

Range extension and species assignment of a rediscovered population of the Black Salamander complex (genus *Aneides*) in California, USA, based on morphological and molecular evidence

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The Black Salamander complex comprises four species of plethodontid salamanders found across northwestern California and Oregon (Reilly and Wake, 2019; Hansen and Shedd, 2025). These salamanders were formerly recognised as a single species, *Aneides flavipunctatus* (*sensu lato*), originally described as *Plethodon flavipunctatus* Strauch, 1870 (Reilly and Wake, 2019). At least three other taxa associated with the Black Salamander complex have been historically named but are no longer recognised, including the widespread recognition of two subspecies now elevated to species (see Frost, 2026 for a detailed review of all names and details about taxonomic issues). Members of the Black Salamander complex display wide phenotypic variation that often overlaps with one another, hence species identification based purely on morphology, without the context of geographic or genetic information, is not reliable (Reilly and Wake, 2019; Hansen and Shedd, 2025).

Early genetic work and morphological analysis of *A. flavipunctatus* (*sensu lato*) in the northern part of its range recognised multiple deeply divergent populations (Larson, 1980; Lynch, 1981). This was later corroborated with additional genetic analysis of mitochondrial markers by Rissler and Apodaca (2007), Reilly et al. (2012), and Reilly and Wake (2015), who detected deep genetic divergence and cryptic diversity within *A. flavipunctatus*. Following the taxonomic

review of *A. flavipunctatus* (*sensu lato*) by Reilly and Wake (2019), the Black Salamander complex includes the species *Aneides niger* Myers and Maslin, 1948 (Santa Cruz Black Salamander), *Aneides iecanus* Cope, 1883 (Shasta Black Salamander), *Aneides klamathensis* Reilly and Wake, 2019 (Klamath Black Salamander), and *Aneides flavipunctatus sensu stricto* Strauch, 1870 (Speckled Black Salamander).

Aneides niger, endemic to the Santa Cruz Mountains of California and historically considered the most distinct member of the Black Salamander complex (Myers and Maslin, 1948), can be identified by genetics, uniform black colouration, and its disjunct distribution (Reilly and Wake, 2019). *Aneides iecanus* is found in the greater Shasta Lake region and differs from other members of the Black Salamander complex by genetics, isolated range, presence of 16 trunk vertebrae, and proportionally shorter legs (Reilly and Wake, 2019; Hansen and Shedd, 2025). *Aneides klamathensis* is found in the Klamath Mountains of southern Oregon and far northwestern California, ranging as far south as the Eel and Van Duzen rivers in southern Humboldt County (Reilly and Wake, 2019; Hansen and Shedd, 2025). *Aneides klamathensis* often displays increased brassy colouration in adulthood (Lynch, 1981; Reilly and Wake, 2019; Hansen and Shedd, 2025), but due to morphological variation it can only be reliably differentiated from other members of the Black Salamander complex by genetics and range (Reilly and Wake, 2019). *Aneides flavipunctatus sensu stricto* is found in the North Coast Range south of the Eel and Van Duzen rivers. It is the most genetically diverse member of the Black Salamander complex (Reilly and Wake, 2015, 2019), and displays a wide variety of patterns and colours ranging from densely spotted to solid black or grey (Reilly and Wake, 2019).

Although the distributions of these four species have been described in detail (Fig. 1), an outstanding

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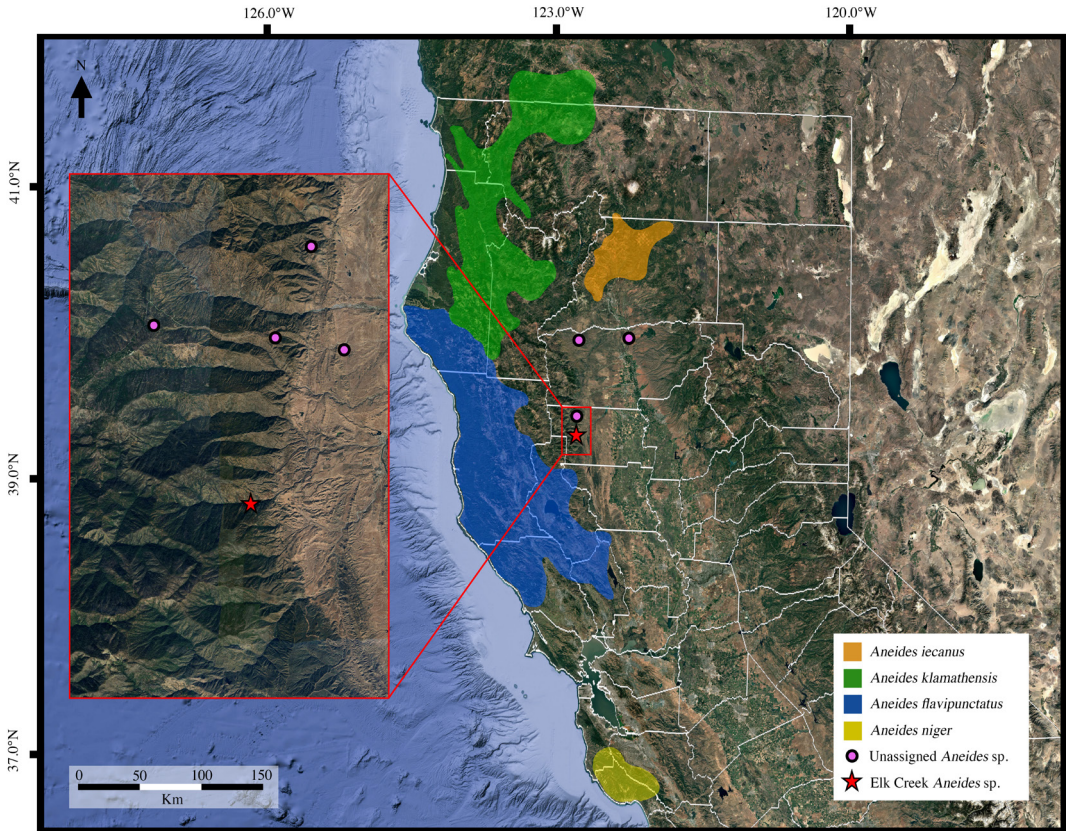


Figure 1. Approximate distribution of the four members of the Black Salamander species complex (adapted from Reilly and Wake [2019] and Hansen and Shedd [2025]). Purple markers represent unassigned *Aneides* sp., and red stars represent the new locality in western Glenn County, California, where we collected three specimens.

knowledge gap exists at the eastern edge of the North Coast Range in Glenn and Tehama Counties, California (Reilly and Wake, 2019; Hansen and Shedd, 2025). Salamander specimens from these two disjunct populations, originally identified as *A. flavipunctatus*, were collected in this region as recently as the early 1970s by J. Lynch (MVZ:Herp:124344–50). However, due to the specimen age and fixation methods, genetic material was unrecoverable (Sean Reilly, pers. comm.) and they could not be assigned to species using morphological characters, largely due to the substantial variation within the Black Salamander complex. The locations of these specimens in Glenn and Tehama Counties are in close proximity to ranges of multiple species (*A. klamathensis*, *A. flavipunctatus*, and *A. iecanus*; Fig. 1), making these populations of significant biogeographic and taxonomic interest.

