NUMBER 472
6 NOVEMBER 1998

CONTRIBUTIONS IN SCIENCE

NEW SPECIES OF SLENDER SALAMANDERS, *BATRACHOSEPS* (AMPHIBIA: PLETHODONTIDAE), FROM THE SIERRA NEVADA OF CALIFORNIA

ELIZABETH L. JOCKUSCH, DAVID B. WAKE,
AND KAY P. YANEV

NATURAL HISTORY MUSEUM OF LOS ANGELES COUNTY

Serial Publications of the Natural History Museum of Los Angeles County

The scientific publications of the Natural History Museum of Los Angeles County have been issued at irregular intervals in three major series; the issues in each series are numbered individually, and numbers run consecutively, regardless of the subject matter.

- Contributions in Science, a miscellaneous series of technical papers describing original research in the life and earth sciences.
- Science Bulletin, a miscellaneous series of monographs describing original research in the life and earth sciences. This series was discontinued in 1978 with the issue of Numbers 29 and 30; monographs are now published by the Museum in Contributions in Science.
- Science Series, long articles and collections of papers on natural history topics.

Copies of the publications in these series are sold through the Museum Book Shop. A catalog is available on request.

The Museum also publishes Technical Reports, a miscellaneous series containing information relative to scholarly inquiry and collections but not reporting the results of original research. Issue is authorized by the Museum's Scientific Publications Committee; however, manuscripts do not receive anonymous peer review. Individual Technical Reports may be obtained from the relevant Section of the Museum.

Natural History Museum of Los Angeles County
900 Exposition Boulevard
Los Angeles, California 90007

Printed at Allen Press, Inc., Lawrence, Kansas
ISSN 0459-8113

New Species of Slender Salamanders, *Batrachoseps* (Amphibia: Plethodontidae), from the Sierra Nevada of California

Elizabeth L. Jockusch,[1] David B. Wake,[1,2] and Kay P. Yanev[1,3]

ABSTRACT. Slender salamanders, genus *Batrachoseps* (Caudata: Plethodontidae), are a taxonomically difficult group widely distributed in California as well as in Oregon and northwestern Baja California. Here we describe four new species from the Sierra Nevada based on analyses of allozymes, mitochondrial DNA, and morphology. Specimens of the new species previously have been assigned to other taxa, most recently to *Batrachoseps nigriventris* and *Batrachoseps pacificus relictus*. One of the new species, a relative of *B. nigriventris*, is distributed along the central and southern Sierra Nevada at low to middle elevations. We recognize *B. relictus*, a taxon currently treated as a subspecies of *B. pacificus*, as a distinct species that ranges from the lower Kern River Canyon and Greenhorn Mountains in northern Kern County to the Sierra Nevada in southern Fresno County. Populations of *Batrachoseps* occurring at low elevations in canyons along the west slopes of the Sierra Nevada from the American River to the Kings River that were previously included in *B. relictus* show substantial biochemical differentiation from that taxon and from each other, and they are described as new species. Although each of the new species is morphologically distinct, the differences are subtle and they are appropriately considered cryptic species.

INTRODUCTION

The slender salamanders, genus *Batrachoseps* Bonaparte, 1841, are widely distributed in the Sierra Nevada of California, and they were long considered to be members of the species *B. attenuatus* (Eschscholtz, 1833), both in formal taxonomic treatments (Hendrickson, 1954) and general works (Stebbins, 1966). The description of three new species from the southern Sierra Nevada and nearby mountains by Brame and Murray (1968) changed the picture radically. In addition to the new taxa, these authors chose to recognize both *B. major* Camp, 1915, and *B. pacificus* Cope, 1865, as taxa distinct from *B. attenuatus*. Sympatry was found between two of the new species (*B. relictus* Brame and Murray, 1968, and *B. simatus* Brame and Murray, 1968) in the lower Kern Canyon. They found no sympatry elsewhere in the Sierra Nevada, although both their *B. attenuatus* and their *B. relictus* occurred from Mariposa County south to the Kern River, the former at lower elevations than the latter, which in turn did not range as far north.

Yanev (1980) separated Brame and Murray's *B. attenuatus* in the Sierra Nevada into northern and southern forms, retaining the former in *B. attenuatus* and placing the latter in *B. nigriventris* Cope, 1869. Furthermore, *B. relictus* was reduced to a subspecies of *B. pacificus*, which was treated as a widespread complex of named and unnamed subspecific entities. Yanev identified an additional area of sympatry in the Sierra Nevada, between *B. nigriventris* and *B. pacificus relictus* in the Kaweah River drainage. Yanev (1978) presented a phenogram of genetic distances based on extensive allozyme surveys that showed relatively large amounts of differentiation within *B. nigriventris* (which also included coastal forms) and *B. p. relictus*. We have conducted DNA sequencing studies (Jockusch, 1996), as well as additional electrophoretic and morphological analyses of the Sierran forms and have concluded that the distinctive populations first identified by Yanev (1980) merit description as species-level taxa. In addition, we believe that *B. pacificus relictus* merits recognition at the species level.

The slender members of the now relatively speciose genus *Batrachoseps* comprise at least four major clades (suggested in the phenogram of Yanev, 1980; our conclusions are based on the analysis of DNA sequence data of Jockusch, 1996; see Jockusch, 1997, and discussion). The species described in this paper belong to two of these clades, which we call the *nigriventris* and *relictus* groups.

Similarity in gross morphology of many of the species, including those described herein, makes the genus *Batrachoseps* taxonomically difficult. Geo-

1. Museum of Vertebrate Zoology and Department of Integrative Biology, University of California, Berkeley, California 94720-3160.

2. Research Associate in Herpetology, Natural History Museum of Los Angeles County, Los Angeles, California 90007.

3. Sequence of authorship alphabetical.

Contributions in Science, Number 472, pp. 1–17
Natural History Museum of Los Angeles County, 1998

graphic variation in morphology can be great within taxa, and past workers (e.g., Hendrickson, 1954) assumed that some of what we now recognize as interspecific variation was intraspecific. Osteological data, useful in distinguishing between taxa at the level of subgeneric clades, are of limited utility in distinguishing species within the subgenus *Batrachoseps,* even between relatively remote relatives (Yanev, 1978). Consequently, molecular data are crucial in determining the limits of species and clades. Once species are identified, some relatively invariant morphological traits usually can be discerned. We have used such traits in the diagnoses and descriptions that follow, but it may be difficult to classify future discoveries in this region without conducting molecular studies.

MATERIALS AND METHODS

Specimens used in this study have been collected since the detailed report of Brame and Murray (1968) and are mainly stored in the Museum of Vertebrate Zoology (MVZ), Berkeley. Paratypes of the new species are deposited in the Natural History Museum of Los Angeles County (LACM). Tissues extracted from these specimens were used for studies of protein variation using starch-gel electrophoresis (detailed methods as in Yanev and Wake, 1981) and for studies of DNA differentiation using direct sequencing of up to 786 base pairs of the mitochondrial gene cytochrome b (detailed methods as in Jackman et al., 1997). Sequences from one individual of each of the five species treated in this paper have been submitted to GenBank (Accession numbers AF057549–AF057553). Results of these studies are contained in two unpublished dissertations (Jockusch, 1996; Yanev, 1978) and are summarized here. Proteins are identified by name, abbreviation, and their Enzyme Commission numbers (see Murphy et al., 1996) on first use, and subsequently by abbreviation. Genetic distances (D_N) for protein comparisons are those of Nei (1972), and divergence in DNA sequence is reported as Kimura two-parameter distances (K2P) (Kimura, 1980). Morphological comparisons are presented in Table 1. All measurements are in millimeters. Standard length (SL) is the distance from the tip of the snout to the posterior angle of the vent. Limb interval is the number of costal folds uncovered when the forelimbs and hind limbs are adpressed toward the trunk. Tooth counts include only visible teeth, and gaps are not counted.

SYSTEMATICS

The genus *Batrachoseps* is a member of the family Plethodontidae and shares with all members of that family many morphological synapomorphies, including lack of lungs, lack of ossified pterygoid bones, and a nasolabial groove (the last two unique to the family). The genus is monophyletic (Jackman et al., 1997), and among the numerous morphological synapomorphies are the presence of a large frontoparietal fontanelle, only four toes on the hind limb, and an attached, projectile tongue that has extremely elongated genioglossal muscles. There are two monophyletic subgenera, *Plethopsis* and *Batrachoseps* (Jackman et al., 1997). The latter subgenus has three osteological synapomorphies: no prefrontal bone, either no or an extremely short preorbital process of the vomer, and a single premaxillary bone (Wake, 1989). These synapomorphies characterize the species described herein; accordingly, they are assigned to *Batrachoseps* (*Batrachoseps*). This assignment is also strongly supported by phylogenetic analysis of mitochondrial DNA sequences (Jockusch, 1996, 1997).

Batrachoseps nigriventris group

***Batrachoseps gregarius,* new species**
Gregarious Slender Salamander
Figure 1

Batrachoseps attenuatus attenuatus (part), Hendrickson, 1954:23.
Batrachoseps attenuatus (part), Brame and Murray, 1968:14.
Batrachoseps relictus (part), Brame and Murray, 1968:7.
Batrachoseps nigriventris (part), Yanev, 1980:535; Stebbins, 1985:55.

HOLOTYPE. MVZ 224581, an adult female from Westfall Picnic Ground east of Highway 41, Sierra National Forest, Madera–Mariposa county line, California, collected by Elizabeth Jockusch and Gabriela Parra-Olla on April 3, 1995. 37° 26′ 43″ N, 119° 39′ 01″ W, 1400 m elevation.

PARATYPES. MVZ 158210–12 (May 25–26, 1980), 224551 (April 25, 1993), 224557–58 (March 25, 1994), 224563, 224576 (April 8, 1994), 224578, 224591–94, 224596, 224606 (April 3, 1995), 224611 (April 28, 1995), 224614 (April 1, 1996), LACM 144219 (April 1, 1996), from same locality as holotype, collected at different times, as indicated.

