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SYSTEMATICS OF THE *PSEUDOEURYCEA BELLII* (CAUDATA: PLETHODONTIDAE) SPECIES COMPLEX

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Abstract: Salamanders of the *Pseudoeurycea bellii* complex are widespread in México north and west of the Isthmus of Tehuantepec. They are the largest terrestrial plethodontids and have distinctive color patterns consisting of a general black ground color with strikingly bold red-orange marks in different patterns on the dorsal surface. At present three species are recognized: *P. bellii* (widespread, with two subspecies), *P. gigantea* (restricted to a small area of eastern México), and *P. naucampatepetl* (known only from the vicinity of the type locality in west-central Veracruz). A phylogenetic analysis based on two mitochondrial genes (cytochrome b and 16S RNA) finds support for three main clades, one including *P. gigantea* and *P. naucampatepetl*, another including the Oaxacan samples, and a third including *P. bellii*. New morphological data, DNA sequences, and limited allozymic information provide evidence that the Oaxacan clade comprises two species. The name *P. boneti* is resurrected from synonymy and applies to northern Oaxacan populations, while a new species is described from extreme western Oaxaca at elevations lower than have been recorded elsewhere for members of the complex.

Key words: México; mitochondrial DNA; Morphology; Phylogenetics; *Pseudoeurycea*; *Pseudoeurycea bellii* complex; Salamanders

Salamanders of the neotropical genus *Pseudoeurycea* have a generalized morphology (Wake, 1966; Wake and Elias, 1983) and the genus is taxonomically difficult. Tanner (1952) considered *Pseudoeurycea* to be the most plesiomorphic of the neotropical plethodontids, and a detailed osteological study (Wake, 1966) revealed that ancestral osteological features are common to most species of this genus. Phylogenetic relationships among species of the genus *Pseudoeurycea* are not fully resolved. Several hypothesized groups of closely related taxa within this genus have been proposed, but, despite the overall differences among the proposals, all have agreed that *P. bellii* is so distinct morphologically that, in the absence of clear affinities with other species, it was placed as the only member of its group (Baird, 1951; Taylor, 1944; Wake and Lynch, 1976). This hypothesis was further supported by Maxson and Wake (1981). A conclusion of their immunological study of albumin was that *P. bellii* is only remotely related to other species of *Pseudoeurycea*. Based on mtDNA sequences, Parra-Olea (2002) proposed that the closest relatives of *P. bellii* are *P. cephalica* and *P. galeanae*.

The *Pseudoeurycea bellii* species complex stands out from all other groups of *Pseudoeurycea* as a distinctive, easily diagnosable morphological entity (Wake and Elias, 1983). Members of this complex are stout animals, exclusively terrestrial, and with vivid and recognizable colorations consisting of a general black ground color, usually with two large red-orange spots on the occipital region and two series of red-orange chevron or half-chevron marks along the dorsum of the trunk. Variation in color pattern occurs within some populations, and some authors have suggested (without studying marked animals) that there may be an ontogenetic change in the pattern of the dorsal spots, with juveniles having fewer marks than adults from the same locality (Martin, 1958). In addition to intrapopulational variation, there are distinct geographically restricted color patterns that differ from the coloration of typical *P. bellii*. These differences in color pattern, together with external morphology, were used by earlier workers to split *P. bellii* into a complex of closely related species or subspecies.

For a long time the only member of the complex recognized was *P. bellii* (Gray, 1850)

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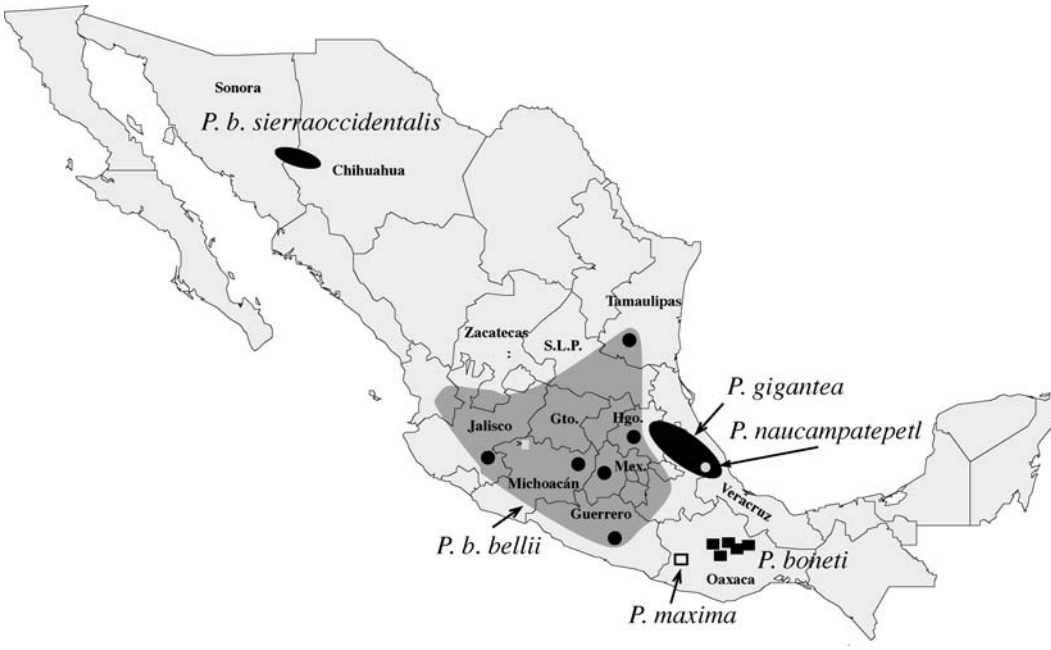

FIG. 1.—Distribution of the members of the *Pseudoeurycea bellii* complex in Mexico. Closed circles represent samples used in this study and shaded area is the range of *P. b. bellii*; closed squares are samples used of *P. boneti*; open square is the type locality and sample used of *P. maxima*; grey circle is the only known locality and sample used of *P. naucampatepetl* as well as the locality of the sample used of *P. gigantea*. The range of *P. gigantea* is shown as a black oval.

(type locality: "Mexico"). Currently, the complex includes three species (*P. bellii*, *P. gigantea*, and *P. naucampatepetl*) (Parra-Olea et al., 2001), one subdivided into two subspecies, *P. bellii bellii* and *P. bellii sierraoccidentalis* (Lowe et al., 1968). *Pseudoeurycea b. bellii* has the widest distribution of any member of the genus (Fig. 1), ranging in the Sierra Madre Occidental from Nayarit and Zacatecas in the northwest through the states of Jalisco and Michoacán, in the Sierra Madre Oriental (Guanajuato, Queretero, Hidalgo, San Luis Potosí, and Tamaulipas), the Sierra Madre del Sur (Guerrero), and along the Trans-Mexican Volcanic Belt (Distrito Federal, Puebla, México, Morelos, Tlaxcala) (Smith and Taylor, 1948). *Pseudoeurycea b. sierraoccidentalis* is known only from the border region of the northern states of Chihuahua and Sonora (Lowe et al., 1968; Tanner, 1989). An old report of the species in Arizona (Dunn, 1926) remains unconfirmed (Lowe et al., 1968). The two subspecies are separated by a large distribution gap (about 1000 km). *Pseudoeurycea gigantea* has a restricted distribution in the Sierra Madre Oriental of Hidalgo, northern Puebla and Veracruz (Smith and Taylor, 1948), and *P. naucampatepetl* is known only from a small area near Cofre de Perote, Veracruz (Parra-Olea et al., 2001). *Pseudoeurycea boneti*, from the Sierra Madre del Sur in the state of Oaxaca, was described by Alvarez and Martin (1967) as a separate species based on general morphology and coloration. This taxon was later synonymized with *P. bellii* by Bogert (1967), but, based on the present analyses, we consider it to be a valid species; consequently, it is treated as such herein.

Members of the *P. bellii* complex are unique among plethodontids in several respects. Compared to all other fully terrestrial plethodontids, they are gigantic, reaching up to 165 mm in snout–vent length (Taylor, 1939). *Pseudoeurycea bellii* not only has one of the widest distributional ranges for a plethodontid salamander, but furthermore, as currently constituted, it lives between the broadest elevational limits of any salamander in the world (750–3300 m) (Feder et al., 1982), occupying a wide variety of habitats including

TABLE 1.—Localities, voucher information, and Genbank accession numbers for the sequences and specimens used in this study. Abbreviations GP: G. Parra field number; MVZ: Museum of Vertebrate Zoology collection.

Sample name	Locality	Voucher	Genbank accession number	
			16S	cyt b
1 *P. bellii bellii*	Guerrero: 72 km NE Atoyac	MVZ143783	AY864704	—
2 *P. bellii bellii*	Guerrero: 72 km NE Atoyac	MVZ143784	AY864703	AY864689
3 *P. bellii bellii*	Hidalgo: 1.3 km SW Durango	MVZ158711	AY864702	—
4 *P. bellii bellii*	Hidalgo: 11.3 km S Durango	MVZ158712	AY864701	—
5 *P. bellii bellii*	Jalisco: 22 km W Ciudad Guzmán	MVZ143795	AY864700	AY864691
6 *P. bellii bellii*	Jalisco: 22 km W Ciudad Guzmán	MVZ143794	AY864699	AY864690
7 *P. bellii bellii*	México: 4.4 km N Raices	MVZ143803	AY864698	AY864687
8 *P. bellii bellii*	México: 4.4 km N Raices	GP254	—	AY864686
9 *P. bellii bellii*	México: Valle de Bravo	MVZ197733	AY864697	—
10 *P. bellii bellii*	Michoacán: Parque Nacional Morelos	GP048	AF451214	AF451194
11 *P. bellii bellii*	Tamaulipas: Gómez Farias	MVZ173433	AY864696	AY864692
12 *P. maxima*	Oaxaca: 6 km S Putla	MVZ194327	AY864693	—
13 *P. maxima*	Oaxaca: 19.5 km NE Putla	MVZ146785	AY862154	AY864684
14 *P. boneti*	Oaxaca: Cerro Zempoaltepetl	MVZ163873	AY864714	—
15 *P. boneti*	Oaxaca: 27.2 km W Zaachila	MVZ137863	AY864713	—
16 *P. boneti*	Oaxaca: 27.2 km W Zaachila	MVZ137861	AY864708	—
17 *P. boneti*	Oaxaca: 1 km E Ayutla	MVZ158714	AY864710	—
18 *P. boneti*	Oaxaca: 5 km W Cerro Machín	MVZ137857	AY864712	AY864688
19 *P. boneti*	Oaxaca: 5 km W Cerro Machín	MVZ137858	AY864711	—
20 *P. boneti*	Oaxaca: Sierra de Monteflor	e43-20	AY864709	—
21 *P. gigantea*	Veracruz: La Joya	GP177	AY451219	AF451198
22 *P. gigantea*	Veracruz: La Joya	MVZ158717	AY864707	—
23 *P. gigantea*	Veracruz: La Joya	MVZ196076	AY864706	—
24 *P. gigantea*	Veracruz: 6 km ENE Chiconquiaco	GP168	AY864705	AY864685
25 *P. naucampatepetl*	Veracruz: Cerro Las Lajas, Las Vigas	MVZ173436	—	AY864683
26 *P. naucampatepetl*	Veracruz: Cerro Las Lajas, Las Vigas	MVZ173435	—	AY864682
27 *P. bellii sierraoccidentalis*	Chihuahua: Ocampo	GP1029	AY864695	AY864680
27 *P. bellii sierraoccidentalis*	Chihuahua: Ocampo	GP1030	AY864694	AY864681

tropical semi-deciduous forests (cafetal and banana plantations), cloud forests, pine and oak forests to high elevation fir forests (but see below). This unusually broad ecological scope (especially for a tropical salamander) suggests that cryptic species may be present. Adults have been found in dry conditions in holes in road banks, whereas subadults and juveniles are usually found under rocks, rotten logs, or other relatively large surface debris.

The present study analyzes phylogenetic structure among taxa and populations of the *P. bellii* species complex, including samples of all taxa of the complex. We used mtDNA sequences from two genes, the 16S ribosomal subunit (16S), and the cytochrome b (cyt b) gene. The goal is to understand the phylogenetic relationships among the populations of *P. bellii* and allied forms (*P. boneti*, *P. gigantea*, *P. naucampatepetl*) and to propose a phylogenetic hypothesis for the members of the group as a whole. Analyses of data gathered require a taxonomic revision of the taxa included in the Oaxacan clade, in which we resurrect one species and describe a distinctive population from southern Oaxaca as a new species.