Extensive field surveys have been conducted in

the region in search of these unassigned *Aneides* sp. However, no specimens were found until early 2025. On 23 February 2025, we collected three *Aneides* sp. in the Elk Creek drainage of Glenn County, California, an area in which they had not been seen since 1971 (MVZ:Herp:124349). Herein, we use a combination of genetic and morphological evidence to determine the species identity of these specimens.

Materials and Methods

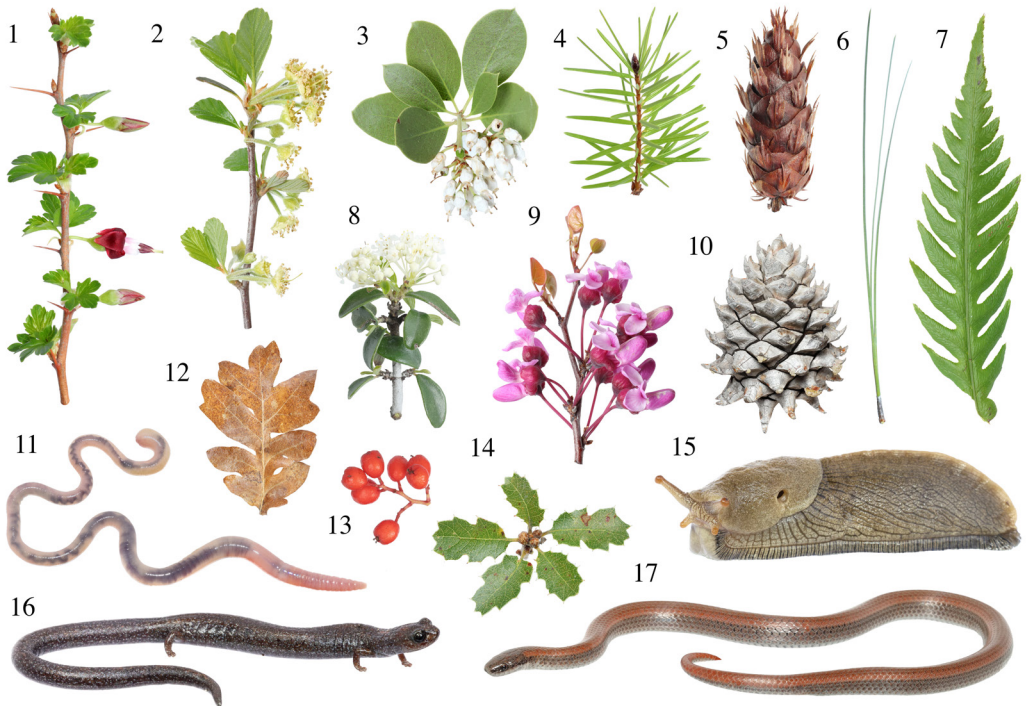
Study area and animal collection. We searched for animals at several locales in Glenn County, California, USA, based on historical records of this species (Fig. 1) collected by J. Lynch (MVZ:Herp:124348–124350). During 22–23 February 2025, we conducted active field surveys for salamanders across several sites in the Alder Springs area, centred around historic sites along Alder Springs Rd in Mendocino National Forest

(39.6568, -122.6046). We spent several hours on 22 February lifting rocks, logs, and fallen branches, and shining flashlights along north-facing embankments at night in search of salamanders, but only found a single salamander species: *Batrachoseps attenuatus*. On 23 February 2025, we shifted our focus approximately 9.8 km south, to search for salamanders along South Fork Elk Creek on Forest Rd 20N01, based on the presence of suitable north-facing mesic habitat, despite a lack of historic records. On Forest Rd 20N01, approximately 3.3 km west from the intersection with Ivory Mill 308 Rd. (39.5694, -122.660) at elevation 720 m, we found three *Aneides* specimens. These individuals were in close proximity under sedimentary stones at the base of a California Buckeye (*Aesculus californica*) growing out of a retaining wall. This site is characterised by steep, north-facing sedimentary talus that flows down from the summit of Tool Cache Ridge, 740 m above, into South Fork Elk Creek, 50 m below (Fig. 2). The vegetation community of this site is dominated by mixed conifer/hardwood forest composed of Douglas Fir (*Pseudotsuga menziesii*), Grey Pine (*Pinus sabiniana*), and Oregon White Oak (*Quercus garryana*), as well as large patches of chaparral composed of Toyon (*Heteromeles arbutifolia*), California Scrub Oak (*Quercus berberidifolia*), and Western Redbud (*Cercis*



Figure 2. Habitat of *Aneides flavipunctatus* along the South Fork Elk Creek Canyon in Glenn County, California, where the three specimens were found in February 2025. Photo by Andre Giraldi.

occidentalis) (Fig. 3). Live animals were transported to California Polytechnic University, Humboldt and photographed (Fig. 4). Photographs of habitat and animals were taken with a Canon EOS R6 Mark II camera. Animals were then euthanized using MS-222 (Gentz, 2007), and a small tip of the tail was taken and preserved in 100% ethanol for genetic analyses. Specimens were then fixed in 10% neutral-buffered



formalin, preserved in 70% ethanol, and accessioned in the Vertebrate Museum at California State Polytechnic University, Humboldt (HSUVM1152, HSUVM1153, HSUVM1154). Deceased specimens were positioned straight on a measuring grid, snout–vent length (SVL) was measured from the tip of the snout to the midpoint of the cloacal opening to the nearest mm.

DNA sequencing. We obtained DNA sequences from two mitochondrial markers from all three specimens: a fragment of the *NADH dehydrogenase 4* [*ND4*] gene and a fragment of the *cytochrome b* [*cyt b*], both of which have been previously used to identify and characterise populations in the Black Salamander complex (Rissler and Apodaca, 2007; Reilly and Wake, 2012, 2015, 2019). Genomic DNA was extracted from tissue samples using the DNeasy Blood and Tissue Kit (QIAGEN, Hilden, Germany). The *ND4* region was amplified with primers *NADH-f(flav)* and *NADH-r(flav)* designed by Rissler and Apodaca (2007). All *ND4* PCRs were carried out in a volume of 25.5 μ L containing 1.5 μ L of genomic DNA, 13 μ L DreamTaq Green PCR Mastermix (2X) (Thermo Fisher Scientific, Waltham, MA, USA.), 1 μ L of each 10 mM primer, and 9 μ L of water. Amplifications were carried out at 95 $^{\circ}$ C for 3 minutes for the initial denaturation step; followed by 30 cycles of 95 $^{\circ}$ C for 30 seconds of denaturation, annealing at 47 $^{\circ}$ C for 30 seconds, and extension at 72 $^{\circ}$ C for 45 seconds; with a final extension at 72 $^{\circ}$ C for 5 minutes.