REFERRED SPECIMENS (specimens used in study of mtDNA). MVZ 224617, same location as holotype; MVZ 224516, Sugar Pine, Madera County, California, 37° 26′ N, 119° 38′ W, 1350 m elevation; MVZ 219157, meadow ca 3.2 km SW Dinkey Mountain, Fresno County, California, 37° 00′ N, 119° 08′ W, 1900 m elevation; MVZ 157350, along South Fork Kaweah River, 7.7 km SE Highway 198, Tulare County, California, 36° 23′ N, 118° 52′ W, 430 m elevation; MVZ 154077, Old Stage Road along Arrastre Creek, 2.3 km SE White River, Tulare County, California, 35° 48′ N, 118° 52′ W, 600 m elevation; MVZ 167636, 4.5 km N Kern River bridge on Rancheria Road, Kern County, California, 35° 28′ N, 118° 49′ W, 350 m elevation.

DIAGNOSIS. A relatively short-bodied (usually 18 trunk vertebrae in males and 19 in females) slender salamander with short limbs (8–11 costal folds between adpressed limbs); distinguished from all other members of the genus by presence of a mental hedonic gland in most adult males and by allozymic and mitochondrial DNA markers. Most closely related to *B. nigriventris,* from which it is distinguished by having a more rapidly migrating variant (a fixed difference) for the protein aspartate ami-

Figure 1. An adult *Batrachoseps gregarius* from near Fountain Springs, Tulare County, California. Photo by M. García Paris, April 1994.

notransferase-1 (AAT-1, #2.6.1.1) and by many differences in the sequence of the mitochondrial gene cytochrome b (K2P ca 9–10%). Distinguished from geographically neighboring species *B. relictus, B. kawia, B. regius,* and *B. diabolicus* by having shorter and narrower hands and feet; from *B. regius* and *B. kawia* by having shorter limbs and fewer maxillary teeth; from *B. regius* and *B. diabolicus* by having a narrower head (Table 1, Fig. 2). Further distinguished from all of these geographically neighboring species by fixed differences at several allozymic loci ($D_N > 1.0$) and by many sequence differences in the DNA of the mitochondrial gene cytochrome b (K2P > 0.12)

DESCRIPTION. *Batrachoseps gregarius* is a small (adults less than 50 mm maximum SL), slender species with a relatively narrow head and only a slightly narrower neck. The facial region (area from the eyes to the snout) is small and inconspicuous, and the eyes are moderately protuberant. The nostrils are small and males have only slight nasolabial protuberances. Barely evident to pronounced mental hedonic glands are present under the chin of most males. Grooving patterns of the head, throat, and neck are typical of the genus. The relatively few vomerine teeth usually are borne in a single row. Small maxillary teeth are borne in a long row extending to the posterior margin of the eye. There are relatively few, small (same size as maxillary teeth) premaxillary teeth in females, and there are fewer teeth in males, but they are relatively enlarged. Males have 17–18 costal grooves between the limbs, and females have 18–19. SL is from 7.9–9.8 ($\bar{x}$ = 8.8) times head width in males and 8.8–10.0 ($\bar{x}$ = 9.5) times in females. The tail is long and tapers toward the tip. The tail is 0.5–1.3 ($\bar{x}$ = 1.0) times SL in males and 1.1–1.5 ($\bar{x}$ = 1.2) times in females. There is no basal tail constriction. The postiliac gland is evident. The limbs are relatively short; SL ranges from 5.6–6.8 ($\bar{x}$ = 6.0) times hind limb length in males and 5.8–7.1 ($\bar{x}$ = 6.6) times in females. The hands and feet are relatively small. The digits are 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 mm). Maximum head width 4.5; snout to gular fold (head length) 7.5; head depth at posterior angle of jaw 2.8; eyelid length 2.1; eyelid width 1.0; anterior rim of orbit to snout 1.4; horizontal orbital diameter 0.9; interorbital distance 1.4; snout to forelimb 9.4; distance separating external nares 1.1; snout projection beyond mandible 0.3; snout to posterior angle of vent (SL) 43.7; snout to anterior angle of vent 41.6; axilla to groin length 29.0; tail length 63.3; tail width at base 3.1; tail depth at base 3.1; forelimb length 5.8; hind limb length 6.4; limb interval 10; width of right hand 1.2; width of right foot 1.4; foot length 1.7; length of third toe 0.6; body width behind forelimbs 3.5. There are 12 premaxillary, 36 maxillary, and 14 vomerine teeth and 19 trunk, 2 caudosacral, and 46 caudal vertebrae.

COLORATION OF THE SPECIES (in alcohol). These are blackish animals with a lighter, generally brownish dorsal band. The ground color of the body and tail is usually black at the lateral borders of the dorsal band grading steadily into a dark to

Table 1. Measurements of the type series and of *B. relictus* from two localities. All measurements are in mm.*

Species	N	Standard length	Vomerine teeth	Maxillary teeth	Premaxillary teeth	Trunk vertebrae	Head width	Limb interval	Hind limb length	Foot width
Males										
B. gregarius	9	35.9 ± 3.1	10.1 ± 2.1	18.6 ± 2.9	4.7 ± 1.8	18	4.1 ± 0.4	9.1 ± 0.9	6.0 ± 0.4	1.3 ± 0.1
		29.9–40.2	7–14	15–24	3–8	18–19	3.5–4.6	8.0–10.5	5.2–6.6	1.1–1.5
B. diabolicus	10	36.2 ± 2.4	12.9 ± 2.0	22.2 ± 6.4	4.1 ± 1.4	20	4.3 ± 0.2	7.9 ± 0.4	6.7 ± 0.5	1.8 ± 0.2
		31.3–39.8	10–17	10–31	2–7	19–20	4.0–4.6	7.5–8.5	5.8–7.4	1.4–2.0
B. regius	10	35.6 ± 1.9	11.8 ± 2.3	30.9 ± 3.3	3.5 ± 1.7	19	4.4 ± 0.2	7.8 ± 0.8	6.7 ± 0.3	1.6 ± 0.2
		33.6–40.1	6–14	25–35	2–6	19–20	4.0–4.6	6.5–9.0	6.3–7.4	1.5–2.1
B. kawia	10	34.9 ± 2.6	13.8 ± 1.2	37.3 ± 3.2	3.3 ± 1.6		4.0 ± 0.3	8.3 ± 0.8	6.5 ± 0.4	1.5 ± 0.1
		31.4–38.8	12–16	32–43	2–7	19–20	3.6–4.5	7.5–9.5	5.9–6.9	1.4–1.7
B. relictus										
Greenhorns	7	37.3 ± 1.7	10.6 ± 2.3	16.0 ± 6.1	4.9 ± 0.4		4.1 ± 0.1	8.2 ± 1.0	5.5 ± 0.2	1.5 ± 0.1
		35.2–40.4	6–13	9–26	4–5	18–19	4.0–4.3	7.0–9.5	5.3–5.8	1.4–1.7
Kern Canyon	7	27.0 ± 4.3	9.9 ± 2.3	20.9 ± 10.6	2.9 ± 0.4	17	3.5 ± 0.5	5.1 ± 1.1	5.1 ± 0.8	1.4 ± 0.3
		20.9–34.8	7–14	5–33	2–3	17–18	2.7–4.4	3.5–7.0	3.8–6.2	0.9–1.9
Females										
B. gregarius	10	42.5 ± 2.5	13.5 ± 1.3	28.2 ± 6.1	9.8 ± 1.9	19	4.5 ± 0.2	10.4 ± 0.7	6.5 ± 0.4	1.4 ± 0.1
		38.4–46.0	12–16	21–37	6–12	18–20	4.3–4.8	9.0–11.0	5.9–7.2	1.2–1.5
B. diabolicus	10	38.9 ± 4.4	13.4 ± 2.5	29.4 ± 7.8	9.6 ± 3.1	20	4.4 ± 0.4	8.4 ± 0.7	6.8 ± 0.4	1.8 ± 0.1
		31.0–44.5	9–17	13–38	5–14	20–21	3.6–4.9	7.0–9.5	6.2–7.3	1.6–2.0
B. regius	10	36.4 ± 3.6	14.5 ± 2.8	41.1 ± 9.3	12.1 ± 3.2	20	4.4 ± 0.3	8.6 ± 0.6	6.7 ± 0.5	1.6 ± 0.2
		32.3–44.9	11–19	24–52	6–16	19–20	4.0–5.1	8.0–10.0	5.7–7.5	1.4–2.1
B. kawia	8	37.6 ± 4.6	18.8 ± 5.0	50.6 ± 10.7	18.6 ± 3.7	20	4.1 ± 0.4	8.6 ± 1.0	6.8 ± 0.6	1.6 ± 0.1
		32.0–46.7	12–28	42–75	15–25	19–20	3.5 ± 4.6	7.5–10.5	6.0–7.5	1.4–1.8
B. relictus										
Greenhorns	8	40.1 ± 3.5	11.8 ± 2.1	21.9 ± 3.3	6.4 ± 2.6		4.3 ± 0.2	8.5 ± 0.8	5.7 ± 0.4	1.7 ± 0.2
		34.4–45.4	9–16	18–28	4–12	18–19	4.0–4.7	7.5–9.5	5.2–6.2	1.4–2.0
Kern Canyon	2	34.0 ± 0.9	14.0 ± 2.8	46.5 ± 0.7	6.0 ± 1.4	17	4.1 ± 0.1	6.8 ± 0.4	6.1 ± 0.1	1.9 ± 0.0
		33.3–34.6	12–16	46–47	5–7	17	4.0–4.1	6.5–7.0	6.0–6.1	1.9

* Measurements are mean ± SD (mode for trunk vertebrae) and range.