MATERIALS AND METHODS

Mitochondrial DNA

This study includes specimens from 19 populations of the *P. bellii* species complex and covers most of its distribution, including representatives of all of the named taxa. Collection localities and museum collection numbers are shown in Table 1.

Samples used in this study include 25 sequences of the large 16S subunit ribosomal mtDNA gene for the following taxa: *P. bellii bellii* (11 samples, 8 populations), *P. bellii sierraoccientalis* (2 samples, 1 population) *P. boneti* (7 samples, 5 populations), *P. gigantea* (4 samples, 2 populations), *P. maxima* sp. nov. (2 samples, 1 population); and a total of 15

sequences of cytochrome b (cyt b) for: *P. bellii bellii* (7 samples, 5 populations), *P. bellii sierraoccidentalis* (2 samples, 1 population), *P. boneti* (1 sample), *P. gigantea* (2 samples, 2 populations), *P. naucampatepetl* (2 samples), *P. maxima* sp. nov. (1 sample) (Table 1). Sequences of one sample of *P. bellii* were published previously (GenBank 451214, 451194) (Parra-Olea, 2002). Published sequences for *P. cephalica* and *P. galeanae* (GenBank AF451196, AF451217, AF451197, AF451218) (Parra-Olea, 2002) and sequences of the related genera *Thorius* and *Batrachoseps* were used as sequential outgroups.

Amplification and sequencing.—Tissues for this study were obtained mostly from the frozen tissue collection of the Museum of Vertebrate Zoology, University of California, Berkeley. Most samples are extracts of ground tissues prepared for protein electrophoresis. Some important samples were obtained from recent field collections.

Whole genomic DNA was extracted from small amounts of frozen or ethanol-preserved tissues or protein extracts using NaCl following a protocol modified from Miller et al. (1988). Overall, we sequenced 554 base pairs of 16S corresponding to positions 2510–3059 in the human mitochondrial genome (Anderson et al., 1981) and 350–570 base pairs of cytb, beginning with codon 7 of the *Xenopus* cyt b gene (Roe et al., 1985). These genes were selected in order to recover maximum phylogenetic information both at the terminal nodes and at the base of the tree. Amplification was done via the polymerase chain reaction (PCR) (Saiki et al., 1988), using the primers "MVZ15" "MVZ18" (Moritz et al., 1992), and "cyt b2" (Kocher et al., 1989) for cyt b, the primers "16Sar" and "16Sbr" (Palumbi et al., 1991) for 16S. The PCR reactions consisted of 38 cycles with a denaturing temperature of 92 C (1 min), annealing at 48–50 C (1 min), and extension at 72 C (1 min) in a Techne PHC-1 thermocycler. The PCR reactions were run in a total volume of 25 μl, using 0.5 pmol of each primer.

Double strand templates were cleaned using a QIAquick PCR purification kit (QIAgen). As a template for cycle sequencing reactions, 5.5 μl of PCR product was used in a 10 μl total volume with the Perkin-Elmer Ready Reaction Kit to incorporate dye-labeled dideoxy terminators. Thermal cycling was performed using standard conditions. Cycle sequencing products were purified using ethanol precipitation and separated on a 6% polyacrylamide gel using an ABI 377 DNA sequencer (Applied Biosystems).

Sequence alignment and analyses.—All sequences were compiled using Sequence Navigator™ version 1.0.1 (Applied Biosystems). The 16S sequences were aligned using Clustal X (Aladdin Systems, Inc., Heidelberg, Germany) with default gap costs and then refined manually by comparing them to published secondary structure models for 16S (Ortí and Meyer, 1997).

Pairwise comparisons of observed proportional sequence divergence (p-distance) and corrected sequence divergence (Kimura 2-parameter; Kimura, 1980), and number of transitions and transversion were obtained using the computer program PAUP*4.0b10 (Swofford, 2002). Corrected sequence divergences were estimated using the Kimura 2-parameter distance (K2p) in order to correct for multiple hits.

To test for the possibility that some types of nucleotide substitutions have become saturated, we plotted p-distance (y) versus corrected (K2p) estimates of proportional sequence divergence (x) for first, second, and third codon positions and for transitions and transversions separately.

Phylogenetic analysis.—Partial sequences of the cyt b and 16S genes were initially analyzed separately because we were unable to obtain sequences of both genes for all samples due to the poor condition of some of the old tissues. A smaller combined data set was also analyzed. We used ModelTest 3.06 (Posada and Crandall, 1998) to find the model of evolution that best fits the data for subsequent maximum likelihood and Bayesian analyses (ML: Felsenstein, 1981; Bayesian: Huelsenbeck and Ronquist, 2001). The GTR+G+I model of evolution with gamma parameter and proportion of invariable positions was used for ML and Bayesian analyses (Gu et al., 1995; Swofford et al., 1996; Yang, 1994). The best fit model likelihood settings were selected by Akaike Information Criterion. The ML analyses with empirical base frequencies were performed using PAUP*. We used nonparametric bootstrapping (bs, 1000 pseudoreplicates) to

assess the stability of internal nodes in the resulting topologies (Felsenstein, 1985).

Unpartitioned Bayesian phylogenetic analyses were conducted with MrBayes 2.0 (Huelsenbeck and Ronquist, 2001). Analyses were initiated with random starting trees and run for 500,000 generations. The Markov chains were sampled each 100 generations. The analysis showed that stationarity was reached after 25,000 generations for 16S and 20,000 for cytb, therefore of the resulting 5000 trees, 250 and 200 trees were discarded as "burn in" respectively.

Maximum parsimony phylogenies were estimated using the heuristic search algorithm (MP; Swofford, 2002). Input order of taxa was randomized 20 separate times to minimize the effect of sequence order on the resulting cladogram topology. The MP analyses were conducted without the steepest descent option and with accelerated character transformation (ACCTRAN) optimization, tree bisection-reconnection (TBR) branch swapping, and zero-length branches collapsed to yield polytomies. We used nonparametric bootstrapping (bs, 1000 pseudoreplicates) and decay indices (d) to assess the stability of internal nodes in the resulting topologies (Bremer, 1994; Felsenstein, 1985; Felsenstein and Kishino, 1993). Nonparametric bootstrap values and decay indices generally are a conservative measure of the probability that a recovered group represents a true clade (Hillis and Bull, 1993; Li, 1997; Zharkikh and Li, 1992). Each base position was treated as an unordered character with four alternate states.

Allozymes

Data from 17 protein loci are available from an incomplete study of allozymic variation by Gloria Wurst and D. B. Wake. The study was left unfinished because additional specimens were desired that were not forthcoming. Included in the study were six population samples: *P. gigantea*, from La Joya, Veracruz, $n = 16$; *P. bellii bellii*, from south of Toluca, México, $n = 8$; *P. bellii bellii*, from 72 km NE Atoyac, Guerrero, $n = 1$; *P. boneti*, from Cerro Machín, Sierra de Juárez, Oaxaca, $n = 2$; *P. boneti*, from Sierra de Cuatro Venados, Oaxaca, $n = 6$; *P. maxima* sp. nov., from Putla, Oaxaca, $n = 1$. Methods were presented in Lynch and Wake (1989). Enzyme systems assayed were: S-Mdh (cystolic) (Enzyme Commission number 1.1.1.37), M-Mdh (mitochondrial) (1.1.1.37), Lap (3.4.11.1), Pep-C (3.4.13.11), S-Aat (1.1.3.15), M-Aat (1.1.3.15), Ldh-1 (1.1.1.27), Ldh-2 (1.1.1.27), Mpi (5.3.1.8), Iddh-1 (1.1.1.14), Iddh-2 (1.1.1.14), Gpi (5.3.1.9), Pgm-1 (5.4.2.2), Pgm-2 (5.4.2.2). Results are summarized as genetic distances (Nei, 1972) because of the substantial volume of such genetic distances that exists for salamanders (Table 2).

TABLE 2.—Nei genetic distance based on 17 loci for six samples of the *Pseudoeurycea bellii* complex.

Species (locality)	Sample size	2	3	4	5	6
1. *P. gigantea* (La Joya)	16	0.24	0.44	0.52	0.60	0.56
2. *P. b. bellii* (Raices)	7		0.50	0.50	0.79	0.95
3. *P. b. bellii* (Guerrero)	1			1.13	0.71	1.08
4. *P. boneti* (Cerro Machin)	2				0.18	0.50
5. *P. boneti* (Cuatro Venados)	6					0.65
6. *P. maxima* (Putla)	1					

Species Description

Description of the new species follows previous taxonomic studies and includes the same basic characters and measurements (Lynch and Wake, 1989). Larger measurements were taken by using dial calipers (accurate to the nearest 0.1 mm). Smaller measurements of feet, toes, and some head dimensions, especially those involving the holotype, were taken by using a stereomicroscope equipped with an eyepiece reticle. All measurements are expressed in mm. Standard length (SL) equals the distance from the tip of the snout to the posterior end of the vent. Limb interval equals the number of costal interspaces between the tips of appressed fore- and hind limbs, measured in one-half increments. A stereomicroscope was used to count teeth. Numbers of maxillary and vomerine teeth are summed for both sides of the head. Color notes are based on living specimens, recorded in the field, and on preserved specimens. Institutional abbreviations are as listed in Leviton et al. (1985).

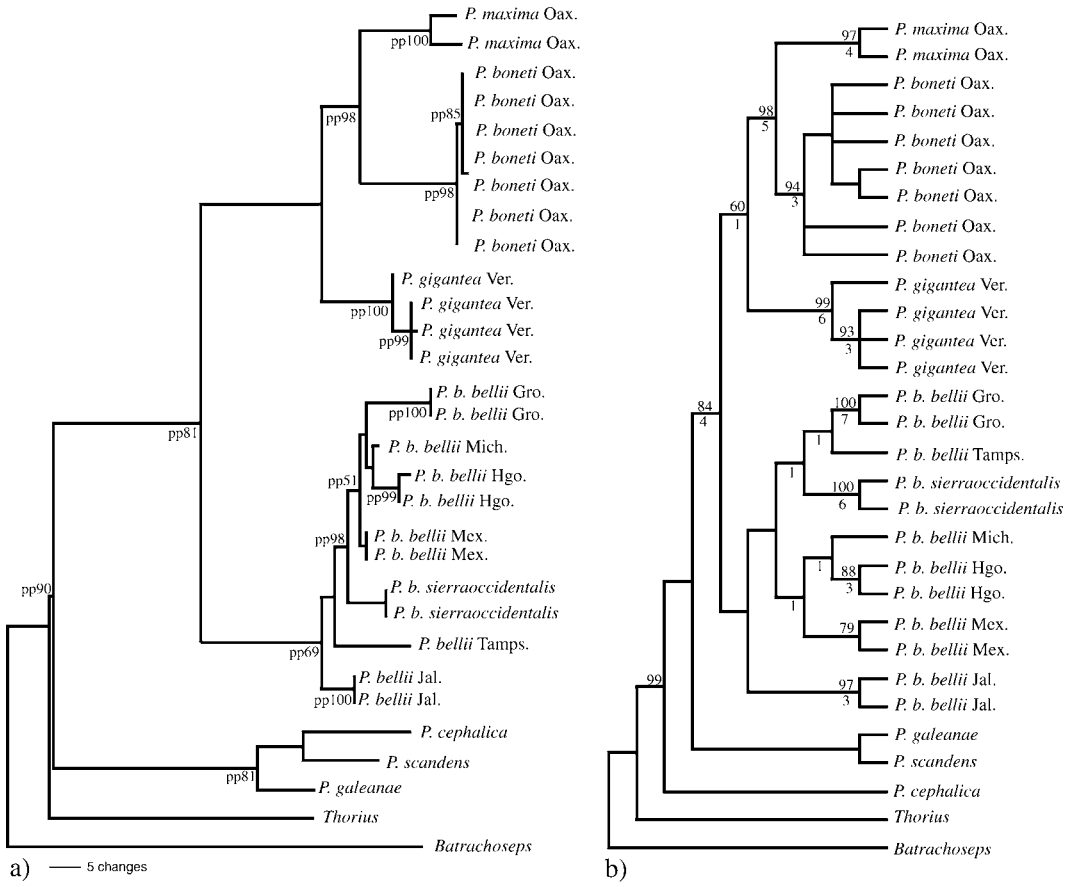

FIG. 2.—16S data set: a) maximum likelihood analysis topology –*ln* L = 2019.020 obtained using the GTR + G + I model; numbers represent posterior probabilites obtained by Bayesian analysis. b) Single most parsimonious tree (L = 274 steps; CI = 0.734; RI = 0.799). Numbers above branches are non-parametric bootstrap values (1000 pseudoreplicates, only values greater than 50% are shown). Numbers below branches correspond to decay index values.