Partial *cyt b* sequences were amplified using the primers *MVZ 15* and *cyt-b2* from Moritz et al. (1992). Amplifications were conducted in a final volume of 24 μ L containing 1.5 μ L of genomic DNA, 12.5 μ L DreamTaq Green PCR Mastermix, 1.5 μ L of 10 mM of each primer, and 7 μ L of water. *Cyt b* PCRs were carried out at 94 $^{\circ}$ C for 3 minutes for the initial denaturation step; followed by 34 cycles of 94 $^{\circ}$ C for 30 seconds of denaturation, annealing at 54 $^{\circ}$ C for 30 seconds, and extension at 72 $^{\circ}$ C for 45 seconds; with a final extension at 72 $^{\circ}$ C for 5 minutes. All PCR amplifications were performed on a Bio-Rad T100 Thermal Cycler.

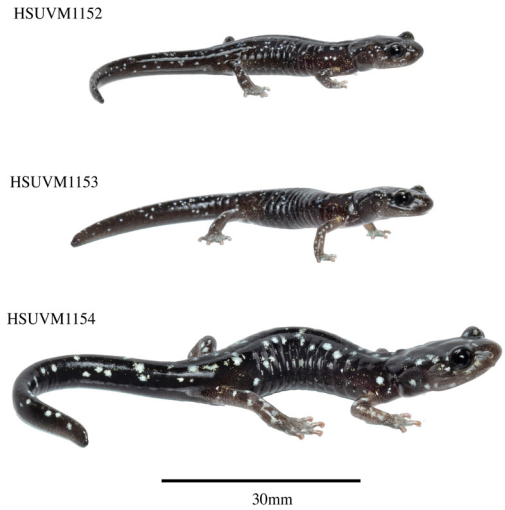


Figure 4. *Aneides flavipunctatus* from South Fork Elk Creek, Glenn County, California. Photos by Andre Giraldi.

The sizes of PCR products were assessed using standard agarose gel electrophoresis and products of the expected size were purified using the QIAquick PCR purification kit (QIAGEN, Hilden, Germany). The purified products were sequenced in both directions using Sanger sequencing conducted by Eurofins Genomics (Louisville, KY). Raw sequence chromatographs were edited using Geneious Prime v2025.2.2 (Dotmatrix, Boston, MA). All new sequences analysed in this study have been deposited in GenBank (PX879114–PX879119).

Phylogenetic analysis. To assess the species identity of Elk Creek *Aneides* sp. specimens ($n = 3$), we compiled *ND4* and *cyt b* DNA sequences available in GenBank from all described members of the Black Salamander complex, including *A. flavipunctatus* ($n = 140$), *A. klamathensis* ($n = 51$), *A. niger* ($n = 7$), and *A. iecanus* ($n = 2$). Additionally, *ND4* and *cyt b* sequences from *Aneides hardii* ($n = 1$) and *A. lugubris* ($n = 1$) were used as outgroups to root the phylogeny. Only specimens



Figure 3. Characteristic flora and fauna found in microhabitat occupied by *Aneides flavipunctatus* in the South Fork Elk Creek Canyon: (1) Gummy Gooseberry (*Ribes lobbii*), (2) Birchleaf Mountain Mahogany (*Cercocarpus betuloides*), (3) Common Manzanita (*Arctostaphylos manzanita*), (4) Douglas Fir (*Pseudotsuga menziesii*), (5) Douglas Fir (*Pseudotsuga menziesii*), (6) Grey Pine (*Pinus sabiniana*), (7) Giant Chain Fern (*Woodwardia fimbriata*), (8) Buckbrush (*Ceanothus cuneatus*), (9) Western Redbud (*Cercis occidentalis*), (10) Grey Pine (*Pinus sabiniana*), (11) Giant Earthworm (*Argilophilini* sp.), (12) Oregon Oak (*Quercus garryana*), (13) Toyon (*Heteromeles arbutifolia*), (14) California Scrub Oak (*Quercus berberidifolia*), (15) Button's Banana Slug (*Ariolimax buttoni*), (16) California Slender Salamander (*Batrachoseps attenuatus*), and (17) Common Sharp-tailed Snake (*Contia tenuis*). Photos by Andre Giraldi.

with sequence data available for both *ND4* and *cyt b* were included in our analysis to minimise the effects of missing data. Sequences for each locus were aligned using the MUSCLE (Edgar, 2004) multiple alignment algorithm implemented in Geneious Prime v2025.2.2. The alignments for the two loci were then concatenated for phylogenetic analysis. The concatenated dataset contained 1120 bp (735 bp *ND4* and 385 bp *cyt b*). Data coverage for each sample used in our phylogenetic analysis can be found in Appendix 1. Maximum-likelihood phylogenetic analyses were conducted in IQ-TREE v1.6.12 (Nguyen et al., 2015; Minh et al., 2020). The concatenated dataset was partitioned by locus (*ND4* and *cyt b*), and the best-fit substitution models for each partition were selected using ModelFinder under the Bayesian Information Criterion in IQ-TREE. We analysed our dataset under a locus partitioning scheme, treating each gene as a separate partition. IQ-TREE identified TN+F+I+G4 as the best model for *ND4* and TPM2u+F+I+G4 for *cyt b*. Nodal support was assessed using 1000 ultrafast bootstrap replicates (UFBoot) and 1000 SH-aLRT tests. For *ND4* independently, nucleotide composition was calculated and corrected pairwise genetic distances were calculated in MEGA 12 (Kumar et al., 2018) using the Kimura 2-parameter (K2P) model (Kumar et al. 2018). For within-clade haplotype comparisons, sequences were trimmed to compare only gene regions obtained for the Elk Creek specimens.

Results and Discussion

Morphological identification. The Elk Creek specimens displayed phenotypes consistent with *A. flavipunctatus*. HSUVM:1152 and HSUVM:1153 measured approximately 30 mm SVL and were identified as juveniles, hence they displayed brassy greenish yellow colouration with fine white spotting consistent with juveniles of all members of the Black Salamander complex (Fig. 4) (Larson, 1980; Lynch, 1981; Reilly and Wake, 2019). HSUVM:1154 was identified as an older individual, measuring approximately 50 mm SVL, and displayed sparsely distributed, white iridophores, and a marked reduction in xanthophore pigmentation (Fig. 4), consistent with southern populations of *A. flavipunctatus* (Lynch, 1981; Reilly and Wake, 2019).