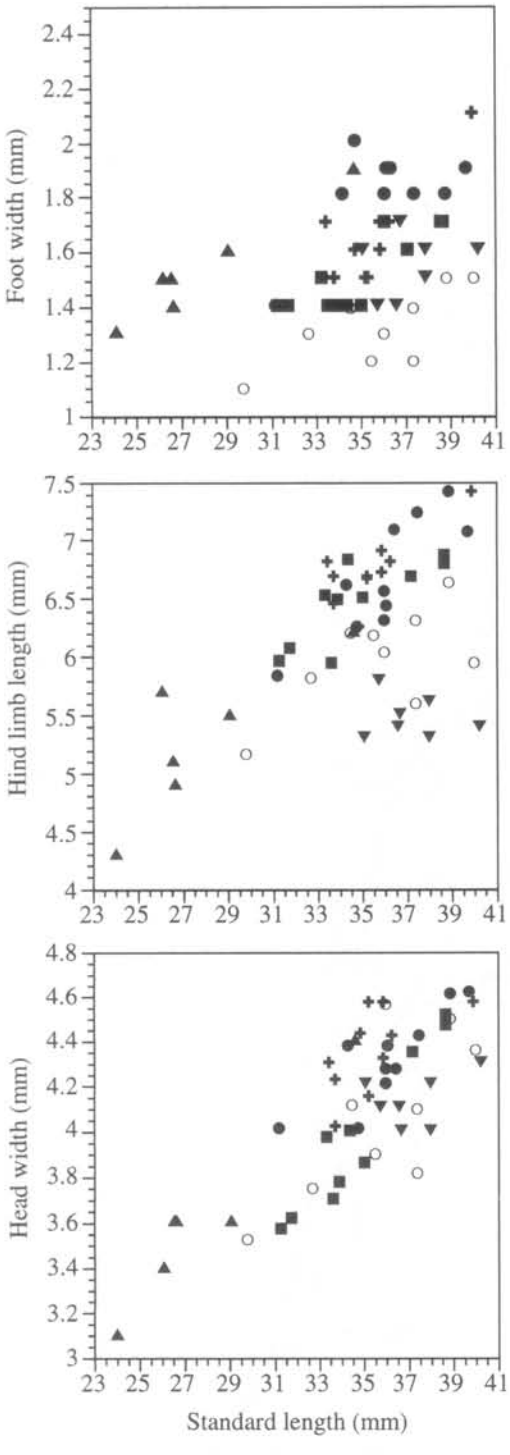

Figure 2. Scatterplots of morphological data for males of species described in this paper. A. Foot width compared to SL. B. Hind limb length compared to SL. C. Head width compared to SL. Symbols: ○ *B. gregarius*, + *B. regius*, ● *B. diabolicus*, ■ *B. kawia*, ▲ *B. relictus* (topotypic), ▼ *B. relictus* (Greenhorn Mountains).

pale gray ventrally. The ground color is heavily marked with small white spots or flecks, especially dorsolaterally where they form a diffuse lateral band. The spots are tiny and well separated ventrally but are most numerous on the gular region and on the tail. In some individuals the midventral region is a flat pale gray, unmarked by white. A distinct dorsal band arises from a zone of golden-brown to tan coloration in the nuchal region. This band occurs well onto the tail, extending nearly to the tip in some specimens. Coloration of the band is variable; in general it is pale brown with golden and tan highlights and some dark flecks. In some individuals the band is very dark and thus relatively obscure. The band is usually streaked or spotted with color that is both lighter and darker than the main band color. In some individuals the band covers the head, extending to the tip of the snout. A dark, inverted triangle that originates near the eyes and ends in the nuchal region is present in some individuals.

HABITAT AND DISTRIBUTION. This species ranges from the southern boundary of Yosemite National Park south nearly to the Kern River on the west slope of the Sierra Nevada and Greenhorn Mountains (Fig. 3). The northernmost localities are Feliciana Mountain and Jerseydale Ranger Station, Mariposa County (specimens in MVZ). In the northern part of its range it occurs from near the eastern margin of the Central Valley up to about 1800 m (in the Dinkey Creek drainage, Fresno County). The species appears to occur only at low elevations on the eastern margins of the Central Valley in the southern parts of its range (below 300 m in the White River drainage, Kern–Tulare county line).

This species occupies a wide range of habitats, from mixed Sierran coniferous forests at high elevation (*Pinus ponderosa, P. lambertiana, Abies concolor, Calocedrus decurrens, Sequoia giganteum, Quercus kelloggii*), to open woodlands, and even to open grasslands at low elevations. In the latter regions it occurs in habitats that would normally be considered highly improbable for plethodontid salamanders, because of the long, very hot and dry summers and low precipitation. A weather station (South Entrance Yosemite National Park; 37° 30′ N, 119° 38′ W, 1560 m) near the type locality, but at a slightly higher elevation, has an average annual precipitation of 113 cm, with some snow often present from early November to the end of April. In sharp contrast, a weather station (Kern River Pump House; 35° 28′ N, 118° 47′ W, 296 m) very near the southernmost sites at which the species has been collected receives only 27 cm of precipitation annually, with no snow, and with average daily temperatures exceeding 27° C (a near lethal temperature even when conditions are moist) for 5.5 months. At this site average monthly rainfall is on the order of 25 mm or less for seven months.

ETYMOLOGY. The name *gregarius* is the Latin word for flock or herd and refers to the habit of

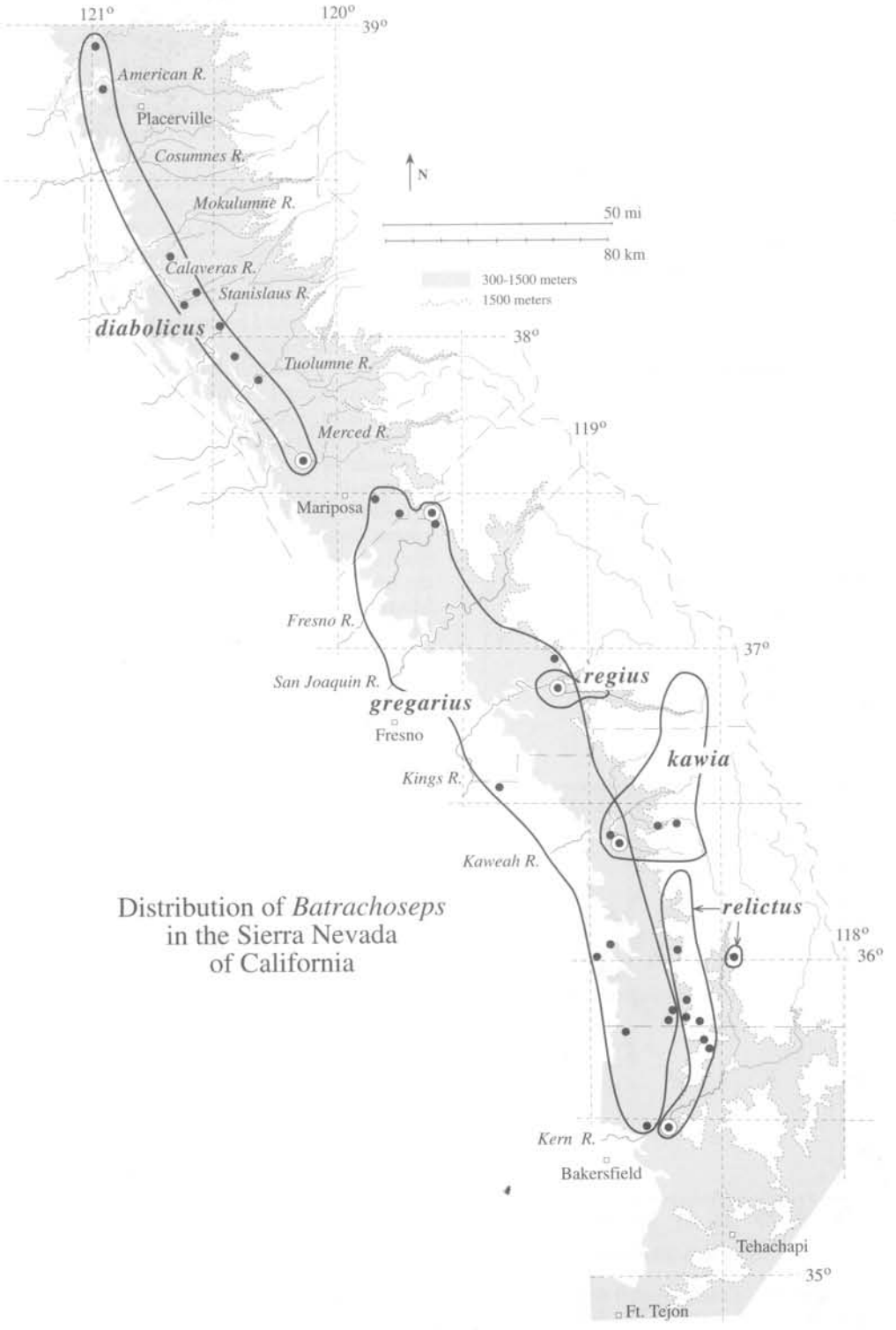

Figure 3. Distribution of four species of salamanders of the subgenus *Batrachoseps* in the Sierra Nevada of California. Type localities are indicated by ⊙, populations sampled for allozymic or DNA sequence analysis are indicated by ●, and estimated ranges are outlined. Additional species occur in the Kern River Canyon region, in the mountains to the south and southwest, and along the eastern edge of the Central Valley north of the Stanislaus River. Illustration by K. Klitz.

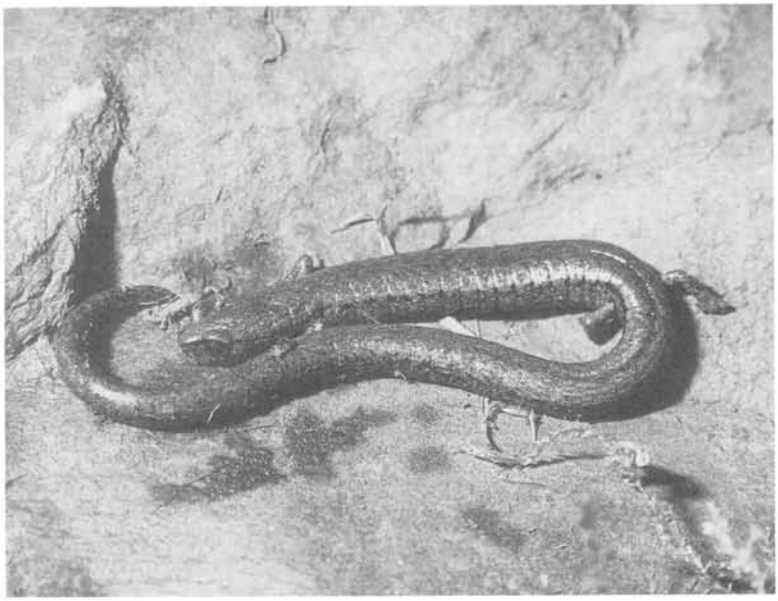

Figure 4. An adult *Batrachoseps diabolicus* from the type locality, in Hell Hollow, Mariposa County, California. Photo by M. García París, April 1994.

this species of laying their eggs communally (Jockusch and Mahoney, 1997).