RESULTS

Phylogenetic Relationships of the P. bellii *Complex*

16S RNA.—Twenty-five sequences of a 536 bp fragment of the 16S gene were analyzed; 158 characters were variable, and 87 of these characters were phylogenetically informative. Sequence divergence within the ingroup was as high as 5.8% (K2-p) between *P. boneti* and *P. b. bellii* from Guerrero. Within a single taxon, the highest divergence was between two populations of *P. b. bellii* (3.9% K2-p; sample from Tamaulipas versus sample from Guerrero). Sequence divergence between samples of *P. b. bellii* and *P. b. sierraoccidentalis* ranged from 2.1% to 3.8% K2-p.

The topology obtained in the ML tree (–ln *L* = 2060.72) of the 16S data set shows three main clades within a monophyletic *P. bellii* complex (Fig. 2a). The first clade includes all of the samples of *P. b. bellii* and *P. b. sierraoccidentalis* (pp69). The westernmost population in the Sierra Madre Occidental of Jalisco is the sister taxon to all others, although there is weak statistical support for the clade as a whole or for structure within it. The samples of *P. bellii sierraoccidentalis* are nested within the *P. bellii bellii* samples, and are sister to a subclade formed by samples from Guerrero, Michoácan, Hidalgo and México (pp98). Basal to this clade is the sample from Tamaulipas. The second clade contains the samples of

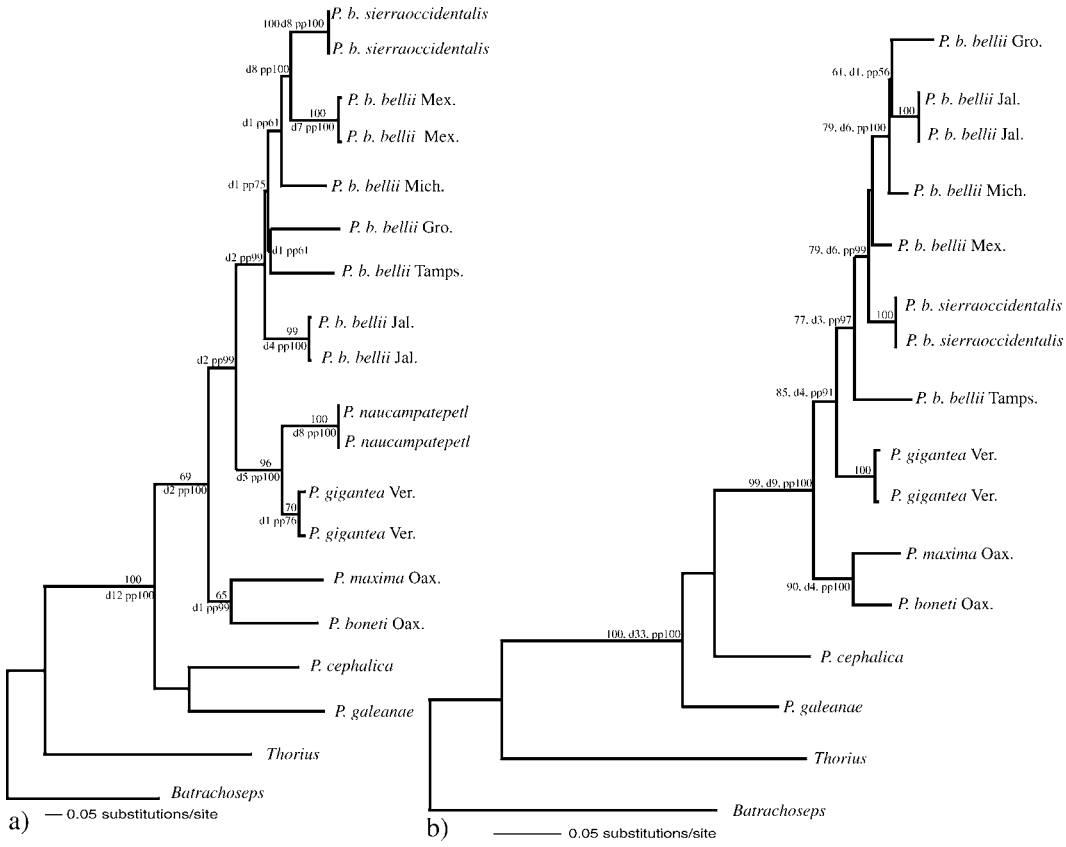

FIG. 3.—a) Cyt b data set. Maximum likelihood analysis topology –*ln* L = 1961.146 obtained using the GTR + G + I model of the cyt b data set. Numbers correspond to bootstrap, decay (d) and posterior probabilities (pp) values. b) Combined data set. Maximum likelihood analysis topology –*ln* L = 4635.119 obtained using the GTR + G + I model of the cyt b data set. Numbers correspond to bootstrap, decay (d) and posterior probabilities (pp) values.

P. gigantea (pp100). The third clade is formed by the populations from Oaxaca (pp98), and there is support for two subclades, one containing all of the samples of *P. boneti* from eastern and northern Oaxaca (pp98) and the second formed by the populations from western Oaxaca (pp100). In this analysis, *P. gigantea* is sister to the Oaxacan clade and these two taxa are sister to *P. bellii*. Bayesian analyses (not shown, but pp shown on ML tree Fig. 2a) resulted in a tree that is very similar to the ML tree, except that the three main clades form a polytomy at the base.

Parsimony analysis produced a single most parsimonious tree (L = 274 steps; CI = 0.734; RI = 0.799). All samples of the *P. bellii* complex form a monophyletic group (d4, bs 84). The topology (Fig. 2b) shows support for two clades: the *P. gigantea* clade (d6, bs99), and the Oaxacan clade (decay 5, bs 98), with two subclades. The first of the subclades includes samples from northern and eastern Oaxaca (d3, bs94) and the second contains samples from western Oaxaca (d4, bs97). Samples of *P. b. bellii* and *P. b. sierraoccidentalis* form a clade in parsimony analysis but it lacks support. In this analysis, *P. gigantea* is sister to the Oaxacan clade (decay 1, bs 60).

Cyt b.—Sequences of cyt b were obtained for 15 samples from 11 populations of the *P. bellii* complex. This analysis differs from that of the 16S data in that it includes sequences of *P. naucampatepetl*, not available for 16S. Sequence divergence among the ingroup was as high as 14.78% (K2-p) between *P. b. sierraoccidentalis* and *P. boneti*. The smallest sequence divergence between species was 4.9% K2-p between *P. gigantea* and *P.*

naucampatepetl. Within species, there was up to 8.9 K2-p between *P. bellii bellii* from Tamaulipas and *P. bellii bellii* from Mexico.

In the ML analysis of the cyt b data set, the tree obtained (–ln L = 2465.101) shows three clades (Fig. 3a). The *Pseudoeurycea gigantea* clade is now formed by *P. gigantea* and *P. naucampatepetl* as sister taxa. All of the populations of *P. b. bellii* plus *P. b. sierraoccidentalis* form a clade. The Oaxacan samples also form a clade. In this analysis, a *P. bellii bellii*+*P. bellii sierraoccidentalis* clade is sister to *P. gigantea* plus *P. naucampatepetl*, and this combined clade is sister to the Oaxacan clade. Parsimony analysis (not shown) of the cyt b data set produced six equally parsimonious trees (TL = 345 steps; 113 characters were parsimony informative; CI = 0.603; RI = 0.619) with similar topology as in our ML analysis, but there is a bootstrap support higher than 50% only for the clades formed by *P. gigantea* and *P. naucampatepetl* (bs 96%), and the Oaxacan clade (bs 65%). The relationships among the components of the *P. bellii* complex are not well resolved, and most of the samples form a basal polytomy.

Combined data set.—The combined data set included 12 samples of the *P. bellii* complex. The ML analysis (–ln L = 4635.119) produced a topology (Fig. 3b) with the same basic structure as other analyses. Samples of *P. bellii bellii* and *P. bellii sierraoccidentalis* form a clade (bs77, d3, pp97), and *P. gigantea* is their sister (bs85, d4, pp91); the Oaxacan clade is the most basal (bs99, d9, pp100). Bayesian analysis (not shown but pp values for clades are indicated on Fig. 3b) is identical to the ML topology. Parsimony analysis of the combined data set produced two equally parsimonious trees (TL = 710 steps; 234 characters were parsimony informative; CI = 0.693; RI = 0.611). The consensus tree (not shown) shows the three clades obtained in the ML analysis and in the separate analysis of the 16S and Cytb data sets, but bootstrap support is slightly higher.

Allozymes

Only an overview of the incomplete allozyme study is presented. Of 17 allozymic loci examined, 14 were variable. The matrix of genetic distance (D_N, Nei, 1972) (Table 2) shows that divergence levels are high. The Oaxacan populations all display large genetic distances ($D_N > 0.50$) from remaining samples. While the two samples of *P. boneti* are relatively little differentiated ($D_N = 0.18$), the large D_N between the Putla sample and the other species is noteworthy. The smallest values of D_N for the Putla sample to others, 0.50 and 0.55, are to *P. boneti*, its closest geographic neighbor. In comparisons with the two large samples, Putla has different alleles than both *P. gigantea* and *P. boneti* (Cuatro Venados) at five loci. We conclude from these preliminary results that allozymic differentiation within the *P. bellii* complex may be great, and that the Oaxacan samples stand out in this respect. The Guerrero population of *P. b. bellii*, although represented here by only a single specimen, is a candidate species based on the large genetic distance from the Raíces sample of the same taxon.

Discussion

The *P. bellii* species complex is geographically structured at both genetic and morphological levels, showing a larger diversity than previously reported, with several taxa included within a common general morphological pattern. Our finding of significant diversification contrasts with previous ideas of a widespread, ubiquitous, single taxonomic unit represented by a relative homogeneous *P. bellii*. Instead, our investigations revealed a complicated geographic pattern of differentiated taxa having unknown interactions along contact zones, in need of further study.

Based on mtDNA sequences, phylogenetic relationships among the components of the *P. bellii* complex are only moderately resolved. In all analyses, there is strong support for the monophyly of the complex (see also Parra-Olea, 2002), and the samples consistently cluster in three clades that correspond to taxonomic entities correlated with the geographic distribution of the populations sampled. *Pseudoeurycea gigantea* and *P. naucampatepetl*, both with small geographic ranges, are always sister taxa. These species have a relatively large cyt b sequence divergence (K2-p = 4–4.9%), which is particularly relevant considering that these species occur within <5 km of each other in similar microhabitats, but at different elevations. *Pseudoeurycea gigantea* has been collected at about 1000–2000 m, while *P. naucampatepetl* has been found only on Cerro

Las Lajas on the slopes of Cofre de Perote, at elevations above 2500 m (Parra-Olea et al., 2001). *Pseudoeurycea naucampatepetl* shows occipital spots, but it has a distinctive triangular shoulder spot, a broad U-shaped spot complex posterior to the hind limb insertion, and has pale pinkish as opposed to dark red or orange coloration. *Pseudoeurycea gigantea* differs from *P. bellii* in external body proportions and is characterized by the absence of the large red spots in the occipital region, although they are occasionally present. The species are not well differentiated in either morphology or ecology, although *P. gigantea* occurs in wetter forests and cloud forests whereas most populations of *P. bellii* occur at higher elevations, often in drier regions. Relationships among populations of *P. b. bellii* are not fully resolved, and in most analysis support for clades is low. Parsimony analyses yields a polytomy, but in the ML analysis of 16S and cyt b, and in the combined analysis, all of the populations form a clade (Figs. 2a, 3a and 3b). Lowe et al. (1968) described the northern populations of *P. bellii* in Sonora as a subspecies (*P. b. sierraoccidentalis*) that differed from *P. b. bellii* in having a much reduced number, and highly irregular arrangement, of dorsal marks. The color pattern gives the impression of being but a fragment of the pattern in *P. b. bellii*. When we collected *P. b. sierraoccidentalis* (Ocampo, Chihuahua, México, 2 September 2003) we were struck with the nontropical nature of the habitat and the distinctive appearance of the animals relative to other members of the *bellii* complex. Although samples of this taxon are differentiated from other members of the complex in mtDNA (K2-p of 2–3% in 16S, and 5–10% in cyt b to other *P. bellii*), they do not form an independent clade but are clustered within *P. bellii*. Accordingly, *P. b. sierraoccidentalis* does not appear to be a genealogical entity. We favor retaining the subspecific designation at present because the taxon is distinctive in coloration and habitat and is very remote geographically in relation to the rest of the complex. Allozymes of this taxon have not been studied as yet, and the mtDNA may not give a complete picture of the situation.