Molecular genetic identification. *ND4* and *cyt b* haplotypes were identical among the three Elk Creek specimens. Pairwise genetic distances (K2P) at the *ND4* locus between Elk Creek specimens and *A. flavipunctatus* specimens previously available on GenBank ranged

from 3.65% to 11.6%, with a mean of 4.93% ($n = 291$ comparisons). Within *A. flavipunctatus*, *ND4* pairwise K2P distances ranged from 0.14% to 12.64%, with a mean of 5.17% ($n = 9653$ comparisons), reflecting substantial mitochondrial divergence consistent with previously recognised deep phylogeographic structure (Rissler and Apodaca, 2007; Reilly and Wake, 2015, 2019). Genetic distances between Elk Creek specimens and other *A. flavipunctatus* (mean K2P \approx 5%) are within the range observed within the species (mean \approx 5.2%; maximum \approx 12.6%) (Rissler and Apodaca, 2007; Reilly and Wake, 2015, 2019).

Elk Creek specimens were recovered to be deeply nested within *A. flavipunctatus* with high support (ML bootstrap = 89%; SH-aLRT = 72.1%) (Fig. 5). The Elk Creek *Aneides* sp. are sister to a clade of diverse localities from northwestern California (Clade 1 in Fig. 5; Appendix 1), although statistical support for this relationship is low (ML bootstrap = 37%; SH-aLRT = 72.7%).

These results strongly support the allocation of Elk Creek specimens to *A. flavipunctatus*, but also suggest that the population represents a genetically structured lineage that is different from the ones previously reported in the literature.

Given the limited taxon sampling and exclusive reliance on mitochondrial loci, we interpret broader relationships within the Black Salamander complex cautiously. Although resolving these relationships is beyond the scope of the present contribution, we do note that we find *A. iecanus* as sister to the remaining members of the Black Salamander complex, with moderate to strong support (ML bootstrap = 86%; SH-aLRT = 76%), a topology that differs from several previous studies. Rissler and Apodaca (2007) inferred relationships within the complex using a concatenated mitochondrial dataset (*ND4* and *12S*), recovering *A. niger* as sister to the remaining taxa with high support. Their tree was rooted with *A. niger* based on unpublished phylogenetic analyses that included a broader taxa sampling (“including other species within the genus *Aneides* and other species within the families Plethodontidae, Ambystomatidae, and Salamandridae”; Rissler and Apodaca, 2007). Reilly and Wake (2015) obtained a similar topology to Rissler and Apodaca (2007) when analysing mitochondrial data and when constructing a mitonuclear concatenated tree, although in both cases, support for the placement of *A. niger* was low. In contrast, the nuclear-only phylogeny by Reilly and Wake (2015) presented *A. iecanus* as sister

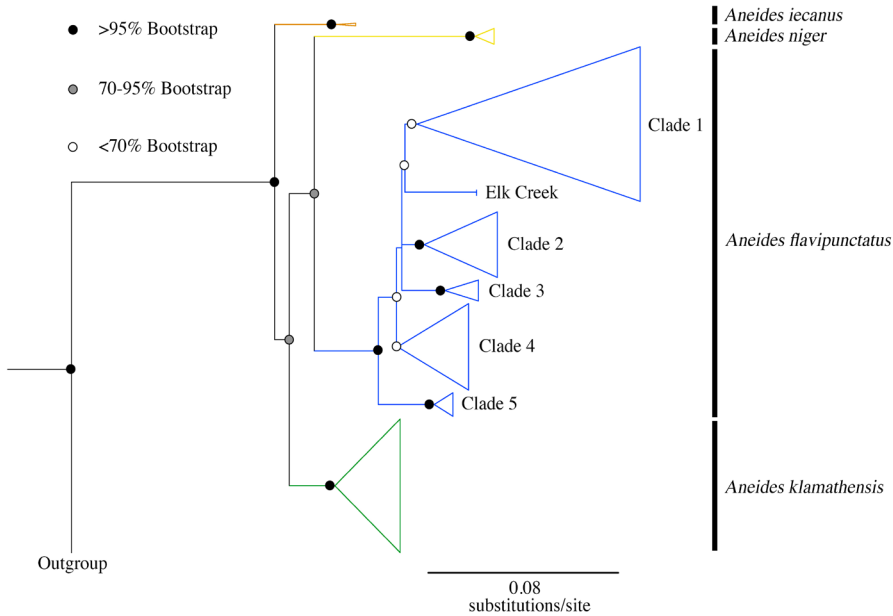


Figure 5. Maximum-likelihood phylogeny of Black Salamander species complex based on concatenated *ND4* and *cyt b* sequences. Node support values are shown as shaded dots representing three tiers of ultrafast bootstrap values. Recovered clades of *Aneides flavipunctatus* are highlighted in blue and numbered Clades 1–5 (see Appendix 1 for clade composition information).

to the rest of the complex, again with low support. Stewart and Wiens (2025) combined phylogenomic (both mitochondrial and nuclear data) and supermatrix approaches and found *A. flavipunctatus* as sister to the rest of the complex with high support, with *A. klamathensis* and *A. iecanus* as sister species. These differences in phylogenetic reconstruction of the Black Salamander complex across studies highlight the need for further research into this species complex to resolve its evolutionary relationships.

Conclusions. The newly collected Elk Creek specimens represent the rediscovery of a disjunct population of *Aneides* in Glenn County, California that has not been seen in more than 50 years, and was not included in the recent taxonomic revision of *Aneides flavipunctatus* by Reilly and Wake (2019). The nearest population of *A. flavipunctatus* molecularly identified to species by Reilly and Wake (2019) is located 73.31 km south of Elk Creek, at Holston Chimney Canyon, Colusa County, California (MVZ232877), and specimens assigned to *A. flavipunctatus* (*sensu stricto*) without published genetic information have been found 22.69 km south of Elk Creek at Fout’s Spring, Colusa County, California (MVZ124359). Hence our specimens represent a northeastern range extension for

A. flavipunctatus and fill an important gap in the known distribution of the genus.

Our findings confirm the presence of *A. flavipunctatus* in Glenn County, which has been the subject of inquiry for several years, yet there still exist major biogeographic questions in relation to the Black Salamander complex, especially in northwestern California. The most obvious of such questions relates to the identity of another geographically disjunct population of *Aneides* sp., in Tehama County, California. This population is only known from four specimens collected in 1972 by David Wake and Bruce Bury (MVZ:Herp:124344–47), and was not assigned to species by Reilly and Wake (2019). As in populations from Glenn County, this population occurs in oak woodlands in the North Coast Range foothills, in close geographic proximity to *A. flavipunctatus*, *A. klamathensis*, and *A. iecanus*, and could belong to any one of these species. Another geographically disjunct population may exist further east in Tehama County, where a single *Aneides* sp. was found along the banks of the Sacramento River in 2008, suggested to be *A. iecanus* but unconfirmed (Hansen and Shedd, 2025).

