tulare counties, California, showing range of coloration found in this population. The bold red coloring is not known from other populations. Refer to the web version of this article for color figure.

Measurements of the holotype (in millimeters). Maximum head width 4.1; snout to gular fold (head length) 8.3; head depth at posterior angle of jaw 1.9; eyelid length 2.5; eyelid width 1.2; anterior rim of orbit to snout 1.3; horizontal orbital diameter 1.7; interorbital distance 1.4; snout to forelimb 10.7; distance separating external nares 1.7; snout projection beyond mandible 0.5; snout to posterior angle of vent (SL) 40.7; snout to anterior angle of vent 37.3; axilla to groin length 23.4; tail length 33.5; tail width at base 1.9; tail depth at base 1.8; forelimb length 6.7; hind limb length 7.2; limb interval 8; width of right hand 1.7; width of right foot 2.2; foot length 2.9; length of third toe 0.8; body width behind forelimbs 2.2. There are 15 premaxillary, 46 maxillary, and 20 vomerine teeth; the row of maxillary teeth on the right appears short, ending at the posterior border of the internal naris. There are 18 costal grooves between the limb insertions.

Coloration of the holotype (in life). Uniformly flat black ventrally with delicate sprinkling of punctate guanophores; ventral guanophores are tiny and rarely connect; most numerous on gular region and at lowest density on tail; guanophores abundant on lateral surfaces, where they become expanded and form irregular patches. On lateral surfaces, guanophores cover more area than ground color. Dorsum metallic silver as a result of numerous expanded guanophores that are especially concentrated dorsolaterally; silvery coloration has metallic brassy-gold highlights; from a distance the silver on black background appears bluish-gray. Guanophores on head more disconnected and less concentrated, but the whole head, including eyelids and snout, is heavily spotted. Pigment most concentrated on nape of neck and front of limbs. Limbs heavily pigmented, especially proximally, but even fingers and toes have extensive spotting. Dark gray iris also has some dorsal guanophores. Only face region has light spotting. Ground color of dorsum slate black to brownish black. Under high magnification, it is clear that the lateral guanophores are more white and superficial, while the dorsal ones are deeper and more metallic. They co-occur on the dorsum, with the superficial cells rare. On the tail, the metallic color becomes gold or brassy and very concentrated.

Morphological variation. Males sampled from the northern end of the range have relatively small adult body size compared to populations from the southern end of the range (mean of 8 males from southern Wofford Heights 42.1 ± 3.6 mm vs. 33.8 ± 3.7 mm for 28 males from northern Packsaddle/Brin/Fairview). No significant differences in female body size across populations were detected; however, sample sizes were <10 for three of four populations from which series were measured. Comparisons between animals from Cannell Creek and the vicinity of the type locality show that chest width is slightly narrower in populations from near the type locality, which accounts for the significant differences in PC2 and PC3, described above. Wofford Heights individuals have insignificant webbing compared to the type series.

Coloration is highly variable both within and between populations (Fig. 5). Many specimens have distinct patches of color over the shoulders; these are usually coppery to gold in color, and do not fully fuse into the dorsolateral stripes. More northern individuals generally exhibit fairly subdued dorsal coloration, but individuals from Cannell Creek can be boldly marked and have reddish as well as metallic coloration.

Habitat and distribution. *Batrachoseps bramei* is known only from the Upper Kern River Canyon, and along the west side of the current Lake Isabella; the southern limit of its distribution is near the original junction of the main and South forks of the Kern River (Fig. 1). The southernmost samples are from Wofford Heights on the west side of the river and ca. 2 km S of the Cannell Creek drainage on the east side of the river. The range extends north at least as far as 1 km N of the confluence of South Falls Creek with the Kern River. Thus, known populations range for about 30 km from south to north. Areas farther north, which are accessible only by trail, have not been thoroughly surveyed; it is likely that the range extends farther north in the Upper Kern River Canyon. *B. bramei* occurs within an elevational range of 860–1280 m, one of the most restricted among all species of *Batrachoseps*. *B. bramei* has not been found in sympatry with any other species of *Batrachoseps*. It closely approaches *B. simatus* in the Lower Kern River Canyon (13 km between Wofford Heights *B. bramei* and Erskine Creek *B. simatus*; 20 km between Cannell Trail *B. bramei* and Erskine Creek *B. simatus*); however, these two species are separated by a major drainage (either the main or South Fork Kern River) as well as by xeric, inhospitable terrain around the confluence of the South Fork and Upper Kern River at Lake Isabella. *B. bramei* also closely approaches *B. robustus*, which has been collected within 7 km of the river, but at much higher elevation (2214 m), and *B. altasierrae* **sp. nov.** (see below).

The range of *B. bramei* on the east side of the Kern River lies entirely within a prominent uplifted ridge of metamorphic rocks paralleling the river. Local populations of salamanders are associated with north-facing slopes and talus. A chaparral plant community consisting of species of the genera *Ceanothus*, *Arctostaphylos*, *Ribes* and *Chrysothamnus*, as well as *Pinus sabiniana* and occasionally *Quercus chrysolepis*, characterizes this area. Most individuals have been found beneath rocks, often on or at the base of talus slopes (Fig. 6). However, specimens have been found in a variety of other habitats and under a variety of cover objects, including under a log in an open sandy flood plain, under logs and rocks in grasslands, in gravel on the river bank, and in leaf litter in protected groves (Fig. 6). Individuals have been observed active at night during winter and early spring and have been collected from beneath surface cover objects under snow. Salamanders have been found under surface cover at body temperatures (inferred from substrate temperatures) of 2.2–16.4°C (mean = 8.4°C, N = 101).

Etymology. Named in honor of Arden H. Brame, Jr., who, along with Keith Murray, was the first to recognize the distinctiveness of *Batrachoseps* in the Kern River Canyon. The species name is formed as a noun in the genitive.

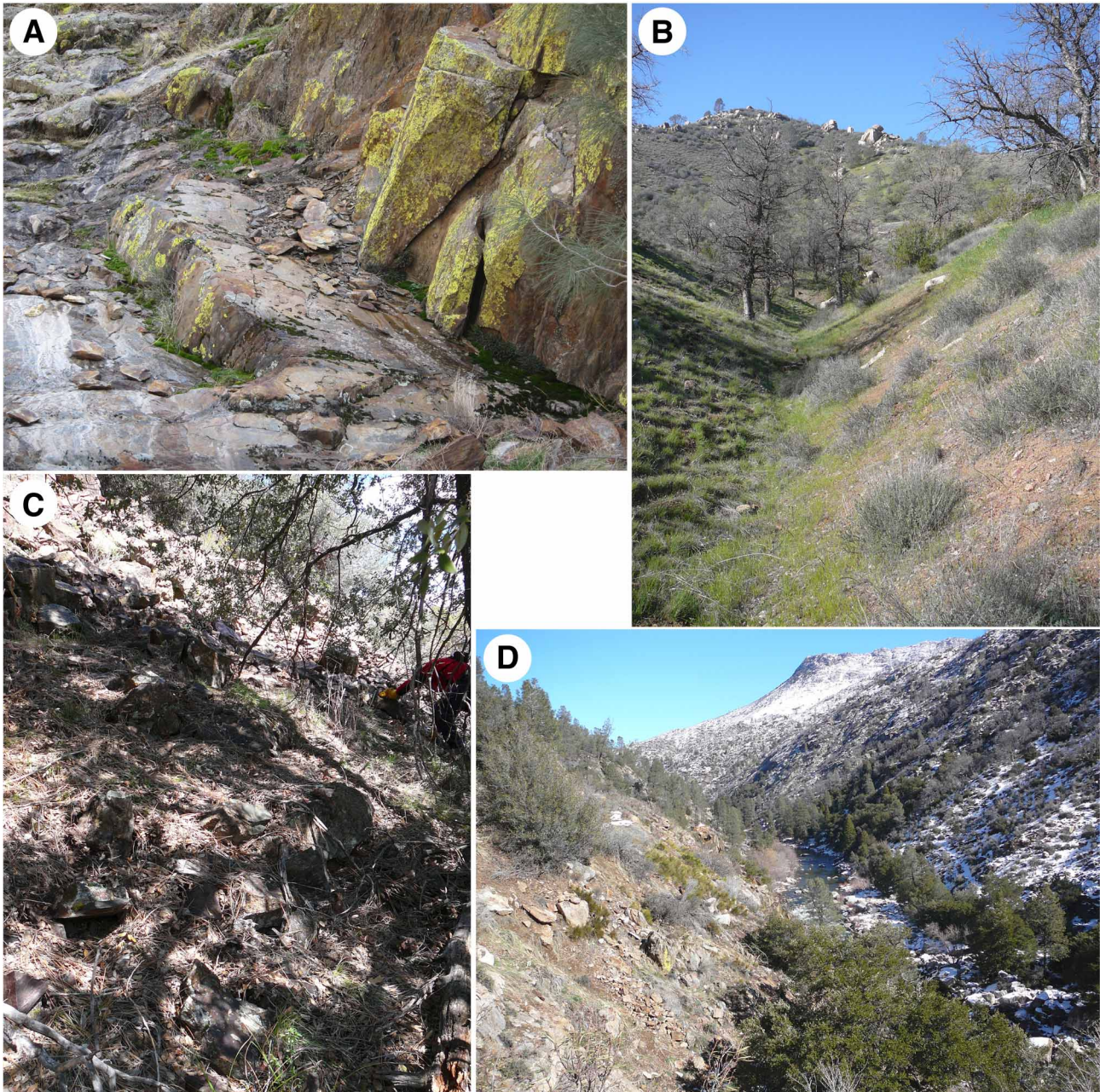


FIGURE 6. Habitats occupied by *B. bramei* in the Kern River Canyon. A. Type locality, Packsaddle Canyon, 1135 m elev., Tulare Co., California (photo taken 5 March 2011). On the east side of the Kern River, salamanders are often associated with metamorphic rock in seepage areas as shown here. B–D. On the west side of the Kern River, *B. bramei* has been found in a diversity of habitats including the following: B. under isolated cover objects in oak grassland S of Kernville, Kern Co., California (photo taken 21 March 2008); C. in pine litter along Tobias Creek, Tulare Co., California (photo taken 22 March 2007) and D. under rocks and in leaf litter N of South Falls Creek, Tulare Co., California (photo taken 28 January 2010; numerous individuals were found on this date). Refer to the web version of this article for color figure.

Comments. In their classic work on *Batrachoseps*, Brame and Murray (1968) referred animals from the Upper Kern River Canyon to *B. simatus*, but noted that they were morphologically different. Stebbins (1985, 2003) followed this taxonomy. Jockusch and Wake (2002) treated populations from the Upper Kern River Canyon, all included here in *B. bramei*, as undescribed lineages based on a phylogeny inferred from mitochondrial DNA sequence data, but the high genetic diversity within the region led them to raise questions about whether more than one species might be present. Jennings (2004) treated populations from the Upper Kern River Canyon as an undescribed species and provided a vernacular name “Fairview slender salamander.” Bartlett and Bartlett (2009) refer to the species as awaiting formal description but provide a short species account, a distribution map, and a color photograph and refer to it as the “Fairview Slender Salamander.”

Until 2004, it was believed that *Batrachoseps* from the canyon proper occurred only on the eastern and southern sides of the river. After specimens were found in the Tillie Creek drainage on the northwest side of the river, exploration intensified on that side, and specimens have now been found at almost all sites north of Tillie Creek that we have sampled with moderate effort (>4 person-hours of searching under good conditions).

Conservation. The range of *B. bramei* lies entirely within plant communities that naturally experience periodic fire. The use of heavy equipment for fire suppression has the potential to negatively impact salamander habitat. Road maintenance of Mountain Highway 99 (Kernville–Johnsontdale) should take into account the intimate proximity of some populations to road-edge habitat. Most known populations occur on public lands administered by Sequoia National Forest. Although *B. simatus* has become difficult to find and is listed as a Threatened Species by the State of California, *B. bramei* appears to be more abundant and can be found consistently throughout its range.