Populations from Oaxaca are all included in a single monophyletic unit well differentiated from all other representatives of the *P. bellii* species group. Our samples from Cerro Zempoaltepetl, Zaachila, Ayutla, Cerro Machín and Sierra de Monteflor (Fig. 1), all form a monophyletic group, share a common morphology and a similar allozyme pattern, and correspond to specimens that closely match the diagnosis of *P. boneti*. The samples from Putla, differ from them in mtDNA, allozymes, and morphology. Based on the degree of morphological and genetic differentiation and its diagnosability, we consider the Oaxacan clade to be represented by two species-level taxa, which are discussed below.

The description of *P. boneti* was based on specimens from Cerro San Felipe and Cerro Zempoaltepetl in central Oaxaca (Alvarez and Martín, 1967) (Fig. 1), which differ from *P. bellii*, *P. gigantea*, and *P. naucampatepetl* in that the dorsal marks do not form two independent rows, but instead are fused to form a series of chevrons that often are connected to one another. Typically, a large pair of scapular spots is present lateral to the row of chevrons, often connected to one or two chevrons. The result is that *P. boneti* has the greatest amount of reddish pigmentation of any of the taxa. Samples from near Putla in western Oaxaca do not show the typical *P. boneti* pattern. Putla specimens have reduced reddish coloration and lack chevron-like marks and scapular spots. They more closely resemble typical *P. bellii*, but with less color, having two rows of dot-like marks along the dorsum with little color and no conjoined spot in the occipital region.

A color pattern similar to that of *P. boneti* has been found in populations in northern México, in the state of Tamaulipas (Martín, 1958). Adults from this population have the spots on the dorsum that form chevrons, just like the color of *P. boneti*, but, according to the description by Martín (1958), there are some morphological differences (especially limb length) between the population from Tamaulipas and the rest of the *P. bellii* complex (including *P. boneti*). In contrast to the variation in coloration within the Tamaulipan population, all the individuals of different sizes examined by Alvarez and Martín (1967) and representing *P. boneti* have the chevron-like dorsal pattern. Alvarez and Martín (1967) suggested the possibility of the presence of two species in Tamaulipas, *P. bellii* (the individuals with the typical pattern) and an

undescribed form closely related to *P. boneti*. The sample available from Tamaulipas for this study is from a single juvenile specimen that shows the typical *P. bellii* pattern. To determine whether two species are present in Tamaulipas, it will be necessary to obtain genetic data from adult specimens of the *P. boneti*-like color pattern.

We consider the differences in morphology, ecology and DNA sequences between samples of *P. boneti* and those from Putla area in western Oaxaca to be sufficient evidence that the Oaxacan clade contains two independent evolutionary trajectories, and that it accordingly is formed by two species. We name the Putla population below.

Description of a New Species

Pseudoeurycea ***maxima*** *sp. nov.*
Southern Giant Salamander
Salamandra Gigante de Putla

Holotype.—Museum of Vertebrate Zoology (MVZ) 106851, an adult male from 6 km south of Putla de Guerrero, Oaxaca, México, approximately 16° 58.7′ N, 97° 53.8′ W; elevation approximately 750 m), collected by T. Papenfuss, 29 December 1972.

Paratypes.—MVZ 137874, MVZ 215973, same data as holotype; UI73862, UI73860, UI65455, UI73863, UI82485, UI65456, UI66653, UI82484, UI82486 from San Vicente Putla, Oaxaca; MVZ 146785 19.5 km NE Putla de Guerrero, Oaxaca, on Highway 125, 1030 m elevation.

Diagnosis.—This is a very large species of the *P. bellii* complex having a characteristic solid black coloration with a series of paired red to orange spots on the dorsum, distinguished from other members of the complex as follows: from *P. bellii* by broader head and longer limbs; from *P. boneti* by broader head, longer limbs, and in having a dorsal color pattern of separated paired spots rather than chevrons and in lacking shoulder markings and a combined pelvic spot complex; from *P. gigantea* by lacking a combined spot at the beginning of the series of dorsal marks; from *P. naucampatepetl* by its much larger size, broader head, and in lacking shoulder spots and a combined pelvic spot complex. It differs from other species in the complex in many allozymic and mtDNA characters.

Description.—This is a very large, robust species; SL in seven adult males 90.0–125. $\bar{x}$ = 101.6), in five adult females 88.8–128.1 ($\bar{x}$ = 111.2). The prominent head is very large and has a broadly rounded but relatively short snout and eyes of moderate size that are moderately protuberant. The head is broad (17.4–19.5%, $\bar{x}$ = 18.4% SL in males; 17.4–19.0, $\bar{x}$ = 17.9% SL in females) and long (21.6–25.5%, $\bar{x}$ = 23.6% SL in males, 22.6–24.4, $\bar{x}$ = 23.3% SL in females). Parotoid glands are not evident. The holotype has a huge, prominent mental gland (11.2 wide, 7.9 long). The smallest adult male paratype (90.0 SL) has only a small mental gland, and the gland is not evident in the next smallest male paratype, suggesting that males do not reach sexual maturity until they reach a size of roughly 90 SL. There are 13 costal grooves, counting one each in the axilla and groin. The limbs are long and robust (combined limb length 42.0–51.5%, $\bar{x}$ = 47.5% SL in males, 42.4–51.6%, $\bar{x}$ = 46/1% SL in females); the limbs fail to overlap when adpressed to the side of the trunk by 1.5–2.5 costal folds in males and by 1.5–3.0 costal folds in females. The digits are well developed but relatively short, with no appreciable basal webbing. Subterminal pads are well developed. Fingers in order of decreasing length: 3-2-4-1; toes in order of decreasing length: 3-4-2-5-1. The tail is large and robust, tapering abruptly to a blunt tip. There is a strong constriction at the base of the tail. Tails are moderately long; complete tails are 77.3–100.5%, SL, $\bar{x}$ = 99.2% SL in males; 72.1–93.8%, $\bar{x}$ = 84.7% SL in females. Premaxillary teeth are slightly enlarged and number 2–10 in males; premaxillary teeth of females are about the same size as the maxillary teeth and number 10–13. Maxillary teeth are small and numerous, ranging from 72–112 ($\bar{x}$ = 88.5). Vomerine teeth are in long rows, 46–78 ($\bar{x}$ = 59). A large number of teeth are present in the paired paravomerine tooth patches, which approach each other closely on the midline.

Coloration.—This species has a variable color pattern, but, in general, it is uniformly black with parallel rows of vivid red-orange to pale orange marks on the head, body, and proximal part of the tail. These spots are discrete and do not join across the midline or along each row. The holotype has the least spotting. There is a single, small occipital spot on the right side, but spots are then absent to

the middle part of the trunk. There are six left and five right spots, all small, but there are only three pairs of spots because the pattern is irregular. The largest spots are a pair in the sacral region just behind the limb insertions. Following these is a single right spot and a final pair on the first caudal segment, followed by a tiny spot on the right of the second caudal segment. All of the paratypes have more color, but, despite variation, there is a general consistency in pattern. All but one specimen have occipital spots that are fragmented or disrupted, but one has a continuous pair of spots of moderate size. None of the specimens has a nuchal unpaired or joined spot, nor are there scapular spots. There are from 10 to 15 pairs of trunk spots, always separated from each other and from neighbors within the row; these are located on the dorsal equivalents of costal interspaces. As many as four unpaired spots are found in some specimens. All specimens have a pair of spots over the sacrum and a pair on each caudosacral segment. These are partly joined to their neighbors in three specimens, but most are separate, as on the trunk and sacrum. The shortened segment at the base of the tail, which marks the autotomy plane (the skin of this segment stays with the tail as part of the wound healing specialization, whereas the inner parts are shed with the tail; Wake and Dresner, 1967), is unmarked. There are spots of color on the tails of all but three of the paratypes but less organized than on the trunk; these markings migrate laterally on the tail, becoming nearly ventral in a few specimens. Tail markings occur as far posteriorly as the 10th caudal segment. The venter is about as black as the dorsum, although the gular region may be a little lighter and there are no ventral markings. The mental glands of males are a little lighter than surrounding tissue. A black and white photograph of the holotype, identified as *P. bellii*, was published by Wake et al. (1992, their Fig. 9). A color photograph of two unlabeled salamanders was published in *Science* magazine (Vol. 305, p. 1397, 2004). A black and white version of another photograph of the same specimens (identified as *Thorius pennatulus* and *Pseudoeurycea bellii*) appears in Wake (1992). The larger of the two is a paratype of *P. maxima* (MVZ 215973).

Measurements of holotype (in mm).—Head width 22.4; head depth 15.0; eyelid length 8; eyelid width 4.5; anterior rim of orbit to snout 7.1; interorbital distance 5.9; distance between corners of eyes 9.2; snout to forelimb 35.6; nostril diameter 0.6; distance between external nares 7.2; projection of snout beyond mandible 0.4; snout to gular fold 26.9; width across shoulders 21.8; snout to posterior angle of vent 122.8; snout to anterior angle of vent 115.6; axilla to groin 67.4; tail length 120.8; tail depth at base 14.7; tail width at base 16.0; forelimb length 24.9; width of hand 8.0; hind limb length 27.6; width of foot 12.0; length of longest (third) toe 3.9; length of fifth toe 2.5. Numbers of teeth: premaxillary 8; maxillary 53/59; vomerine 30/36.

Habitat and geographic distribution.—This species occurs at lower elevations than have been recorded for any other members of this complex. The type locality is recorded on the collector's tag as 750 m, but has been reported as 730 m (Feder et al., 1982). Other members of this complex occur at higher elevations; however, there is one report of a *P. gigantea* at 3000 ft. (approximately 930 m) (Reese and Firschein, 1950). All other records for this complex are from much higher elevations, usually above 2000 m. The known geographic range of *P. maxima* is in far western and southern Oaxaca, close to the border with Guerrero. It extends from a few km south of Putla to a few km north and east of the city. We presume that the location cited for a series of paratype specimens, San Vicente Putla, is an alternative name for the town, also known as Putla de Guerrero. The climate in the vicinity of Putla (which translates to place of fog) is wet tropical, and the type specimen was collected in an agricultural zone where bananas are grown. The type specimen (which weighed 58 g upon capture) was found by digging out a hole in a road bank. The specimen was about 30 cm deep in the hole. In the immediate vicinity, nine specimens of *Bolitoglossa riletti* were obtained. Two additional specimens of *P. maxima* from the type locality, collected at a later date, were found in the same road bank hole.

Comparisons.—The largest individual in the entire *P. bellii* complex that is known to us is a specimen of *P. gigantea*, which is 161 SL with a tail of 115 (Taylor and Smith, 1945). We measured 10 adult males and 10 adult females from a single site in northwestern Oaxaca (near

Tejocotes, series from AMNH). Males averaged 95.8 SL with the largest being 100.7 SL; females were substantially larger, averaging 110.2 with the largest being 115.6. A series of *P. bellii* from a single site in Jalisco (series from MVZ) consisted of six adult males averaging 76.5 SL with the largest being 93.8 SL, and 10 adult females averaging 104.0 SL with the largest being 139.9 SL. Smith (1949) reports a specimen of *P. bellii* 146 SL, with a total length of 291. However, *P. maxima* marginally has the largest average size (males 101.6, females 110.6). The smallest species is *P. naucampatepetl* (largest individual recorded is 82.9 SL).

The vivid color pattern of the different species is subject to considerable variation, but each species has a distinctive and characteristic pattern. Occipital spots are typically absent in *P. gigantea*, but occasionally are present, whereas these spots are typically present in all other species but occasionally absent in *P. bellii* (Duellman, 1965), particularly *P. b. sierraoccidentalis*. Scapular spots are typically present in *P. boneti*, and a different kind of scapular spot is present in *P. naucampatepetl*. The trunk spots are typically in parallel rows of separated spots, but they are often joined to form chevrons in *P. bellii*. Such chevrons are large and prominent in *P. boneti*, in which they frequently join anterior or posterior chevrons. This species displays more reddish coloration than any of the others. The reddish coloration is greatly reduced in *P. b. sierraoccidentalis*; the rows of dorsal marks are incompletely and highly asymmetric, with only a few usually small marks in each row, representing only a remnant of the pattern found elsewhere in the complex.