While our results allow us to confidently identify Elk Creek specimens as *A. flavipunctatus*, they are

not sufficient to make precise inferences on the evolutionary relationships between our specimens and other populations of *A. flavipunctatus*, which would require additional genetic data. Specific biogeographic questions, such as the age of the Elk Creek population and which populations they are closest related to, are also avenues that warrant further investigation.

Acknowledgments. The handling and processing of these specimens was approved by the Institutional Animal Care and Use Committee of California State Polytechnic University, Humboldt (protocol 2025B22-A) and specimens were collected under a California Department of Fish and Wildlife permit (S-230940001-23097-001). We thank Sean Reilly for advice and communication throughout this project. We thank Alyssa Semerdjian for museum accession of specimens. We thank Max Laubstein and Brandon Kong for meaningful discussions that helped guide this project. Funding for fieldwork was provided by a grant from the Humboldt Emeritus & Retired Faculty & Staff Association to PP.

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Appendix 1. Museum vouchers, and localities of *Aneides* data used in phylogenetic reconstruction. Clade assignments recovered from Fig. 5 are provided in the last column. “—” indicate two samples that have identical museum vouchers and sequence data.

Sample ID	Species group	State	Museum ID	Locality	Lat decimal	Long decimal	Clade #
PLVP3367	<i>flavipunctatus</i>	California	HSUVM1152	Glenn	39.5694	-122.6603	Elk Creek
PLVP3368	<i>flavipunctatus</i>	California	HSUVM1153	Glenn	39.5694	-122.6603	Elk Creek
PLVP3369	<i>flavipunctatus</i>	California	HSUVM1154	Glenn	39.5694	-122.6603	Elk Creek
Af_V	<i>flavipunctatus</i>	California	MVZ264011	Humboldt	40.2290	-123.8539	Clade 1
Af_2	<i>flavipunctatus</i>	California	MVZ269410	Humboldt	40.2362	-123.8340	Clade 1
Af_3	<i>flavipunctatus</i>	California	MVZ269396	Humboldt	40.1272	-123.8671	Clade 1
Af_4	<i>flavipunctatus</i>	California	MVZ263996	Humboldt	40.0452	-123.7843	Clade 4
Af_5	<i>flavipunctatus</i>	California	MVZ264050	Mendocino	39.9583	-123.7758	Clade 4
Af_7	<i>flavipunctatus</i>	California	MVZ269432	Mendocino	39.8291	-123.6517	Clade 4
Af_8	<i>flavipunctatus</i>	California	MVZ264054	Mendocino	39.3887	-123.4318	Clade 2
Af_13	<i>flavipunctatus</i>	California	MVZ264021	Mendocino	39.3839	-123.4740	Clade 2
Af_14	<i>flavipunctatus</i>	California	MVZ264022	Mendocino	39.3503	-123.5107	Clade 2
Af_15	<i>flavipunctatus</i>	California	MVZ264023	Mendocino	39.3972	-123.6979	Clade 2
Af_16	<i>flavipunctatus</i>	California	MVZ264024	Mendocino	39.3972	-123.6979	Clade 2
Af_17	<i>flavipunctatus</i>	California	MVZ264025	Mendocino	39.6688	-123.6681	Clade 4
Af_18	<i>flavipunctatus</i>	California	MVZ264026	Mendocino	39.4608	-123.3447	Clade 3
Af_19	<i>flavipunctatus</i>	California	MVZ264027	Mendocino	39.5194	-123.3863	Clade 2
Af_20	<i>flavipunctatus</i>	California	MVZ264028	Mendocino	39.5552	-123.4201	Clade 4
Af_21	<i>flavipunctatus</i>	California	MVZ264029	Mendocino	39.6172	-123.4625	Clade 3
Af_22	<i>flavipunctatus</i>	California	MVZ264030	Mendocino	39.6172	-123.4625	Clade 3
Af_23	<i>flavipunctatus</i>	California	MVZ264031	Mendocino	39.7680	-123.5439	Clade 4
Af_24	<i>flavipunctatus</i>	California	MVZ269434	Mendocino	39.7847	-123.5439	Clade 4
Af_25	<i>flavipunctatus</i>	California	MVZ264032	Mendocino	39.8197	-123.5641	Clade 4
Af_29	<i>flavipunctatus</i>	California	MVZ269414	Mendocino	39.9682	-123.7996	Clade 4
Af_30	<i>flavipunctatus</i>	California	MVZ264033	Mendocino	39.9412	-123.7797	Clade 4
Af_31	<i>flavipunctatus</i>	California	MVZ264034	Mendocino	39.8640	-123.7213	Clade 4
Af_32	<i>flavipunctatus</i>	California	MVZ264035	Mendocino	39.7680	-123.5439	Clade 4
Af_33	<i>flavipunctatus</i>	California	MVZ264036	Mendocino	39.6597	-123.5265	Clade 4
Af_34	<i>flavipunctatus</i>	California	MVZ264037	Mendocino	39.6597	-123.5265	Clade 4
Af_35	<i>flavipunctatus</i>	California	MVZ264038	Mendocino	39.7986	-123.5427	Clade 4
Af_36	<i>flavipunctatus</i>	California	MVZ269397	Humboldt	40.4119	-124.3931	Clade 1
Af_37	<i>flavipunctatus</i>	California	MVZ269401	Humboldt	40.4477	-124.0404	Clade 1
Af_38	<i>flavipunctatus</i>	California	MVZ264039	Mendocino	39.8560	-123.7299	Clade 4
Af_39	<i>flavipunctatus</i>	California	MVZ269426	Mendocino	39.8605	-123.7502	Clade 4
Af_40	<i>flavipunctatus</i>	California	MVZ264040	Mendocino	39.8061	-123.8041	Clade 4
Af_41	<i>flavipunctatus</i>	California	MVZ264041	Mendocino	39.4419	-123.7247	Clade 2
Af_42	<i>flavipunctatus</i>	California	MVZ264042	Mendocino	39.2835	-123.6647	Clade 2
Af_43	<i>flavipunctatus</i>	California	MVZ264043	Mendocino	39.2767	-123.6510	Clade 2
Af_44	<i>flavipunctatus</i>	California	MVZ264044	Mendocino	39.2767	-123.6510	Clade 2
Af_45	<i>flavipunctatus</i>	California	MVZ264045	Mendocino	39.2311	-123.4758	Clade 2
Af_46	<i>flavipunctatus</i>	California	MVZ264046	Mendocino	39.2470	-123.4154	Clade 2
Af_47	<i>flavipunctatus</i>	California	MVZ264047	Mendocino	39.2305	-123.3479	Clade 2
Af_48	<i>flavipunctatus</i>	California	MVZ264048	Mendocino	39.2093	-123.3046	Clade 2
Af_49	<i>flavipunctatus</i>	California	MVZ264049	Mendocino	39.5552	-123.4201	Clade 2
Af_50	<i>flavipunctatus</i>	California	MVZ264051	Mendocino	39.8297	-123.6132	Clade 4
Af_54	<i>flavipunctatus</i>	California	MVZ269394	Humboldt	40.1711	-123.6046	Clade 4

Appendix 1. Continued.