***Batrachoseps altasierrae* new species**

Suggested common name: Greenhorn Mountains Slender Salamander

Figure 7A–C

Batrachoseps attenuatus (part) Grinnell and Camp 1917:137

Batrachoseps attenuatus attenuatus (part) Dunn 1926:232

Batrachoseps relictus (part) Brame and Murray 1968:5

Batrachoseps pacificus relictus (part) Yanev 1980:535

Batrachoseps relictus (part) Jockusch, Wake and Yanev 1998:13

Holotype. Museum of Vertebrate Zoology (MVZ) 59909, an adult male from 1.5 mi [2.4 km] SE Alta Sierra [Greenhorn Mountains], Kern Co., California, USA (35.732°N, 118.538°W), collected on 26 June 1952 by Keith Murray.

Paratypes. MVZ 190953 and MVZ 190955, both from Hwy. 155, 0.85 km N (by road) Greenhorn Summit, Kern Co., California, USA (35.744°N, 118.557°W), collected 28 May 1984 by Robert W. Hansen; MVZ 190961–190963 and MVZ 190965–190967, all from Hwy. 155, 1 km ESE (by road) Greenhorn Summit, Kern Co., California, USA (35.735°N, 118.554°W), collected 24 June 1984 by Robert W. Hansen and D. C. Holland; MVZ 190976–190979 from Tiger Flat Rd. (=U.S. Forest Service Rd. 23S16), 0.7 km N (by road) junction Hwy. 155 at Greenhorn Summit, Kern Co., California, USA (35.744°N, 118.554°W), collected 24 June 1984 by Robert W. Hansen and D. C. Holland; MVZ 190983–190985 from Tiger Flat Rd. (=U.S. Forest Service Rd. 23S16), 0.85 km N (by road) junction Hwy. 155 at Greenhorn Summit, Kern Co., California, USA (35.745°N, 118.553°W), collected 24 June 1984 by Robert W. Hansen and D. C. Holland.

Referred specimens. Paratypes of *Batrachoseps relictus*: Los Angeles County Museum (LACM) 33082–33090 and MVZ 184906–184908 from 1.2 mi [1.9 km] S White River Camp Grounds, Tulare Co., California, USA (35.840°N, 118.649°W), collected 31 March 1957 by A. H. Brame, Jr. (MVZ 184906–8 are described as “3 cleared & stained specimens in D. B. Wake collection” by Brame and Murray (1968)); LACM 33091–33093 from 1 mi [1.6 km] above O’Quinn Meadow, Tulare Co., California, USA (35.863°N, 118.628°W), collected 31 March 1957 by L. Hughes and A. H. Brame, Jr.; LACM 33080–33081 from White River Camp Grounds (Lower Camp), Tulare Co., California, USA (35.845°N, 118.636°W), collected 31 March 1957 by A. H. Brame, Jr. Additional referred specimens: MVZ 156360–156365 from Quaking Aspen Meadows along Hwy. 190, South Fork of Middle Fork Tule River, Tulare Co., California, USA (36.118°N, 118.544°W); MVZ 156366–156392 from the upper reaches of Spear Creek along U.S. Forest Service Rd. 24S06, 6.9 km SE junction of 23S05 and 3.2 km NE Portuguese Pass, Tulare Co., California, USA (35.821°N, 118.583°W); MVZ 156393–156399 from White River drainage, at Sugarloaf Peak, U.S. Forest Service Rd. 23S05, 0.6 km N junction U.S. Forest Service Rd. 24S06, Tulare Co., California, USA (35.843°N, 118.614°W), all collected 29 July 1975 by Kay P. Yanev and Samuel S. Sweet; MVZ 224835 from road to Sugarloaf Village, 1.0 km SE Sugarloaf Village, Tulare Co., California, USA (35.820°N, 118.641°W), collected 26 March 1994 by Elizabeth L. Jockusch, David B. Wake and others; MVZ 158244 and MVZ 158226–158228 from 9.5 km E Cherry Hill Road on Sherman Pass Road, Tulare Co., California, USA (35.983°N, 118.381°W), collected 11 July 1980 and 28 July 1979, respectively, by Robert W. Hansen; MVZ 224810 from Hwy. 155, 9.0 km NW Lake Isabella and ca. 2.2 km SE Alta Sierra, Tulare Co., California, USA (35.724°N, 118.527°W), collected 27 March 1994 by Elizabeth L. Jockusch, David B. Wake and others.

Diagnosis. A small, relatively slender species (SL of 8 adult males $38.0 \text{ mm} \pm 2.4 \text{ mm}$; of 8 adult females $40.1 \text{ mm} \pm 3.5 \text{ mm}$) distinguished from other species of *Batrachoseps* in the southern Sierra Nevada and vicinity as follows: from *B. relictus* by a relatively longer trunk, a relatively narrower head, shorter limbs, and smaller feet, and by females having many fewer maxillary teeth; from *B. simatus* by its smaller adult body size with a relatively narrower head and chest and fewer trunk vertebrae (mode 18–19 versus 20–21 in *B. simatus*); from *B. bramei* by a relatively shorter and narrower head, longer trunk, shorter limbs, narrower feet and longer tail, and smaller number of maxillary and vomerine teeth; from *B. gregarius* by its more robust morphology including a relatively longer and broader head, shorter trunk, longer limbs, and larger hands and feet; from *B. stebbinsi* by its less robust morphology and much smaller size; from *B. campi* and *B. robustus* by its less robust morphology, much smaller size, different color pattern and unpaired premaxillary bone. Distinguished from its close relative *B. kawia* by having fewer maxillary teeth; from *B. regius* Jockusch, Wake and Yanev 1998 by having a relatively longer trunk and tail, shorter head, shorter limbs, smaller feet and fewer maxillary teeth; and from *B. diabolicus* Jockusch, Wake and Yanev 1998 by having fewer trunk vertebrae, a relatively narrower head, shorter limbs and smaller feet.

Description. *Batrachoseps altasierrae* is a small (adults less than 50 mm standard length), slender species with a relatively narrow head and short limbs. The facial region is relatively narrow, and the eyes are not generally protuberant enough to be seen in ventral view. Mental gland is not visible under the chin of males. Grooving patterns of the head, throat, and neck are typical of the genus. Standard length ranges from 8.4–9.5 (mean = 9.0) times head width in males and 8.2–9.9 (mean = 9.3) times head width in females. There are relatively few teeth, especially on the maxilla: 4–5 (mean = 4.8) premaxillary teeth in males, 4–12 (mean = 6.4) in females; 9–27 (mean = 17.4) maxillary teeth in males, 18–28 (mean = 21.9) in females; 6–13 (mean = 10.9) vomerine teeth in males, 9–16 (mean = 11.8) in females. Vomerine teeth are arranged somewhat patchily. Small maxillary teeth are borne in a long row extending about two thirds of the way through the eye in males and to the posterior end of the eye in females. In females, premaxillary teeth are the same size as maxillary teeth; in males, the premaxillary teeth are enlarged. Males and females both have 18–19 trunk vertebrae and 17–18 costal grooves between the limb insertions. The tail is long and fairly cylindrical, tapering at the tip. The tail is 1.3–1.5 (mean = 1.4) times SL in males and 1.1–1.6 (mean = 1.3) times in females in specimens lacking evidence of tail regeneration. There is no basal tail constriction. The postiliac gland is present. The limbs are relatively short in length, and limb interval ranges from 7–9.5 (mean = 8.3) in males and 7.5–9.5 (mean = 8.5) in females. SL ranges from 6.2–7.5 (mean = 6.8) times hind limb length in males and 6.3–7.8 (mean = 7.1) times in females. The hands and feet are relatively narrow; foot width ranges from 1.4–1.7 mm (mean 1.6 mm) in males and 1.4–2.0 mm (mean 1.7 mm) in females. The digits are short, well formed and discrete with expanded tips that bear subterminal pads. Webbing is insignificant. Fingers and toes in order of decreasing length are 3-2-4-1.

Measurements of the holotype (in millimeters). Maximum head width 4.9; snout to gular fold (head length) 7.4; head depth at posterior angle of jaw 2.7; eyelid length 2.2; eyelid width 1.2; anterior rim of orbit to snout 1.4; horizontal orbital diameter 1.8; interorbital distance 1.9; snout to forelimb 9.3; distance separating external nares 1.6; snout projection beyond mandible 0.5; snout to posterior angle of vent (SL) 42.5; snout to anterior angle of vent 36.9; axilla to groin length 25.6; tail tip broken after 33.5; tail width at base 3.3; tail depth at base 2.7; forelimb length 6.3; hind limb length 6.8; limb interval 9; width of right hand 1.2; width of right foot 1.6; foot length 2.1; length of third toe 0.9; body width behind forelimbs 2.9. There are 4 premaxillary, 27 maxillary, and 13 vomerine teeth; vomerine teeth are arranged somewhat patchily. There are 17 costal grooves between the limb insertions.

Coloration of the holotype (in alcohol). The ground color is dark blackish brown dorsally and laterally (and in the limbs), and fades to lighter brown ventrally. A prominent dorsal stripe ranging in color from dark brown to reddish brown is present and is well demarcated on its lateral borders.

Habitat and distribution. This species is restricted to higher elevations in the southern Sierra Nevada. The elevational range is from 900–2440 m. Populations extend from the higher elevations on the northern side of the Lower Kern River Canyon to the Tule River drainage and upper elevations of the Little Kern River drainage in Kern and Tulare counties, California, USA (Fig. 1). Two populations, separated by just over 1 km, are known from the western margin of the Kern Plateau, Tulare Co., California, USA. Most populations are found in coniferous forest containing a mixture of pine, fir and incense cedar (Fig. 7F).