The species all are similar in proportions, but *P. maxima* appears to have the broadest head (averaging about 18% SL versus 17% in *P. gigantea* and about 16% or less in the other species). Heads are 16.7% SL in male *P. boneti* and 15.9% in females, and in the Jalisco sample of *P. bellii* heads are 16.5% SL in males and 15.6% in females. Limbs appear to be shorter in *P. bellii* and *P. boneti* (combined limb length 45% SL or less) than in the other species (46–47% in *P. maxima*, up to 55% in *P. gigantea*, and up to 51% in *P. naucampatepetl*).

Divergence levels for 16S mtDNA (K2p) for *P. maxima* relative to other species are: 4.3–5.4 to *P. bellii*, 3.9–4.8 to *P. gigantea*, and 1.4–2.5 to *P. boneti*. Cyt b divergences are 11.5–12.9 to *P. bellii*, 13.3 to *P. gigantea*, 11.5 to *P. naucampatepetl*, and 9.9 to *P. boneti*. As outlined above, *P. maxima* differs from all other species in allozymes studied by a genetic distance (Nei, 1972) of 0.50 or greater.

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NEWLY DISCOVERED POPULATIONS OF SALAMANDERS FROM SISKIYOU COUNTY CALIFORNIA REPRESENT A SPECIES DISTINCT FROM *PLETHODON STORMI*

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ABSTRACT: *Plethodon stormi* and *Plethodon elongatus* are two closely related species of plethodontid salamanders that are restricted to the Klamath Province of northwestern California and southwestern Oregon. Discovery of three localities south of the Klamath River, in the Scott River drainage, not assignable to either *P. elongatus* or *P. stormi*, motivated closer examination of this complex. We describe molecular (mitochondrial DNA) and morphological variation among specimens collected from the three newly discovered populations and compare these to populations of *P. elongatus* and *P. stormi* from Siskiyou County, California and Jackson and Josephine Counties, Oregon. Analyses of mitochondrial sequence data from the ATPase 6 and cytochrome b genes recovered clades corresponding to *P. elongatus*, *P. stormi* and the Scott River populations. Multivariate analyses indicate that Scott River drainage animals are morphologically distinct from *P. elongatus* and *P. stormi*. Because both genetic and morphological data indicate that the Scott River populations are distinctive, we provide a description of a new species, *Plethodon asupak*, to reflect the evolutionary history of this group and facilitate species management.

Key words: California; Mitochondrial DNA; Morphological variation; New species; Plethodontidae; *Plethodon asupak*; *Plethodon elongatus*; *Plethodon stormi*; Siskiyou County

PLETHODON STORMI and *Plethodon elongatus* are closely related species of plethodontid salamanders (Highton and Larson, 1979; Mahoney, 2001, 2004) with restricted ranges in northern California and southern Oregon (Petranka, 1998). Species identification, however, continues to be problematic in Siskiyou County, California and the taxonomic status of *P. stormi* is in need of clarification. Some

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authors treat *P. stormi* as a subspecies of *P. elongatus* (Stebbins, 1985, 2003) whereas others treat *P. stormi* as a full species (Brodie, 1970; Highton and Brame, 1965; Nussbaum, 1974; Nussbaum et al., 1983, Petranka, 1998). Variation in body length and pigmentation along an east-west cline from coastal *P. elongatus* to interior *P. stormi* (Bury, 1973, 1999) suggests *Plethodon* along the Klamath River between Seattle Creek and Seiad Valley may be hybrids. These observations are concordant with allozyme data indicating *P. elongatus* and *P. stormi* may be merging upon secondary contact (R. Highton, personal communication), but discordant with a study of mitochondrial DNA variation indicating *P. stormi* extends across this range (Mahoney, 2004). Finally, recently discovered populations of *Plethodon* from near the confluence of the Klamath and Scott Rivers (hereafter referred to as "Scott River" populations) are not readily assignable based on morphology to either *P. elongatus* or *P. stormi* and raise the question of a previously unknown species or a second region of hybridization.

The taxonomic treatment of *P. stormi* from Siskiyou County and the newly discovered Scott River animals depends on the criteria used to define groups and the choice of species concept. Shared characters, whether molecular, biochemical or morphological, can indicate common ancestry, hybridization or convergence. Different views regarding the implications of genetic divergence and complex patterns of gene flow at species boundaries can also lead to different interpretations of available data (Highton, 1998, 2000; Wake and Schneider, 1998). Interpretation of geographic patterns of genetic and phenotypic variation has considerable impact on conservation. Federal agencies manage large portions of the ranges of both *P. elongatus* (65%) and *P. stormi* (84%) (Nauman and Olson, 1999). In addition, the California State Endangered Species Act provides protection for *P. stormi* but not *P. elongatus* on private lands. Because *P. elongatus* has a much larger range than *P. stormi*, species protections are greater for *P. stormi* than *P. elongatus* on federal, state and private lands. The lack of reliable field characters and continuing taxonomic uncertainty of the specific status of populations, particularly in Siskiyou County, California, has caused difficulties in land management planning. We use mtDNA sequence data and morphological characteristics to clarify the status of the Scott River populations and refine the distribution of *P. stormi* and *P. elongatus*.

Materials and Methods

Species and Populations Studied

This study included populations of *P. elongatus* and *P. stormi* from a total of 38 localities (Table 1, Fig. 1) in Siskiyou County, California and Jackson and Josephine Counties, Oregon. In addition, we included samples from three recently discovered populations in the Scott River drainage south and east of the known range of *P. stormi*. We collected tail tips from individuals in the field and preserved tissue in 95% ethanol. Animals used for the morphological study were transported to Oregon State University and euthanized by topical application of a solution of 20% ethyl-P amino benzoate dissolved in polyethylene glycol.

Samples used to assess molecular variation included partial sequences of the mitochondrial gene ATPase 6 for 38 *P. elongatus* representing 17 populations, 43 *P. stormi*, representing 21 populations and 18 samples of a potential new species from the three Scott River populations (Appendix I). This region of the mitochondrial genome has proven useful for fine scale phylogeography (Vitt et al., 1997), and was previously used by Mahoney (2004) to examine broad phylogeographical patterns in *P. elongatus* and *P. stormi*. We also obtained a 370 bp region of the mtDNA cyctochrome *b* gene for 36 of these individuals (*P. elongatus*: $n = 16$; *P. stormi*: $n = 14$; Scott River populations: $n = 6$) to estimate pairwise differences and calculate percent sequence divergence within and between the major clades identified in our phylogenetic analysis. We used cytochrome *b* for these estimates to compare divergence estimates to other plethodontids (Jackman et al., 1997; Mahoney, 2004; Mead, 2001; Moritz et al., 1992).

We surveyed morphological variation among populations of *Plethodon elongatus*, *P. stormi*, and the Scott River populations. We collected salamanders from 13 localities (Table 1). We examined a total of 15 adult Scott River

TABLE 1.—Localities for samples used in this study * Indicates sample(s) from this locality includes individuals used in morphological analysis.

Number	Locality	County	State	Latitude	Longitude
1	Rainie Falls	Josephine	OR	42.647	−123.608
2	Graves Creek	Josephine	OR	42.651	−123.583
3	Near Fiddler Gulch	Josephine	OR	42.255	−123.760
4	Powell Creek	Josephine	OR	42.275	−123.80
5	Cave Creek	Josephine	OR	42.109	−123.428
6	Ferris Gulch	Jackson	OR	42.238	−123.218
7	Nine Mile Creek*	Jackson	OR	42.139	−123.163
8	Grouse Creek	Jackson	OR	42.148	−122.998
9	Carberry Creek	Jackson	OR	42.040	−123.181
10	China Gulch	Jackson	OR	42.062	−123.157
11	Gravel Pit	Jackson	OR	42.097	−123.018
12	Yellow Jacket	Jackson	OR	42.017	−122.948
13	East Fork Indian	Siskiyou	CA	41.937	−123.411
14	Thompson Creek	Siskiyou	CA	41.935	−123.349
15	Horse Camp Trail*	Siskiyou	CA	41.972	−123.183
16	Applegate Bridge*	Siskiyou	CA	41.984	−123.176
17	Hutton Guard Station	Siskiyou	CA	42.000	−123.140
18	Joe Creek	Siskiyou	CA	41.995	−123.130
19	Elliot Creek	Siskiyou	CA	41.986	−123.031
20	Seiad Creek	Siskiyou	CA	41.905	−123.141
21	Horse Creek Northern*	Siskiyou	CA	41.900	−123.104
22	Horse Creek Southern*	Siskiyou	CA	41.881	−123.090
23	Seattle Creek	Siskiyou	CA	41.843	−123.301
24	Joe Miles	Siskiyou	CA	41.813	−123.294
25	Evans Mountain*	Siskiyou	CA	41.833	−123.265
26	West Grider	Siskiyou	CA	41.848	−123.231
27	Walker Gulch	Siskiyou	CA	41.827	−123.143
28	Muck-a-Muck*	Siskiyou	CA	41.774	−123.031
29	Mill Creek*	Siskiyou	CA	41.743	−122.958
30	Ottley Gulch*	Siskiyou	CA	41.771	−123.336
31	Grider Creek	Siskiyou	CA	41.787	−123.205
32	Clear Creek*	Siskiyou	CA	41.727	−123.519
33	Elk Creek/Twin Creek*	Siskiyou	CA	41.726	−123.365
34	Seiad Valley (Walker Creek)	Siskiyou	CA	41.750	−123.175
35	Independence River Access	Siskiyou	CA	41.596	−123.397
36	Dillon Creek	Siskiyou	CA	41.573	−123.543
37	Dobbins Creek*	Siskiyou	CA	41.539	−123.538
38	Sandy Bar Creek	Siskiyou	CA	41.487	−123.513
39	Somes Bar (2 mi. NW)	Siskiyou	CA	41.402	−123.515
40	Sawyers Bar	Siskiyou	CA	41.297	−123.064
41	DeadMan East	Siskiyou	CA	41.894	−123.435

drainage animals from two populations, 22 adult *P. elongatus* from four populations, and 19 adult *P. stormi* from seven populations (Appendix I). Thirty-five of these individuals also were used in molecular analyses described above. Six sampled *P. elongatus* and *P. stormi* populations (nos. 24, 25, 30, 32, 33, and 37) occurred across the region reported by Bury (1973) as showing clinal variation. Voucher specimens were deposited at the University of Michigan Museum of Zoology. In addition, two juveniles (one from population 28 and one from population 29) were examined for the type series description.

DNA Extraction, Amplification, Sequencing and Analysis

We extracted total genomic DNA from frozen and ethanol preserved tissue following a standard proK digestion and phenol/chloroform/isoamyl alcohol extraction (Hillis et al., 1996). The polymerase chain reaction was used to amplify a 667 base-pair mitochondrial DNA segment containing a portion of the ATPase 6 gene using primers L9252 (5′-AACCTGACCATGAACCTAAGCT-3′) and H9923 (5′-TAGGAGTGTGCTTGGTGTGC-CAT-3′) (Vitt et al., 1997). Amplifications were