Sample ID	Species group	State	Museum ID	Locality	Lat decimal	Long decimal	Clade #
Af_55	<i>flavipunctatus</i>	California	MVZ269405	Humboldt	40.1736	-123.6024	Clade 4
Af_56	<i>flavipunctatus</i>	California	MVZ269406	Humboldt	40.1736	-123.6024	Clade 4
Af_57	<i>flavipunctatus</i>	California	MVZ269395	Humboldt	40.1983	-123.5923	Clade 4
Af_59	<i>flavipunctatus</i>	California	MVZ269419	Mendocino	39.8197	-123.5641	Clade 4
Af_62	<i>flavipunctatus</i>	California	MVZ269402	Humboldt	40.4477	-124.0404	Clade 1
Af_63	<i>flavipunctatus</i>	California	MVZ269415	Mendocino	39.0567	-123.2438	Clade 1
Af_64	<i>flavipunctatus</i>	California	MVZ269416	Mendocino	39.0567	-123.2438	Clade 1
Af_65	<i>flavipunctatus</i>	California	MVZ269417	Mendocino	39.0567	-123.2438	Clade 1
Af_66	<i>flavipunctatus</i>	California	MVZ269427	Mendocino	38.9060	-123.3260	Clade 1
Af_67	<i>flavipunctatus</i>	California	MVZ269472	Sonoma	38.6556	-123.3598	Clade 1
Af_68	<i>flavipunctatus</i>	California	MVZ269466	Sonoma	38.6630	-123.3301	Clade 1
Af_69	<i>flavipunctatus</i>	California	MVZ269467	Sonoma	38.6630	-123.3301	Clade 1
Af_70	<i>flavipunctatus</i>	California	MVZ269468	Sonoma	38.6630	-123.3301	Clade 1
Af_71	<i>flavipunctatus</i>	California	MVZ269469	Sonoma	38.6630	-123.3301	Clade 1
Af_72	<i>flavipunctatus</i>	California	MVZ269459	Sonoma	38.7424	-122.8326	Clade 1
Af_73	<i>flavipunctatus</i>	California	MVZ269460	Sonoma	38.7424	-122.8326	Clade 1
Af_74	<i>flavipunctatus</i>	California	MVZ269461	Sonoma	38.7424	-122.8326	Clade 1
Af_76	<i>flavipunctatus</i>	California	MVZ269403	Humboldt	40.4477	-124.0404	Clade 1
Af_77	<i>flavipunctatus</i>	California	MVZ264053	Mendocino	39.6172	-123.4625	Clade 4
Af_78	<i>flavipunctatus</i>	California	MVZ269449	Sonoma	38.8518	-123.0286	Clade 1
Af_79	<i>flavipunctatus</i>	California	MVZ269450	Sonoma	38.8518	-123.0286	Clade 1
Af_80	<i>flavipunctatus</i>	California	MVZ269420	Mendocino	39.8197	-123.5641	Clade 4
Af_82	<i>flavipunctatus</i>	California	MVZ269451	Sonoma	38.8518	-123.0286	Clade 1
Af_83	<i>flavipunctatus</i>	California	MVZ269452	Sonoma	38.8518	-123.0286	Clade 1
Af_84	<i>flavipunctatus</i>	California	MVZ264055	Mendocino	39.2612	-123.1126	Clade 2
Af_85	<i>flavipunctatus</i>	California	MVZ264056	Mendocino	39.2612	-123.1126	Clade 2
Af_86	<i>flavipunctatus</i>	California	MVZ264057	Mendocino	39.2612	-123.1126	Clade 2
Af_87	<i>flavipunctatus</i>	California	MVZ264058	Mendocino	39.2612	-123.1126	Clade 2
Af_88	<i>flavipunctatus</i>	California	MVZ264059	Mendocino	39.2612	-123.1126	Clade 2
Af_96	<i>flavipunctatus</i>	California	MVZ269457	Sonoma	38.7457	-122.8329	Clade 1
Af_97	<i>flavipunctatus</i>	California	MVZ269458	Sonoma	38.7457	-122.8329	Clade 1
Af_98	<i>flavipunctatus</i>	California	MVZ269418	Mendocino	39.0849	-123.4857	Clade 1
Af_99	<i>flavipunctatus</i>	California	MVZ269423	Mendocino	39.1201	-123.5997	Clade 1
Af_100	<i>flavipunctatus</i>	California	MVZ264019	Mendocino	39.6639	-123.3457	Clade 3
Af_101	<i>flavipunctatus</i>	California	MVZ264020	Mendocino	39.6054	-123.3821	Clade 3
Af_102	<i>flavipunctatus</i>	California	MVZ269433	Mendocino	39.7680	-123.5439	Clade 4
Af_116	<i>flavipunctatus</i>	California	MVZ269436	Napa	38.7071	-122.4624	Clade 5
Af_118	<i>flavipunctatus</i>	California	MVZ264002	Humboldt	40.0541	-123.9873	Clade 1
Af_119	<i>flavipunctatus</i>	California	MVZ264017	Lake	38.7107	-122.6288	Clade 5
Af_120	<i>flavipunctatus</i>	California	MVZ264014	Lake	38.7107	-122.6288	Clade 5
Af_124	<i>flavipunctatus</i>	California	MVZ263999	Humboldt	40.4477	-124.0404	Clade 1
Af_127	<i>flavipunctatus</i>	California	MVZ264067	Mendocino	38.9720	-123.0212	Clade 1
Af_128	<i>flavipunctatus</i>	California	MVZ264018	Lake	38.9937	-122.9538	Clade 1
Af_129	<i>flavipunctatus</i>	California	MVZ264005	Humboldt	40.2218	-123.7966	Clade 4
Af_130	<i>flavipunctatus</i>	California	MVZ264012	Humboldt	40.2046	-123.7840	Clade 4
Af_131	<i>flavipunctatus</i>	California	MVZ264013	Humboldt	40.1786	-123.7813	Clade 4

Appendix 1. Continued.