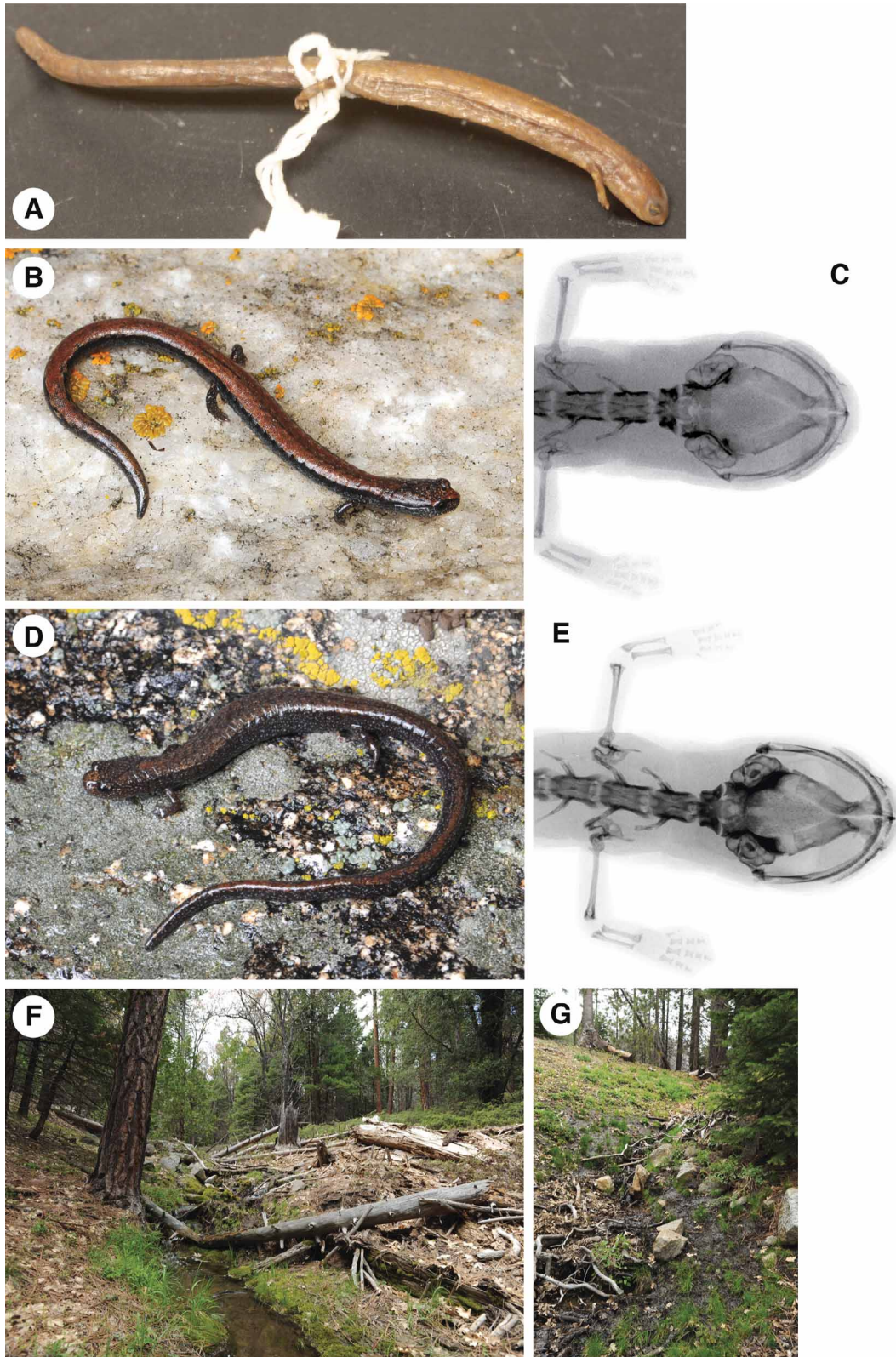


FIGURE 7. *B. altasierrae* and *B. relictus*. A. Holotype of *B. altasierrae*. B. *B. altasierrae* from the type locality in life (no voucher). C. Digital x-ray of the skull and forelimbs of an adult female *B. altasierrae* (MVZ 156394, SL = 39.9 mm) from Sugarloaf Peak, Tulare Co., California. D. Adult *B. relictus* from Breckenridge Mountain, Kern Co., California in life (no voucher). E. Digital x-ray of the skull and forelimbs of an adult (SL = 45.3 mm) female *B. relictus* from Breckenridge Mountain (MVZ 267114). F. Type locality of *B. altasierrae*. G. High elevation site on Breckenridge Mountain, Kern Co., California at which specimens referred here to *B. relictus* were discovered. Nearly all individuals at this locality have been found within the seep shown in the foreground. Habitat photos taken on 28 May 2011. Refer to the web version of this article for color figure.

B. altasierrae has not been found in sympatry with any other species of *Batrachoseps*. It closely approaches *B. gregarius* at lower elevations on the western edge of its range. *B. gregarius* is found within 5 km of *B. altasierrae* along the Middle and North Forks of the Tule River, where *B. altasierrae* occurs as low as 900 m. In the Greenhorn Mountains, *B. gregarius* is known from as high as 1460 m (MVZ 266680–266683; 35.745°N, 118.598°W) and *B. altasierrae* from as low as 1460 m (California Academy of Sciences (CAS) 224163, identified by morphology; 36.176°N, 118.692°W) in the vicinity of Cedar Creek; these populations are separated by less than 1.5 km. On the eastern edge of its range, *B. altasierrae* closely approaches *B. bramei*, but the two species appear to be separated by elevation, although the highest-elevation samples of *B. bramei* are from less than 200 m below the lowest-elevation samples of *B. altasierrae* in the Greenhorn Mountains. The ranges of these species are separated by less than 10 km between Alta Sierra and Wofford Heights. They may also approach each other along the Upper Kern River Canyon. *B. altasierrae* on Sugarloaf Peak, Tulare Co., California occurs less than 10 km away from *B. bramei* along Tobias Creek, Tulare Co., California. *B. altasierrae* is known from Peppermint Creek, Tulare Co., California; this locality is about 2 km from the Kern River. The nearest known *B. bramei* is about 10 km south; however, the range of *B. bramei* likely extends north from there along the Kern River. On the Kern Plateau, east of the Kern River, *B. altasierrae* has been found 1.6 km from the nearest population of *B. robustus* in habitat typical of both species (Wake *et al.* 2002) and less than 10 km from the nearest *B. bramei*.

At higher elevations to the north of the Tule River drainage, *B. altasierrae* is replaced by its sister species, *B. kawia*. To date, all robust specimens (that is, not *B. gregarius*) from the South Fork of the Kaweah River identified with molecular data belong to *B. kawia*, whereas robust specimens from the Tule River drainage carry mitochondrial DNA from *B. altasierrae*. The northernmost specimen of *B. altasierrae* is from Mountain Home State Forest, Tulare Co., California, USA (CAS 214814; 36.269°N, 118.668°W), which is 12 km southeast of the southernmost *B. kawia* from entrance to Soldiers Cave near South Fork entrance Sequoia National Park, Tulare Co., California, USA (MVZ 237285; 36.345°N, 118.7568°W). Very few specimens are available from the higher elevations separating these drainages. Molecular sequence data show that some populations along the North Fork of the Middle Fork of the Tule River have genes from both *B. altasierrae* and *B. kawia*, but the contact zone appears to be narrow (Jockusch *et al.* unpublished).

Etymology. Named for the mountain hamlet of Alta Sierra, located at the summit of the Greenhorn Mountains, an area where this species is particularly common. The species name is formed as a noun in the genitive.

Comments. Jockusch *et al.* (1998) included a rediagnosis of *B. relictus* based on specimens from both the Lower Kern River Canyon and the Greenhorn Mountains. We use their series from the Greenhorn Mountains as the paratypes for *B. altasierrae*.

All molecular data identified in previous publications as coming from *B. pacificus relictus* (Yanev 1978, 1980) or *B. relictus* (Jockusch 1996; Jockusch *et al.* 1998; Jockusch & Wake 2002) are from *B. altasierrae* or from more northern members of its species group. Thus, the molecular diagnoses provided by Jockusch *et al.* (1998) to distinguish *B. relictus* from other species all in fact apply to *B. altasierrae* rather than to *B. relictus*. Molecular differentiation within *B. altasierrae* is limited. Yanev (1978) included six populations of *B. altasierrae*, ranging from the vicinity of the type locality in the Greenhorn Mountains to Quaking Aspen Meadow, on the South Fork of the Middle Fork of the Tule River near the northern end of the range, in her allozyme study of the genus. These populations were separated by a maximum Nei's (1972) genetic distance (D) of 0.075. Mitochondrial DNA (*cob*) data also show a low level of variation (Jockusch & Wake 2002).

Conservation. By virtue of their previous inclusion in *B. relictus*, populations of *B. altasierrae* have been listed as a Species of Special Concern by the State of California and Sensitive Species by the U.S. Forest Service. Our impression is that populations of *B. altasierrae* are healthy, both in the Greenhorn Mountains and within the Tule River drainage; individuals have been found in large numbers when surface conditions were appropriate. For example, about 60 individuals were seen along a single stream in the vicinity of Sugarloaf Village, Greenhorn Mountains, Tulare Co., California in a few hours of searching in August 1995. *B. altasierrae* was also abundant in the Tule River drainage, Tulare Co., California in April 2008: individuals were found at numerous sites, and at Moorehouse Creek, along Hwy. 190 (36.153°N, 118.657°W), 10 individuals were found in an area of a few square meters of moist pine needle litter in less than 10 min.

The Status of *B. relictus*

With the description of *B. altasierrae*, the only populations included in *B. relictus* by Brame and Murray (1968) that are still recognized as *B. relictus* are those from the type locality and vicinity in the Lower Kern River Canyon region of California. Thus, *B. relictus* (Fig. 7D, E) becomes a Breckenridge Mountain endemic, with an elevational range from 480 m along the Kern River to at least 2000 m. Despite this large elevational range, the total area occupied by the species is very small, with a maximum distance of 15 km separating historic populations, and less than 5 km separating the only two known extant populations, giving *B. relictus* the smallest known range for a described species of *Batrachoseps* (Fig. 1). Molecular data from these populations show that *B. relictus* is a member of the *nigriventris* group (Fig. 2), not the Sierran clade called the *relictus* group by Jockusch *et al.* (2002). Historically, *B. relictus* occurred in sympatry with another member of the *nigriventris* group, *B. simatus*, at low elevations in the Kern River Canyon. Although sympatry between species is relatively common in *Batrachoseps* (though typically limited in geographic extent), this is only the second instance of sympatry that involves members of the same species group.

Information on the natural history of *B. relictus* is limited, because virtually all published reports about *B. relictus* are from populations placed here in *B. altasierrae*. At the two known high elevation Breckenridge Mountain sites (1700 and 2000 m), individuals have been found active on the surface from May to early October. The most distinctive aspect of its biology, relative to other species of *Batrachoseps*, may be its association with water. Brame and Murray (1968) described *B. relictus* from the Kern River Canyon as “semiaquatic” and Hilton (1948) noted that specimens were “in a slightly unusual situation for them [*Batrachoseps*]; in a very wet place, in and out of the water of a spring.” Hilton (1948) identified the locality as “Walker’s Pass, west”; in conversation with D. Wake in 1958, he confirmed that the locality was the Lower Kern River Canyon. D. Wake (pers. obs.) saw *B. relictus* in the Kern River Canyon twice (11 April 1960, 22 February 1970), both times actually in water. As at low elevations, at high elevations, animals were also found in very close association with water. At one of its extant localities (E of Squirrel Meadow, 2000 m elev.), we typically find *B. relictus* directly associated with a small seep (Fig. 7G). Salamanders have been found here beneath rocks with water underneath, typically on a sandy-gravel substrate. On 13 June 1979, RWH found a communal nest here consisting of about 125 eggs in a large mass beneath a rock resting in seepage; approximately 20 adult salamanders were present (first reported by Stebbins (1985) in his account of *B. simatus*). Several females collected on this date contained large eggs, suggesting that oviposition was still in progress. One female deposited six eggs in the lab two days post-collection. A female found on 23 May 2010 had partially yolked ova. Rarely, salamanders have been found beyond surface water; two adults were found within a moist log about 45 m upslope from the seep area. At the second Breckenridge Mountain site (headwater seepage of Lucas Creek, 1665–1700 m elev.), all specimens have been found under cover objects directly beside the stream over a distance of about 750 m.

The populations from higher elevations on Breckenridge Mountain have been discussed by several authors. Stebbins (1985) placed them in *B. simatus*, along with populations we assign here to *B. bramei*, noting “animals from Breckenridge Mt. and Fairview are somewhat different from those in Kern River Canyon and are therefore tentatively included in this species [*B. simatus*].” Stebbins (2003) repeated this statement, adding the Cottonwood Creek population from the mouth of the Kern River Canyon. (We assign the Cottonwood Creek population to *B. gregarius*. Its enigmatic genetic makeup will be treated elsewhere.) On the basis of mtDNA sequence data, Jockusch and Wake (2002) treated one of the Breckenridge Mountain populations in their discussion of *B. simatus*, but noted that it is differentiated in other respects, thus rendering its taxonomic status uncertain. Jennings (1996, 2004) treated the Breckenridge Mountain populations as an undescribed species and provided a vernacular name “Breckenridge Mountain slender salamander.” Bartlett and Bartlett (2009) provide a short species account, a color photograph, and a distribution map and refer to the “Breckenridge Slender Salamander” as “formal description pending.”

A fossil trackway from the Miocene of Tuolumne Co., California is consistent with *Batrachoseps* (Peabody & Savage 1959) and was referred to *B. relictus* by Brame and Murray (1968) on the basis of its limb proportions and locality. Given the current geographic range of *B. relictus*, the occurrence of other species (*B. attenuatus* and *B. diabolicus*) in the northern Sierra Nevada, and the combination of morphological conservatism and homoplasy observed in *Batrachoseps*, this fossil trackway cannot be assigned to species.