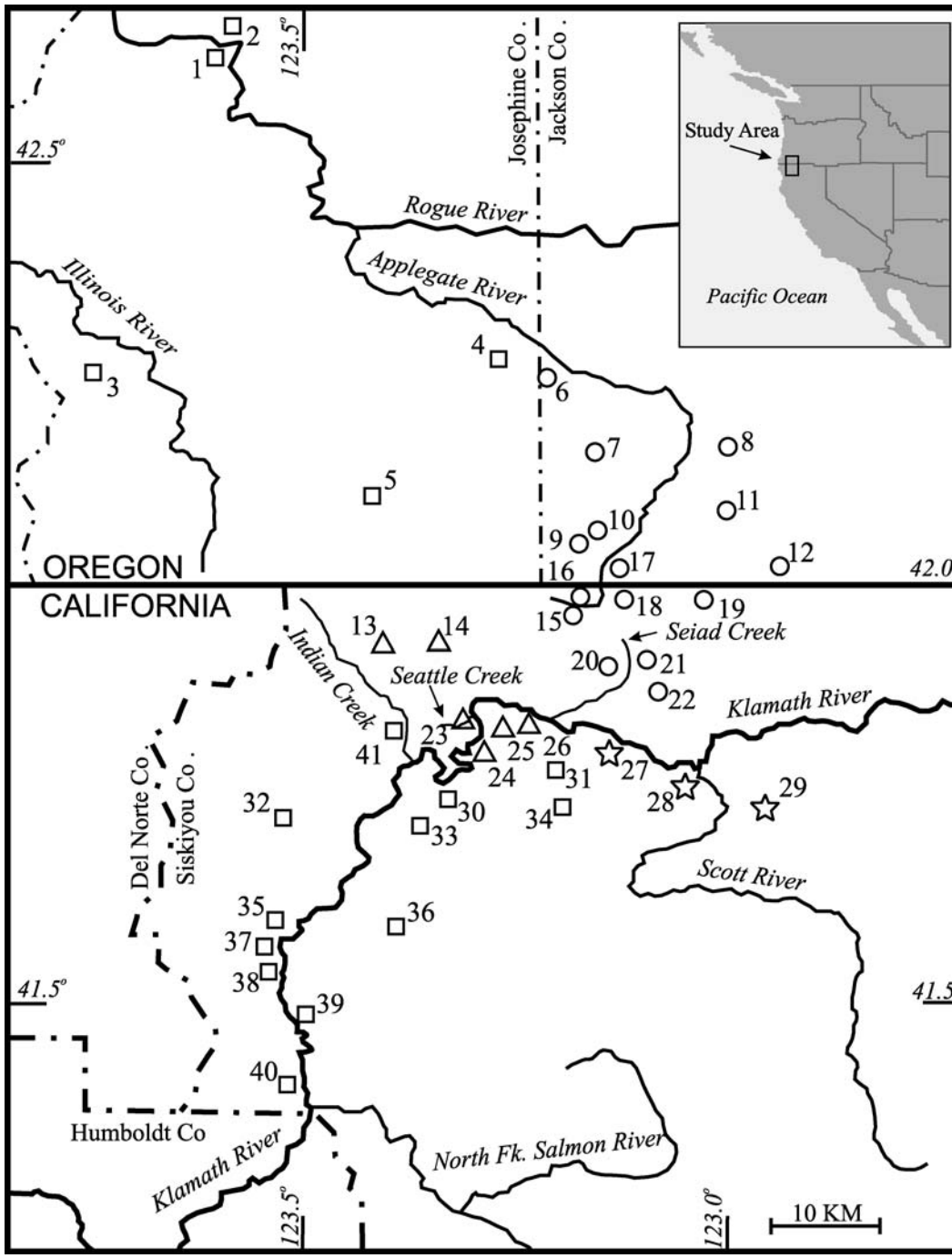

FIG. 1.—Map showing sampled populations included in analyses. The shade and shape of markers for populations in all figures correspond to the clades resolved in our phylogenetic analyses as follows: filled circles: clade I; filled triangles: clade II; black stars: clade III; unfilled squares: clade IV.

performed in 50 μl reactions using 1 μl of genomic DNA, 5 μl 10× PCR buffer, 5 μl 8mM dNTP mix, 5 μl of each 2 μM primer, 3 μl of 25 mM $MgCl_2$, 0.2 μl Taq DNA polymerase (Life Technologies, Carlsbad, CA) and water to final volume. Reactions consisted of an initial denaturation step followed by 40 cycles of 92 C for 45 s, 48 C for 45 s, and 72 C for 90 s. QIAquick PCR purification kit (Qiagen, Valencia, CA) was used to purify the PCR product. The DNA sequence of purified PCR products was obtained using an Applied Biosystems Inc. (Foster City, CA) ABI 377 automated DNA sequencer using Big-Dye Terminator Cycle Sequencing. PCR fragments were sequenced in both directions to check accuracy. The same protocol was used to obtain a 370 bp region of the cytochrome *b* gene using primers MVZ15 (5′-GAACTAATGGCCCACACWWTACG-3′) and CYTB2 (5′-CCCCTCAGAATGATATTTGTCCTCA-3′) (Moritz et al., 1992) and the following PCR reaction protocol: an initial denaturation step followed by 35 cycles of 96 C for 30 s, 54 C for 60 s, and 72 C for 90 s.

Mitochondrial DNA sequences (Appendix I) were aligned by eye using the programs SEQED (Applied Biosystems Inc., Foster City, CA) and a Clustal W algorthm implemented in MegAlign (DNASTAR Inc., Madison, WI). We analyzed ATPase 6 and cytochrome *b* gene sequences separately. Sequence variation was calculated as the percent of variable sites in pair-wise comparisons of unique haplotypes (genetic variants) for both ATPase 6 and cytochrome *b* sequences.

We examined ATPase 6 variation using maximum parsimony (MP) and maximum likelihood (ML) implemented in the computer program PAUP* version 4.0b-10 (Swofford, 1999) and Bayesian estimation (MB) performed with MrBayes version 3 (Huelsenbeck and Ronquist, 2001). Because ML analyses are computationally time consuming with large datasets, we used a subset of haplotypes for the ML analysis only, randomly subsampling a total of 31 individuals from among the unique haplotypes in each major clade (I, II, III, and IV). A model of nucleotide substitution was selected by the hierarchical likelihood ratio tests implemented by Modeltest version 3.0 (Posada and Crandall, 1998). This analysis selected the GTR+I+Γ substitution model (Yang, 1994) with empirical base frequencies, 6 rate parameters ([A–C] = 0.86187, [A–G] = 4.09176, [A–T] = 0.56107, [C–G] = 0.46517, [C–T] = 6.79798, [G–T] = 1.0), proportion of invariant sites equal to 0.3323, and a gamma distribution shape parameter equal to 0.8874. For the ML and MP analyses heuristic searches were conducted with 10 random additions and branch swapping by tree bisection rearrangement. Support was assessed using nonparametric bootstrap with 100 pseudoreplicates under the heuristic method. We used the mtDNA sequences of *P. dunni* and *P. vandykei* as outgroups based on previous analysis of molecular variation within the genus *Plethodon* (Mahoney, 2001).

Bayesian analyses were conducted with a random starting tree, run 5.0×10^6 generations and sampled every 100 generations. Base frequencies and initial starting values for specific nucleotide model parameters (including base frequencies, rate matrix, gamma shape distribution, and proportion of invariant sites) were estimated from the data assuming a general time reversible model + I + Γ. We determined stationarity of the Markov chain as the point when sampled log likelihood values plotted against generation time reached a stable mean equilibrium value. Data sampled from generations preceding this point were discarded (Huelsenbeck and Ronquist, 2001). We estimated nodal posterior probabilities, mean log likelihood scores, and a summary phylogeny using all data collected at stationarity.

Morphological Analysis

Morphological measurements were made to the nearest 0.1 mm using digital calipers. The following measurements were taken from each specimen: standard length from the snout to the posterior margin of the vent (SVL); head length from anterior end of the snout to posterior end of gular fold (HL); head width between angle of jaws (HW); interocular distance, measured between medial margins of eyelids (IO); internarial distance (IN), measured between the medial margins of the nares; eye-naris distance, measured between the medial and anterior margin of the eye to the medial and posterior margin of the naris (EN); forelimb length, measured from the anterior point of contact between the forelimb and body to the tip of the third digit (FLB);

hindlimb length, measured from the anterior point of contact between the hindlimb and the body to the tip of the third digit (HLB). We also recorded the number of vomerine teeth (VM) and the number of premaxillary and maxillary teeth (PMM) using a dissecting microscope, and the number of costal grooves (COS) and intercostal folds between adpressed limbs (INCOS). We determined sex of specimens by direct examination of the gonads.

Statistical analyses were performed using SAS version 8e (SAS Institute, Cary, NC). Only sexually mature specimens were used for statistical analyses. To determine if variances were the same within each of the groups defined by the independent variable (SPECIES), we used Levene's tests for homogeneity of variances between traits among species. We assigned each individual to a species according to phylogenetic results (*P. stormi* = clade I and II; *P. elongatus* = clade IV; Scott River = clade III). We also performed a one-way ANOVA to examine the total variation in response (dependent) variables (SVL, HL, HW, IO, IN, EN, FLB, HLB, VM, PMM, COS, INCOS) due to the effects of the classification (independent) variable (SPECIES). Additionally, a variable was chosen (SEX), which separates the data set into groups of observations.

We used univariate Analysis of Covariance (ANCOVA) with SVL as the covariate to test for sexual dimorphism. Each species was analyzed separately. SAS was used to perform an ANOVA with HL, HW, IO, IN, EN, FLB, HLB, PMM, VM, COS, and INCOS as dependent variables, SEX as the classification variable and SVL as the covariate. SVL was used as a covariate to adjust for any effects of size on the dependent variables, thereby reducing the variance of the error term. An interaction between SEX and SVL was also specified to account for variation in the dependent variable not explained by only main effects and covariates.

In all multivariate analyses, we analyzed males and females separately. We performed a Canonical Discriminant Analysis (CDA) on z-transformed data. The z-transformation generates scores that equalize differences in magnitudes among variables by transforming the raw score to a value that corresponds to the deviation from its distribution's mean, expressed in units of its standard deviation. CDA is a type of discriminant function analysis used to discriminate between two or more naturally occurring groups by deriving functions that optimally combine variables such that the first function provides the most overall discrimination among groups, the second provides second most, and so on. Computation of two canonical variables was specified in the CDA based on linear functions using Mahalanobis distances to compute squared distances between class means based on pooled within-class covariance matrices. We also examined how well we could predict group membership using classification functions. Classification criteria were based on the pooled covariance matrix (yielding a linear function) and accounted for prior probabilities of group membership. We set prior probabilities proportional to the sample sizes.

Results

Our survey revealed 51 unique haplotyes. Identical haplotypes were found in samples from nine populations and three haplotypes were shared across populations. Of the 285 variable sites in our alignment, 176 were parsimony informative. Thirty-six individuals from 33 populations were sequenced for the cytochrome *b* gene, yielding 31 unique haplotypes.

Phylogenetic Analyses

The equally weighted maximum parsimony analysis using all haplotypes resulted in one most parsimonious tree of 435 steps (Fig. 2A). Phylogenetic reconstruction of the subset of unique haplotypes using maximum likelihood resulted in a single ML tree with a likelihood score of –3401.149 (Fig. 2B). The tree resulting from the Bayesian analysis also exhibited similar topological structure to the MP tree in Fig. 2A. The Markov chain appeared to reach stationarity by the 1×10^5 generation. We used a conservative burn-in 1×10^6 generations, resulting in 4.0×10^6 generations considered for estimations of mean likelihood score (–4901.37). Posterior probabilities were comparable to bootstrap support values for most nodes (Fig. 2A).

All our analyses resolved four monophyletic groups. One cluster of haplotypes, correspond-

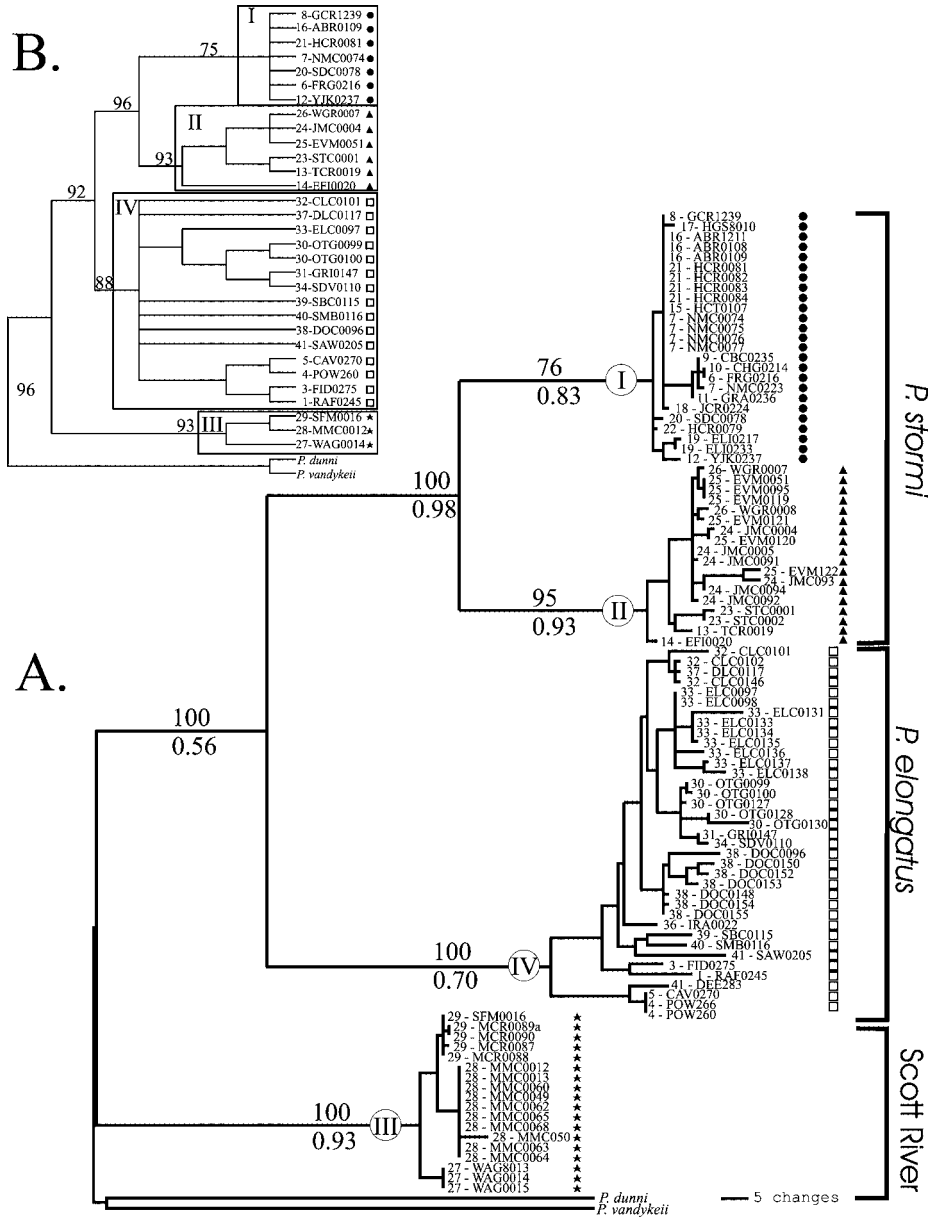

FIG. 2.—A. Single tree retained from maximum parsimony analysis for ATPase 6 sequences. Tree was rooted with *P. vandykei* and *P. dunni*. Bootstrap values are given above each branch. Bayesian posterior probabilities are given below each branch. Four large haplotype clades are designated by roman numerals. Each haplotype includes the population number from which it was sampled followed by individual identification number. B. Strict bootstrap consensus tree for maximum likelihood analysis.

