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Ichthyology & Herpetology 109, No. 3, 2021, 836–850

A New Relict Species of Slender Salamander (Plethodontidae: *Batrachoseps*) with a Tiny Range from Point Arguello, California

Samuel S. Sweet[1,¶] and Elizabeth L. Jockusch[2,3,¶]

Among western North American amphibian lineages, the plethodontid salamander genus *Batrachoseps* has undergone the most extensive radiation. Here we describe a new species in the genus from the vicinity of Point Arguello, central California. This lineage falls within the *B. pacificus* group, but it is differentiated from other species in the group by both molecular sequence data and morphology. It is geographically disjunct from its close relatives, with a tiny range in unlikely habitat along a narrow strip of the Pacific Coast, where it is entirely surrounded by *B. nigriventris*, a distant relative. Although intraspecific molecular variation is almost entirely absent, some population structure was detected across the 4 km extent of its range. Because of its tiny range and limited genetic variation, the impacts of any potential modifications to its known habitat should be evaluated to ensure the species' continued conservation.

NEW vertebrate species continue to be discovered even in well-studied regions with long histories of faunistic investigations. While, in many cases, species discovery results from molecular analyses that show high genetic divergence or other evidence of reproductive isolation among known populations previously classified as a single species (Jackman, 1998; Shaffer et al., 2004; Kuchta, 2007; Feldman and Hoyer, 2010; Jackson et al., 2017; Bingham et al., 2018), other cases involve the discovery of distinctive new taxa (Köhler et al., 2005; Mead et al., 2005; Graham et al., 2018). Both processes have played substantial roles in the large increase in number of recognized amphibian species (Köhler et al., 2005), including in the plethodontid salamander genus *Batrachoseps*. *Batrachoseps* is the most species-rich amphibian clade on the west coast of North America; the number of recognized species has increased from four prior to the work of Brame and Murray (1968) to 21, including one with two morphologically divergent subspecies, at present (Jockusch et al., 2015). Six of these species represented newly discovered lineages, primarily from outside the known range of the genus at the time of discovery, that were also straightforward to diagnose morphologically (Brame and Murray, 1968; Marlow et al., 1979; Wake, 1996; Wake et al., 2002). The rest resulted from analyses of molecular data, which led to the recognition of substantial additional genetic diversity and sharp contact zones within a relatively conserved morphology (Yanev, 1980; Jockusch et al., 1998, 2001, 2012).

On 8 May 2006, Mr. Wes Fritz discovered a large *Batrachoseps* under debris at the abandoned Coast Guard station at Point Arguello, California, USA on Vandenberg Space Force Base. This region is within the range depicted for *Batrachoseps nigriventris* (Fig. 1), but ManTech biologist Morgan Ball recognized that this animal was not morphologically consistent with this species and urged a wider search. Base biologist Nancy Sandburg collected a second individual at the same site on 22 May and brought the two individuals to Sweet's lab at the University of California, Santa Barbara. In size, coloration, and pattern, these individuals were sufficiently similar to *Batrachoseps pacificus* from the northern Channel Islands that we initially suspected an introduction in materials transported by the Coast Guard from their facilities on Anacapa or Santa Cruz Islands to the mainland station at Point Arguello. Tissue samples assayed by Jockusch confirmed that these were members of the *B. pacificus* group. However, sequences of the mitochondrial gene *cytochrome b* (*cytb*) did not place the Point Arguello samples within *B. pacificus*, ruling out a human-mediated introduction from any known population. Instead, these sequences grouped with those of *B. minor* (Jockusch et al., 2015). *Batrachoseps minor* is a distant relative of *B. pacificus* within the *pacificus* group, one of five strongly supported subgeneric groups within the genus (Yanev, 1980; Jockusch et al., 2015). However, the nearest known population of *B. minor* is >90 km away, and *cytb* sequences differed by an average of 4.9% between samples of *B. minor* and the haplotype from Point Arguello.

The mitochondrial differentiation and geographic isolation of this newly discovered population led to an expanded search for animals, guided in part by attention to the complex tectonics of the southern Coast and western Transverse Ranges. It also led us to characterize genetic diversity at multiple nuclear loci. The molecular results, in combination with geography and morphology, support the conclusion that these populations merit recognition as a new species. Here we describe this attractive and distinctive species, and name it in honor of David Wake. This species is a member of the *B. pacificus* species group, but geographically disjunct from the other taxa within this clade. The geographic isolation from close relatives, tiny known range, and near absence of genetic diversity suggest that this lineage is an evolutionary relict.

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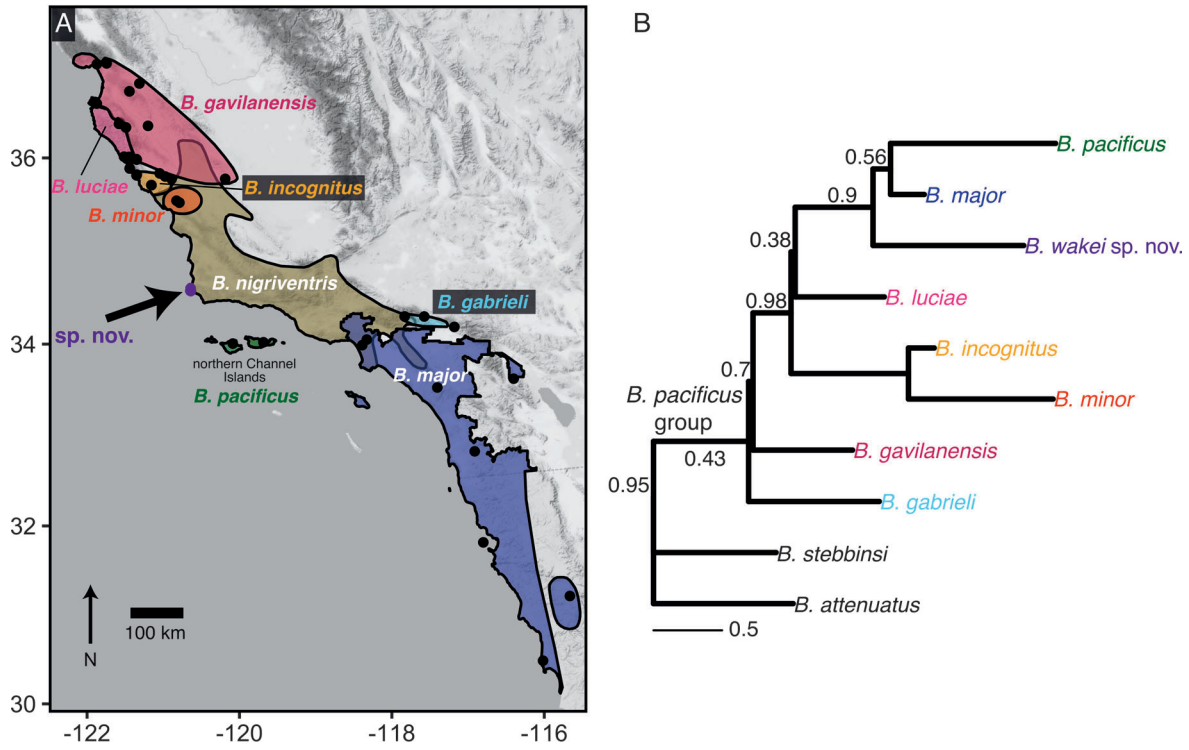


Fig. 1. Distribution and relationships of the *B. pacificus* group. (A) Colored polygons show the ranges of the seven species currently recognized in *B. pacificus* (based on IUCN range polygons), and black dots show the locations of molecular samples included in this study; brown polygon shows range of *B. nigriventris*. Arrow and purple circle indicate the locality of the species described here. Map tiles by Stamen Design, under a CC BY 3.0 license. Map data by OpenStreetMap under ODbL. (B) Species tree for the *B. pacificus* group and selected outgroups inferred from coalescent analysis of seven nuclear and one mitochondrial marker. Support values are ASTRAL-III's local posterior probabilities; branch lengths are in coalescent units. See Data Accessibility for tree file.

MATERIALS AND METHODS

Morphology.—Voucher specimens were preserved in 10% formalin following standard protocols and then transferred to 70% ethanol for long-term storage. Morphological measurements were taken to the nearest 0.1 mm with a Helios dial caliper by SSS, with the aid of a dissecting microscope for smaller measurements. Clearing and staining with alizarin red (for bone) and alcian blue (for cartilage) followed standard protocols (Hanken and Wassersug, 1981). All specimens used for morphological descriptions have been deposited in museum collections (see species description); collections abbreviations follow Sabaj (2020).

Table 1. Known populations of *B. wakei*. Pop is population number shown on Figure 7; *n* is the number of individuals sampled for at least one molecular marker.

Pop	Descriptive locality	Lat (N)	Long (W)	*n*
1	0.5 km SE Arguello Coast Guard Station, along railroad tracks, Santa Barbara Co., CA, USA	34.58	−120.64	2
2	Point Arguello, vicinity of abandoned Coast Guard Station, Santa Barbara Co., CA, USA	34.58	−120.64	8
3	0.9 km NNE Arguello Coast Guard Station, along railroad tracks, Santa Barbara Co., CA, USA	34.59	−120.64	8
4	Honda Point (= Point Pedernales), Santa Barbara Co., CA, USA	34.60	−120.64	7

Taxon sampling and sequence generation.—The species-tree analyses of Jockusch et al. (2015) identified *B. wakei*, new species (there called *B.* sp. nov.), as a member of the *B. pacificus* group. We retained all representatives of the *B. pacificus* group from this dataset ($n = 6$ for widespread species and $n = 2$ for narrowly distributed ones, including *B. wakei*, new species; Fig. 1A), as they were selected to span genetic and geographic diversity within the clade, and greatly increased the sampling of *B. wakei*, new species (Table 1). The final sample size for *B. wakei*, new species, varied from 18–25 individuals per marker, except for one marker that had limited variation across the *pacificus* group, for which only two individuals were sequenced. For *cytb*, the only available sequence of *B. (m.) aridus* was also added to the dataset. Supplemental Table S1 (see Data Accessibility) provides voucher and GenBank accession numbers (MW651987–MW652170) for all molecular sequences from *B. wakei*, new species.