Conservation.—*B. relictus* is listed as a Species of Special Concern by the State of California and as a Sensitive Species by the U.S. Forest Service. However, these designations confer no legal protection. On the basis of its limited range, small number of known populations, apparent small population sizes, and apparent extirpation at lower elevations, we consider formal protection measures warranted. All recent sightings of *B. relictus* have been from two small high elevation populations in pine-fir forest. The first site (E of Squirrel Meadow) was discovered in 1979, but was later severely degraded by the construction of a logging road through the seepage area. A subsequent fire and timber harvest further compromised this site, and salamanders were not found here despite multiple searches over the next 22 years. Recent visits suggest that the population has rebounded somewhat, but prime seep habitat is quite small. Efforts should be undertaken to locate additional populations of this species. Intermediate elevations on Breckenridge Mountain are virtually unexplored for *Batrachoseps*. Given that *B. relictus* occurs both at river level and at high elevations, streamside and seep habitats that drain the northern slope of Breckenridge Mountain into the Kern River should be surveyed.

Molecular methods and results

Allozyme data. Two allozyme datasets address relationships of populations in the Kern River Canyon and vicinity. The larger study scored 27 variable loci for representatives of *B. altasierrae*, *B. kawia* and all *nigriventris* group species from the vicinity of the Kern River Canyon, including 10 individuals from a Breckenridge Mountain population of *B. relictus* and 18 total individuals from two populations of *B. bramei* (Table 3; loci and methods are as described in Wake *et al.* (2002)). The balanced minimum evolution algorithm (Desper & Gascuel 2002), as implemented in the R package APE (Paradis *et al.* 2004), was used to infer relationships among populations. These data highlight the deep divergence between *B. altasierrae* and its closest relative *B. kawia* (average Nei's (1978) D (D_{Nei}) = 0.59, 11 fixed differences) and confirm that *B. altasierrae* and *B. kawia* are only distantly related to the *nigriventris* group ($D_{\text{Nei}} > 0.935$ for all comparisons; Table 4), including *B. relictus* from Breckenridge Mountain (average D_{Nei} = 1.26, 18 fixed differences) and *B. bramei* (average D_{Nei} = 1.05, 15 fixed differences). *B. altasierrae* from the Greenhorn Mountains and Kern Plateau, on opposite sides of the Kern River Canyon, display relatively little differentiation (D_{Nei} = 0.083). *B. relictus* falls within the *nigriventris* group (Fig. 8), but it is highly divergent from all other species, with average D_{Nei} = 0.52 and a minimum of 9 fixed differences to five populations of *B. simatus*, average D_{Nei} = 0.63 and 13 fixed differences to two populations of *B. bramei*, average D_{Nei} = 0.72 to a geographically close population of *B. gregarius*, and average D_{Nei} = 0.97 to two populations of *B. stebbinsi* (Table 4). The two populations of *B. bramei* are separated by only 1.5 km, are barely differentiated from each other (D_{Nei} = 0.004) and are most similar to *B. gregarius* (average D_{Nei} = 0.52; Fig. 8). They are separated from *B. simatus* by average D_{Nei} of 0.611 and 11 fixed differences. Maximum differentiation within *B. simatus* was 0.19.

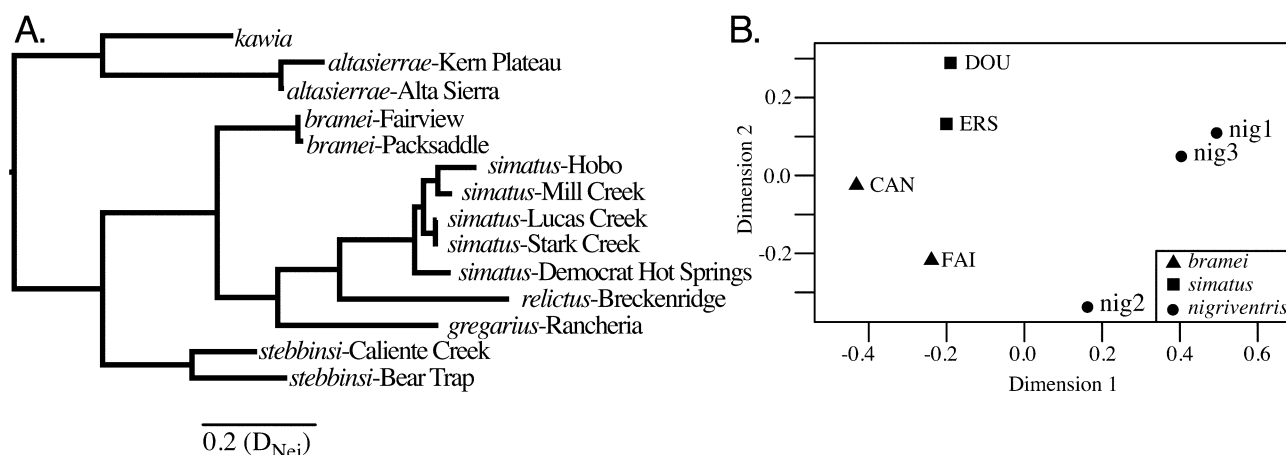


FIGURE 8. Allozyme analyses. A. Minimum evolution tree showing the relationships of 14 populations based on Nei's (1978) D (Table 4) for 27 variable loci included in allozyme study 1. The topology of the neighbor-joining tree is identical. B. Multidimensional scaling of Nei's (1978) D (Table 5) for eight populations from the *nigriventris* group based on allozyme study 2. Note the intermediate position of the Cannell Creek (CAN) population between *B. bramei* from Fairview (FAI) and *B. simatus* from Erskine Creek (ERS). See Table 5 for abbreviations. Sampling localities are listed in Table 3.

TABLE 3. Localities and sample sizes (N) for allozyme studies 1 and 2.

Species	Locality	Lat (°N)	Long (°W)	N
study 1				
<i>B. altasierrae</i>	Kern Plateau, 7.3 mi NE (air) Fairview, Tulare Co.	35.99	118.39	3
<i>B. altasierrae</i>	Hwy. 155, 0.5 mi SE Greenhorn Summit at Alta Sierra, Kern Co.	35.74	118.55	12
<i>B. kawia</i>	South Fork Kaweah River, Tulare Co.	36.38	118.86	6
<i>B. bramei</i>	Fairview, Kern River Canyon, Tulare Co.	35.93	118.49	11
<i>B. bramei</i>	Packsaddle Canyon at Kern River Canyon, Tulare Co.	35.94	118.48	7
<i>B. simatus</i>	Hobo Hot Springs, Kern River Canyon, Kern Co.	35.57	118.53	4
<i>B. simatus</i>	Hwy. 178, 1 mi. SW Democrat Hot Spring turnoff, Kern River Canyon, Kern Co.	35.52	118.67	1
<i>B. simatus</i>	Mill Creek, just above Kern River, Kern Co.	35.54	118.62	1
<i>B. simatus</i>	Lucas Creek at Hwy. 178, Kern River Canyon, Kern Co.	35.48	118.71	10
<i>B. simatus</i>	Stark Creek, just above Hwy. 178, Kern River Canyon, Kern Co.	35.48	118.72	1
<i>B. relictus</i>	Breckenridge Mountain, vicinity Squirrel Meadow, Kern Co.	35.48	118.57	10
<i>B. stebbinsi</i>	Caliente Creek Rd., 10.4 mi E Caliente, Tehachapi Mountains, Kern Co.	35.30	118.48	4
<i>B. stebbinsi</i>	2 mi (air) N Bear Trap Canyon, Tehachapi Mountains, Kern Co.	34.91	118.69	14
<i>B. gregarius</i>	Rancheria Rd, 2.8 mi N Kern River bridge, Kern Co.	35.46	118.83	9
study 2				
<i>B. bramei</i>	Fairview & Packsaddle Canyons, Kern River Canyon, Tulare Co.	35.93	118.49	2
<i>B. bramei</i>	Cannell Creek, Kern and Tulare counties	35.79	118.42	4
<i>B. simatus</i>	Dougherty Creek at Hwy 178, Kern River Canyon, Kern Co.	35.47	118.71	2
<i>B. simatus</i>	Erskine Creek Canyon, Kern Co.	35.57	118.40	3
<i>B. nigriventris</i>	Mt. Pinos, border of Kern and Ventura counties	34.81	119.10	2
<i>B. nigriventris</i>	Santa Rita-Old Creek Rd., San Luis Obispo Co.	35.52	120.77	2
<i>B. nigriventris</i>	Bear Trap Canyon, Tejon Ranch, Kern Co.	34.89	118.75	2

A subsequent survey of allozyme variation included 28 loci, and small samples ($N = 2-4$) from multiple populations of *B. bramei*, *B. simatus* and *B. nigriventris* (see Table 3 for localities and sample sizes). Pairwise divergences between populations within the Kern River Canyon all exceeded 0.2 (Table 5). The two *B. bramei* populations, from Fairview and Cannell Creek, were most similar to each other ($D_{\text{Nei}} = 0.219$), but were separated by 5 fixed differences. Divergence between *B. bramei* from Fairview and *B. simatus* from Erskine Creek was high (0.500, 10 fixed differences), paralleling the results from the larger study, but the divergence between the Cannell Creek and Erskine populations was substantially lower (0.226, 4 fixed differences). These are the geographically closest populations of *B. bramei* and *B. simatus* known on the southeastern side of the river (Fig. 9). Multidimensional scaling (R v. 2.7.2, R Development Core Team 2008) highlights the intermediate position of the Cannell Creek population (Fig. 8B). The average divergence between *B. bramei* and *B. nigriventris* was 0.72.

Phylogenetic analysis of mtDNA data. A portion of the mitochondrial gene *cytochrome b* (*cob*) flanked by the primers MVZ15 and MVZ16 was amplified and sequenced following standard methods (Jockusch & Wake 2002; Martínez-Solano *et al.* 2007). After trimming of the ends because of low sequence quality, 677 base pairs (bp) were analyzed for 62 sequences from the *B. nigriventris* group (Fig. 9, Table 6). Some of these sequences were also included in the analyses of Jockusch and Wake (2002). The dataset analyzed here contains all sequences generated for specimens from within Kern River Canyon (25 specimens representing *B. bramei* and 11 specimens representing *B. simatus*) and Breckenridge Mountain ($N = 11$, all referred to *B. relictus*). Additional specimens were chosen to represent the known mitochondrial and geographic diversity of the other three *nigriventris* group species (7 *B. nigriventris*, 6 *B. gregarius*, and 2 *B. stebbinsi*). For samples from outside the Kern Canyon region, the data matrix was essentially complete. For samples from within Kern River Canyon, a smaller portion of *cob* was sequenced for

TABLE 4. Nei's (1978) genetic distances for allozyme study 1 based on 27 loci for 14 populations from the vicinity of the Kern River Canyon. Figure 8A shows the minimum evolution tree derived from this distance matrix.

	1-alt	2-alt	3-kaw	4-bram	5-bram	6-sim	7-sim	8-sim	9-sim	10-sim	11-rel	12-steb	13-steb
1. <i>B. altasierrae</i> (Kern Plateau)	--												
2. <i>B. altasierrae</i> (Alta Sierra)	0.083	--											
3. <i>B. kawia</i>	0.631	0.550	--										
4. <i>B. bramei</i> (Fairview)	1.042	0.935	0.808	--									
5. <i>B. bramei</i> (Packsaddle)	1.092	0.977	0.849	0.004	--								
6. <i>B. simatus</i> (Hobo)	1.045	1.144	1.273	0.578	0.574	--							
7. <i>B. simatus</i> (Democrat)	0.993	1.077	1.200	0.656	0.653	0.189	--						
8. <i>B. simatus</i> (Mill Creek)	1.060	1.161	1.298	0.582	0.579	0.094	0.122	--					
9. <i>B. simatus</i> (Lucas Creek)	1.016	1.108	1.317	0.611	0.608	0.125	0.098	0.068	--				
10. <i>B. simatus</i> (Stark Creek)	1.026	1.118	1.317	0.601	0.598	0.125	0.099	0.060	0.002	--			
11. <i>B. relictus</i> (Breckenridge)	1.204	1.315	1.427	0.628	0.644	0.541	0.468	0.554	0.509	0.508	--		
12. <i>B. stebbinsi</i> (Caliente Creek)	1.081	0.958	0.700	0.624	0.646	0.831	1.053	0.962	0.991	0.994	1.069	--	
13. <i>B. stebbinsi</i> (Bear Trap)	1.122	0.997	0.864	0.731	0.754	0.849	0.972	0.933	0.898	0.904	0.864	0.285	--
14. <i>B. gregarius</i> (Rancheria Rd.)	1.154	1.264	1.417	0.527	0.514	0.582	0.611	0.584	0.462	0.462	0.722	0.856	0.796

a subset of samples; in all cases, these sequences were identical or virtually identical to another, more complete, sequence. For a subset of Kern River Canyon samples (5 *B. bramei*, 2 *B. relictus* from Breckenridge Mountain, and 3 *B. simatus*) and 12 of the 15 other *nigriventris* group samples, we also obtained sequences of a portion of three other mtDNA genes: 16S ribosomal DNA (16S), *cytochrome oxidase 1 (cox1)* and *NADH dehydrogenase 4 (nad4)* following the methods of Martínez-Solano *et al.* (2007). Alignment of 16S was straightforward, with gaps 1–2 nucleotides in length introduced at only seven positions. Combined with the *cob* data, this produced an aligned dataset of 2298 nucleotides, the 4-gene dataset, which had essentially no missing data.