ing to clade I (Fig. 2), comprised eight unique haplotypes distributed from the Applegate River drainage in Oregon to a region in Siskiyou County, California, just south of the Applegate River drainage and north of the Klamath River (Fig. 1). Clade I received 75–76% bootstrap support upon resampling of data. This cluster of haplotypes was most closely related to the monophyletic group of 12 unique haplotypes (clade II, Fig. 2) found

TABLE 2.—Range and average of percent sequence divergence at mtDNA cytochrome *b* gene for within (on diagonal) and between (above diagonal) clade comparisons and ATPase 6 gene between clades (below diagonal). Sample sizes in top row are for cytochrome b and in first column are for ATPase 6.

	Clade I *P. stormi* $n = 5$	Clade II *P. stormi* $n = 9$	Clade III Scott River $n = 6$	Clade IV *P. elongatus* $n = 16$
Clade I *P. stormi* $n = 25$	**0.0–0.73 (0.317)**	1.83–2.93 (2.22)	10.9–11.7 (11.5)	8.06–9.89 (8.62)
Clade II *P. stormi* $n = 18$	0.225–4.5 (3.19)	**0.0–2.56 (1.01)**	10.6–13.2 (11.68)	6.59–9.15 (7.59)
Clade III Scott River $n = 18$	11.7–13.9 (12.7)	11.9–13.5 (12.9)	**0.0–1.09 (0.58)**	11.35–14.28 (12.85)
Clade IV *P. elongatus* $n = 38$	7.7–11.7 (9.5)	8.4–12.5 (10.4)	11.24–13.19 (13.0)	**0.15–6.49 (3.34)**

in seven populations in northern California between Indian Creek and Seiad Creek on the north side of the Klamath River and between Elk Creek and Grider Creek on the south side of the Klamath River (Fig. 1). Bootstrap support for this clade ranged from 93–95%. Clades I and II formed a monophyletic group which received 96–98% bootstrap support in all analyses (Fig. 2) and appeared as the sister group to *P. elongatus* (clade IV, Fig. 2).

Samples from the Scott River populations appeared as a basal monophyletic group (clade III, Fig. 2) of six unique haplotypes (representing 18 sampled individuals). Bootstrap support for clade III was strong, ranging from 93–100% in all analyses (Fig. 2). Placement of this clade as the sister group to a clade containing *P. stormi* and *P. elongatus* also received strong support (92–100%) in MP and ML analyses. Posterior probability of this node in Bayesian analysis was 0.56 (Fig. 2).

Genetic Diversity

The major clades obtained in our analysis showed variable levels of within-clade divergence, with average percent sequence divergence in cytochrome *b* ranging from 0.3% to 3.34% (Table 2). Between-clade estimates of average percent sequence ranged from 2.22 between *P. stormi* clades I and II to 12.85 between clade III (Scott River) and clade IV (*P. elongatus*). These values were similar to estimates of divergence obtained using ATPase 6 sequences (Table 2). Between-clade estimates of average percent sequence for ATPase 6 ranged from 3.19 between clade I and II (*P. stormi*) to 13.0 between clade III (Scott River) and clade IV (*P. elongatus*) (Table 2).

Morphological Analysis

Morphometric measurements and tooth counts appear in Table 3. The ranges of most morphological measurements show overlap among species. We found no significant heterogeneity in variances among groups (Appendix II). The one-way ANOVA indicated differences among species in SVL, HL, HW, IO, IN, FLB, HLB, COS, INCOS for males and SVL, HL, HW, IO, EN, IN, FLB, HLB, COS, and INCOS for females (Appendix II). Because many morphological measurements vary with body size and sex, we also used analysis of covariance with snout–vent length (SVL) as the covariate to test for sexual dimorphism within species. For characters that differed in intercept but not slope we also performed a test of the least-square adjusted means (Appendix III). Most characters did show differences between the sexes. However, once differences attributable to SVL were accounted for, within *P. elongatus*, there was significant sexual dimorphism for HW and VM. *Plethodon stormi* exhibited significant sexual dimorphism for INCOS. Among specimens collected from Scott River only PMM and INCOS varied sexually (Appendix III).

Canonical discriminant analysis for males identified SVL, HW, and FLB as contributing most to discrimination among species along the first canonical axis (Table 4), which accounted for 96.7% of the total dispersion ($F = 4.99$, $P = 0.0058$). The second canonical axis produced loadings that were much more uniform (Table 4) although HLB and INCOS provided some discrimination among species. For females, the first variable explained 97.7% of the dispersion among species with SVL, IN, and FLB contributing most to CAN1 ($F = 4.94$, $P = 0.002$). Along the second canonical axis HLB and SVL contributed to variation among species (Table 4). Ordination of individual specimens on the first two canonical axes indicates good separation based on individual canonical scores

TABLE 3.—Comparison of morphometric variation and number of teeth in adults of *P. elongatus*, *P. stormi*, and Scott River populations. Mean ± SE (above) and range (below); all measurements in mm. Modal numbers are given for costal grooves and intercostals folds.

	P. elongatus		*P. stormi*		Scott River	
Variable	Males $n = 9$	Females $n = 13$	Males $n = 6$	Females $n = 13$	Males $n = 7$	Females $n = 8$
Snout–vent length	50.2 ± 3.42	47.7 ± 3.7	62.9 ± 2.2	58.2 ± 2.2	60.7 ± 3.0	67.2 ± 2.0
	(30.6–63.0)	(27.5–64.9)	(53.5–67.9)	(48.6–72.3)	(48.4–71.3)	(54.9–72.0)
Tail length	41.4 ± 3.82	49.7 ± 5.1	54.0 ± 3.7	52.9 ± 3.0	50.6 ± 2.3	57.8 ± 2.0
	(25.9–62.0)	(27.5–65.0)	(46.3–59.3)	(37.0–48.0)	(34.9–58.9)	(48.8–51.5)
Head length	12.1 ± 0.72	11.3 ± 0.7	14.5 ± 0.4	13.7 ± 0.5	14.4 ± 0.8	15.3 ± 0.8
	(7.5–14.4)	(7.8–13.8)	(12.4–15.3)	(11.3–15.8)	(11.7–17.4)	(11.4–17.9)
Head width	7.1 ± 0.42	6.5 ± 0.3	8.7 ± 0.3	8.1 ± 0.3	8.9 ± 0.4	9.6 ± 0.4
	(4.7–8.7)	(4.9–8.0)	(7.4–9.9)	(6.8–10.0)	(7.3–10.3)	(8.0–10.9)
Interocular distance	2.2 ± 0.1	1.9 ± 0.1	2.6 ± 0.1	2.4 ± 0.1	2.9 ± 0.1	2.8 ± 0.1
	(1.6–2.7)	(1.2–2.6)	(2.3–2.9)	(1.6–3.1)	(2.4–3.2)	(2.4–3.3)
Internarial distance	2.0 ± 0.1	1.9 ± 0.1	2.6 ± 0.1	2.4 ± 0.1	2.9 ± 0.1	2.9 ± 0.1
	(1.3–2.6)	(1.3–2.6)	(1.9–2.9)	(1.9–2.7)	(2.6–3.5)	(2.7–3.2)
Eye–nostril distance	1.5 ± 0.1	1.3 ± 0.1	1.9 ± 0.1	1.8 ± 0.1	1.8 ± 0.1	2.0 ± 0.1
	(0.9–2.1)	(0.9–2.6)	(1.4–2.2)	(1.4–2.0)	(1.3–2.2)	(1.7–2.6)
Forelimb length	8.8 ± 0.5	8.6 ± 0.5	11.5 ± 0.5	10.9 ± 0.3	12.5 ± 0.4	12.9 ± 0.2
	(5.7–10.8)	(6.0–11.2)	(9.7–12.5)	(9.7–13.3)	(10.7–14.3)	(11.6–13.7)
Hindlimb length	10.4 ± 0.7	10.0 ± 0.6	13.8 ± 0.4	13.1 ± 0.3	14.3 ± 0.6	15.5 ± 0.4
	(6.2–13.2)	(5.9–12.9)	(12.3–14.8)	(11.6–15.1)	(11.7–16.0)	(13.1–16.4)
Maxillary-premaxillary teeth	46.7 ± 1.8	50.8 ± 1.2	43.5 ± 3.1	54.8 ± 1.9	50.3 ± 2.9	59.8 ± 2.7
	(40–55)	(45–56)	(31–52)	(45–67)	(44–65)	(42–67)
Vomerine teeth	14.1 ± 0.9	11.6 ± 0.5	12.2 ± 0.7	13.2 ± 0.6	13.2 ± 0.7	13.4 ± 0.7
	(9–18)	(9–14)	(10–14)	(9–17)	(10–15)	(11–16)
Costal grooves	18 (17–18)	18 (17–19)	17 (17–18)	17 (16–18)	16 (16–17)	17 (16–17)
Intercostal folds	6 (5–7)	6 (4–7)	5 (5–6)	5 (4–6)	3 (2–3)	3 (3–5)

(Fig. 3). Males from all three groups clearly separated along CAN1 (Fig. 3, top). Fore- and hindlimb length, head width, and number of intercostal folds between adpressed limbs provide good diagnostic differences between groups. Females also showed good separation on the first canonical axis (Fig. 3, bottom), indicating measurements of fore- and hindlimb length, internarial distance and intercostal folds are important diagnostic differences between groups.

TABLE 4.—Canonical variate coefficients for the Canonical Discriminant Analysis of adult males and females.

	Males		Females	
Variable	CAN 1	CAN 2	CAN 1	CAN 2
Snout–vent length	−7.825	−2.412	2.667	−4.798
Head length	1.835	−0.797	0.348	−0.789
Head width	4.529	−0.567	0.116	1.658
Interocular distance	0.629	−1.661	−0.321	−0.003
Internarial distance	−0.235	0.352	3.182	−1.933
Eye-nostril distance	−2.238	1.125	−0.133	−0.282
Forelimb length	5.047	2.108	−1.187	1.361
Hindlimb length	1.215	3.572	−1.009	4.189
Vomerine teeth	0.656	0.203	0.421	−0.121
Premaxillary-maxillary	−0.214	1.266	0.914	−0.412
Costal grooves	−0.651	−0.752	−0.229	−0.347
Intercostal folds	−2.202	2.524	−3.859	1.375

Linear discriminant functions generated for the classification analysis indicated SVL, HW, FLB, and HLB were most important in discriminating all three groups for males. Additionally, HL discriminated *P. stormi* and INCOS and EN discriminated individuals from Scott River. For females, IN and INCOS best discriminated all three groups. Snout–vent length (SVL) was important in discriminating both *P. stormi* and Scott River populations. Examination of posterior probability of group membership indicated the functions succeeded in differentiating the three groups. Classification of males and females was entirely accurate.