COMMENTS. This species is widely distributed in the Sierran foothills and extends out onto the floor of the Central Valley in some places, especially in the White River drainage on the Tulare–Kern county line and just north of the Kern River in northern Kern County. It occurs in sympatry with *B. kawia* along the South Fork of the Kaweah River and may occur in sympatry with other species as well. Its range comes close to that of *B. relictus* in Tulare County, to *B. regius* in Fresno County, and to *B. diabolicus* in central Mariposa County. It may occur in sympatry with some or all of these species, but it should be easily distinguished from them using morphological characters. Although it does not occur in sympatry with *B. attenuatus,* it is similar in morphology to that species and it would be difficult to distinguish the taxa on morphological grounds, even though they are extremely different for congeneric taxa in both allozymes and mtDNA gene sequences. Sympatry of *B. gregarius* occurs with *Aneides lugubris* near Coarsegold and *Ensatina eschscholtzii* at several places in Fresno and Madera counties. *Batrachoseps gregarius* and *B. nigriventris* are allopatric. The range of *B. nigriventris* most closely approaches that of *B. gregarius* along the western slopes of the Tehachapi Mountains, but there is a geographic gap of undetermined magnitude (on the order of 30–35 km) between the two species in central Kern County, where the valley of the Kern River separates them.

This taxon varies geographically in morphology as well as in allozymes and mtDNA sequences (see below). Populations lying west of the main Sierra Nevada range, in northern Kern and southern Tulare counties, are more slender than topotypic specimens, with more trunk vertebrae, shorter limbs, and smaller hands and feet. They also live in much more open habitat that is subject to intense and prolonged summer heat and drought. By contrast, populations near the type locality occur in closed-canopy forest in areas of relatively high rainfall and mild temperatures. In addition, members of the lowland populations lay eggs in the fall, whereas those in the vicinity of the type locality lay eggs in the spring.

We considered the possibility that the two population segments might be sufficiently distinct to warrant the recognition of two species rather than one. However, the conflicting placement of geographically intermediate populations in analyses of allozymic and DNA sequence data suggests that gene flow has occurred locally, leaving geographically distant populations differentiated. Thus we have chosen to recognize only a single species (see Discussion).

Batrachoseps relictus group

Batrachoseps diabolicus, new species
Hell Hollow Slender Salamander
Figure 4

Batrachoseps relictus (part), Brame and Murray, 1968:7.

Batrachoseps pacificus relictus (part), Yanev, 1980: 535.

HOLOTYPE. MVZ 95446, an adult male from Hell Hollow, at the junction with the Merced River at Lake McClure on California Highway 49, Mariposa County, California, collected on February 6, 1971, by S.B. Ruth. 37° 36′ 33″ N, 120° 08′ 10″ W, ca 300 m elevation.

PARATYPES. MVZ 95444–45, 95449–51, 95454–55, 156572 (all collected February 6, 1971), 156586, 156588, 156598–99 (March 6, 1974), 156601, 156603–604 (January 27, 1973), 224836–38 (March 25, 1994), LACM 144218 (January 27, 1973), from same locality as holotype.

REFERRED SPECIMENS (specimens used in study of mtDNA). MVZ 156543, 7.9 km SE Vallecitos, Calaveras County, California, 38° 03′ N, 120° 28′ W, 425 m elevation; MVZ 172281, Highway 12 at intersection with Highway 49, 2.4 km NW San Andreas, Calaveras County, California, 38° 12′ N, 120° 42′ W, 250 m elevation; MVZ tissue number S-10450, Ponderosa Way, ca 6.5 km (air) S Mountain Ranch, Calaveras County, California, 38° 10′ N, 120° 32′ W, ca 450 m elevation; MVZ 158260, 4.2 km NW Cool, El Dorado County, California, 38° 55′ N, 121° 02′ W, 245 m elevation; MVZ 224840, SE side Middle Fork, American River, at Highway 49, El Dorado County, California, 38° 55′ N, 121° 03′ W, 200 m elevation; MVZ 224848, NW side Middle Fork, American River, at Highway 49, Placer County, California, 38° 55′ N, 121° 03′ W, 200 m elevation.

DIAGNOSIS. A relatively short-bodied (usually 19–21, mode 20, trunk vertebrae) slender salamander with limbs of moderate length (7–9½ costal folds between adpressed limbs). Distinguished from its geographical neighbor *B. gregarius* by males lacking a mental hedonic gland and by having longer limbs and larger hands and feet. It differs from its sympatric associate *B. attenuatus* in having longer limbs with broader hands and feet, a broader head, and a more robust body (in addition, the taxa show fixed differences for 12 of 19 proteins surveyed, and nearly fixed differences in two other proteins). Most closely related to three geographically neighboring species from which it differs as follows: from *B. relictus* in having longer limbs; from *B. kawia* and *B. regius* by having fewer maxillary teeth (Table 1, Fig. 2). Further distinguished from these three species by fixed differences in the following proteins: a more slowly migrating variant of lactate dehydrogenase-1 (LDH-1, #1.1.1.27) and more rapidly migrating variants of malate dehydrogenase (MDH-2, #1.1.1.37); it differs additionally from *B. relictus* by fixed differences in isocitrate dehydrogenase (IDH-2, #1.1.1.42) (a more rapidly migrating variant), leucine aminopeptidase (LAP, #3.4.11)(a more rapidly migrating variant), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH, #1.2.1.12) (a more slowly migrating variant); from *B. kawia* by phosphogluconate dehydrogenase (PGDH, #1.1.1.44) (two more slowly migrating variants, but also a rare more rapidly migrating variant), LAP (a more rapidly migrating variant), and GAPDH (a more slowly migrating variant); from *B. regius* by PGDH (three variants, one faster than either of two *regius* variants, one of intermediate mobility, and one slower than either variant), IDH-2 (a more rapidly migrating variant), and mannose-6-phosphate dehydrogenase (MPI, #5.3.1.8) (mainly a more rapidly migrating variant, but also an uncommon more slowly migrating variant). Distinguished from all other members of the genus by allozymic and mitochondrial DNA markers ($D_N > 0.49$; $K2P > 0.096$).

DESCRIPTION. *Batrachoseps diabolicus* is a small (adults less than 45 mm maximum SL), slender species with a relatively broad head and only a slightly narrower neck. The facial region (area from the eyes to the snout) is small and inconspicuous, and the eyes are moderately protuberant. The nostrils are small, and males have only slight nasolabial protuberances. No mental hedonic glands are observed in males. Grooving patterns of the head, throat, and neck are typical of the genus. The relatively few vomerine teeth usually are borne in patches. Small to very small maxillary teeth are borne in a long row that extends to the posterior margin of the eye. Premaxillary teeth are more numerous in females than in males and are dimorphic in size (enlarged in males but the same size as maxillary teeth in females). Males have 18–19 costal grooves between the limbs, and females have 19–20. SL is from 7.8–8.7 ($\bar{x}$ = 8.4) times head width in males and from 8.2–9.3 ($\bar{x}$ = 8.8) times in females. The tail is long, slender, and cylindrical and often shows evidence of having been regenerated. The tail is 1.3–1.6 ($\bar{x}$ = 1.4) times SL in males and 1.0–1.5 ($\bar{x}$ = 1.3) times in females. There is no basal tail constriction. The postiliac gland is evident. Limbs are moderately long. SL ranges from 5.2–5.6 ($\bar{x}$ = 5.4) times hind limb length in males and 4.9–6.5 ($\bar{x}$ = 5.7) times in females. The hands and feet are large for the slender members of the genus. The digits are 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 mm). Maximum head width 4.3; snout to gular fold (head length) 7.4; head depth at posterior angle of jaw 2.8; eyelid length 1.8; eyelid width 1.0; anterior rim of orbit to snout 1.6; horizontal orbital diameter 1.3; interorbital distance 1.1; snout to forelimb 9.3; distance separating external nares 1.4; snout projection beyond mandible 0.5; snout to posterior angle of vent (SL) 36.5; snout to anterior angle of vent 34.2; axilla to groin length 21.9; tail length 51.4; tail width at base 3.1; tail depth at base 2.7; forelimb length 6.5; hind limb length 7.1; limb interval 7½; width of right hand 0.9; width of right foot 1.9; foot length 2.4; length of third toe 0.7; body width behind forelimbs 3.3. There are 4 premaxillary, 26 maxillary, and 12 vomerine teeth and 20 trunk, 2 caudosacral, and 41 caudal vertebrae.

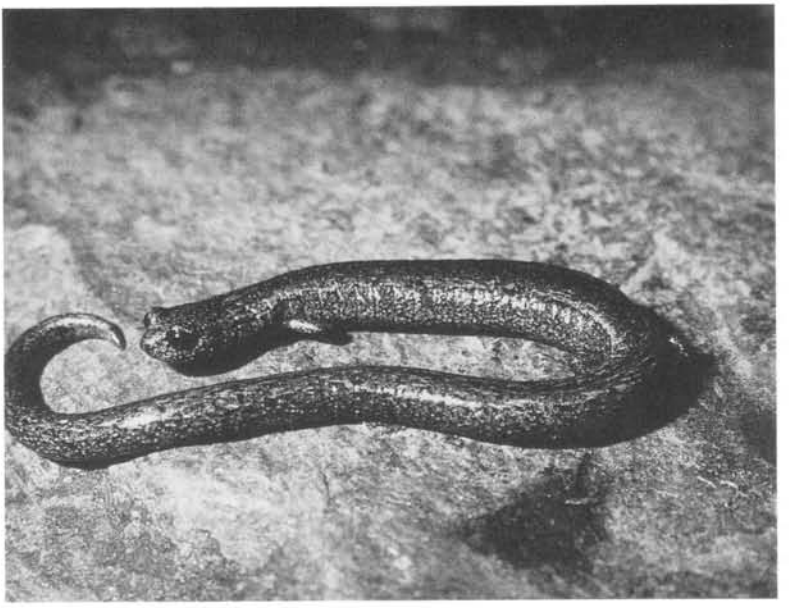

Figure 5. An adult *Batrachoseps regius* from the type locality, along the North Fork of the Kings River, Fresno County, California. Photo by M. García París, April 1994.