TABLE 5. Nei's (1978) genetic distances for allozyme study 2 based on 28 loci for seven populations in the *nigriventris* group. Figure 8B shows the results of multidimensional scaling of these values.

	FAI	CAN	DOU	ERS	<i>nig1</i>	<i>nig2</i>
<i>B. bramei</i> -Fairview (FAI)	--					
<i>B. bramei</i> -Cannell (CAN)	0.219	--				
<i>B. simatus</i> -Dougherty (DOU)	0.448	0.253	--			
<i>B. simatus</i> -Erskine (ERS)	0.500	0.226	0.231	--		
<i>B. nigriventris</i> -Mt Pinos (<i>nig1</i>)	0.813	0.943	0.671	0.663	--	
<i>B. nigriventris</i> -San Luis Obispo (<i>nig2</i>)	0.433	0.617	0.744	0.557	0.496	--
<i>B. nigriventris</i> -Tejon Ranch (<i>nig3</i>)	0.702	0.818	0.604	0.727	0.209	0.491

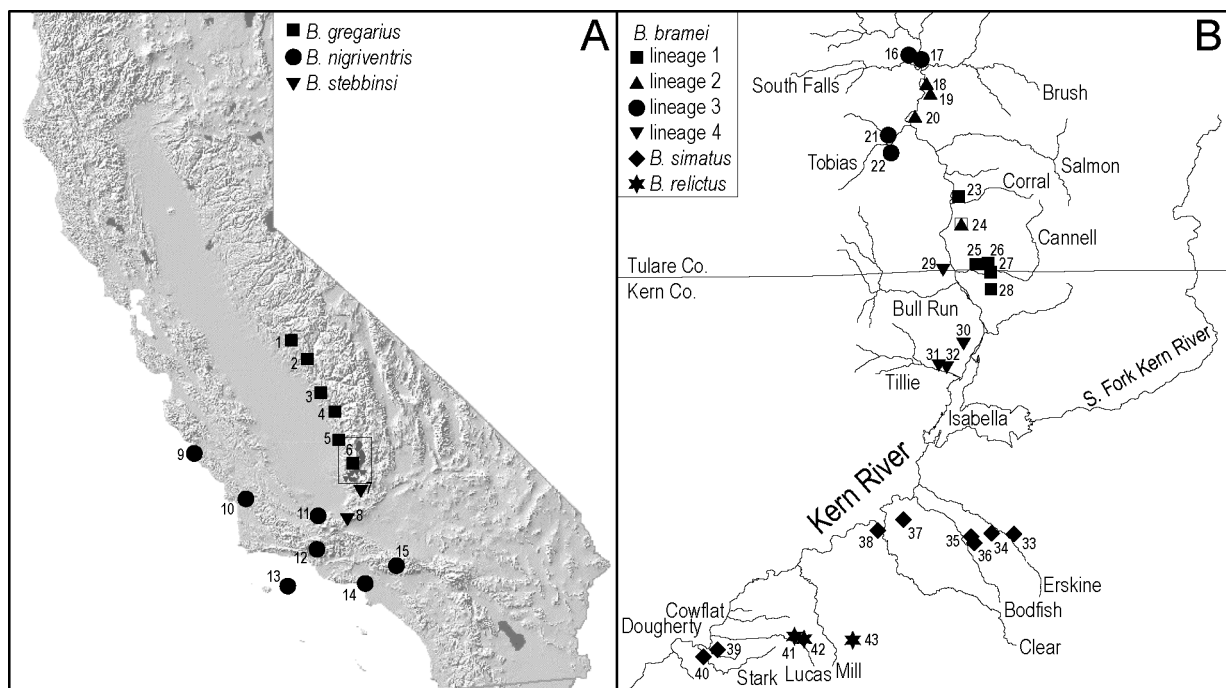


FIGURE 9. Samples included in the *cob* phylogeny of the *Batrachoseps nigriventris* group. A. Map of California, showing topographic relief, with localities for specimens from outside of the Kern River Canyon (black symbols). Box shows approximate area of panel B. B. Kern River Canyon, showing localities for specimens from the Kern River Canyon and Breckenridge Mountain. Major side drainages are labeled. For clarity, streams on the north side of the Lower Kern River Canyon are omitted.

When analyzed as part of a larger dataset (Jockusch & Wake 2002), a subset of *B. nigriventris* samples (“northern *B. nigriventris*”) is confidently resolved as the sister taxon to the rest of the *nigriventris* group. This lineage was represented by four specimens here, which were treated as a monophyletic outgroup to root the trees.

Analyses of both the *cob* and 4-gene datasets included equally-weighted maximum parsimony (MP) (with support estimated using 100 bootstrap replicates, 5 random addition replicates/bootstrap replicate), as implemented in PAUP 4.1b10 (Swofford 2003), Bayesian inference, as implemented in MrBayes v. 3.1 (Huelsenbeck & Ronquist 2001), and maximum likelihood (with support estimated using 100 bootstrap replicates), as implemented in the

TABLE 6. Voucher, locality and Genbank accession numbers for *Batrachoseps* specimens sequenced for mtDNA. Lineage refers to monophyletic subspecific clade. Pop indicates the population number in Figure 9. For individuals in the 4-gene dataset, Genbank accession numbers are listed in the order *cob/cox1/nad4/16S*.

Voucher	Species	Line-age	Pop	County	Descriptive Locality	Lat (°N)	Long (°W)	Genbank Accession Number(s)
MVZ266690	<i>gregarius</i>	N	1	Mariposa	Westfall Picnic Ground, ca. 10 km S Wawona	37.44	119.65	JQ035781/JQ035685/ JQ035706/JQ035727
MVZ251725	<i>gregarius</i>	N	2	Fresno	Jose Basin, Italian Creek	37.13	119.38	JQ035782/JQ035681/ JQ035702/JQ035723
MVZ266674	<i>gregarius</i>	N	3	Fresno	Sand Creek Rd.	36.69	119.16	JQ035783/JQ035684/ JQ035705/JQ035726
MVZ266720	<i>gregarius</i>	N	4	Tulare	Kaweah River, South Fork Rd.	36.42	118.91	JQ035774/JQ035683/ JQ035704/JQ035725
MVZ266717	<i>gregarius</i>	S	5	Tulare	South Fork of Tule River	36.02	118.82	JQ035785/JQ035686/ JQ035707/JQ035728
MVZ230925	<i>gregarius</i>	S	6	Kern	Alder Creek campground, Greenhorn Mts.	35.72	118.61	JQ035775/JQ035682/ JQ035703/JQ035724
MVZ267155	<i>stebbinsi</i>		7	Kern	Last Chance Canyon, Caliente Creek Rd.	35.30	118.47	JQ035785/JQ035686/ JQ035707/JQ035728
MVZ169121	<i>stebbinsi</i>		8	Kern	S side Pastoria Creek	34.91	118.69	JQ035789/JQ035670/ JQ035691/JQ035712
MVZ266842	<i>nigriventris</i>	N	9	Monterey	Redwood Gulch above Hwy. 1	35.84	121.39	JQ035788/JQ035669/ JQ035690/JQ035711
MVZ266895	<i>nigriventris</i>	N	10	San Luis Obispo	Lopez Lake	35.19	120.45	JQ035786/JQ035666/ JQ035687/JQ035708
IMSZ387	<i>nigriventris</i>	N	11	Kern	San Emigdio Creek	34.98	119.19	JQ035787
MVZ266997	<i>nigriventris</i>	N	12	Ventura	Ojai Valley, Santa Ynez Mountains	34.47	119.22	EU011253/EU011254/ EU011255/EU011252
DBW6459	<i>nigriventris</i>	S	13	Santa Barbara	Santa Cruz Island	34.00	119.72	JQ035741/JQ035668/ JQ035689/JQ035710
MVZ225708	<i>nigriventris</i>	S	14	Los Angeles	Baldwin Hills	34.01	118.37	JQ035740/JQ035667/ JQ035688/JQ035709
MVZ222695	<i>nigriventris</i>	S	15	Los Angeles	Coldbrook Camp	34.29	117.84	JQ035688/JQ035709
MVZ267138	<i>bramei</i>	3	16	Tulare	Upper Kern River Canyon at M99 Bridge, W side of River	35.97	118.49	JQ035770
MVZ224858	<i>bramei</i>	3	17	Tulare	Upper Kern River Canyon at M99 Bridge, E side of River	35.97	118.49	JQ035771/JQ035677/ JQ035698/JQ035719
MVZ224859	<i>bramei</i>	3	17	Tulare	Upper Kern River Canyon at M99 Bridge, E side of River	35.97	118.49	JQ035769

continued next page

TABLE 6. (continued)

Voucher	Species	Line-age	Pop	County	Descriptive Locality	Lat (°N)	Long (°W)	Genbank Accession Number(s)
MVZ220477	<i>bramei</i>	2	18	Tulare	between Brin and Limestone Canyons	35.96	118.48	JQ035767
MVZ168760	<i>bramei</i>	2	19	Tulare	Brin Canyon	35.95	118.47	JQ035768
MVZ224854	<i>bramei</i>	2	20	Tulare	Fairview, Kern River Canyon	35.92	118.50	JQ035765
MVZ244087	<i>bramei</i>	2	20	Tulare	Fairview, Kern River Canyon	35.93	118.49	JQ035766/JQ035680/ JQ035701/JQ035722
MVZ267142	<i>bramei</i>	3	21	Tulare	Tobias Creek	35.90	118.52	JQ035773
MVZ267140	<i>bramei</i>	3	22	Tulare	Tobias Creek	35.90	118.52	JQ035772
MVZ267158	<i>bramei</i>	1	23	Tulare	Corral Creek at M99, Kern River Canyon	35.86	118.45	JQ035732
MVZ220454	<i>bramei</i>	1	23	Tulare	Corral Creek at M99, Kern River Canyon	35.86	118.45	JQ035739
MVZ220479	<i>bramei</i>	2	24	Tulare	1 mi ENE Hospital Flat Campground	35.83	118.44	JQ035764
MVZ226706	<i>bramei</i>	1	24	Tulare	1 mi ENE Hospital Flat Campground	35.83	118.44	JQ035731
MVZ226707	<i>bramei</i>	1	24	Tulare	1 mi ENE Hospital Flat Campground	35.83	118.44	JQ035730
MVZ226708	<i>bramei</i>	1	24	Tulare	1 mi ENE Hospital Flat Campground	35.83	118.44	JQ035729
MVZ226709	<i>bramei</i>	1	24	Tulare	1 mi ENE Hospital Flat Campground	35.83	118.44	JQ035733/JQ035678/ JQ035699/JQ035720
MVZ224864	<i>bramei</i>	1	25	Tulare	Cannell Creek	35.79	118.42	JQ035737
MVZ217948	<i>bramei</i>	1	26	Tulare	Cannell Creek	35.79	118.42	JQ035738
MVZ267156	<i>bramei</i>	1	27	Tulare	Cannell Creek	35.79	118.43	JQ035735/JQ035679/ JQ035700/JQ035721
MVZ267157	<i>bramei</i>	1	27	Tulare	Cannell Creek	35.79	118.43	JQ035734
MVZ226712	<i>bramei</i>	1	28	Kern	Cannell Trail	35.78	118.43	JQ035736
MVZ267154	<i>bramei</i>	4	29	Tulare	N of Bull Run Creek	35.79	118.45	JQ035776
MVZ267118	<i>bramei</i>	4	30	Kern	Plater Rd., just N jcn. Burlando Rd., W of Kernville	35.74	118.43	JQ035777
MVZ251745	<i>bramei</i>	4	31	Kern	Tillie Creek drainage, vic. Wofford Heights	35.71	118.48	JQ035778/JQ035676/ JQ035697/JQ035718
MVZ251741	<i>bramei</i>	4	32	Kern	Wofford Heights	35.71	118.46	JQ035779
MVZ158457	<i>simatus</i>	33	33	Kern	Erskine Creek Canyon, Piute Mts.	35.57	118.40	JQ035753
ELJ1922	<i>simatus</i>	34	34	Kern	Erskine Creek Canyon, Piute Mts.	35.57	118.40	JQ035754/JQ035671/ JQ035692/JQ035713
MVZ232842	<i>simatus</i>	35	35	Kern	Bodfish Creek Canyon, N slope Piute Mts.	35.56	118.44	JQ035763/JQ035672/ JQ035693/JQ035714

continued next page

TABLE 6. (continued)