Jockusch et al. (2015) included data from six gene regions: the mitochondrial protein-coding marker *cytb*, two protein-coding nuclear markers (portions of *recombination activating gene 1* [*rag1*] and *pro-opiomelanocortin* [*pomc*]), and three largely intronic nuclear markers (*parvalbumin* [*pvalb*], *myosin light chain, phosphoralatable, fast skeletal muscle* [*mylpf*], and *interleukin enhancer binding factor 3* [*ilf3*]). Methods for DNA extraction, PCR, sequencing, and alignment are as described in Jockusch et al. (2015). Sampling of *pomc* was not expanded because of its overall low level of variation across the genus. *rag1* was rephased for *B. wakei*, new species, using PHASE 2.1.1 and following the methods of Jockusch et al. (2015), as

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the additional sequencing revealed additional alleles in the homozygous form. These sequences added support to the previous computational phasing, which had inferred that one individual was heterozygous for two novel alleles.

We added one additional marker, a largely intronic region of *glyceraldehyde-3-phosphate dehydrogenase* (*gapdh*). This marker was amplified using the primers GapdL890 and GapdH950 (Friesen et al., 1997) and sequenced with either these primers or three newly designed sequencing primers (gapdLseq: 5′–attgcCCTCAATGACCACTTT–3′; gapdHseq: 5′–ctgtaACCGCACTCATTGTCAT–3′; luciae_gdH3′seq: 5′–ATCACTAAAACACATGCCTGGG–3′). The first two are in the exon region and partially overlap the amplification primers; luciae_gdH3′seq is within the intron and was designed to bypass a very long (20+) polyT stretch at the 3′ end of the intron in a subset of taxa. The amplification mix for *gapdh* contained 1X ExTaq buffer (Takara), 190 μM each dNTP, 0.25 μM each primer, and 0.25 U ExTaq DNA polymerase (Takara), along with 80 ng of template DNA in a 10 μl volume. This mix was cycled using a touchdown program: 94°C for 3 minutes initial denaturation, then 16 cycles of denaturation at 94°C for 30 seconds, annealing at 70°C for 30 seconds, decreasing by 0.5°C/cycle, and extension at 72°C for 1 minute, followed by 24 cycles with a constant annealing temperature of 62°C. Products were checked by gel electrophoresis, cleaned enzymatically using a combination of alkaline phosphatase and an exonuclease, and sequenced using BigDye version 1.1 or 3.1, with varying recipes. A typical reaction mix contained 1 μl of cleaned PCR product as template, 0.5 μl of BigDye, and 1.5 pmol primer in a 7.5 μl reaction. Products were separated on an ABI 3100 or ABI 3130xl Genetic Analyzer and checked by eye in Sequencher v. 5.1 (Gene Codes Corporation). Indel differences between alleles within an individual were common, and resulted from variation in the length of a polyG tract. Chromatogram traces were compared to call the alleles present in each individual. Phasing for the full dataset was completed computationally using Phase v2.1 (Stephens et al., 2001; Stephens and Scheet, 2005), as in our previous work, and the resulting sequences were deposited in GenBank (MW652171–MW652283).

Batrachoseps attenuatus was selected as the outgroup for all markers, because it was the closest relative that was consistently strongly supported as outside of the full *B. pacificus* group in gene trees including complete representation of the genus (Jockusch et al., 2015). *Batrachoseps stebbinsi* was also included, as it is a much closer outgroup in 6 of the 7 markers. However, *pvalb* lacked support for monophyly of the *pacificus* group relative to *B. stebbinsi*, so *B. stebbinsi* was not formally treated as an outgroup. For *B. attenuatus* and *B. stebbinsi*, the 'a' allele of each specimen in Jockusch et al. (2015) was arbitrarily chosen. Both alleles were retained for members of the *pacificus* group.

Genetic diversity in* B. wakei*, new species.—AMOVA (Excoffier et al., 1992) was used to test for population structure and quantify the proportion of the variation that was distributed among populations of *B. wakei*, new species. This analysis was run on the allelic data for *gapdh* and *rag1* combined, as these were the only loci to show variation. Missing data were excluded in a pairwise fashion, with distances between populations calculated as percent of alleles that differed using the diss.dist command in poppr v. 2.8.1 (Kamvar et al., 2014) run in R v. 3.5.1 (R Core Team, 2018). We obtained data for at least one of the two variable loci from 23 individuals: two individuals from the southernmost population (RR tracks south), and six to eight individuals from each of the other three populations. AMOVA was run in the R package pegas v. 0.11 (Paradis, 2010), with 1,000 permutations to estimate significance. Haplotype networks were also inferred for sequences of *B. wakei*, new species, from these two loci in the R package pegas v. 0.11. For *gapdh*, gaps were treated as equal to substitutions (accomplished using false coding of indels as nucleotide variants).

Phylogenetic analysis methods.—Because relationships may differ across genes as a consequence of multiple evolutionary processes, we used coalescent-based species tree methods to infer the placement of *B. wakei*, new species. PartitionFinder v. 1.0.1 (Lanfear et al., 2012) was used to select models of evolution for each locus and, where applicable, to test whether *a priori* candidate partitions should be modeled separately or together. Because different model sets are available in the phylogenetic analysis software packages we used, PartitionFinder was run twice, once comparing all models and once restricting the model set to those available in MrBayes (Ronquist et al., 2012). The Bayesian Information Criterion was used for model selection. For *cytb*, *rag1*, and *pomc*, each codon position was treated as a candidate partition. For *ilf3*, exonic regions and the two introns were candidate partitions. *pvalb*, *mylpf*, and *gapdh* each consist of a single intron with a very small number of bases in the flanking exonic regions, so they were each analyzed as a single partition. In all PartitionFinder analyses, sites that are coded as gaps in all *pacificus* group samples, and thus were present only in the included non-*B. pacificus* group lineages (i.e., *B. attenuatus* and *B. stebbinsi*), were coded as a separate partition; these sites were excluded in the phylogenetic analyses. When choosing among partitioning schemes, only those in which these 'excluded' sites were treated as a separate partition were considered, to ensure that the model selected was the best model for the sites included in the final analyses.

Gene trees were inferred using maximum likelihood (ML) using GARLI v. 2.0 (Zwickl, 2006) and Bayesian inference (using MrBayes v. 3.2.4–pre 3.2.7) on each marker alignment. For ML analyses, ten replicate searches with different taxon addition orders were conducted. Support was estimated with 1,000 non-parametric bootstrap replicates, with one random addition replicate per bootstrap replicate. For MrBayes, the compound Dirichlet tree length prior was used (Zhang et al., 2012), with parameter values $(1,\beta_T,1,1)$. Tree lengths, but not topology or branch supports, are sensitive to the prior mean on tree length, so β_T was set empirically based on the length of the maximum likelihood tree. As a result, values of β_T ranged from 0.5 for *cytb* to 10.8 for *rag1*. Topology and branch lengths were linked across partitions; all other parameters were unlinked. When multiple partitions were present, the command Ratepr=variable was used; this allows different rates for each partition but maintains a common set of relative branch lengths across partitions. Each analysis was run for 10 million generations, using the default value of two independent runs, each with four chains. Examination of estimated sample sizes for all parameters, the standard deviations of split frequencies, and plots of each parameter against generation indicated that the burn-in and run length

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were sufficient for all analyses. Stationarity was achieved rapidly, and a standard burn-in of 10% was used in all analyses.

Additionally, we inferred haplotype networks for two nuclear markers in which *B. wakei*, new species, formed part of a large comb in phylogenetic analysis, following the methods described above. For *pomc*, the network was inferred using the most probable pair of haplotypes for each individual, as this more accurately depicts the allelic diversity. For *ilf3*, only two unresolved polymorphisms remained in the ingroup; these were ignored.

Relationships among species were inferred using the quartet-based inference approaches in ASTRAL-III (Zhang et al., 2018), which uses inferred gene trees, and SVDQuartets, which uses site patterns (Chifman and Kubatko, 2014). These two methods are based on the coalescent and thus incorporate incomplete lineage sorting as a cause of discordance across loci; they also accommodate multiple individuals per species (Chifman and Kubatko, 2014; Rabiee et al., 2019). To account for gene tree error, ASTRAL-III was run on three different gene tree sets: the ML trees, ML trees with branches receiving <10% bootstrap support collapsed (Zhang et al., 2018), and a set of 1,000 bootstrap replicates. SVDQuartets was run in PAUP* v. 4.0a168 (Swofford, 2003), with 100 bootstrap replicates to estimate support. To produce the data matrix, all 'a' alleles from a given individual were concatenated, as were all 'b' alleles. The *cytb* sequence was concatenated to both the 'a' and 'b' alleles to maximize the information available in each tip sequence. Taxa sequenced at only 1–2 loci were excluded (or when justified by geography or phylogeny, combined with sequence from another individual to produce a composite sequence), as were sites that were present only in the outgroup. The SVD quartets data matrix contained 116 tips (representing 10 species) and 6,346 sites (5,562 nuclear and 784 mitochondrial), including 1,446 variable sites (1,149 nuclear and 297 mitochondrial). Given the possibility that the mtDNA may have been acquired by introgression, all species tree analyses were run both including and excluding the mitochondrial locus.