COLORATION OF THE SPECIES (in alcohol). The coloration of the type series has faded, but in general these are blackish animals with a lighter brownish dorsal band. The dorsal band is generally pale tan to light brown and is brighter at the lateral edges than in the center. The band continues onto the head. The dorsal band is more obvious in some individuals than in others and may be obscure. The band is variously flecked or streaked with both darker and brighter coloration than the main band color. A dark, inverted triangle that originates near the eyes and ends in the nuchal region is present in some individuals. There is extensive white spotting laterally and ventrally, with the spots most numerous laterally. The gray ventral surfaces are much lighter than the lateral ground color but fade in alcohol to a light tan.

HABITAT AND DISTRIBUTION. Known from the Merced River drainage to the American River at elevations below 300 m (Fig. 3). In general this species occurs in open, brushy areas on the margins of often dense chaparral. Typically the only trees present are scattered pines (usually *Pinus sabiniana,* occasionally *P. ponderosa*), oaks (both live oaks and deciduous oaks of several species) and buckeye (*Aesculus californica*).

This species generally is found in areas subject to long periods of hot, dry weather. The weather station nearest the type locality (Cathay Bull Run Ranch, 37° 24′ N, 120° 03′ W, 436 m) receives 50.5 cm of rainfall annually, with six summer months recording less than 25 mm and 4.5 months of average daily temperatures in excess of 27° C. A weather station very near the northernmost station for the species (Auburn, 38° 54′ N, 121° 04′ W, 396 m) receives 77 cm of rainfall annually, with over four months of average daily temperatures in excess of 27° C.

ETYMOLOGY. The name of the species is derived from the Greek word *diabolos,* devil, with reference to the name of the type locality, Hell Hollow.

COMMENTS. This species occurs in sympatry with *B. attenuatus* at the northern end of its range on the north slopes of the American River at Highway 49. Although the ranges of both species extend southward (both occur as far south as Calaveras County), these species have not been found in sympatry elsewhere. We have found *B. diabolicus* in sympatry with *Hydromantes brunus* along the Merced River and with *Aneides lugubris* and *Ensatina eschscholtzii xanthoptica* at several sites in Calaveras County.

Batrachoseps regius, new species
Kings River Slender Salamander
Figure 5

Batrachoseps pacificus relictus (part), Yanev, 1980: 535; Stebbins, 1985:58.

HOLOTYPE. MVZ 94029, an adult male from the south bank of the North Fork, Kings River, 1.6 km N (by road) of the Kings River, Fresno County, California, collected on December 5, 1970, by J. Crim, J.L. Edwards, S.B. Ruth, and S.S. Sweet. 36° 52′ 46″ N, 119° 07′ 30″ W, ca 335 m elevation.

PARATYPES. MVZ 94022–23, 94025–26, 94030–32, 94034, 94036, 94038–39, 94047–48,

94056–57, LACM 144216, same data as holotype; MVZ 224799–224800, 224803 same vicinity as holotype, collected on March 26, 1994.

DIAGNOSIS. A relatively short-bodied (usually 19 trunk vertebrae in males and 20 in females), slender salamander with limbs of moderate length (6½–10 costal folds between adpressed limbs). Distinguished from its geographical neighbor *B. gregarius* by males lacking a mental hedonic gland and by having a broader head, longer limbs, larger hands and feet, and more maxillary teeth. Distinguished from close relatives as follows: from *B. relictus* by more maxillary teeth; from *B. kawia* by its somewhat more robust habitus, with a longer and wider head and somewhat fewer maxillary teeth; from *B. diabolicus* by more maxillary teeth. The following fixed protein difference distinguishes it from these close relatives: MPI (a variant that migrates more rapidly than any except an even more rapidly migrating variant in *B. diabolicus*); it differs additionally from *B. relictus* by fixed differences in peptidase (PEP, #3.1.1.-) (a more slowly migrating variant), LAP (a more rapidly migrating variant), and GAPDH (a more slowly migrating variant); from *B. kawia* and *B. regius* by LAP (a more rapidly migrating variant), GAPDH (a more slowly migrating variant), and MDH-2 (a more slowly migrating variant); additionally from *B. kawia* by IDH-2 [a more rapidly migrating variant (common) and a unique variant of intermediate mobility (uncommon)], and from *B. regius* by IDH-2 [a more slowly migrating variant (common) and a unique variant of intermediate mobility (uncommon)]; from *B. diabolicus* by LDH-1 (a more rapidly migrating variant) and MDH-2 (a more slowly migrating variant). Distinguished from all other members of the genus by allozymic and mitochondrial DNA markers ($D_N > 0.37$; $K2P > 0.10$).

DESCRIPTION. *Batrachoseps regius* is a small (adults less than 45 mm maximum SL), slender species with a relatively broad head and a distinct neck. The facial region (area from the eyes to the snout) is small and inconspicuous, and the eyes are only slightly protuberant. The nostrils are small, and males have only slight nasolabial protuberances. Males lack mental hedonic glands. Grooving patterns of the head, throat, and neck are typical of the genus. The relatively few vomerine teeth are borne in distinct patches. Small to very small maxillary teeth are moderate in number and borne in a long row extending to the posterior margin of the eye in males, but in patchy or irregular rows in females. There are relatively many small premaxillary teeth (same size as maxillary teeth) in females, but fewer and larger teeth in males. Both sexes have 18–19 costal grooves between the limbs. SL is from 7.8–8.8 ($\bar{x}$ = 8.2) times head width in males and 7.6–8.8 ($\bar{x}$ = 8.3) times in females. The tail is long, slender, and cylindrical and often shows evidence of having been regenerated. The tail is 1.0–1.5 ($\bar{x}$ = 1.2) times SL length in males and 1.0–1.4 ($\bar{x}$ = 1.2) times in females. There is no basal tail constriction. The postiliac gland is inconspicuous. Limbs are relatively long; SL ranges from 4.9–5.6 ($\bar{x}$ = 5.3) times hind limb length in males and 5.0–6.5 ($\bar{x}$ = 5.4) times in females. The hands and feet are of moderate size for the slender members of the genus; the digits are 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 mm). Maximum head width 4.4; snout to gular fold (head length) 7.5; head depth at posterior angle of jaw 2.4; eyelid length 1.9; eyelid width 1.0; anterior rim of orbit to snout 1.6; horizontal orbital diameter 1.4; interorbital distance 1.1; snout to forelimb 9.1; distance separating external nares 1.6; snout projection beyond mandible 0.2; snout to posterior angle of vent (SL) 36.4; snout to anterior angle of vent 33.1; axilla to groin length 21.2; tail length 38.5; tail width at base 2.5; tail depth at base 2.0; forelimb length 6.2; hind limb length 6.8; limb interval 7½; width of right hand 1.3; width of right foot 1.7; foot length 2.2; length of third toe 0.7; body width behind forelimbs 3.1. There are 2 premaxillary, 34 maxillary, and 12 vomerine teeth and 19 trunk, 2 caudosacral, and 43 caudal vertebrae.

COLORATION OF THE SPECIES (in alcohol). This is a blackish species with little pattern. While specimens nearly always have a dorsal band, it is relatively obscure and may be only slightly paler than the dark gray-black lateral ground color. However, in some specimens the dorsal band is relatively broad and is light brown to tan in coloration. A blackish, inverted triangle that originates near the eyes and ends in the nuchal region is present in some individuals. Once abundant white spots have faded but are evident on the lateral and ventral surfaces. The ventral surface is generally light gray, much lighter than other surfaces.

HABITAT AND DISTRIBUTION. Known only from the lower drainage of the Kings River system in Fresno County, California (Fig. 3). The type locality is a well-shaded, north-facing slope in an area of mixed chaparral with *Aesculus californicus, Umbellularia californica,* and *Quercus wislizenii.* There are also scattered *Pinus ponderosa, Pinus sabiniana,* and *Quercus douglasii.* Salamanders were found under rocks in areas of talus near the roadside. The species occurs in sympatry with *Ensatina eschscholtzii platensis* at the type locality, and this is the lowest elevation at which a blotched form of *Ensatina* has been found in the Kings River drainage and is close to the lowest locality known for the taxon.

A weather station (Balch Power House, 36° 54′ N, 119° 06′ W, 533 m) near the type locality but at somewhat higher elevation receives 77 cm of rainfall annually, with more than four months of average daily temperatures in excess of 27° C.

ETYMOLOGY. The name *regius* is derived from the Latin word *rex,* for king, in reference to the

region of the Kings River, the only known habitat of the species.

COMMENTS. Although *B. gregarius* and *B. relictus* have been collected in close geographic proximity, we know of no instances of sympatry with *B. regius*. Although *B. regius* is known only from the vicinity of the type locality, a population of some member of the *B. relictus* group is known from high elevations in the Kings River drainage (Summit Meadow, Kings Canyon National Park, Fresno County; herein assigned to *B. kawia*, see below). Morphologically these specimens are similar to high-elevation specimens of *B. kawia*, but we do not have a good understanding of geographic variation in morphology, and it is possible that this population might represent a high-elevation population of *B. regius*.

Brame and Murray (1968) examined one specimen of this species and identified it as *B. relictus* (MVZ 33109, Kings River Experimental Range, Fresno County). This specimen and another from the vicinity share some morphological characters with *B. regius* and other characters with *B. gregarius*. Since *B. gregarius* is now the common species at these localities, we tentatively assign these specimens to that taxon.

Batrachoseps kawia, new species

Sequoia Slender Salamander

Batrachoseps relictus (part), Brame and Murray, 1968:6.

Batrachoseps pacificus relictus (part), Yanev, 1980: 535; Stebbins, 1985:58.

HOLOTYPE. MVZ 94134, an adult male from the west side of the South Fork, Kaweah River, Tulare County, California, collected on December 6, 1970, by James Edwards, Samuel Sweet, David Wake, and Richard Wassersug. 36° 22′ 57″ N, 118° 52′ 15″ W, ca 430 m elevation.

PARATYPES. MVZ 94126–30, 94133, 94135–37, 94139, 94141, 94144–45, 94148, 94152–53, LACM 144217, same data as holotype.

REFERRED SPECIMENS. MVZ 178626 (specimen used in study of mtDNA), MVZ 17820–25, 17827–28, along Mineral King Rd., 1.3 km ESE Silver City, East Fork Kaweah River, Tulare County, California, 36° 27′ N, 118° 40′ W, 2200 m elevation; MVZ 178629–30, along Mineral King Rd., 10.6 km ENE Lookout Peak Ranger Station, East Fork Kaweah River drainage, Tulare County, California; MVZ 17996, 400 m SE Powerhouse #3, Sequoia National Park, Tulare County, California, 490 m elevation; MVZ 58280–81, 9.7 km NNE Three Rivers, Tulare County, California, 823 m elevation; MVZ 94103, 94115, 58282, Grunigan Creek, at W boundary Sequoia National Park, ca 12.9 km ENE Three Rivers, Tulare County, California, ca 1100 m elevation.