Sample ID	Species group	State	Museum ID	Locality	Lat decimal	Long decimal	Clade #
Af_132	<i>flavipunctatus</i>	California	MVZ263995	Humboldt	40.1563	-123.7956	Clade 4
Af_134	<i>flavipunctatus</i>	California	MVZ264068	Napa	38.5996	-122.2683	Clade 1
Af_135	<i>flavipunctatus</i>	California	MVZ264069	Napa	38.5996	-122.2683	Clade 1
Af_136	<i>flavipunctatus</i>	California	MVZ264070	Napa	38.5996	-122.2683	Clade 1
Af_137	<i>flavipunctatus</i>	California	MVZ264071	Napa	38.5996	-122.2683	Clade 1
Af_138	<i>flavipunctatus</i>	California	MVZ264072	Napa	38.5996	-122.2683	Clade 1
Af_147	<i>flavipunctatus</i>	California	MVZ264015	Lake	39.3886	-123.0443	Clade 2
Af_148	<i>flavipunctatus</i>	California	MVZ264016	Lake	39.3886	-123.0443	Clade 2
Af_139	<i>flavipunctatus</i>	California	MVZ264060	Mendocino	39.5194	-123.3863	Clade 2
Af_141	<i>flavipunctatus</i>	California	MVZ264061	Mendocino	39.6054	-123.3825	Clade 3
Af_142	<i>flavipunctatus</i>	California	MVZ264062	Mendocino	39.6337	-123.3433	Clade 3
SBR23	<i>flavipunctatus</i>	—	= Af_100	—	—	—	Clade 3
SBR24	<i>flavipunctatus</i>	—	= Af_100	—	—	—	Clade 3
Af_145	<i>flavipunctatus</i>	California	MVZ264065	Mendocino	39.2612	-123.1126	Clade 2
Af_146	<i>flavipunctatus</i>	California	MVZ264066	Mendocino	39.2612	-123.1126	Clade 2
SBR68	<i>flavipunctatus</i>	California	MVZ269437	Napa	38.6126	-122.5956	Clade 5
SBR69	<i>flavipunctatus</i>	California	MVZ269438	Napa	38.6126	-122.5956	Clade 5
SBR70	<i>flavipunctatus</i>	California	MVZ269439	Napa	38.6126	-122.5956	Clade 5
SBR71	<i>flavipunctatus</i>	California	MVZ269440	Napa	38.6126	-122.5956	Clade 5
SBR72	<i>flavipunctatus</i>	California	MVZ269441	Napa	38.6126	-122.5956	Clade 5
SBR73	<i>flavipunctatus</i>	California	MVZ269453	Sonoma	38.8487	-123.0763	Clade 1
SBR74	<i>flavipunctatus</i>	California	MVZ269411	Mendocino	38.9190	-123.2907	Clade 1
SBR75	<i>flavipunctatus</i>	California	MVZ269412	Mendocino	38.9190	-123.2907	Clade 1
SBR76	<i>flavipunctatus</i>	California	MVZ269413	Mendocino	38.9190	-123.2907	Clade 1
SBR78	<i>flavipunctatus</i>	California	MVZ269428	Mendocino	38.8926	-123.3620	Clade 1
SBR79	<i>flavipunctatus</i>	California	MVZ269429	Mendocino	38.8926	-123.3620	Clade 1
SBR80	<i>flavipunctatus</i>	California	MVZ269430	Mendocino	38.8926	-123.3620	Clade 1
SBR81	<i>flavipunctatus</i>	California	MVZ269431	Mendocino	38.8884	-123.3949	Clade 1
SBR82	<i>flavipunctatus</i>	California	MVZ269421	Mendocino	38.8881	-123.4089	Clade 1
SBR83	<i>flavipunctatus</i>	California	MVZ269422	Mendocino	38.8881	-123.4089	Clade 1
SBR84	<i>flavipunctatus</i>	California	MVZ269424	Mendocino	38.8686	-123.4228	Clade 1
SBR85	<i>flavipunctatus</i>	California	MVZ269425	Mendocino	38.8686	-123.4228	Clade 1
SBR86	<i>flavipunctatus</i>	California	MVZ269465	Sonoma	38.6521	-123.3653	Clade 1
SBR87	<i>flavipunctatus</i>	California	MVZ269473	Sonoma	38.6630	-123.3301	Clade 1
SBR88	<i>flavipunctatus</i>	California	MVZ269474	Sonoma	38.6630	-123.3301	Clade 1
SBR89	<i>flavipunctatus</i>	California	MVZ269454	Sonoma	38.6614	-123.2202	Clade 1
SBR90	<i>flavipunctatus</i>	California	MVZ269462	Sonoma	38.6704	-123.2114	Clade 1
SBR91	<i>flavipunctatus</i>	California	MVZ269463	Sonoma	38.6704	-123.2114	Clade 1
SBR92	<i>flavipunctatus</i>	California	MVZ269455	Sonoma	38.6867	-123.0132	Clade 1
SBR93	<i>flavipunctatus</i>	California	MVZ269456	Sonoma	38.6867	-123.0132	Clade 1
SBR100	<i>flavipunctatus</i>	California	MVZ269443	Napa	38.6288	-122.4886	Clade 5
SBR223	<i>flavipunctatus</i>	California	MVZ269464	Sonoma	38.4426	-122.9894	Clade 1
SBR224	<i>flavipunctatus</i>	California	MVZ269435	Napa	38.6144	-122.2876	Clade 1
SBR225	<i>flavipunctatus</i>	California	MVZ269442	Napa	38.6444	-122.5141	Clade 5
SBR226	<i>flavipunctatus</i>	California	MVZ269470	Sonoma	38.5455	-122.9810	Clade 1
SBR227	<i>flavipunctatus</i>	California	MVZ269471	Sonoma	38.5455	-122.9810	Clade 1

Appendix 1. Continued.