Voucher	Species	Line- age	Pop	County	Descriptive Locality	Lat (°N)	Long (°W)	Genbank Accession Number(s)
MVZ232843	<i>simatus</i>		36	Kern	Bodfish Creek Canyon, N slope Piute Mts.	35.56	118.44	JQ035762
MVZ267110	<i>simatus</i>		37	Kern	SW of Sandy Flat Campground, Kern River Canyon	35.58	118.52	JQ035761/JQ035673/ JQ035694/JQ035715
ELJ1672	<i>simatus</i>		38	Kern	W of Clear Creek, Kern River Canyon	35.57	118.53	JQ035757
ELJ1673	<i>simatus</i>		38	Kern	W of Clear Creek, Kern River Canyon	35.57	118.53	JQ035760
MVZ168529	<i>simatus</i>		38	Kern	W of Clear Creek, Kern River Canyon	35.57	118.53	JQ035759
MVZ220498	<i>simatus</i>		38	Kern	W of Clear Creek, Kern River Canyon	35.57	118.53	JQ035758
MVZ218033	<i>simatus</i>		39	Kern	Dougherty Creek, Kern River Canyon	35.47	118.71	JQ035756
MVZ167629	<i>simatus</i>		40	Kern	Stark Creek, Kern River Canyon	35.47	118.72	JQ035755
CAS219746	<i>relictus</i>		41	Kern	Breckenridge Mt., Lucas Creek	35.48	118.63	JQ035748/JQ035675/ JQ035696/JQ035717
CAS219748	<i>relictus</i>		41	Kern	Breckenridge Mt., Lucas Creek	35.48	118.63	JQ035749
MVZ233571	<i>relictus</i>		42	Kern	Breckenridge Mt., Lucas Creek	35.48	118.62	JQ035750
MVZ233572	<i>relictus</i>		42	Kern	Breckenridge Mt., Lucas Creek	35.48	118.62	JQ035746
MVZ233573	<i>relictus</i>		42	Kern	Breckenridge Mt., Lucas Creek	35.48	118.62	JQ035745
MVZ233574	<i>relictus</i>		42	Kern	Breckenridge Mt., Lucas Creek	35.48	118.62	JQ035747
MVZ233575	<i>relictus</i>		42	Kern	Breckenridge Mt., Lucas Creek	35.48	118.62	JQ035751
MVZ233576	<i>relictus</i>		42	Kern	Breckenridge Mt., Lucas Creek	35.48	118.62	JQ035752
MVZ267162	<i>relictus</i>		43	Kern	Breckenridge Mt., vicinity Squirrel Meadow	35.48	118.57	JQ035744/JQ035674/ JQ035695/JQ035716
MVZ163661	<i>relictus</i>		43	Kern	Breckenridge Mt., vicinity Squirrel Meadow	35.48	118.57	JQ035742
MVZ163663	<i>relictus</i>		43	Kern	Breckenridge Mt., vicinity Squirrel Meadow	35.48	118.57	JQ035743

Abbreviations: CAS—California Academy of Sciences herpetology collection; DBW—David B. Wake catalogue; ELJ—Elizabeth L. Jockusch tissue collection; IMSZ—Ifiño Martínez-Solano catalogue; MVZ—Museum of Vertebrate Zoology herpetology collection

Garli-partition testing version 0.96 (Zwickl 2006). For maximum likelihood and Bayesian analyses, the *cob* data were partitioned into two subsets: 1st and 2nd codon positions together versus 3rd codon positions. The extremely limited variation at 2nd codon positions meant that there was not enough information in our dataset to estimate parameters for 2nd codon positions alone. The 4-gene dataset was partitioned into noncoding (16S), 1st codon position, 2nd codon position and 3rd codon position. This partitioning scheme was supported over three others (single partition, three partitions (with 1st and 2nd codon positions combined) and ten partitions (16S and 1st, 2nd and 3rd codon positions for each gene)) by results of stabilized stepping-stone analyses (Fan *et al.* 2011) obtained using Phycas (Lewis *et al.* 2010). An appropriate model for each data partition was selected based on the AIC criterion as implemented in ModelTest (Posada & Crandall 1998). Parameter estimates were unlinked across partitions, except for branch lengths, which were constrained to be proportionate (using `prset ratepr = variable` in MrBayes and `subset-specific rates = 1` in Garli), and tree topology. Default settings were used for analyses, with the following exceptions. In parsimony, the settings `nchuck = 10` and `chucklen = 1` were used to prevent the *cob* analyses from spending extensive computational time exploring trees that differed only in the arrangement of very short branches. After 1,000 random-addition replicates, a strict consensus of shortest trees was constructed and 10,000 additional random-addition replicates were done using this tree as a constraint to ensure that it represented the strict consensus of all maximally parsimonious trees. For the MP bootstrap analysis of the *cob* dataset, the settings `nchuck = 100` and `chucklen = 1` were used. DeBry and Olmstead (2000) showed that this procedure does not bias estimates of support for well-supported nodes. In MrBayes, the prior on alpha was set to an exponential distribution with a mean of 1. Use of the default branch length prior led to trees that were more than an order of magnitude longer than the ML tree with likelihoods far from the ML estimate and several unrealistic parameter estimates. Thus, the prior on branch lengths was set to an exponential distribution with a mean of 106.5 in the *cob* analysis and 51.0 in the 4-gene analysis following the suggestion of Brown *et al.* (2010) that half the prior weight fall below the mean branch length, which was estimated from the maximum likelihood tree.

Genetic diversity and phylogenetic relationships of *B. relictus*. Differentiation of mtDNA in *B. relictus* on Breckenridge Mountain is limited. The eight samples from Lucas Creek have only two haplotypes, which differ at a single site. Divergence is also limited at the second locality, while divergence between haplotypes from the two populations ranges from 0.9–1.2% (Kimura-2-parameter sequence divergence; this distance measure was used because it has been reported in previous studies of mitochondrial divergence in *Batrachoseps*). Monophyly of *B. relictus* from Breckenridge Mountain is strongly supported in all analyses (Fig. 10). Divergence between *B. relictus* and *B. simatus* haplotypes ranges from 2.2–3.5% (mean 2.9%). This interspecific divergence is substantially less than the deepest divergence observed at *cob* in any of the other species of the *nigriventris* group. Monophyly of a larger group comprising mtDNA from all *B. simatus* and *B. relictus* samples is strongly supported in all analyses. Although the phylogenies based on *cob* alone do not resolve relationships among *B. relictus*, *B. simatus* from Erskine Creek in the Piute Mountains, and a clade comprising all other samples of *B. simatus* (ranging along the Kern River Canyon from Bodfish Creek, also in the Piute Mountains, to Stark Creek), the 4-gene dataset shows that the basal split is between *B. simatus* from Erskine Creek and a clade formed by *B. relictus* and other populations of *B. simatus*. Thus, *B. relictus* renders *B. simatus* paraphyletic in the mtDNA tree.

Given only the mtDNA data, one might question the distinctiveness of *B. relictus*. However, the allozyme results, showing 9 fixed differences, are further supported by nuclear intron sequence data, which do not recover a close relationship between *B. relictus* and *B. simatus* within the *nigriventris* group (Martínez-Solano *et al.* unpublished). Additionally, the species are morphologically distinct and were found in sympatry in the recent past (Brame & Murray 1968). Thus, we hypothesize that the mtDNA of *B. simatus* introgressed into *B. relictus* at some point following the earliest diversification of *B. simatus*; all sampled individuals of *B. relictus* carry *B. simatus*-like mtDNA. In no case did we find mitochondrial haplotypes shared across species, however, suggesting that at present, the species are fully isolated.

These data show that *B. relictus* is a member of the *nigriventris* group. *B. altasierrae*, formerly treated as *B. relictus*, is not a member of the *nigriventris* group, and is instead more closely related to *B. kawia*, *B. regius* and *B. diabolicus* (Fig. 2). We suggest that the clade formed by these four taxa, which was formerly called the *relictus* group, be known henceforth as the *diabolicus* group.

Genetic diversity and phylogenetic relationships of *B. bramei*. High levels of sequence divergence in the mitochondrial gene *cob* and strong geographic structuring characterize *B. bramei*. The 30 km range (linear distance along the Kern River Canyon) of this species is occupied by four geographically restricted mtDNA lineages, each