DIAGNOSIS. A relatively short-bodied (19 or 20 trunk vertebrae) slender salamander with limbs of moderate length (7½–10½ costal folds between adpressed limbs). Distinguished from its geographical neighbor *B. gregarius* by males lacking a mental hedonic gland and by having a broader head, longer limbs, larger hands and feet, and more maxillary teeth. Distinguished from close relatives as follows: from *B. relictus* by having longer limbs (at least in geographically neighboring sites) and many more maxillary teeth; from *B. regius* and *B. diabolicus* by its more slender habitus and in having more maxillary teeth (Table 1, Fig. 2). The following fixed protein differences distinguish it from these close relatives: LAP (a variant that migrates more rapidly than any found in *B. regius* and *B. diabolicus*, but more slowly than any in *B. relictus*) and MDH-2 (a more rapidly migrating variant than in *B. regius* and *B. relictus* and a more slowly migrating variant than in *B. diabolicus*); it differs additionally from *B. regius* by GAPDH (a more rapidly migrating variant), MPI (two more slowly migrating variants), IDH-2 [a rapidly migrating variant of intermediate mobility (uncommon)], from *B. diabolicus* by LDH-1 (a more rapidly migrating variant), GAPDH (a more rapidly migrating variant), and PGDH (its single variant migrates more rapidly that the common variant and a rare variant in *B. diabolicus*, but more slowly than another uncommon variant). Distinguished from all other members of the genus by allozymic and mitochondrial DNA markers ($D_N > 0.47$; K2P > 0.087).

DESCRIPTION. *Batrachoseps kawia* is a small (adults less than 50 mm maximum SL), slender species with a relatively broad head and a distinct neck. The facial region (area from the eyes to the snout) is small and inconspicuous, and the eyes are moderately protuberant. The nostrils are small and males have only slight nasolabial protuberances. Males lack mental hedonic glands. Grooving patterns of the head, throat, and neck are typical of the genus. The relatively numerous vomerine teeth usually are borne in distinct patches. Small to very small, numerous maxillary teeth are variously arranged in a single smooth row, a ragged row, or a double row. The premaxillary teeth are small (same size as maxillary teeth) and numerous in females, but far less numerous and enlarged in males. Males have 18–19 and females 18–20 costal grooves between the limbs. SL is from 8.4–9.1 ($\bar{x}$ = 8.8) times head width in males and 8.6–10.2 ($\bar{x}$ = 9.2) times in females. The tail is long, slender, and cylindrical and often shows evidence of having been regenerated. The tail is 0.9–1.6 ($\bar{x}$ = 1.2) times SL in males and 1.1–1.5 ($\bar{x}$ = 1.3) times in females. There is no basal tail constriction. The postiliac gland is evident. Limbs are moderately long; SL ranges from 5.1–5.7 ($\bar{x}$ = 5.4) times hind limb length in males and 5.1–6.2 ($\bar{x}$ = 5.5) times in females. The hands and feet are of moderate size for the slender members of the genus; the digits are 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 mm). Maximum head width 4.5; snout to gular fold (head length) 7.8; head depth at posterior angle of jaw 2.3; eyelid length 1.8; eyelid width 1.0; anterior rim of orbit to snout 1.6; horizontal orbital diameter 1.2; interorbital distance 1.2; snout to forelimb 10.3; distance separating external nares 1.8; snout projection beyond mandible 0.4; snout to posterior angle of vent (SL) 38.8; snout to anterior angle of vent 37.6; axilla to groin length 24.0; tail length 50.4; tail width at base 2.2; tail depth at base 2.0; forelimb length 6.3; hind limb length 6.8; limb interval 9½; width of right hand 1.4; width of right foot 1.7; foot length 2.3; length of third toe 0.8; body width behind forelimbs 3.2. There are 3 premaxillary, 36 maxillary, and 13 vomerine teeth and 19 trunk, 2 caudosacral, and 43 caudal vertebrae.

COLORATION OF THE SPECIES (in alcohol). These are generally blackish animals with a lighter brownish black dorsal band that is usually not prominent and may be obscure. The dorsal band is generally brown, usually brighter at the lateral edges than in the center. The band may continue onto the head but usually ends near the nuchal region at a dark blackish spot that marks the apex of an inverted triangle that originates near the eyes. There is extensive white spotting laterally and ventrally, with the spots most numerous laterally. The gray ventral surfaces are lighter than the lateral ground color.

HABITAT AND DISTRIBUTION. Known only from the drainage of the Kaweah River system from relatively low elevations (below 500 m) to high elevations (2200 m) in Tulare County, California (Fig. 3). Only scattered trees are found in the habitats in which this species occurs, but the type locality is a protected, relatively mesic site in a generally xeric area and is shaded by oaks (*Quercus douglasii* and *Q. wislizenii*), California Sycamore (*Platanus racemosa*), White Alder (*Alnus rhombifolia*), California Buckeye (*Aesculus californica*), and Fremont Cottonwood (*Populus fremontii*), as well as Western Redbud (*Cercis occidentalis*). At higher elevations the species occurs in mixed coniferous forest.

A weather station (Three Rivers Hammond, 36° 28′ N, 118° 51′ W, 341 m) near the type locality but at slightly lower elevation receives 60 cm of rainfall annually, with 5.5 months of daily average temperatures in excess of 27° C. At high elevations it is much colder and wetter and the dry season is short.

ETYMOLOGY. The name *kawia* is derived from the name of the Native Americans who inhabited this region.

COMMENTS. This species occurs in sympatry with *B. gregarius* at the type locality, and both species occur throughout the drainage. No case of sympatry between *B. kawia* and *B. relictus* has been identified, but both occur at high elevations in Tulare County, and sympatry may occur. The northernmost populations that we have identified as *B. relictus* occur in the Tule River drainage, about 40 km south of the nearest populations of *B. kawia*. The highland populations of *B. kawia* were not studied for protein variation, and the lowland populations were not studied for mtDNA sequences. However, with respect to both proteins and mtDNA sequences, the relationship to *B. relictus* is the same—*B. kawia* and *B. relictus* are sister taxa. Accordingly, we believe that both taxa display unusual elevational range.

We tentatively assign specimens (MVZ 62530–34) from Summit Meadow, Kings Canyon National Park, Fresno County, California, 2470 m elevation to *B. kawia*.

Status of *Batrachoseps relictus*

A major factor that has delayed taxonomic revision of the slender salamanders of the southern Sierra Nevada has been our inability to locate living representatives of *B. relictus* in the lower Kern River Canyon. The most recent sighting of this species at the type locality was by DBW in 1971, and all subsequent efforts have failed to uncover any specimens. We suspect that the species is extinct in the canyon. Accordingly, we have had no tissue available for our genetic studies. Brame and Murray (1968) believed that there were four widespread geographic units of this species; these four units do not correspond to the four taxa described herein. Yanev (1980) subsequently reduced the species to a subspecies of *B. pacificus* and restricted *B. p. relictus* to only one of Brame and Murray's (1968) geographic units, that occurring in the Sierra Nevada. We have further restricted the taxon both taxonomically and geographically by describing *B. diabolicus, B. kawia,* and *B. regius,* but even with this restriction *B. p. relictus* has a moderately extensive distribution. Brame and Murray (1968) demonstrated geographic variation in *B. relictus* in the Sierra Nevada and selected the population that was the most distinctive morphologically (shortest trunk, broadest hands and feet) as the type. Because the long and complicated original diagnosis was for a compound species including representatives of a number of species we now recognize as distinct, we present a revised diagnosis for *B. relictus,* parallel to that for the newly described species. Our molecular data are derived from populations in the Greenhorn Mountains on the north and west sides of the Kern River. Thus, all molecular diagnostic characters relate to this population and not necessarily to populations at and near the type locality. Morphological diagnostic characters are provided that will distinguish both of these populational units from other species. As revised, *B. relictus* is the common high-elevation species in the southern Sierra Nevada.

Batrachoseps relictus Brame and Murray, 1968

Relictual Slender Salamander

Batrachoseps attenuatus (part) Grinnell and Camp, 1917:137.

Batrachoseps relictus (part) Brame and Murray, 1968:5.

Batrachoseps pacificus relictus (part) Yanev, 1980: 535, Stebbins, 1985:58.

HOLOTYPE. LACM 34360, an adult female from "about 150 yards above the junction of State Hwy. 178 and the road turnoff to Democrat Hot Springs Resort, above the upper dirt road, 2400 feet elevation, in the Kern River Canyon" (Brame and Murray, 1968), Kern County, California, 35° 31′ 43″ N, 118° 39′ 25″ W.

REFERRED SPECIMENS (specimens used in mtDNA study). MVZ 190983, 0.9 km N Greenhorn Summit near Alta Sierra, Kern County, California, 35° 45′ N, 118° 33′ W, 1890 m elevation; MVZ 224835, along road to Sugarloaf Village, Tulare County, California, 35° 49′ N, 118° 53′ W, 1500 m elevation; MVZ 158244, 9.5 km E Cherry Hill Road on Sherman Pass Road, Tulare County, California, 35° 58′ N, 118° 23′ W, 2400 m elevation.

REVISED DIAGNOSIS. A relatively small (generally less than 45 mm SL), short-bodied slender salamander with moderately long limbs. There are 17–18 (mode 17) (type locality) to 18–19 trunk vertebrae, and 3½–7 (type locality), or 7–9½ costal folds between adpressed limbs. Distinguished from geographically neighboring species as follows: from *B. gregarius* by larger hands and feet and by males lacking a mental hedonic gland; from *B. simatus* by a shorter trunk, smaller size, and smaller hands and feet; from *B. kawia* by fewer teeth and shorter limbs; from *B. regius* by shorter limbs and fewer maxillary teeth; from *B. diabolicus* by shorter limbs and a narrower head (Table 1, Fig. 2). Distinguished from all members of the genus by allozymic and mitochondrial DNA markers ($D_N > 0.27$; K2P > 0.087) (see other diagnoses in this paper for specific comparisons to close relatives).