RESULTS

Batrachoseps wakei, new species

urn:lsid:zoobank.org:act:15A84C27-4042-4585-8D2A-843A5C02F0A8

Suggested common name: Arguello Slender Salamander

Figures 2–7

Holotype.—MVZ:Herp:293138 (original number SSS 32478), adult male, collected under boards lying against the northwest wall of the abandoned U.S. Coast Guard housing block at Point Arguello on Vandenberg Space Force Base, Santa Barbara County, California, 34.58°N, 120.64°W, 32 m elevation, Samuel S. Sweet, 7 March 2010. Precise locality information for the holotype and other specimens is held by the collections in which they were deposited.

Paratypes.—14 specimens. LACM:Herps:192005–192014 (original numbers SSS 33428–33437), 1 male, 1 female, 8 smaller individuals of undetermined sex, MVZ:Herp:293140–293142 (original numbers SSS 32706–32708), 2 males, 1 female, Honda Point, S base of Destroyer Rock, 30 m elevation, 34.60°N, 120.64°W, 2.8 km N of type locality, Alice Abela, Morgan Ball, Christopher Evelyn, John LaBonte, and Samuel Sweet, 9 January 2011 (MVZ series), 30 April 2012 (LACM series); MVZ:Herp:293139 (original number SSS 32479; Fig. 2A), adult male, collected under debris 40 m NNE of the holotype at 34.58°N, 120.65°W, 33 m elevation, Samuel S. Sweet, 7 March 2010.

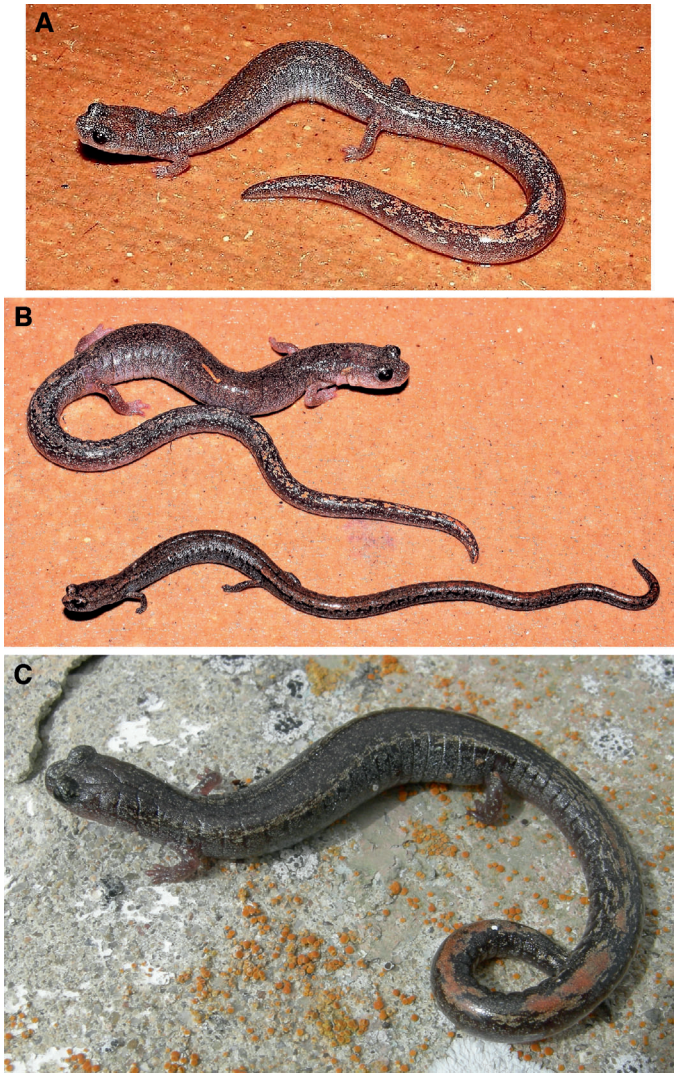


Fig. 2. Photos of *Batrachoseps wakei*. (A) Paratype (MVZ 293139, adult male, SVL = 60.4 mm) in life; (B) paratype of *B. wakei* (MVZ 293140, adult male, SVL = 63.0 mm) contrasted with *B. nigriventris* from Honda Canyon, Santa Barbara Co., California, in close parapatry with *B. wakei*; (C) ELJ 1662. Photos A, B by Sam Sweet; photo C © Iñigo Martínez-Solano, used with permission.

Referred specimens.—17 specimens: CCBER:Herps:32844 (original number SSS 32223), abandoned Coast Guard housing structure 0.3 km E of Point Arguello, 35 m elevation, 34.58°N, 120.64°W, Wes Fritz, 8 May 2006; CCBER:Herps:32845 (original number SSS 32229), same locality as CCBER:Herps:32844, Nancy Sandburg, 22 May 2006; CCBER:Herps:32846 (original number SSS 32384), W side of railroad tracks, 1.4 km NE Point Arguello, 51 m elevation, 34.59°N, 120.64°W, Alice Abela, 1 March 2009; CCBER:Herps:32847–32857 (original numbers SSS 33415–33425), Honda Point, gravel pit at S base of Destroyer Rock, 30 m

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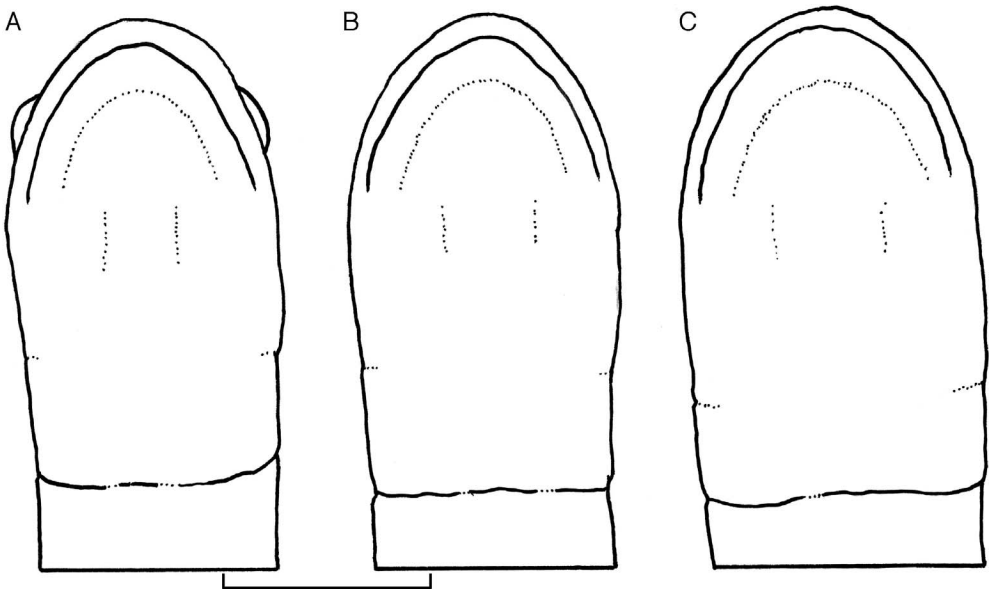


Fig. 3. Head shape in ventral view of (A) *Batrachoseps wakei* (CCBER 32844, adult female, SVL = 67.6 mm); (B) *Batrachoseps major* (SSS 34390, adult female, SVL = 66.5 mm); and (C) *Batrachoseps pacificus* (SBMNH 654, adult female, SVL = 67.0 mm); scale bar = 5 mm.

elevation, 34.60°N, 120.64°W, Alice Abela, Morgan Ball, Christopher Evelyn, John LaBonte, and Samuel Sweet, 26 March 2012; SBMNH:HE:2733 (original number SSS 32385), E side of railroad tracks, 1.3 km NE of Point Arguello, 53 m elevation, 34.59°N, 120.64°W, Christopher Evelyn, 1 March 2009; SBMNH:HE:2734–2735 (original numbers SSS 32476–32477), W side railroad tracks, 0.5 km SE Arguello Coast Guard Station, 51 m elevation, 34.58°N, 120.64°W, Samuel Sweet, 1 March 2009.

Diagnosis.—The species is assigned to the genus *Batrachoseps* in having elongate genioglossus muscles attached to the midsection of the mandible, a large dorsal fontanelle in the skull, four toes on the hind feet, and genetic attributes that assign it to that genus. It is assigned to the subgenus *Batrachoseps* and differs from members of the subgenus *Plethopsis* (*B. campi*, *B. robustus*, and *B. wrighti*) in having fused premaxillary bones and more trunk vertebrae (21–22 vs. 17–19). *Batrachoseps wakei* is a large, long-bodied member (to 67.6 mm SVL, with 21–22 trunk vertebrae) of the *B. pacificus* group (Jockusch and Wake, 2002; Martínez-Solano et al., 2012), sharing with *B. major* and *B. pacificus* large body size, a dorsal pattern of longitudinally vermiculate tan markings on a medium brown ground color, few white iridophores either dorsally or ventrally, a tendency to show orange patches on the distal tail, and a pale venter (Fig. 2). *Batrachoseps wakei* differs from both species in having a smaller head, with the eyes protruding laterally beyond the margin of the upper jaw (Figs. 3–4), in having ca. 50% fewer melanophores on the throat and chest (Table 2), in often having extensive patches of orange pigment on the distal half of the tail (Fig. 2), in lacking melanophores in the peritoneal lining, and in genetic attributes described below. It further differs from *B. pacificus* in having a longer tail. It further differs from *B. major* in having proportionally longer limbs, larger feet, and a shorter tail (Fig. 4). *Batrachoseps wakei* has a proportionally smaller head and longer tail than *B. m. aridus*, a morphologically distinctive lineage variably treated as a subspecies of *B. major* (Wake and Jockusch, 2000) or as a species (e.g., AmphibiaWeb, 2020), the ventral trunk is pale, and the dorsal pattern is of pale longitudinal vermiculations rather than a dense suffusion of gold and silver iridophores as seen in *B.* (*m.*) *aridus*.