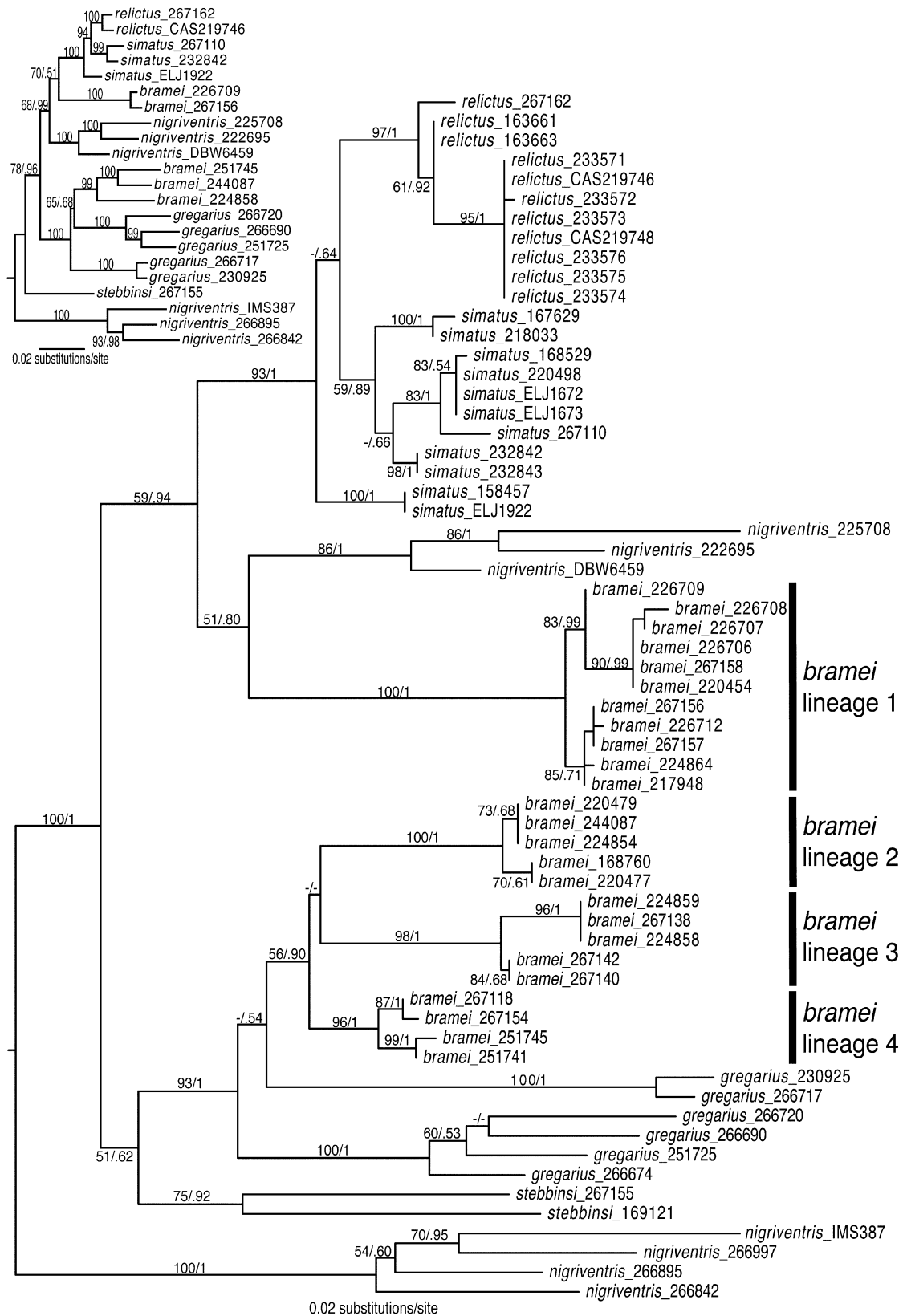


FIGURE 10. Phylogeny of the *Batrachoseps nigriventris* group. Tree resulting from maximum likelihood (ML) analysis of *cob* (LnL = -3032.9164; preferred model was GTR + I + G for the 1st + 2nd codon partition and GTR + G for the 3rd codon partition). Inset tree in upper left shows ML tree resulting from the analysis of the 4-gene dataset (LnL = -8158.2306). Samples are named by species followed by specimen voucher. Sampling localities are listed in Table 6. Numbers along branches are maximum likelihood bootstrap values/Bayesian posterior probabilities; - indicates support value below 50%. In the inset tree, the posterior probability was 1 for all interior branches except those for which the posterior probability is shown.

of which is strongly supported in all analyses (Fig. 10). These four lineages are distributed as follows (Fig. 9): lineage 1 is restricted to the E side of the Kern River extending from Cannell Creek to Corral Creek; lineage 2 (topotypic) is also restricted to the E side of the river, extending from Hospital Flat (where it is sympatric with lineage 1) to Brin Canyon; lineage 3 is known from both sides of the river just north of its confluence with South Falls Creek, where a bridge on Mountain Hwy 99 (M99) crosses the river; its range extends at least as far south as Tobias Creek on the W side of the river; lineage 4 is restricted to the W side of the river extending from the Tillie Creek drainage, which enters Lake Isabella, to north of Bull Run Creek along the Upper Kern River. Divergence within each of these lineages is relatively limited, ranging up to a maximum of 2.1% in lineage 1, 0.6% in lineage 2, 1.2% in lineage 3 and 1.1% in lineage 4. The within-lineage diversity generally results from fine-scale geographic structure. Jockusch and Wake (2002) reported relatively high divergence between two lineage 1 populations, Cannell and Corral Creeks (their localities 63–64 vs. 65). We have since determined that a portion of that Corral Creek sequence was from a contaminant. Divergence between samples from Corral Creek and other lineage 1 samples is less than 1.5%. Usually, samples were more closely related to geographically distant samples on the same side of the river than they were to geographically closer samples from the opposite side of the river, suggesting that the Kern River poses a barrier to mitochondrial gene flow. The exception is lineage 3, in which identical haplotypes characterized individuals from both sides of the M99 bridge.

Lineages 2–4 of *B. bramei* are all more closely related to each other and also to *B. gregarius* than they are to conspecific lineage 1. Average divergence between lineages 2–4 ranged from 3.6–4.1%. Divergences between these lineages and lineage 1 averaged 7.4%, while their average divergence from *B. gregarius* was 5.8%. Monophyly of the mtDNA of *B. bramei* can be confidently rejected, because a clade comprising lineages 2, 3 and 4 of *B. bramei* and all samples of *B. gregarius* received moderate or strong support in all analyses. Within this clade, however, monophyly of a group giving rise to the three *B. bramei* lineages was neither strongly supported nor strongly rejected based on analysis of *cob* alone. The 4-gene dataset confirms that *bramei* lineages 2–4 form a monophyletic group, but does not resolve the trichotomy formed by this clade and two lineages comprising *B. gregarius*. Lineage 1 of *B. bramei* was more closely related to *B. simatus*, *B. relictus* from Breckenridge Mountain and the southern lineage of *B. nigriventris* than it was to conspecific lineages 2–4 in all analyses (Fig. 10). The average divergence between lineage 1 of *B. bramei* and *B. simatus* was 6.3%. Even the 4-gene dataset was unable to resolve the trichotomy among southern *B. nigriventris*, *B. bramei* lineage 1 and the clade comprising *B. simatus* and *B. relictus*. The clades in this trichotomy demonstrate a genetic connection between coastal southern California and the southern end of the Sierra Nevada. Similar connections have been found in several other amphibians, including *Ensatina eschscholtzii* (Moritz *et al.* 1992; Pereira & Wake 2009), *Taricha torosa* (Kuchta & Tan 2005), *Pseudacris regilla* (Recuero *et al.* 2006) and *Rana muscosa* (Macey *et al.* 2001; Vredenburg *et al.* 2006). These geographic regions are connected by the geologically complex Transverse Ranges (Chatzimanolis & Caterino 2007), which are currently occupied by *B. nigriventris* carrying mtDNA from the northern *nigriventris* lineage (Jockusch & Wake 2002).

Discussion

How many species of *Batrachoseps* are in the Upper Kern River Canyon? We accept the argument of de Queiroz (2007) that taxonomists all work within the framework of the same general species concept, what he terms the General Lineage Concept. In our work we are attempting to identify independently evolving lineages, and this entails the deployment of several different criteria (concepts of different authors). Sympatry is the clearest criterion, but is not present here; in the case of allopatric populations we proceed in an integrative manner, considering degree of morphological divergence, pattern and degree of allozyme differentiation, pattern and degree of DNA sequence variation and habitat/geographic range change within versus between taxa. Given the substantial allozyme and mitochondrial diversity found in the upper portion of the Kern River Canyon, one question is whether there might in fact be more than one species in this region. In particular, the Cannell Creek population carries a divergent lineage of mtDNA and is almost as close allozymically to *B. simatus* as it is to *B. bramei* from Fairview. Although there is some variation across populations, *B. bramei* (including the Cannell Creek population) is morphologically cohesive (Fig. 3), and well differentiated genetically from both *B. gregarius* (to which the clade formed by three of the four *B. bramei* mtDNA lineages is most closely related) and *B. simatus* (to which the other

B. bramei mtDNA lineage is closely related) (Fig. 8, 10). Additionally, populations of all four mtDNA lineages assigned to *B. bramei* form a monophyletic group in analyses of DNA sequence data from two of four nuclear introns we have examined (Martínez-Solano *et al.* unpublished). The evidence thus suggests that there has been no recent gene flow between *B. simatus* and populations in the Upper Kern River Canyon and additionally that there has been gene flow connecting all populations in the Upper Kern River Canyon. Thus, we conclude that assignment of all populations in the Upper Kern River Canyon to *B. bramei* is supported by the available evidence.

We think it likely that two factors have contributed to the high differentiation among populations in the Upper Kern River Canyon, and in particular to the divergence of the Cannell Creek populations. First, strong geographic structuring is common in *Batrachoseps* (e.g., Jockusch & Wake 2002; Martínez-Solano *et al.* 2007; Martínez-Solano & Lawson 2009), consistent with the extremely low vagility measured in mark-recapture studies (Hendrickson 1954; Maiorana 1978). The largely xeric conditions of the upper canyon likely reduce vagility even further and may lead to patchy distributions. The second factor hypothesized to promote high divergence among populations of *B. bramei* is historical gene flow from *B. simatus* into geographically neighboring populations of *B. bramei*. This is suggested by two lines of evidence: 1) the intermediate genetic distance of the Cannell Creek population between *B. bramei* from Fairview and *B. simatus* from Erskine Creek; and 2) the occurrence of an mtDNA lineage most closely related to *B. simatus* in the populations of *B. bramei* that are geographically closest to *B. simatus*, including the Cannell Creek population. However, it is unlikely that gene flow is currently occurring between *B. simatus* and the Cannell Creek population. They are separated by a geographic gap of >20 km on the east side of the Kern River. This gap includes more open habitat of the Kern River Valley around its confluence with the South Fork of the Kern River (Fig. 1). Additionally, at the DNA sequence level, no allele sharing across species has been observed. Thus, we conclude that only a single species is present in the Upper Kern River Canyon and that the Cannell Creek population is most appropriately treated as *B. bramei*.

Species diversity in the Kern River Canyon. The Kern River Canyon at the southern end of the Sierra Nevada has both a high level of endemism and of diversity within the California biodiversity hotspot (Lapointe & Rissler 2005). As a result of the endemism for both species and subspecific lineages, this area was identified as “irreplaceable” in an analysis of California biodiversity (Rissler *et al.* 2006). The description of two new species of *Batrachoseps* from this region brings the number of *Batrachoseps* in and around Kern River Canyon to six, representing more than 25% of the diversity of this genus. Four of the six *nigriventris* group species occur in or around the Kern River Canyon, as do one species each from the *diabolicus* group and the subgenus *Plethopsis*. Two more widespread species have ranges that closely approach the canyon: *B. gregarius* (which occurs at the mouth of the canyon and in the Greenhorn Mountains, generally at lower elevations than *B. altasierrae*), and *B. altasierrae* (occurring in the Greenhorn Mountains to the north of the canyon, and extending north to the Tule River drainage, and on the Kern Plateau). The other four species are endemic to the Kern Canyon region: *B. bramei* (endemic to the Upper Kern River Canyon), *B. simatus* (endemic to the Lower Kern River Canyon), *B. relictus* (endemic to Breckenridge Mountain and the Lower Kern River Canyon) and *B. robustus* (endemic to the Kern Plateau and Scodie Mountains). Thus, this xeric canyon represents a center of diversification for *Batrachoseps*.

Acknowledgments

Many individuals have contributed to exploring the Kern River Canyon region for *Batrachoseps* through the years. We thank Brad Alexander, Chris Evelyn, Mario García-París, Ron Gonzales, Vance Harper, Charles Heim, Dan Holland, Carolina Martín, Jonathan Richmond, Maxi Richmond, Zach Sturbaum, Elizabeth Timpe and Jens Vindum for assistance in collecting or information about sightings. We are especially grateful to Brad Alexander for his hospitality and for sharing his intuitions and intimate knowledge of the region and to Kay Yanev and Dom Nguyen for collecting the allozyme data. Thanks to David Wagner and Sean Rovito for the photographs in Figure 5B and 7A respectively, and to Tobias Landberg for the digital x-ray images. Digital x-rays were taken at the Museum of Comparative Zoology, Harvard; we appreciate the assistance of Jim Hanken, José Rosado, and Jon Woodward in arranging access and providing training. Jens Vindum (California Academy of Sciences) and Carol Spencer (Museum of Vertebrate Zoology, University of California, Berkeley) assisted with specimen loans. Elizabeth Timpe and an anonymous reviewer provided helpful comments on a draft of this manuscript. Phylogenetic

analyses were conducted using the Bioinformatics Facility of the University of Connecticut. The California Department of Fish and Game provided collecting permits. This work was conducted under approved Institutional Animal Care and Use Protocols at the University of California, Berkeley and the University of Connecticut. Partial funding for this work was provided by NSF grant DEB 0543446 to ELJ and several earlier NSF grants to DBW.