HABITAT AND DISTRIBUTION. The type locality lies at relatively low elevation (730 m), and other populations in the lower Kern Canyon once were found as low as 500 m. Outside of the canyon, populations occur at substantially higher elevation (1200–2500 m). At these higher elevations the species occurs mainly in heavily forested areas in mixed pine-fir-incense cedar forest, sometimes with substantial numbers of deciduous oaks. In lower Kern Canyon, tree cover is scanty and consists mainly of deciduous and live oaks with scattered pines and buckeyes and a few sycamores in creek bottoms. The species ranges from the lower Kern River Canyon in Kern County, California, to highlands drained by the Tule River and Kern River in central Tulare County, California (in the vicinity of Quaking Aspen Meadow). It also is known from one site on the western margins of the Kern Plateau, east of the Kern River (Fig. 3).

COMMENTS. Since topotypic *B. relictus* has not been available for biochemical studies, taxonomic assignment of other populations currently included in the species is problematic. Topotypic *B. relictus* has fewer trunk vertebrae (usually 17, sometimes 18) than any other of the slender species. Specimens from the Greenhorn Mountains are within 20 km of the type locality, and these have been studied genetically. As noted by Brame and Murray (1968), populations from areas north of Kern River Canyon have more vertebrae, but the range of variation overlaps that of the type series. These populations differ from the Kern Canyon populations in having shorter limbs (Fig. 2). The localities in the lower Kern Canyon are at a lower elevation (to about 500 m) than any of the other populations assigned to this species. Other than the Kern Canyon populations, populations of this species are generally upland forms that occur as high as 2500 m in the mountains.

BIOCHEMICAL DATA

Protein data are based on 19 allozymic loci studied by starch-gel electrophoresis (Yanev, 1978, 1980). That study included 105 populations from species of *Batrachoseps* that were all compared with each other in a single analysis. Included in the analysis were 10 populations of *B. gregarius* totaling 155 individuals (population sample sizes range from 2 to 26), six populations of *B. relictus* totaling 85 individuals (population sample sizes range from 5 to 28), and one population each of *B. diabolicus* (20 individuals), *B. regius* (nine individuals), and *B. kawia* (13 individuals). In addition, eight populations were identified as *B. diabolicus* through limited electrophoretic studies using only a subset of diagnostic loci (to separate it from *B. attenuatus,* with which its range overlaps to the north). In Figure 3 we indicate the geographic distribution of populations that have been identified by molecular markers. DNA sequence data are from study of approximately 750 base pairs of the mitochondrial gene cytochrome b (Jockusch, 1996). More than 200 populations for the genus have now been sampled, and relevant to the present study are single individuals from six populations of *B. gregarius,* seven populations of *B. diabolicus,* three populations of *B. relictus,* and one population each of *B. regius* and *B. kawia.*

Batrachoseps nigriventris group

Both protein electrophoretic and mtDNA sequence data support the distinctiveness of *B. gregarius* relative to *B. nigriventris* (type locality Fort Tejon, Kern County, California). The type locality of *B. gregarius* is near the northern limit of the range of the species. We selected this locality because of

some uncertainty as to the assignment of populations that occur at low elevations on the margins of the Central Valley in southern Tulare and northern Kern counties, north of the Kern River. The mtDNA haplotype found in topotypic *B. gregarius* belongs to a well-supported clade containing populations from the Sierra Nevada extending from the type locality south to the Kaweah River, central Tulare County (Jockusch, 1996). Sequence divergence in this clade (which we identify as group 1) reaches a maximum of 5.2% (K2P). Two populations from the eastern margins of the Central Valley in northern Kern and southern Tulare counties (which we call group 2) share a distinctive mtDNA haplotype 7.2–8.2% divergent from group 1. In phylogenetic analyses, groups 1 and 2 are always more closely related to each other than either is to the clade containing *B. nigriventris* from the vicinity of the type locality (8.9–9.8% divergent; Jockusch, 1996). However, in many of these analyses, groups 1 and 2 are not each other's closest relatives. Instead, they form part of a clade containing two additional haplotypes (group 3) from within the upper Kern Canyon.

A UPGMA phenogram of genetic distances (Yanev, 1978, 1980) also identifies two differentiated units of *B. gregarius* in the Sierra Nevada. However, the break between these units is not concordant with the break between groups 1 and 2 defined by mtDNA. Three populations (all representing mtDNA group 1) extending from the vicinity of topotypic *B. gregarius* south to Negro Creek at the northern edge of Tulare County form a geographically and allozymically cohesive group ($\bar{D}_N$ = 0.06). Seven populations from the Kaweah River south, including one each representing groups 1 and 2 (the remainder are suspected to be group 2 based on geography) form a second cohesive group ($\bar{D}_N$ = 0.04, maximum D_N = 0.08). Genetic distances between populations in the two groups range from 0.12 to 0.33 ($\bar{D}_N$ = 0.20), with the greatest distances between the geographically most remote populations. The lowest D_N between populations representing the two mtDNA groups is 0.04. There is a range of genetic distances separating individual populations from topotypic *B. nigriventris* (D_N = 0.16–0.31, $\bar{D}_N$ = 0.20 from the northern cluster, D_N = 0.33–0.40, $\bar{D}_N$ = 0.36 from the southern cluster). Both clusters share one fixed difference relative to *B. nigriventris*. Each cluster is separated from *B. nigriventris* by two additional fixed differences, in addition to some major frequency differences. No fixed differences distinguish the two clusters.

The borders between the differentiated groups of *B. gregarius* recognized by the different molecular markers are not concordant. We believe that genetic interchange has taken place in the recent past and may be continuing. We provisionally recognize all populations from the Sierra Nevada that were included in Yanev's *B. nigriventris* as *B. gregarius*. This decision leads to the recognition of a species which may be paraphyletic (based on our analysis of mtDNA) with respect to *Batrachoseps* from the upper Kern River Canyon (group 3). High genetic distances (D > 0.4, Wake, unpublished) and relatively high mtDNA divergence indicate that there has been no recent contact between populations of *B. gregarius* and unassigned populations in Kern Canyon. The history of *Batrachoseps* in the Kern Canyon region is clearly complex. The upper Kern Canyon populations are so distinctive relative to both *B. gregarius* and *B. nigriventris* (one of these populations was tentatively referred to *B. simatus* by Brame and Murray, 1968) that they will be included in a separate report of molecular markers and morphology. At present we consider them to represent a distinct and unnamed taxon.

With the description of *B. gregarius*, the taxon *B. nigriventris* is more tightly circumscribed than was previously the case. However, *B. nigriventris* remains somewhat heterogeneous: populations in southern California and on Santa Cruz Island are biochemically well differentiated from those near the type locality (which are similar to populations occurring northwestward along the coast) in both allozymes and mtDNA (unpublished data; Jockusch, 1996).

Batrachoseps relictus group

The taxa *B. diabolicus, B. kawia, B. regius,* and *B. relictus* are considered together because they are close relatives, based on the assessments by Brame and Murray (1968) on morphology and both Yanev (1978, 1980) and Jockusch (1996) on biochemical data (Fig. 6). Yanev (1978) studied six populations assignable to *B. relictus*. These populations are very similar in proteins ($\bar{D}_N$ = 0.03; maximum D_N = 0.07). The other three species are represented by only a single population each. *Batrachoseps relictus* is most similar to *B. kawia* ($\bar{D}_N$ = 0.26), next to *B. regius* ($\bar{D}_N$ = 0.39), and then to *B. diabolicus* ($\bar{D}_N$ = 0.41) The other three species are distinct from each other: D_N for *B. kawia* to *B. regius* is 0.35, for *B. kawia* to *B. diabolicus* is 0.42, and for *B. regius* to *B. diabolicus* is 0.34. Genetic distances to other nearby species are high, in excess of D_N = 1.0 to *B. attenuatus, B. nigriventris,* and *B. gregarius*. There are also substantial numbers of fixed differences between the species from the Sierra Nevada. In addition to the fixed differences noted in the diagnoses, there are numbers of loci at which nearly fixed differences or major differences in frequencies occur.

These four taxa also differ substantially in cytochrome b sequences (Jockusch, 1996). Three haplotypes of *B. relictus* are from 1.2 to 2.2% divergent; they are 8.7–9.0% from the *B. kawia* haplotype, 10.1–11.2% from the *B. regius* haplotype, and 9.6–13.5% divergent from five haplotypes of *B. diabolicus*. *Batrachoseps kawia* is 13.3% divergent from the haplotype of *B. regius* and 11.9 to 15.7% from the haplotypes of *B. diabolicus*. *Batra-*

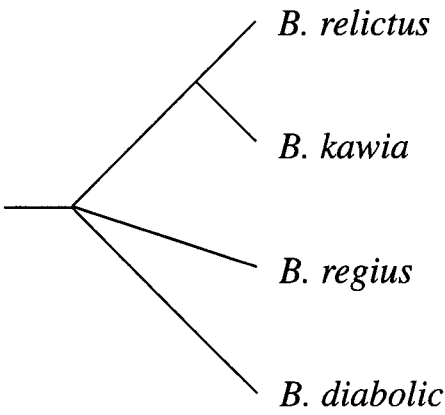

Figure 6. Hypothesis of phylogenetic relationships among the species of the *B. relictus* group, based on a consensus of allozymic and mitochondrial DNA sequence data (detailed analysis to be published elsewhere). A phenetic analysis of allozymic data (Yanev, 1980) clustered *B. diabolicus* (her Merced River sample) with *B. regius* (her Kings River sample) and *B. relictus* with *B. kawia* (her Kaweah River sample). This is in agreement with a Neighbor Joining analysis of the DNA data, but a parsimony analysis fails to resolve the basal polytomy.

choseps regius is 10.6 to 12.4% divergent from the haplotypes of *B. diabolicus*.

The five haplotypes of *B. diabolicus* are highly divergent from each other and fall into two distinct clades. One clade spans the entire geographic range, with maximum divergence of 5%. The other, found only in Calaveras County, includes two haplotypes that differ from each other by 1.6%. The two clades are 8.0–11.8% (mean 9.6%) divergent, although they approach each other closely in Calaveras County. A strict consensus of trees derived from phylogenetic analysis of the mtDNA data gives the tree in Figure 6. It is unclear from these analyses whether *B. regius* is the sister group of *B. diabolicus* or of the clade containing the sister taxa *B. regius* and *B. kawia*.