Other species in the *B. pacificus* group (*B. gavilanensis*, *B. incognitus*, *B. luciae*, and *B. minor*) occurring to the north in central coastal California are smaller, with proportionally smaller heads, shorter limbs, narrower feet, and longer tails. They are darker both dorsally and ventrally, with their venters typically being dark gray or black, often with conspicuous white guanophores. The final member of the *B. pacificus* group, *Batrachoseps gabrieli*, from the central and eastern Transverse Ranges, is a smaller, darker animal with more gracile proportions (narrower head, smaller limb interval resulting from longer limbs, and a long, thin tail), typically with lichenose coppery markings on the dorsum.

Batrachoseps wakei is nearly sympatric with the widespread congener *Batrachoseps nigriventris*, from which it differs in its larger size, proportionally larger head, limbs, and feet, proportionally shorter tail, and pale ventral trunk and tail (Fig. 2B compares the two). The same features serve to

Table 2. Melanophore patterns. Density of punctate melanophores on the midline of the throat (gular) and pectoral region of large species in the *Batrachoseps pacificus* group. Counts are per mm^2.

Species	Population	*n*	Gular mean	Range	Pectoral mean	Range
B. wakei	Point Arguello	6	40.7	29–50	58.7	30–67
B. major	Los Angeles basin	6	80.8	76–90	101.3	67–130
B. pacificus	Santa Cruz Island	5	86.4	80–95	93.0	84–98
B. pacificus	Santa Rosa Island	6	116.5	108–125	115.3	100–124
B. pacificus	San Miguel Island	6	75.0	46–92	65.0	46–77

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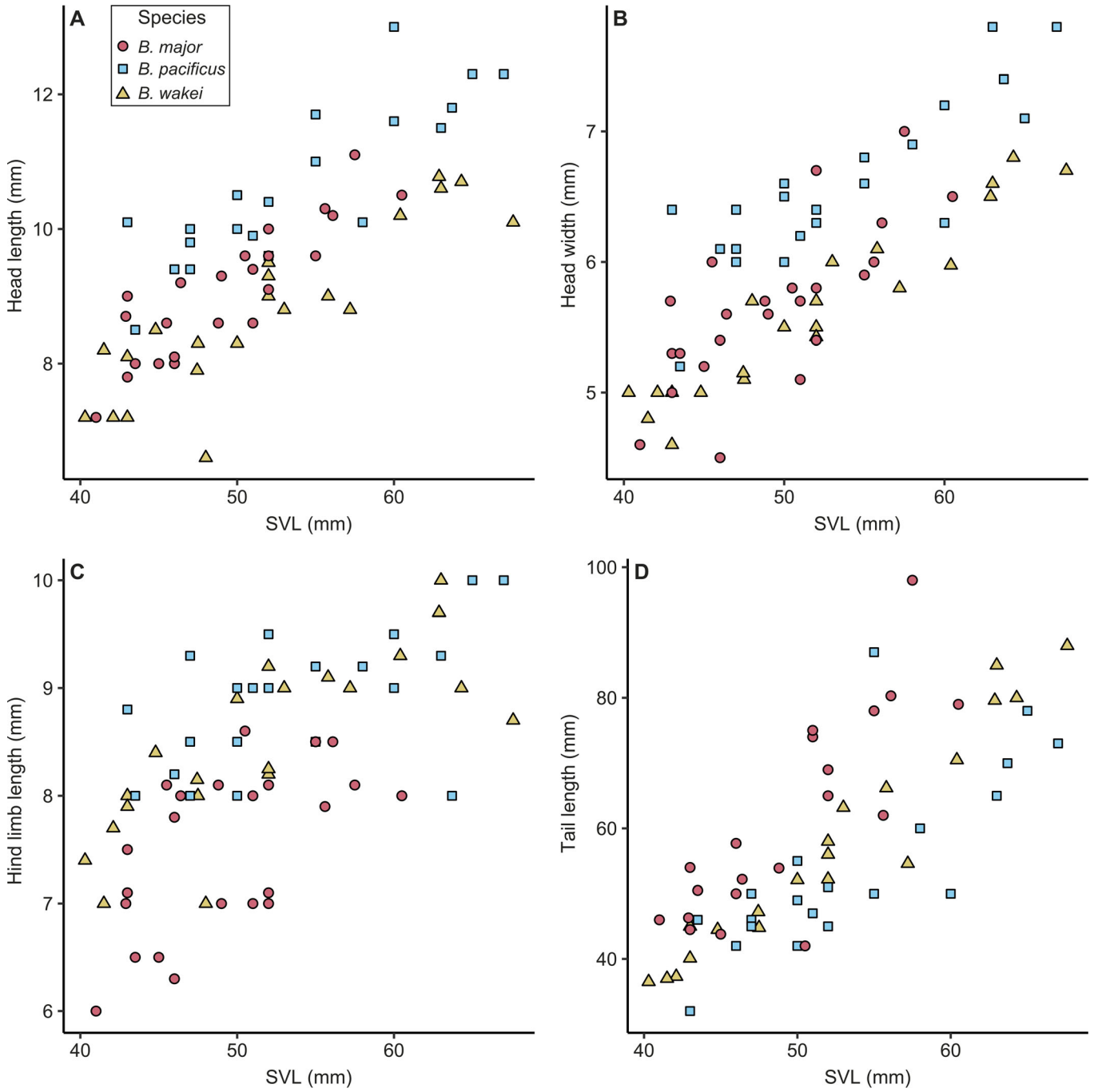


Fig. 4. Comparisons of body proportions in *B. wakei* and its closest relatives. (A) Head length (snout to gular fold); (B) maximum head width; (C) hind limb length; and (D) tail length versus snout–vent length (SVL); color and shape indicate species.

distinguish *B. wakei* from *Batrachoseps attenuatus* and *Batrachoseps gregarius*.

There are several species in the subgenus *Batrachoseps* in the Tehachapi Mountains and Sierra Nevada that are unlikely to be easily confused with *B. wakei*. The largest and most robust species, *B. stebbinsi*, is the only other species to approach *B. wakei* in standard length. It differs in having 18–19 trunk vertebrae (vs. 21–22), longer limbs, and larger feet. *Batrachoseps simatus* overlaps with *B. wakei* in vertebral number (having 20–21 trunk vertebrae) and also often having a dorsal pattern of longitudinal tan vermiculations. However, *B. simatus* is a smaller, more slender species with proportionally smaller feet, a longer but thinner tail, and a medium gray venter (vs. light tan). *Batrachoseps bramei* is much smaller than *B. wakei*, with a proportionally longer head and fewer trunk vertebrae (18–19). *Batrachoseps relictus* is also a much smaller species, with proportionally shorter limbs, smaller feet, and a longer tail than *B. wakei*.

There are also four smaller Sierran species, all belonging to the *B. diabolicus* group: *B. altasierrae*, *B. diabolicus*, *B. kawia*, and *B. regius*. These are all small (less than 50 mm SVL), relatively slender species with fewer trunk vertebrae (19–20, except 20–21 in female *B. diabolicus*), shorter limbs, relatively

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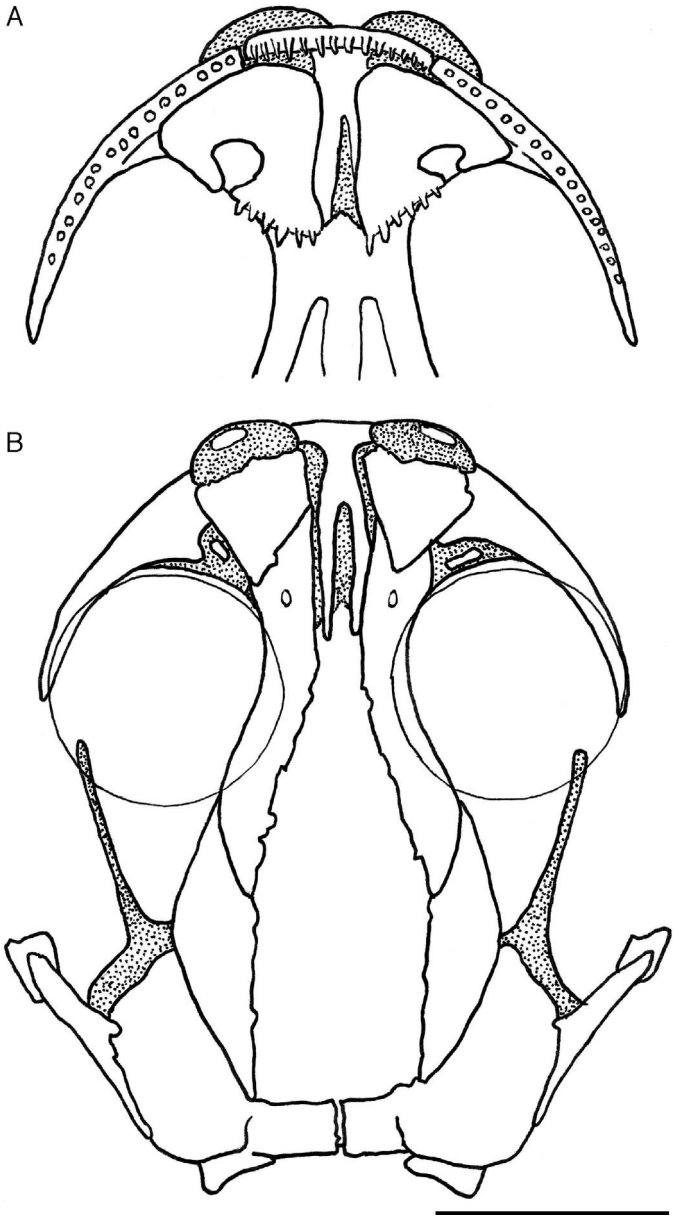


Fig. 5. Skull of an adult female *Batrachoseps wakei* (CCBER 32848, SVL = 53.0 mm); anterior ventral (top) and dorsal (bottom) views; bones, teeth, and cartilage (stippled) are shown. Scale bar = 2 mm.