References

- Bartlett, R.D. & Bartlett, P.P. (2009) *Guide and Reference to the Amphibians of Western North America (North of Mexico) and Hawaii*. University of Florida Press, Gainesville, 217 pp.
- Brame, A.H. Jr. & Murray, K.F. (1968) Three new slender salamanders (*Batrachoseps*) with a discussion of relationships and speciation within the genus. *Bulletin of the Natural History Museum of Los Angeles County*, 4, 1–35.
- Brown, J.M., Hedtke, S.M., Lemmon, A.R. & Lemmon, E.M. (2010) When trees grow too long: investigating the causes of highly inaccurate Bayesian branch length estimates. *Systematic Biology*, 59, 145–161.
- Camp, C.L. (1915) *Batrachoseps major* and *Bufo cognatus*, new Amphibia from southern California. *University of California Publications in Zoology*, 12, 327–334.
- Chatzimanolis, S. & Caterino, M.S. (2007) Toward a better understanding of the “Transverse Range break”: lineage diversification in southern California. *Evolution*, 61, 2127–2141.
- Cope, E.D. (1869) A review of the species of the Plethodontidae and Desmognathidae. *Proceedings of the Academy of Natural Sciences of Philadelphia*, 21, 93–118.
- de Queiroz, K. (2007) Species concepts and species delimitation. *Systematic Biology*, 56, 879–886.
- DeBry, R.W. & Olmstead, R.G. (2000) A simulation study of reduced tree-search effort in bootstrap resampling analysis. *Systematic Biology*, 49, 171–179.
- Desper, R. & Gascuel, O. (2002) Fast and accurate phylogeny reconstruction algorithms based on the minimum-evolution principle. *Journal of Computational Biology*, 9, 687–705.
- Dunn, E.R. (1926) *The Salamanders of the Family Plethodontidae*. Smith College, Northampton, Massachusetts, 456 pp.
- Fan, Y., Wu, R., Chen, M.-H., Kuo, L. & Lewis, P.O. (2011) Choosing among partition models in Bayesian phylogenetics. *Molecular Biology and Evolution*, 28, 523–532.
- Grinnell, J. & Camp, C.L. (1917) A distributional list of the amphibians and reptiles of California. *University of California Publications in Zoology*, 17, 127–208.
- Hansen, R.W. & Wake, D.B. (2005a) *Batrachoseps simatus* Brame and Murray, 1968. In: Lannoo, M. (Ed.), *Amphibian Declines: the Conservation Status of United States Species*. University of California Press, Berkeley, pp. 691–693.
- Hansen, R.W. & Wake, D.B. (2005b) *Batrachoseps relictus* Brame and Murray, 1968. In: Lannoo, M. (Ed.), *Amphibian Declines: the Conservation Status of United States Species*. University of California Press, Berkeley, pp. 688–690.
- Hendrickson, J.R. (1954) Ecology and systematics of salamanders of the genus *Batrachoseps*. *University of California Publications in Zoology*, 54, 1–46.
- Hilton, W.A. (1948) Salamander notes from the Northwest. *Herpetologica*, 4, 120.
- Huelsenbeck, J.P. & Ronquist, F. (2001) MrBayes: Bayesian inference of phylogeny. *Bioinformatics*, 17, 754–755.
- Jennings, M.R. (1996) Status of amphibians. In: *Sierra Nevada Ecosystem Project, Final Report to Congress, Volume II. Assessments and Scientific Basis for Management Options*. University of California, Davis, Wildlife Resources Center Report 37, pp. 921–944.
- Jennings, M.R. (2004) An annotated check list of the amphibians and reptiles of California and adjacent waters. *California Fish and Game*, 90, 161–213.
- Jennings, M.R. & Hayes, M.P. (1994) *Amphibian and Reptile Species of Special Concern in California*. California Department of Fish and Game, Rancho Cordova, California, 255 pp.
- Jockusch, E.L. (1996) *Evolutionary Studies in Batrachoseps and other Plethodontid Salamanders: Correlated Character Evolution, Molecular Phylogenetics, and Evolution of Reaction Norms*. Ph.D. Dissertation, University of California, Berkeley, 220 pp.
- Jockusch, E.L. (1997) Geographic variation and phenotypic plasticity of number of trunk vertebrae in slender salamanders, *Batrachoseps* (Caudata: Plethodontidae). *Evolution*, 51, 1964–1980.
- Jockusch, E.L., Wake, D.B. & Yanev, K.P. (1998) New species of *Batrachoseps* (Caudata: Plethodontidae) from the Sierra Nevada, California. *Los Angeles County Museum Contributions in Science*, 472, 1–17.
- Jockusch, E.L., Yanev, K.P. & Wake, D.B. (2001) Molecular phylogenetic analysis of slender salamanders, genus *Batrachoseps* (Amphibia: Plethodontidae), from central coastal California with descriptions of four new species. *Herpetological Monographs*, 15, 54–99.
- Jockusch, E.L. & Wake, D.B. (2002) Falling apart and merging: the diversification of slender salamanders (Plethodontidae: *Batrachoseps*) in the American West. *Biological Journal of the Linnean Society*, 76, 361–391.
- Kuchta, S.R. & Tan, A.M. (2005) Isolation by distance and post-glacial range expansion in the rough-skinned newt, *Taricha granulosa*. *Molecular Ecology*, 14, 225–244.
- Lapointe, F.J. & Rissler, L.J. (2005) Congruence, consensus, and the comparative phylogeography of codistributed species in California. *American Naturalist*, 166, 290–299.
- Lawson, R., Wake, D. B. & Beck, N.T. (1971) Tooth replacement in the red-backed salamander, *Plethodon cinereus*. *Journal of Morphology*, 134, 259–269.

- Lewis, P.O., Holder, M.T. & Swofford, D.L. (2010) Phycas v. 1.1.2. Software available from <http://phycas.org>.
- Macey, J.R., Strasburg, J.L., Brisson, J.A., Vredenburg, V.T., Jennings, M. & Larson, A. (2001) Molecular phylogenetics of western North American frogs of the *Rana boylei* species group. *Molecular Phylogenetics and Evolution*, 19, 131–143.
- Maiorana, V.C. (1978) Difference in diet as an epiphenomenon: space regulates salamanders. *Canadian Journal of Zoology*, 56, 1017–1025.
- Marlow, R.W., Brode, J.M. & Wake, D.B. (1979) A new salamander, genus *Batrachoseps*, from the Inyo Mountains of California, with a discussion of relationships in the genus. *Natural History Museum of Los Angeles County Contributions in Science*, 308, 1–17.
- Martínez-Solano, I., Jockusch, E.L. & Wake, D.B. (2007) Extreme population subdivision throughout a continuous range: phylogeography of *Batrachoseps attenuatus* (Caudata, Plethodontidae) in western North America. *Molecular Ecology*, 16, 4335–4355.
- Martínez-Solano, I. & Lawson, R. (2009) Escape to Alcatraz: evolutionary history of slender salamanders (*Batrachoseps*) on the islands of San Francisco Bay. *BMC Evolutionary Biology*, 8, 38.
- Martínez-Solano, I., Peralta-García, A., Jockusch, E.L., Wake, D.B., Vázquez-Domínguez, E. & Parra-Olea, G. (2012). Molecular systematics of *Batrachoseps* (Caudata, Plethodontidae) in southern California and Baja California: mitochondrial-nuclear DNA discordance and the evolutionary history of *B. major*. *Molecular Phylogenetics and Evolution*. doi: 10.1016/j.ympev.2011.12.026
- Moritz, C., Schneider, C.J. & Wake, D.B. (1992) Evolutionary relationships within the *Ensatina eschscholtzii* complex confirm the ring species interpretation. *Systematic Zoology*, 41, 273–291.
- Nei, M. (1972) Genetic distances between populations. *American Naturalist*, 106, 283–292.
- Nei, M. (1978) Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics*, 89, 538–590.
- Paradis, E., Claude, J. & Strimmer, K. (2004) APE: analyses of phylogenetics and evolution in R language. *Bioinformatics*, 20, 289–290.
- Peabody, F.E. & Savage, J.M. (1959) Trackways of living and fossil salamanders. *University of California Publications in Zoology*, 63, 1–72.
- Pereira, R.J. & Wake, D.B. (2009) Genetic leakage after adaptive and nonadaptive divergence in the *Ensatina eschscholtzii* ring species. *Evolution*, 63, 2288–2301.
- Posada, D. & Crandall, K.A. (1998) MODELTEST: testing the model of DNA substitution. *Bioinformatics*, 14, 817–818.
- Pyron, R.A. & Wiens, J.J. (2011) A large-scale phylogeny of Amphibia including over 2,800 species, and a revised classification of extant frogs, salamanders, and caecilians. *Molecular Phylogenetics and Evolution*, 61, 543–583.
- R Development Core Team (2008) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Software available from <http://www.R-project.org>.
- Recuero, E., Martínez-Solano, I., Parra-Olea, G. & García-París, M. (2006) Phylogeography of *Pseudacris regilla* (Anura: Hylidae) in western North America, with a proposal for a new taxonomic rearrangement. *Molecular Phylogenetics and Evolution*, 39, 293–304.
- Rissler, L.J., Hijmans, R.J., Graham, C.H., Moritz, C. & Wake, D.B. (2006) Phylogeographic lineages and species comparisons in conservation analyses: a case study of California herpetofauna. *American Naturalist*, 167, 655–666.
- Stebbins, R.C. (1985) *A Field Guide to Western Reptiles and Amphibians*. Second edition, revised. Houghton Mifflin Co., Boston, 336 pp.
- Stebbins, R.C. (2003) *A Field Guide to Western Reptiles and Amphibians*. Third edition, revised. Houghton Mifflin Co., Boston, 533 pp.
- Stebbins, R.C. & McGinnis, S.M. (in press) *Field Guide to Amphibians and Reptiles of California, revised edition*. University of California Press, Berkeley, 544 pp.
- Swofford, D.L. (2003) *PAUP*4.0b: Phylogenetic Analysis Using Parsimony*. Sinauer Associates, Sunderland, Massachusetts.
- Vieites, D.R., Román, S.N., Wake, M.H. & Wake, D.B. (2011) A multigene perspective on phylogenetic relationships in the largest family of salamanders, the Plethodontidae. *Molecular Phylogenetics and Evolution*, 59, 623–635.
- Vredenburg, V.T., Bingham, R., Knapp, R., Morgan, J.A.T., Moritz, C. & Wake, D. (2006) Concordant molecular and phenotypic data delineate new taxonomy and conservation priorities for the endangered mountain yellow-legged frog. *Journal of Zoology*, 271, 361–374.
- Wake, D.B. (1989) Phylogenetic implications of ontogenetic data. In: David, B., Dommergues, J.-L., Chaline, J. & Laurin, B. (Eds.), *Ontogenèse et Évolution*. *Geobios*, Mémoire Spécial 12, pp. 369–378.
- Wake, D.B. & Jockusch, E.L. (2000) Detecting species borders using diverse data sets: plethodontid salamanders in California. In: Bruce, R.C., Jaeger, R.G. & Houck, L.D. (Eds.), *The Biology of the Plethodontidae*. Kluwer Academic/Plenum Publishers, New York, pp. 95–119.
- Wake, D.B., Yanev, K.P. & Hansen, R.W. (2002) New species of slender salamander, genus *Batrachoseps*, from the southern Sierra Nevada of California. *Copeia*, 2002, 1016–1028.
- Yanev, K.P. (1978) *Evolutionary Studies of the Plethodontid Salamander Genus Batrachoseps*. Ph.D. Dissertation, University of California, Berkeley, 242 pp.
- Yanev, K.P. (1980) Biogeography and distribution of three parapatric salamander species in coastal and borderland California. In: Power, D.M. (Ed.), *The California Islands: Proceedings of a Multidisciplinary Symposium*. Santa Barbara Museum of Natural History, Santa Barbara, California, pp. 531–550.
- Zwickl, D.J. (2006) *Genetic Algorithm Approaches for the Phylogenetic Analysis of Large Biological Sequence Datasets under the Maximum Likelihood Criterion*. Ph.D. Dissertation, University of Texas, Austin, 115 pp.