Limited studies of allozyme variation in this region using only loci showing fixed differences between *B. attenuatus* and *B. diabolicus* failed to reveal deep differentiation within *B. diabolicus*. While these data are insufficient to determine if nuclear gene flow is occurring among populations displaying the two differentiated mtDNA haplotype groups in Calaveras County, we prefer to be conservative and to treat all populations of putative *B. diabolicus* as conspecific.

Populations representing the four taxa under consideration were included in *B. relictus* by Brame and Murray (1968) and in *B. pacificus relictus* by Yanev (1980). Other representatives of Brame and Murray's *B. relictus* occur along the central California coast, and these were placed in *B. pacificus* (although not in *B. p. relictus*) by Yanev. Genetic distances (from allozyme data) from the Sierra Nevada taxa to those along the central California coast are high (mean $\bar{D}_N$ = 0.40–0.68), and divergence of mitochondrial sequences is great (in excess of 15%), suggesting a long history of independent evolution. Furthermore, a sister group relationship between these taxa is not always indicated in phylogenetic analysis of the mtDNA data (Jockusch, 1996). The taxa on the central California coast will be treated in a separate paper.

DISCUSSION

The salamander fauna of the Sierra Nevada has been greatly undervalued in terms of biodiversity. The southern Sierra Nevada and adjoining mountains, in particular, have been areas of significant evolutionary diversification, and with the description of the new species presented here the extent of that diversity will begin to emerge. *Batrachoseps* is spottily distributed in the Sierra Nevada and can be difficult to find, especially in the central Sierra Nevada. We suspect that there is greater differentiation than we have described, but this cannot be fully resolved without additional sampling. The present paper is one of a series that will deal with the taxonomy of this difficult genus.

Species of *Batrachoseps* can be placed into five species groups, each of which is thought to be monophyletic (based on phylogenetic analysis of unpublished molecular data and Jockusch, 1996). Four of these species groups occur in the Sierra Nevada.

The robust group, which has been recognized as the subgenus *Plethopsis* by Jackman et al. (1997), is represented in the Sierra Nevada by an undescribed species that occurs on the Sierran Plateau, east of the Kern River.

The slender members of the genus, representing the subgenus *Batrachoseps,* are placed in at least four species groups based on analyses of proteins (Yanev, 1978, 1980) and mitochondrial DNA sequences (Jockusch, 1996). The only member of the *attenuatus* group is *B. attenuatus,* which in the Sierra Nevada occurs from Butte to Calaveras counties. This species is widespread in coastal regions, where its range extends from the Monterey Bay area and inland Monterey County northward into southwestern Oregon.

The *nigriventris* group includes *B. nigriventris, B. simatus* (type locality in the lower Kern River Canyon, Kern County), *B. stebbinsi* Brame and Murray, 1968 (type locality along Caliente Creek, Piute Mountains, Kern County), and *B. gregarius,* as well as two or three undescribed taxa. The group occurs from central and southern Monterey County south to southern Orange County and southwestern Riverside County in the coastal zone and from central Mariposa County south to the Kern/Los Angeles county border in the inland zone.

We define a new *relictus* group to include *B. relictus, B. diabolicus, B. kawia,* and *B. regius.* We choose to recognize *B. relictus* as a species distinct

from *B. pacificus* based on many fixed genetic differences and large genetic distances (e.g., distances to *B. pacificus major* and *B. pacificus pacificus* are on the order of $D_N > 0.4$, Yanev, 1978), large differences in mitochondrial DNA sequences, and phylogenetic analysis of those data. As revised, *B. relictus* is the common high elevation species in the southern Sierra Nevada, ranging at least as far north as Quaking Aspen Meadow, Tulare County. The three remaining species of the *relictus* group occur in canyons along the western slopes of the Sierra Nevada from Tulare to Placer counties. Although they resemble each other closely in morphology, populations from the type localities can be distinguished, and they differ greatly in biochemical characters. Highland populations of *Batrachoseps* belonging to the *relictus* group occur as far north as Summit Meadow in Kings Canyon National Park, central Fresno County. With the exception of a population from near Silver City, northern Tulare County, which we assign to *B. kawia,* these highland populations have not been examined biochemically, and current assignments are tentative. While *B. regius* has a small known range, those of *B. kawia, B. relictus,* and especially *B. diabolicus* are extensive (Fig. 3).

The *pacificus* group is the only major clade of *Batrachoseps* that does not occur in the Sierra Nevada. As revised here it contains *B. pacificus, B. aridus* Brame, 1970, and possibly also *B. gabrieli* Wake, 1996. With the elevation of *B. relictus* to species level, *B. pacificus* is restricted to the coastal members of Yanev's (1980) *B. pacificus* complex: two named taxa, *B. p. pacificus* on the northern Channel Islands and *B. p. major* in the Los Angeles Basin and to the south and east, and two unnamed taxa in central coastal California.

In 1954, Hendrickson recognized only a single species of *Batrachoseps* in California. While some of the currently recognized species represent subdivision of his species based on new biochemical analyses, many of them (e.g., *B. aridus, B. campi, B. gabrieli, B. simatus, B. stebbinsi,* and *B. kawia*) represent new discoveries. Given its complex terrain and habitats, it is likely that California will continue to yield novel plethodontid salamanders.

ACKNOWLEDGMENTS

We thank Monica Frelow for laboratory assistance with electrophoretic studies conducted after the completion of Yanev's dissertation that helped define borders for some of the species described herein. Bob Hansen provided critical specimens from the Kern Canyon region, habitat information, and a detailed and helpful critique of the manuscript. We appreciate the thoughtful and constructive reviews of the manuscript by R. Highton and an unidentified reviewer. This work was supported by grants from NSF (DEB 9408347 and DEB 9321323) and cooperative agreement PNW 94-0618 with the U.S. Forest Service. ELJ was supported by predoctoral fellowships from NSF and the University of California, Berkeley, during much of this work.

LITERATURE CITED

Bonaparte, C.L.J.L. 1841. *Iconographia della fauna Italica per le quattrao classi degli animali vertebrati.* Rome: Salviucci.

Brame, A.H., Jr. 1970. A new species of *Batrachoseps* (slender salamander) from the desert of southern California. *Contributions in Science* 200:1–11.

Brame, A.H., Jr., and K.F. Murray. 1968. Three new slender salamanders (*Batrachoseps*) with a discussion of relationships and speciation within the genus. *Science Bulletin, Museum of Natural History, Los Angeles County* 4:1–35.

Camp, C.L. 1915. *Batrachoseps major* and *Bufo cognatus,* new amphibia from southern California. *University of California Publications in Zoology* 12: 327–329.

Cope, E.D. 1865. Third contribution to the herpetology of tropical America. *Proceedings of the Academy of Natural Sciences, Philadelphia* 17:185–198.

Cope, E.D. 1869. A review of the species of Plethodontidae and Desmognathidae. *Proceedings of the Academy of Natural Sciences, Philadelphia* 21:93–118.

Eschscholtz, F. 1833. *Zoologischer Atlas, enthaltend Abbildungen und Beschreibungen neuer Thierarten während des Flottcapitains von Kotzebue zweiter Reise um die Welt, auf der Russisch-Kaiserlichen Kriegsschlupp Predpriaetie in den Jahren 1823–1826,* part V, viii + 28 pp., pls. XXI–XXII. Berlin.

Grinnell, J., and C.L. Camp. 1917. A distributional list of the amphibians and reptiles of California. *University of California Publications in Zoology* 17:127–208.

Hendrickson, J.R. 1954. Ecology and systematics of salamanders of the genus *Batrachoseps. University of California Publications in Zoology* 54:1–46.

Jackman, T.R., G. Applebaum, and D.B. Wake. 1997. Phylogenetic relationships of bolitoglossine salamanders: a demonstration of the effects of combining morphological and molecular data sets. *Molecular Biology and Evolution* 14:883–891.

Jockusch, E.L. 1996. *Evolutionary studies in* Batrachoseps *and other plethodontid salamanders: Correlated character evolution, molecular phylogenetics, and reaction norm evolution.* Ph.D. Thesis in Integrative Biology, University of California, Berkeley, CA.

Jockusch, E.L. 1997. Geographic variation and phenotypic plasticity of number of trunk vertebrae in slender salamanders, *Batrachoseps* (Caudata: Plethodontidae). *Evolution* 51:1966–1982.

Jockusch, E.L., and M.J. Mahoney. 1997. Communal oviposition and lack of parental care in *Batrachoseps nigriventris* (Caudata: Plethodontidae) with a discussion of the evolution of breeding behavior in plethodontid salamanders. *Copeia* 1997:697–705.

Kimura, M. 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution* 2:87–90.

Murphy, R.W., J.W. Sites, Jr., D.G. Buth, and C.H. Haufler. 1996. Proteins: isozyme electrophoresis. In *Molecular systematics,* chap. 4, 2nd edn., ed. D.M. Hillis, C. Moritz, and B.K. Mable, 51–120. Sunderland, MA: Sinauer Associates.

Nei, M. 1972. Genetic distance between populations. *American Naturalist* 106:283–292.

Stebbins, R.C. 1966. *A field guide to western reptiles and amphibians.* Boston: Houghton Mifflin, xiv + 279 pp.

Stebbins, R.C. 1985. *A field guide to western reptiles and amphibians,* 2nd edn., revised. Boston: Houghton Mifflin, xiv + 336 pp.

Wake, D.B. 1989. Phylogenetic implications of ontogenetic data. Geobios, mémoire spécial 12:369–378.

Wake, D.B. 1996. A new species of *Batrachoseps* (Amphibia: Plethodontidae) from the San Gabriel Mountains, Southern California. *Contributions in Science* 463:1–12.

Yanev, K.P. 1978. *Evolutionary studies of the plethodontid salamander genus* Batrachoseps. Ph.D. Thesis in Zoology, University of California, Berkeley, CA.

Yanev, K.P. 1980. Biogeography and distribution of three parapatric salamander species in coastal and borderland California. *The California islands: Proceedings of a multidisciplinary symposium,* ed. D.M. Power, 531–550. Santa Barbara, CA: Santa Barbara Museum of Natural History.

Yanev, K.P., and D.B. Wake. 1981. Genic differentiation in a relict desert salamander, *Batrachoseps campi. Herpetologica* 37:16–28.

Received 16 July 1997; accepted 22 May 1998.