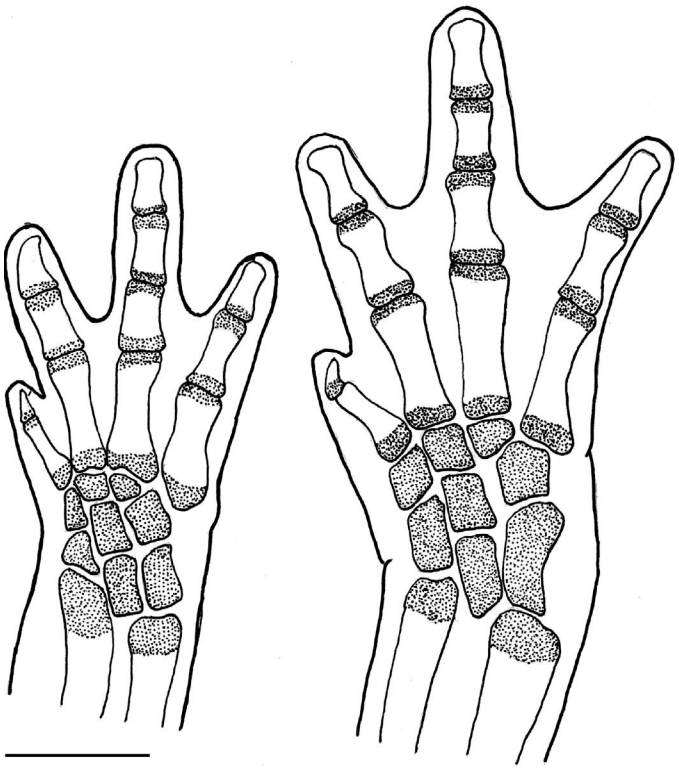


Fig. 6. Dorsal view of the right front and hind foot of an adult male *Batrachoseps wakei* (CCBER 32847, SVL = 55.8 mm); cartilage is stippled. Scale bar = 2 mm.

small feet, and proportionally longer tails, and none is likely to be confused with *B. wakei*.

Description.—*Batrachoseps wakei* is a large, moderately robust species, the largest male in the series being 65.0 mm SVL and the largest female 67.6 mm SVL, with a rounded head that is not distinct from the neck; further, the eyes extend beyond the outline of the head when viewed from below (Fig. 3). The head width to hind limb length ratio of eight males with SVL $\geq$ 50 mm ranges from 0.60–0.76, mean 0.66, and in the four females with SVL $\geq$ 50 mm it ranges from 0.66–0.77, mean 0.70. Hind limb length averaged 16.0% of SVL in males (range 14.0–18.0%) and 15.4% of SVL in females (range 12.9–15.0%). Relative tail length increases with body size, from 0.57 of SVL at 21 mm to 1.27 SVL in the largest male and 1.37 SVL in the largest female. Regenerating tails and limbs are relatively uncommon, being evident in only 2 and 1 of 32 specimens, respectively.

Five of seven males and three of four females have 22 trunk vertebrae; the others have 21. Costal groove counts (one less than vertebral numbers) are 24 individuals with 21 and 8 with 20 costal grooves. There are usually nine costal grooves between adpressed limbs. The pattern of skin grooves (indentations) on the head demarcating muscle masses is typical of the genus. Neither a mental gland nor a postiliac gland is evident. Six adult males have 5–8 (mean 6.3) enlarged premaxillary teeth that perforate the upper lip. Cleared and stained specimens (CCBER 32847–32849) have one sacral, two caudosacral, and 39–43 caudal vertebrae. These specimens have 11–14 premaxillary, 13–15 and 14–17 maxillary teeth, 5–7 and 6–7 vomerine teeth, and ca. 250 small teeth in the elongate parasphenoid patches (Fig. 5). Small preorbital processes are present on the vomers.

Measurements of the holotype (in mm).—Standard length (= SVL, snout to posterior angle of vent) 64.3; tail length 80.0; maximum head width 6.8; snout to gular fold 10.8; head depth at posterior angle of jaw 4.9; eyelid length 3.0; eyelid width 2.1; anterior rim of orbit to snout 1.8; orbit diameter 2.0; separation of external nares 2.5; snout projection beyond mandible 0.4; snout to forelimb insertion 13.7; axilla to groin length 40.2; forelimb length 8.0; hind limb length 9.0; limb interval between adpressed limbs 22.0; width of right hand 1.6; width of right foot 3.0; length of third toe 1.4; body width behind forelimbs 5.3. There are 21 costal grooves between the limb insertions, and 11 between adpressed limbs. There are 12 premaxillary, 30 maxillary, and 13 vomerine teeth (left and right sides combined), plus about

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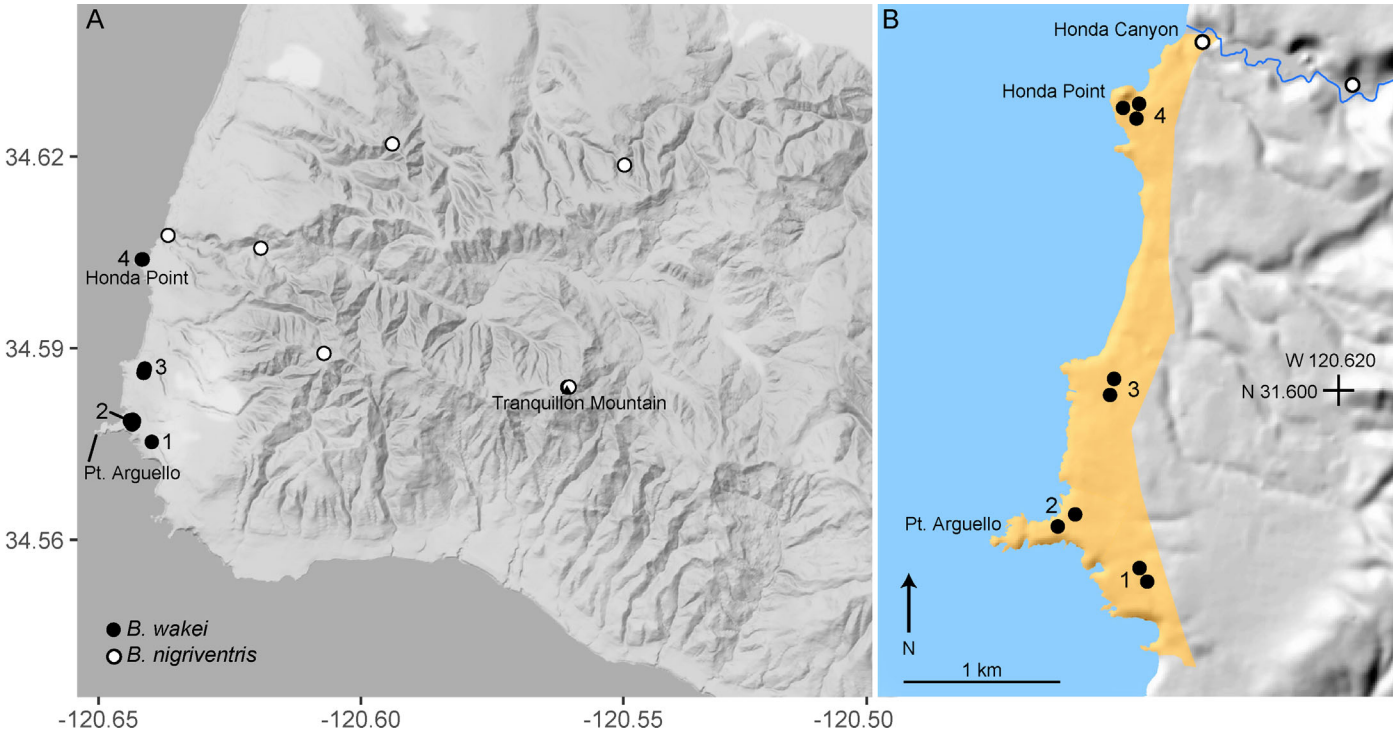


Fig. 7. Distribution of *B. wakei*. (A) Topographic relief map, with black dots marking the four localities at which *B. wakei* has been found; population numbers are as in Table 1. White dots indicate nearby localities for *B. nigriventris* on Vandenberg Space Force Base. Map tiles by Stamen Design, under a CC BY 3.0 license. Map data by OpenStreetMap under ODbL. (B) Inset of coastal region showing hypothesized range of *B. wakei*.

250 small teeth in the paired parasphenoid patches. Eight premaxillary teeth are enlarged and pierce the upper lip.

Coloration (in alcohol).—Adult *B. wakei* in ethanol are uniformly medium brown above, slightly darker on the head and in the dorsal third of each costal groove. Melanophores are deployed in rings around the openings of the skin glands, with the degree of envelopment decreasing slightly on the upper sides and then abruptly at the ventrolateral line. Melanophores become punctate and many fewer on the venter, almost absent on the ventral midline of the throat and trunk, and slightly denser on the pectoral region, along the cloacal lips, and the venter of the tail. The limbs are pigmented like the body, dark dorsally and paler beneath. The iris is dark, and the eyelids have dense melanophores along their edges.

Coloration in life.—Life coloration was examined in 26 individuals, including three small juveniles (21.0, 28.4, and 29.0 mm standard length). The dorsal ground color was light brown, created by melanophores grouped tightly around the openings of each skin gland. These became less dense at the ventrolateral line, and sparse melanophores were tightly condensed on the ventral surfaces, which were light tan with a faint orange cast on the tail. There were tiny white guanophores on the snout, anterior half of the eyelids and lateral surface of the face, neck, and trunk that did not reach the middle third of the venter behind the pectoral region. The dorsal ground color was overlain on the neck, trunk, and tail by tan to dull golden iridophores distributed as longitudinal streaks, usually less concentrated on the dorsal midline and so appearing to form irregular pale dorsolateral bands. Overlying these were patches of coppery orange iridophores, increasing in extent posteriorly on the dorsum of the tail (Fig. 2). These orange patches enlarge with size and may cover the entire dorsum of the distal third of the tail in large adults. There seems to be no sexual dimorphism in this feature. Most individuals had small orange patches dorsally on the thighs. The iris is black with two thin golden arcs bordering the dorsal third of the pupil.