Sample ID	Species group	State	Museum ID	Locality	Lat decimal	Long decimal	Clade #
MVZ_137133	<i>flavipunctatus</i>	California	MVZ137133	Mendocino	39.2497	-123.1197	Clade 2
MVZ_219969	<i>flavipunctatus</i>	California	MVZ219969	Mendocino	39.8477	-123.7080	Clade 4
MVZ_238551_	<i>flavipunctatus</i>	California	MVZ238551	Mendocino	39.6855	-123.6581	Clade 4
Af_6	<i>flavipunctatus</i>	California	MVZ264052	Mendocino	39.8291	-123.6517	Clade 4
Af_117	<i>iecanus</i>	California	MVZ269358	Shasta	40.6782	-122.6345	
MVZ_145014_	<i>iecanus</i>	California	MVZ145014	Shasta	40.7535	-122.4593	
Af_9	<i>klamathensis</i>	California	MVZ269351	Humboldt	40.9979	-123.6266	
Af_10	<i>klamathensis</i>	California	MVZ269352	Humboldt	41.1403	-123.6814	
Af_11	<i>klamathensis</i>	California	MVZ269353	Humboldt	41.2282	-123.7714	
Af_12	<i>klamathensis</i>	California	MVZ269354	Humboldt	41.2473	-123.7720	
Af_26	<i>klamathensis</i>	California	MVZ269339	Del Norte	41.5221	-123.9971	
Af_27	<i>klamathensis</i>	California	MVZ269340	Del Norte	41.5221	-123.9971	
Af_28	<i>klamathensis</i>	California	MVZ269341	Del Norte	41.5249	-124.0084	
Af_51	<i>klamathensis</i>	California	MVZ269369	Trinity	40.8601	-123.5069	
Af_52	<i>klamathensis</i>	California	MVZ269370	Trinity	40.7901	-123.4539	
Af_53	<i>klamathensis</i>	California	MVZ269371	Trinity	40.7808	-123.1290	
Af_58	<i>klamathensis</i>	California	MVZ269409	Humboldt	40.3344	-123.6796	
Af_60	<i>klamathensis</i>	California	MVZ269407	Humboldt	40.7054	-123.8458	
Af_61	<i>klamathensis</i>	California	MVZ269408	Humboldt	40.7044	-123.8464	
Af_75	<i>klamathensis</i>	California	MVZ269342	Del Norte	41.5249	-124.0084	
Af_89	<i>klamathensis</i>	California	MVZ269343	Del Norte	41.7501	-123.9920	
Af_90	<i>klamathensis</i>	California	MVZ269344	Del Norte	41.7501	-123.9920	
Af_91	<i>klamathensis</i>	California	MVZ269345	Del Norte	41.7501	-123.9920	
Af_92	<i>klamathensis</i>	California	MVZ269346	Del Norte	41.7501	-123.9920	
Af_93	<i>klamathensis</i>	California	MVZ269348	Del Norte	41.7501	-123.9920	
Af_94	<i>klamathensis</i>	California	MVZ269347	Del Norte	41.7501	-123.9920	
Af_95	<i>klamathensis</i>	California	MVZ269349	Del Norte	41.7501	-123.9920	
Af_103	<i>klamathensis</i>	California	MVZ269355	Humboldt	40.9979	-123.6266	
Af_104	<i>klamathensis</i>	California	MVZ269356	Humboldt	41.2970	-123.5542	
Af_105	<i>klamathensis</i>	California	MVZ269357	Humboldt	41.2970	-123.5542	
Af_106	<i>klamathensis</i>	California	MVZ269359	Siskiyou	41.3811	-123.4886	
Af_107	<i>klamathensis</i>	California	MVZ269360	Siskiyou	41.3811	-123.4886	
Af_108	<i>klamathensis</i>	California	MVZ269361	Siskiyou	41.5407	-123.5258	
Af_109	<i>klamathensis</i>	California	MVZ269362	Siskiyou	41.7108	-123.4491	
Af_110	<i>klamathensis</i>	California	MVZ269363	Siskiyou	41.8172	-123.3061	
Af_111	<i>klamathensis</i>	California	MVZ269364	Siskiyou	41.8172	-123.3061	
Af_112	<i>klamathensis</i>	California	MVZ269365	Siskiyou	41.8270	-123.1390	
Af_113	<i>klamathensis</i>	California	MVZ269350	Del Norte	41.7499	-123.9907	
Af_114	<i>klamathensis</i>	California	MVZ269398	Humboldt	40.8340	-123.9849	
Af_115	<i>klamathensis</i>	California	MVZ269475	Trinity	40.4596	-123.5241	
SBR94	<i>klamathensis</i>	California	MVZ269399	Humboldt	40.4782	-123.9222	
SBR95	<i>klamathensis</i>	California	MVZ269400	Humboldt	40.4782	-123.9222	
A1	<i>klamathensis</i>	Oregon	MVZ269372	Jackson	42.1477	-122.6974	
C1	<i>klamathensis</i>	Oregon	MVZ269373	Jackson	42.0680	-123.1659	
E1	<i>klamathensis</i>	Oregon	MVZ269374	Jackson	42.1609	-122.6092	
E2	<i>klamathensis</i>	Oregon	MVZ269375	Jackson	42.1609	-122.6092	

Appendix 1. Continued.

Sample ID	Species group	State	Museum ID	Locality	Lat decimal	Long decimal	Clade #
H1	<i>klamathensis</i>	California	MVZ269366	Siskiyou	41.9967	-122.6087	
H2	<i>klamathensis</i>	California	MVZ269367	Siskiyou	41.9967	-122.6087	
H3	<i>klamathensis</i>	California	MVZ269368	Siskiyou	41.9967	-122.6087	
HG1	<i>klamathensis</i>	Oregon	MVZ269376	Jackson	42.0884	-123.0282	
HG2	<i>klamathensis</i>	Oregon	MVZ269377	Jackson	42.0884	-123.0282	
HG3	<i>klamathensis</i>	Oregon	MVZ269378	Jackson	42.0884	-123.0274	
HG4	<i>klamathensis</i>	Oregon	MVZ269379	Jackson	42.0884	-123.0274	
T2	<i>klamathensis</i>	Oregon	MVZ269380	Jackson	42.1546	-122.8836	
T3	<i>klamathensis</i>	Oregon	MVZ269381	Jackson	42.1543	-122.8837	
W1	<i>klamathensis</i>	Oregon	MVZ269382	Jackson	42.1346	-122.8356	
W2	<i>klamathensis</i>	Oregon	MVZ269383	Jackson	42.1346	-122.8356	
MVZ_219973	<i>klamathensis</i>	California	MVZ219973	Siskiyou	41.4336	-123.5052	
Af_1	<i>niger</i>	California	MVZ264073	Santa Cruz	36.9991	-122.0565	
Af_133	<i>niger</i>	California	MVZ264074	Santa Cruz	37.0455	-122.0929	
SBR97	<i>niger</i>	California	MVZ269447	Santa Cruz	36.9989	-122.0572	
SBR98	<i>niger</i>	California	MVZ269448	Santa Cruz	36.9989	-122.0572	
SBR99	<i>niger</i>	California	MVZ269446	Santa Clara	37.0860	-121.7927	
SBR101	<i>niger</i>	California	MVZ269444	Santa Clara	37.1827	-121.8719	
SBR102	<i>niger</i>	California	MVZ269445	Santa Clara	37.1827	-121.8719	
Anha_AY728226	<i>hardii</i>	New Mexico	MVZ226110	Otero	32.7972	-105.7921	
A.lugSBR220	<i>lugubris</i>	California	MVZ269481	Sonoma	38.4426	-122.9894	