Small juvenile *B. wakei* were uniformly dark brown dorsally and tan ventrally, without tan dorsal vermiculations or orange iridophore patches, but with dense, punctate white guanophores on all lateral surfaces.

Osteology.—CCBER 32847–32849 (55.8, 53.0, and 50.0 mm SVL) were cleared and stained for comparison with series of the most similar congeners, *Batrachoseps major* (Los Angeles basin and Orange County) and *B. pacificus* (Santa Cruz Island). The skull (Fig. 5) is relatively wide, with a large fontanelle between the frontals and parietals, as is typical of the genus. The premaxillae are fused anteriorly, their frontal processes remaining separate and loosely meeting the median edges of the nasals and the anterior extensions of the frontals, which do not contact one another. There are prominent foramina in the anterior frontals for the nasolacrimal ducts. Small prefrontal bones are present in some specimens, embedded in connective tissue between the frontals and the frontal processes of the maxillae. The cartilaginous olfactory capsules are only slightly protuberant beyond the outline of the upper jaw. The maxillae and premaxillae describe an arc that is exactly one-half a circle extending slightly posterior to the middle of the eyes, with small teeth that are absent from the posterior 10% of the maxillae. The pterygoid cartilages are thin and rodlike, extending forward to underlie the rear margin of the eye. The short parietals are widely separated anteriorly but approach each other posteriorly. Crests are absent on the otic

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capsules, although shallow grooves accommodate the thin posterior processes of the slender squamosals. The quadrates are small, and the lower jaw elements are relatively thin and flexible. There appears to be no mineralized or bony rod on the columella. Ventrally, the vomers contact the inner surfaces of the upper jaw elements but are widely separate from each other. Posteromedially, the vomers are strongly developed and thickened, bearing 6–7 stout, posteriorly directed teeth. A small preorbital process is present on each vomer, extending laterally to approximately the midpoint of the internal nares; this is an unusual trait for the subgenus *Batrachoseps* (Wake, 1966; Marlow et al., 1979). The body of the vomer also extends beyond the lateral margin of the internal nares. As a result, the internal nares are largely surrounded by bone, rather than being open posteriorly, which is the more typical condition in the genus. Paired subtriangular patches of ca. 120–130 tiny teeth lie on the ventral surface of the parasphenoid, becoming broader to the rear.

The hyobranchial apparatus (not illustrated) is typical for the genus, being entirely cartilaginous with a flattened basibranchial with thin, backward-sweeping, divergent radii anteriorly, and long epibranchials that angle up over the shoulder to terminate slightly behind the forelimb insertion. No urohyal is present.

There are 21–22 trunk vertebrae behind the atlas, only the last lacking ribs, and a single sacral followed by two caudosacral vertebrae. The tails have 39–43 caudal vertebrae, more of them ossified in larger individuals. The frequency of obviously regenerated tails (<10%) seems lower in *B. wakei* than in other species in the subgenus (e.g., *B. major*, ca. 33% [Cunningham, 1960]; *B. attenuatus*, 25–74% [Maiorana, 1977; Meneghin, 1977]).

The limb elements are relatively stout, but tibial spurs are generally absent, even on large adults. Phalangeal formulas are 1-2-3-2 for fore- and hind limbs alike, with the first digit barely free of the footpad in both (Fig. 6). Terminal phalanges are not much expanded. Carpal and tarsal elements are typical for the genus save that in all three individuals examined, the ulnare and intermedium are separate elements bilaterally, as is the case in most *B. major* and *B. pacificus* examined.

Etymology.—The species is named in honor of David Burton Wake, Professor of the Graduate School and emeritus Director of the Museum of Vertebrate Zoology at the University of California, Berkeley, in recognition of his immense contributions to the knowledge of California salamanders, as well as his broader roles in both amphibian conservation and evolutionary studies in western North America. David Wake has set an example of scholarship and integrity that has inspired his colleagues and his many graduate students (of whom we are two) by his insightful and never routine work on the evolution of salamanders, spanning the range of morphology, systematics, ecology, molecular genetics, and phylogeography. The complexity of the genus *Batrachoseps* has been progressively revealed by his embrace of the most current analytical techniques, and this is one of a minority of the 22 species in the genus that he has not had a hand in discovering or naming. As Museum of Vertebrate Zoology Director from 1971–1998, David Wake nurtured the museum's focus on discovering the diversity and evolutionary history of western North American tetrapods. He also played a critical early role in focusing attention on the emerging declining amphibian crisis (e.g., Blaustein and Wake, 1990) and, as a founder of AmphibiaWeb, in promoting a collaborative vision for the amphibian research community and making high quality information about amphibians easily accessible to all.

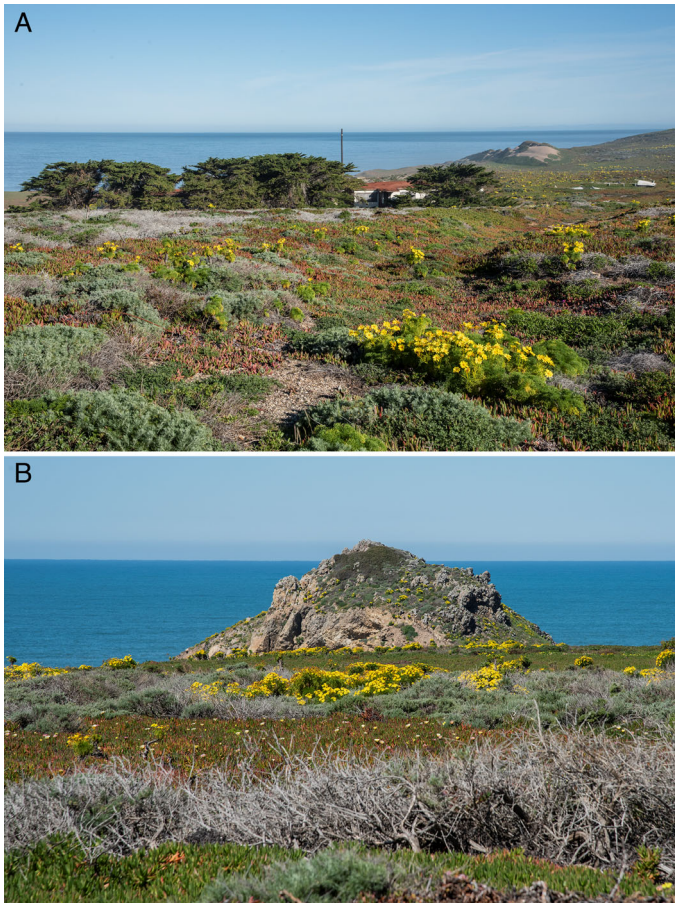


Fig. 8. Habitat of *B. wakei*. (A) Type locality at Point Arguello, California, USA; (B) northernmost locality at Honda Point, California, USA. Photographs © Ivan Parr, used with permission.

Habitat and distribution.—As far as is known, *Batrachoseps wakei* has a very restricted distribution on a narrow marine terrace centered on Point Arguello. It is known from the south rim of Honda Canyon to an old railroad siding north of Rocky Point, from the coastal cliffs inland to the Union Pacific Railroad tracks, at elevations of 20–60 m (Fig. 7). This zone is no more than 400 m wide and 4 km long, comprising about 130 ha. Within this region, it has been found at four sites (Table 1). The western end of the Transverse Ranges here juts out into the Pacific Ocean, extending up to 10 km farther west than does the rest of the local coastline from Point Sal south to Point Conception, and rises to 650 m at Tranquillon Peak 8 km inland. This results in strong but very local enhancement of onshore winds, fog, and rainfall. The prevailing NW wind transports beach sand up onto the terrace, and decomposition of the relatively thick low vegetation, dominated by non-native Iceplant (*Carpobrotus edulis*) and including stands of the arborescent Giant Coreopsis, *Leptosyne gigantea* (Fig. 8), creates a dark, sandy loam soil that is not found elsewhere on the coastal terraces. This soil holds burrows, unlike the sand deposits on less-

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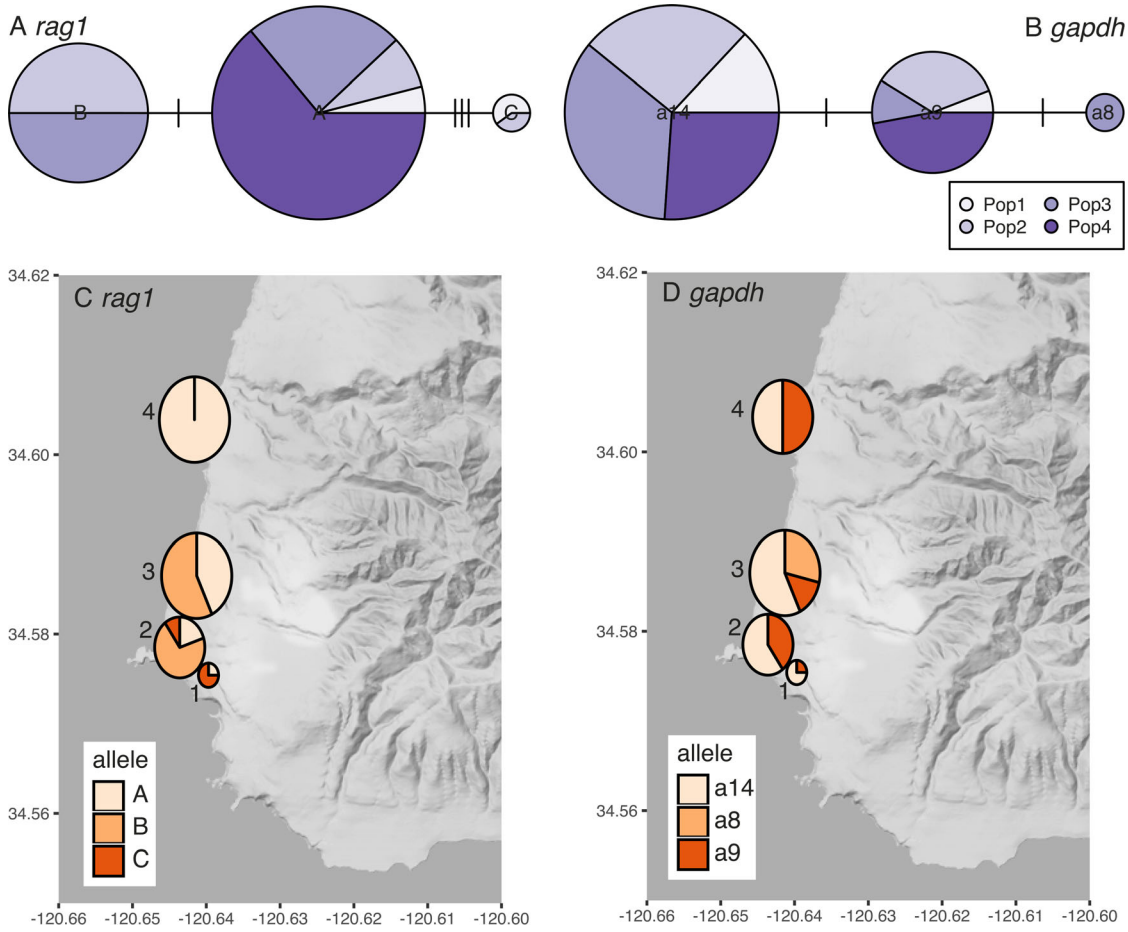


Fig. 9. Haplotype networks and geographic variation in allele frequencies for the two loci showing variability in *B. wakei*. Haplotype networks for *rag1* (A) and *gapdh* (B), with circle size scaled to allele frequency and shading indicating the four source populations. Distribution of alleles across geography for *rag1* (C) and *gapdh* (D), with circle size scaled to sample size from the population, and shading distinguishing the three alleles.

exposed terraces nearby. To the north the coast is mostly dune fields for 60 km, and south of Point Conception the coast turns eastward for over 100 km and experiences much less fog and rainfall.

The crest of the western terminus of the Santa Ynez Range at Point Arguello is composed of early Miocene rhyolitic tuffs of the Tranquillon Volcanics Formation, unconformably overlying the Rincon Shale and overlain by the Monterey Formation, both deepwater marine deposits (Dibblee, 1950, 1988; Cole and Stanley, 1998). Volcanic activity corresponds to the transition from a converging (subducting) to a transform (sliding) boundary as the Farallon Plate contacted North America, culminating in the origin of the San Andreas Fault Zone (Atwater, 1970), and dates to about 14 mya. The rocks composing Honda Point (also known as Point Pedernales and including Destroyer Rock, the site of the catastrophic grounding of seven U.S. Navy destroyers in 1923) are Tranquillon volcanics, while to the south Point Arguello is underlain by Monterey shale.

The amphibian and reptile community recorded at the site reflects the strong maritime influence. *Batrachoseps nigriventris* avoids the coastal terrace but occupies the canyon of Honda Creek to within 400 m of the nearest records of *B. wakei*, and also occurs in rocky soft chaparral within about 1.1 km to the east. *Batrachoseps nigriventris* has been found in sympatry with six species in the *B. pacificus* group (*B. gabrieli*, *B. pacificus*, *B. major*, *B. minor*, *B. incognitus*, and *B. gavilanensis*; Yanev, 1978; Wake, 1996; Jockusch et al., 2001; Jockusch and Wake, 2002), so sympatry with *B. wakei* where their habitats meet would not be surprising. The plethodontid salamanders *Aneides lugubris* and *Ensatina eschscholtzii* are each microsympatric with *B. wakei*, as is the frog *Pseudacris hypochondriaca*, with *Rana draytonii* in Honda Creek 0.5 km away. Among lizards, *Sceloporus occidentalis*, *Plestiodon skiltonianus*, *Elgaria multicarinata*, and *Anniella pulchra* are microsympatric with *B. wakei*. Among snakes, only *Diadophis punctatus* and *Pituophis catenifer annectens* have been recorded on-site, with *Crotalus helleri* nearby.

***Genetic diversity in* B. wakei.**—Overall genetic diversity in *B. wakei* is low. Despite relatively large samples (18 mtDNA samples, 40–50 allele copies for each nuclear locus) spanning the full known range of the species, no variation within *B. wakei* was found in five of the seven analyzed markers, including the mitochondrial gene. The exceptions were *rag1* and *gapdh*, each of which had three sampled alleles. Geographic structuring was present (Fig. 9), with AMOVA on the multilocus data showing that 31.2% of the variation was distributed among populations ($P < 0.001$). When run on the individual loci, no structure was detected in *gapdh* ($P = 0.60$), while 52.1% of the variation at *rag1* occurred between populations ($P < 0.0001$).

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Variation in *gapdh* involves the length of an intronic mononucleotide repeat, which has 8, 9, or 14 Gs; such motifs are expected to have a high mutation rate. Multiple alleles were observed in all populations (Fig. 9). The 8-nucleotide repeat was private to one population in these samples, while the other two repeat lengths were observed in all four populations. Each of three *rag1* alleles was found in at least two populations, and multiple alleles were sampled in three of four populations (Fig. 9). The most widespread allele was fixed in the sample of seven specimens from the northernmost population.

***Phylogenetic relationships of* B. wakei.**—Supplemental Table S2 (see Data Accessibility) gives alignment information and the selected models and partitioning scheme for each marker. Gene trees for each marker are shown in Supplemental Fig. S1 (maximum likelihood; see Data Accessibility) and Supplemental Fig. S2 (Bayesian; see Data Accessibility); haplotype networks are provided for loci where *B. wakei* formed part of a multispecies polytomy (Supplemental Fig. S3; see Data Accessibility). No allele sharing across species was observed, with the exception of one allele in *pomc*, the least variable locus. Additionally, species-level structuring was strong, with most species being monophyletic or nearly so at most markers. However, relationships strongly conflicting with monophyly of conspecifics were not uncommon.

In all species-tree analyses, *B. wakei* belonged to a clade comprising three species: *B. major*, *B. wakei*, and *B. pacificus*, which received high support in most analyses (Fig. 1B; Supplemental Table S3; see Data Accessibility). Relationships within this clade depended on inference method and were not strongly supported, with *B. wakei* inferred to be the sister taxon to a clade comprising the other two lineages in all analyses using ASTRAL and with it instead inferred to be the sister taxon to *B. major* with SVDQuartets (Supplemental Table S3; see Data Accessibility). This southern affinity of *B. wakei* is reflected in the relationships in five of seven gene trees or haplotype networks; in the other two loci (*rag1*, *cytb*), *B. wakei* shows central coastal, rather than southern, affinities (Supplemental Figs. S1–S3; see Data Accessibility).

DISCUSSION

The description of *B. wakei* brings to 23 the number of taxa recognized within the genus *Batrachoseps*, 22 species and a morphologically divergent subspecies within *B. major* that is also sometimes treated as a species, *B. m. aridus*; nine of these taxa belong to the *B. pacificus* group, making it the most diverse of the five subgeneric groups recognized. The eight taxa previously known within the *B. pacificus* group occur in three geographic regions (Jockusch and Wake, 2002; Jockusch et al., 2015): coastal southern California and northern Baja California (home to *B. gabrieli*, *B. major*, and *B.* (*m.*) *aridus*); the northern Channel Islands (*B. pacificus*); and the Coast Ranges of central California (*B. gavilanensis*, *B. luciae*, *B. incognitus*, and *B. minor*). Within each of the mainland regions, the *B. pacificus* group species are parapatrically distributed, or narrowly allopatric (Fig. 1A). However, neither set of geographically contiguous species constitutes a monophyletic group; this non-monophyly is believed to reflect a biogeographic history in which the central coastal taxa were transported north separately on distinct pieces of land by tectonic activity (Yanev, 1980; Wake, 2006).

The geographic gap between the central and southern distribution of the *B. pacificus* group was previously thought to extend from central San Luis Obispo County to the Los Angeles Basin and San Gabriel Mountains, a region occupied by *B. nigriventris* (Fig. 1A). Among *B. pacificus* group species, *B. wakei* falls squarely within this gap. It is geographically closest to populations from the northern Channel Islands. However, multiple lines of evidence suggest that these islands have been isolated from the mainland, likely for their entire history, by a saltwater barrier that constitutes a very substantial barrier to dispersal by amphibians (Yanev, 1980). The new species is separated from the nearest documented population in the central coast region by 84 km and from the nearest mainland population in the southern portion of the range by 202 km. The region separating *B. wakei* from its mainland *B. pacificus* group relatives is occupied only by *B. nigriventris*.

***Biogeography of* B. wakei *and the* B. pacificus *group*.**—The inability to find other populations of *B. wakei* has delayed naming the species for a number of years, during which time extensive sampling efforts both near and far were undertaken. The tectonic history of the Santa Ynez Mountains is quite complex and involves over 100 degrees of rotation of the western end, from a position aligned N–S against the coast of what is now northern San Diego County to its current WNW–SSE alignment projecting into the Pacific Ocean (Luyendyk, 1991). Much of this movement has occurred during the estimated times of divergence of species in the *B. pacificus* group (Wake, 2006), although the areas and durations of submerged and emergent portions of the Santa Ynez range and northern Channel Islands are complex and poorly established. To the north this rotation is compressing the triangular Los Osos Domain (Lettis et al., 2004), creating a series of NW-trending ridges and valleys that closed off the Santa Maria Embayment and elevated the Santa Lucia Range that extends SE from Big Sur to the Cuyama River. *Batrachoseps incognitus* and *B. minor* now occupy this ridge, where they occur in microsympatry with *B. nigriventris*.

The occurrence of *B. wakei* in a very restricted coastal terrace habitat raises questions about when and how it reached this habitat. A major fault zone parallel to the San Andreas system (the Hosgri Fault) originates at Honda Point and extends NW mostly offshore, but has three more coastal fragments at Point Sal, Point Buchon, and Point Piedras Blancas, the latter within 5 km of localities for *B. incognitus*. However, searches at these sites and throughout the Irish Hills inland of Point Buchon have yielded only *B. nigriventris*. Extensive fieldwork on the distribution of the northern members of the *pacificus* group and *B. nigriventris* in the Santa Lucia Mountains leads us to conclude that *B. wakei* is very likely a true relict species with a tiny range.

***Phylogenetic relationships and genetic diversity of* B. wakei.**—Although the placement of *B. wakei* is variable across different gene trees, in most markers it shows a southern affinity, as expected given its placement in a clade with *B. pacificus* and *B. major* (Fig. 1). The exceptions to this are the mitochondrial marker *cytb* and one nuclear marker, *rag1*, where *B. wakei* instead appears more closely related to a central coast species (Supplemental Figs. S1–S3; see Data Accessibility). A key question is what process(es) are responsible for the variable placement of *B. wakei* across

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different gene trees. This variation could be explained either by incomplete lineage sorting or by post-speciation gene flow between species (Maddison, 1997; Degnan and Rosenberg, 2009). Adjacent short internodes, as observed in the species tree, provide conditions under which incomplete lineage sorting is expected. The tendency for clades that violate species monophyly to involve geographic neighbors, and samples from range margins, suggests a role for gene flow as well. Additional investigation with a much larger number of markers is needed to robustly test the role of post-divergence gene flow in the evolution of this clade. Given its geographically intermediate position and current isolation, evidence that *B. wakei* hybridized with *B. minor* after its separation from the more southern lineages would be particularly interesting, especially since these two species presently occupy very different environments (exposed marine terrace vs. mesic, rocky montane woodland).

The genetic homogeneity of mitochondrial DNA and other markers in *B. wakei* is striking, especially in the context of genetic diversity within the genus. The complete absence of variation in *cytb* across 18 individuals from four populations is particularly unusual and contrasts with the general pattern found in other species of *Batrachoseps* (Martínez-Solano et al., 2007, 2012; Martínez-Solano and Lawson, 2009; Jockusch et al., 2012, 2020). For example, Martínez-Solano et al. (2007) found multiple haplotypes in the majority of populations of *B. attenuatus* from which only two individuals were sampled and eight of nine populations from which at least three individuals were sampled. The only exception was a northern population, in a region that shows a signature of a recent range expansion. Similarly, populations of *B. attenuatus* from islands in San Francisco Bay and the surrounding headlands generally harbored two or more *cytb* haplotypes, with the only exception hypothesized to have resulted from an anthropogenic introduction (Martínez-Solano and Lawson, 2009). Intraspecific variation has been studied in less depth in other species, which show the same general pattern of extensive variation, both within and between populations, especially in mitochondrial *cytb* sequences (Jockusch et al., 2001, 2012, 2020; Martínez-Solano et al., 2012). The extremely low level of genetic diversity in *B. wakei* extends to the nuclear genome and is low even in comparison to single-population samples of other species of *Batrachoseps*.

Extremely low levels of genetic diversity result from genetic drift in association with certain demographic processes. One scenario is a bottleneck in the recent past, followed by an extended period at a low population size (Nei et al., 1975). Low genetic diversity may also be a consequence of a recent range expansion. Serial subsampling of diversity as a population expands leads to loss of diversity along the expanding range front (Slatkin and Excoffier, 2012). For this scenario to explain the lack of diversity, the species must have been more widespread, with either extinction eliminating the source populations or, alternatively, these persisting and awaiting discovery. The much lower sea levels during Pleistocene glacial maxima provided an expanded area of potential occupancy. At the estimated sea level minimum during the last glacial cycle of greater than 100 m below present with the California coast estimated to have been located more than 10 km seaward of its current location off Point Arguello (Reeder-Myers et al., 2015). Additionally, if the species is restricted to marine terrace habitats, sea level fluctuations would be expected to result in serial subsampling.

***Conservation status of* B. wakei.**—Two features of *B. wakei* are important to consider in making decisions that affect its habitat and conservation: the lack of genetic diversity and its small range size. The limited genetic diversity signals that the effective population size is very low and is expected to limit the adaptive potential of the species. Small range size is a major predictor of endangerment in many taxa, including amphibians (Sodhi et al., 2008; Pincheira-Donoso and Hodgson, 2018). A recent analysis (Tietje and Rödel, 2018) found that range size was far more important than other tested variables (measures of body size, abundance, and latitude) in predicting lineage duration in amphibians. The tiny known range of *B. wakei* is at the extreme lower end of amphibian range sizes.

Range estimates calculated from IUCN Red List data (v. 6.1 shape files) identified nine salamander taxa (of 564 included species) with a range size below 5 km^2 (IUCN, 2018). Of these, three (*Plethodon ainsworthi*, *Nototriton tapanti*, and *Oedipina paucidentata*) are poorly known; each appears to have only been collected once (i.e., at one locality on one day; Brame, 1968; Good and Wake, 1993; Lazell, 1998), with no new specimens since their descriptions, and the validity of *P. ainsworthi* has been questioned (Himes and Beckett, 2013). Three additional species are in highly specialized, geographically restricted habitats: a single cave system (*Gyrinophilus subterraneus*; Besharse and Holsinger, 1977), a 4.8 km^2 marine island (*Oedipina maritima*; García-París and Wake, 2000), and at high elevations on a volcanic peak in Lake Nicaragua (*Bolitoglossa insularis*; Stark et al., 2014). The other three species (*Cryptotriton monzoni*, *Bolitoglossa indio*, and *Urspelerpes brucei*) resemble *B. wakei* in having been collected on multiple occasions, and not having obvious geographic restrictions on their distributions. They have all been discovered at additional localities since the IUCN assessment was completed and are now known to be considerably more widely distributed (Sunyer et al., 2012; McCranie and Rovito, 2014; Pierson et al., 2016; Pierson, pers. comm.). Thus, *B. wakei* falls in the bottom 1% for salamander range size.

In 13 years since the first individuals of this species were discovered, we and others have surveyed an area encompassing 475 km^2 surrounding the known range of this species. This effort has managed to extend the range by a total of just 3.5 km, within a very narrow elevational band. Under good surface conditions, *B. wakei* can be found at moderate abundance within this area. This makes *B. wakei* unusual in being a species that can be found consistently under good conditions in its tiny range. In this regard, it resembles *G. subterraneus* (Niemiller et al., 2010) and *Dendrotriton xolocalcae* (Taylor and Smith, 1945). We predict that continued efforts to find additional populations may be successful, as somewhat similar habitat exists along the coast to both the north and south. Thus, survey efforts along the coast should continue to include coastal terrace habitat at Point Conception (20 km SSE), where an area of approximately 10,000 hectares was recently protected through acquisition by The Nature Conservancy and is not open to the public, relict dune sheets on the north side of Point Sal (36 km NNE), and the coastal slopes of the Irish Hills north of Point Buchon (75 km N). Additionally, the restricted marine terrace habitat

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along the Harmony Headlands coast south of Cambria (110 km NNW) should be searched; this area contains habitat similar to that occupied by *B. wakei* in the geographic vicinity of central coast members of the *B. pacificus* group.

Despite its small range and lack of genetic diversity, *B. wakei* is inherently well protected, and so long as its habitat is not disturbed, it is likely to persist. Land ownership is divided between the United States Space Force, the U.S. Coast Guard, and the Union Pacific Railroad Corporation, and the governmental entities are expected to be supportive of management practices that aid in conservation of this species. At present, most of the habitat is undeveloped dunes and none of the habitat is accessible to the general public. The dune flora is heavily impacted by non-native Iceplant, which was originally planted for erosion control but has become invasive and is thus a target of management. However, the Iceplant does not appear to negatively affect the salamanders, which may in fact benefit from the increased retention of surface moisture. Significantly, all entry to the area is strictly regulated and monitored because Vandenberg Space Force Base hosts a set of dispersed launch complexes used to send military and civilian satellites into polar orbits.

DATA ACCESSIBILITY

Supplemental material is available at https://www.ichthyologyandherpetology.org/h2020027. All sequences newly reported in this study have been deposited in GenBank (accession numbers MW651987–MW652283). Supplemental Table S1 provides additional information on tissue samples of *B. wakei*, including population of origin, genotypes for *gapdh* and *rag1*, and GenBank accession numbers. Additional phylogenetic analysis details are included in Supplemental Tables S2 and S3. Trees in Newick format from Supplemental Figures S1 and S2 are included in Supplemental Files S1 and S2; species trees in nexus format from the ASTRAL and SVDQuartets analyses are included in Supplemental File S3. Unless an alternative copyright or statement noting that a figure is reprinted from a previous source is noted in a figure caption, the published images and illustrations in this article are licensed by the American Society of Ichthyologists and Herpetologists for use if the use includes a citation to the original source (American Society of Ichthyologists and Herpetologists, the DOI of the *Ichthyology & Herpetology* article, and any individual image credits listed in the figure caption) in accordance with the Creative Commons Attribution CC BY License. ZooBank publication urn:lsid:zoobank.org:pub:E7D1EE65-D4F5-41B5-96B5-F4AB1EF51048.

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