DISCUSSION

Molecular and morphological variation support recognition of three distinct groups: *P. elongatus*, *P. stormi*, and the Scott River populations. Molecular variation in mitochondrial DNA detect four distinct clades distributed across Jackson, Josephine and Siskiyou counties. Clades I and II correspond to *P.*

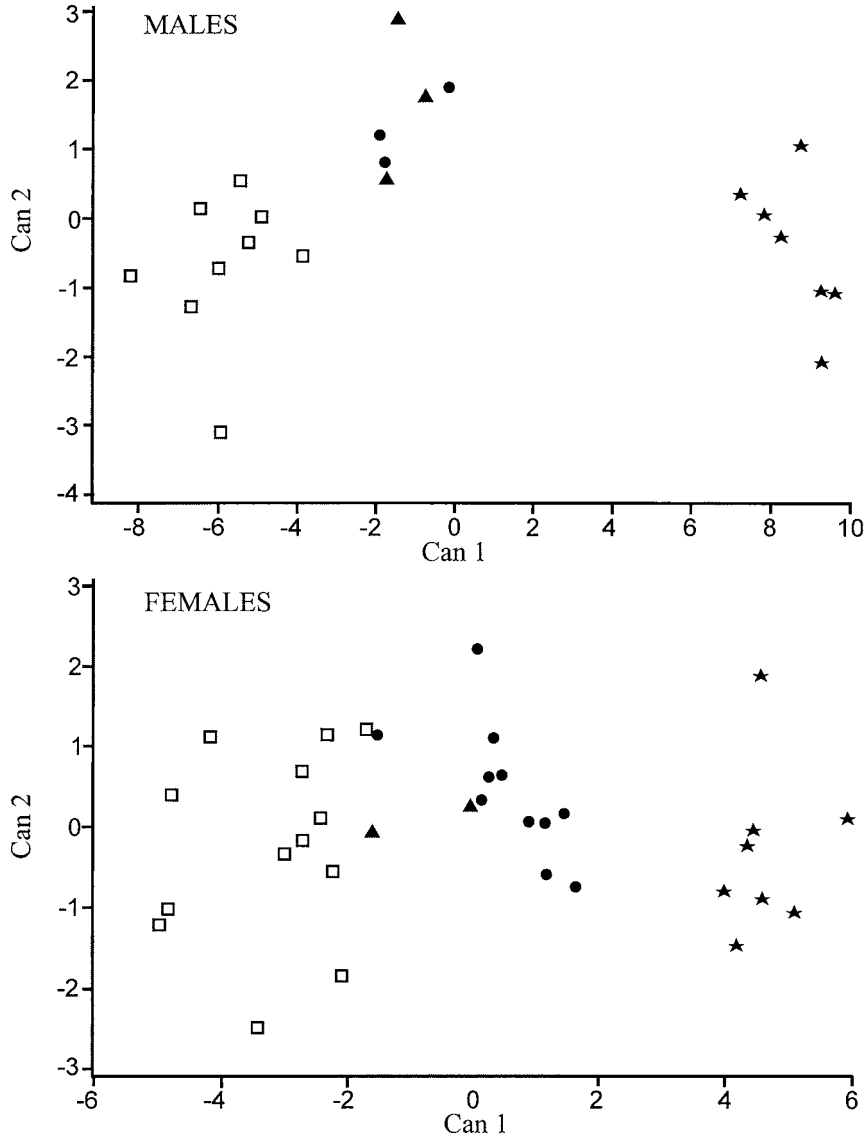

FIG. 3.—Projections of individuals on the two canonical variate axes derived from morphological measurements on adults. *Plethodon elongatus*: open squares; *P. stormi*: filled circles (clade I) and triangles (clade II); Scott River: filled stars.

stormi, clade IV to *P. elongatus*, and clade III to Scott River populations. These results are consistent with those of Mahoney (2004). *Plethodon elongatus* and *P. stormi* are differentiated from one another by 7–10% divergence at both ATPase 6 and cytochrome *b*. Similar estimates were obtained from ATPase 6, cytochrome *b*, and ND4 by Mahoney (2004). Furthermore, populations from Scott River are genetically distinct, exhibiting 10 to 14% divergence (based on uncorrected percent difference at the cytochrome *b* mitochondrial gene) from both *P. stormi* and *P. elongatus*. Levels of divergence between Scott River animals and *P. elongatus* and *P. stormi* are higher than the divergence between *P. elongatus* and *P. stormi* and comparable to divergence between reproductively isolated members of the *Ensatina eschscholtzii* complex (Moritz et al., 1992), morphologically

distinct species of desmognathine salamanders (Mead, 2001), and genera within both the Bolitoglossini and Plethodontini tribes (Jackman et al., 1997). Morphological analyses further reinforce genetic data and support recognizing three distinct groups distributed throughout Siskiyou County, California. Groups show significant morphological variation in a number of characters, with *P. elongatus* and *P. stormi* appearing more similar to one another than either is to Scott River populations. Canonical discriminant analysis provides good discrimination between *P. elongatus*, *P. stormi*, and Scott River populations (Fig. 3). Furthermore, morphological characters traditionally used to distinguish *P. elongatus* and *P. stormi* can also be used to identify Scott River individuals (see below).

Phylogeography

Our analyses also reveal a major phylogeographic subdivision within *P. stormi*. One clade is distributed throughout the Applegate River valley in southern Oregon and extends south into northern Siskiyou County, California. A second clade (*P. stormi* clade II) is distributed across part of Siskiyou County, California north and south of the Klamath River from east of Indian Creek to west of Seiad Creek. The range of *P. stormi* clade II corresponds to the area where there has been disagreement in field identification of *Plethodon*. Historically, individuals found in Siskiyou County, California between Indian Creek and Seiad Valley were referred to *P. elongatus* (e.g., specimens from Seattle Creek, Siskiyou County, California Museum of Vertebrate Zoology nos. 181501–12, collected 23 April 1983), although Brodie (1970) and Bury (1973) reported individuals sampled from this region exhibited characteristics intermediate between *P. stormi* and *P. elongatus*. Our mitochondrial DNA phylogeny indicates Seattle Creek (population 23) as well as all other samples east of Indian Creek (populations 13 and 14) and nearby populations north and immediately south of the Klamath River (populations 23, 24, 25 and 26) are *P. stormi* (clade II). All phylogenetic analyses support clade II as most similar and most closely related to *P. stormi* clade I. Multivariate analyses of morphological data also show clade II males cluster as *P. stormi* (Fig. 3). Female *P. stormi* from clade II are more intermediate morphologically between *P. stormi* I and *P. elongatus* and may explain why some have interpreted the clinal variation in morphological characters along the Klamath River to represent a broad region of hybridization.

It is difficult to decipher the historical events that have led to the observed pattern of divergence since the geological history of the Klamath-Siskiyou region is poorly understood (Atwater, 1970; Earnst, 1981). However, high levels of population subdivision and Pliocene level divergences have been found in other vertebrate taxa from this region (Loiron et al., 2000; Pfrender et al., 2004). The existence of two divergent *P. stormi* clades and the finding of no mixing of *P. elongatus* and *P. stormi* haplotypes in populations distributed in the region of Seattle Creek strongly supports the scenario that *P. stormi* II diverged from *P. stormi* clade I and represents an isolated group of populations that are intermediate in color and morphology between *P. elongatus* and *P. stormi*, rather than a broad region of hybridization. We focused on populations of *Plethodon* in Siskiyou County, California and Jackson and Josephine Counties, Oregon, however, and did not include known localities of *P. elongatus* further south, in Humboldt County, California or further north in Coos and Curry Counties, Oregon. Current understanding of the molecular phylogenetics of *P. elongatus* and *P. stormi*, inclusive of all *P. elongatus*, are that *P. stormi* and *P. elongatus* form two distinct, monophyletic groups (Mahoney, 2004).

Molecular data also confirm a zone of parapatry between *P. elongatus* and *P. stormi*. In Siskiyou County, California *P. stormi* clade II haplotypes occur just east of Indian Creek (populations 13 and 14) and a haplotype clustering within *P. elongatus* occurs just west of Indian Creek (population 41). The distribution of haplotypes reinforces the possibility of an extremely narrow region of secondary contact across Indian Creek (Mahoney, 2004). Plethodontid salamanders are known to exhibit complex patterns of gene flow across species boundaries (e.g., Mead and Tilley, 2000; Mead et al., 2001; Tilley and Mahoney, 1996; Wake, 1997; Wake and Schneider, 1998; Wake and Jockusch, 2000) and molecular variation at mitochondrial genes should be

interpreted cautiously. Examination of the contact zone between *P. elongatus* and *P. stormi* using nuclear molecular markers is currently in progress (DeGross et al., 2002, 2004).

Scott River

Potential gene flow between *P. elongatus* and *P. stormi* does not influence our conclusions about the unique nature of the Scott River animals. Phylogenetic analyses reinforce that the group of populations along the Scott River are an independent lineage, distinct from *P. elongatus* and *P. stormi*. As noted earlier, costal grooves and intercostals folds between adpressed limbs differentiate *P. elongatus* and *P. stormi* (Highton and Brame, 1965). Scott River animals also differ from *P. elongatus* by having a distinct number of costal grooves and from both *P. elongatus* and *P. stormi* by having one less intercostal fold between adpressed limbs. Studies indicate number of costal grooves correlates with the number of vertebrae (Highton, 1957) and therefore Scott River animals may also differ from *P. elongatus* in number of vertebrae. Relative limb lengths (i.e., FLB/SVL) also indicated animals from Scott River are more robust, with longer limbs and corresponding fewer intercostal folds between adpressed limbs. Small sample sizes for both *P. stormi* and Scott River animals could exaggerate differences. However, variation within *P. elongatus* indicates larger sample sizes would not significantly affect measurable differences between Scott River animals and *P. stormi* or *P. elongatus*, assuming levels of variation are comparable in each group.

It is clear from molecular and morphological data that individuals sampled from Scott River represent a unique, basal lineage with a high degree of divergence from both *P. stormi* and *P. elongatus*. We believe the level of morphological and molecular divergence and basal position of this group suggests an evolutionarily distinct lineage that warrants specific status. Our data on genetic divergence indicate these lineages show sufficient divergence so that they no longer form a cohesive, interbreeding group. As currently known, this new group may have the most restricted range of any species of western *Plethodon* salamander.

Conservation

Our assessment of population structure and the degree of genetic cohesion between lineages has important implications for biodiversity conservation. The rarity, restricted distributions, and ecological sensitivity to land management practices and natural disturbances place these species at risk. We have confirmed that *P. elongatus* and *P. stormi* are distinct evolutionary lineages, potentially having been on different evolutionary trajectories. We have also detected a new, unique basal lineage corresponding to the Scott River populations. Our phylogeographic analyses suggest a long history of fragmentation and restricted gene flow among these three lineages.

Taxonomy

The discovery of cryptic species has become a recurring pattern in phylogenetic studies across numerous taxonomic groups (e.g., Dawood et al., 2002; Masta et al., 2002; Mayer and von Helversen, 2001; Wilke and Pfenninger, 2002). Given concordance between morphology and molecules, there are two alternatives for treatment of the Scott River populations. First, all three groups can be collapsed into a single species, *P. elongatus*. This option would create a single species exhibiting extensive genetic and morphological variation, with more variation across its range than many other plethodontid salamanders (Chippendale et al., 2000; Mead et al., 2001; Moritz et al., 1992). Alternatively, the Scott River populations can be described as a new species, concordant with the existence of distinct mtDNA clades that correspond to geographically defined groups of populations, suggesting a history of independent evolution. We choose to recognize groups that are clearly on independent evolutionary trajectories. We therefore treat *Plethodon* from Scott River as a new species as we believe this option best represents the evolutionary history of the group, recognizes the unique nature of the Scott River animals, and provides the best concordance between taxonomy and our current understanding of species boundaries.

Plethodon ***asupak****, sp. nov.*

Holotype.—UMMZ (University of Michigan Museum of Zoology) 262026, MEP field tag

0040, an adult female 70.7 mm SVL (snout to posterior margin of vent), collected at Muck-a-Muck Creek (41.774 N, 123.031 W) above Scott Bar, Siskiyou County California at the confluence of the Scott and Klamath Rivers on April 2 2001 by Louise S. Mead, Richard S. Nauman, Doug DeGross, and Alyssa Zastopil.

Paratypes.—UMMZ 232002 and 232003, MEP field tags 0038 and 0045, same location as holotype; UMMZ 232004 and 232005, field tag MEP 0070 and MEP 0072, collected from Mill Creek, Siskiyou County, California (41.743 N, 122.958 W).

Diagnosis.—*Plethodon asupak* is a medium size salamander of the western *Plethodon* group that closely resembles *P. elongatus* and *P. stormi* but typically is more robust, with a wider head and longer limbs. *Plethodon asupak* is distinguished from *P. elongatus* by having a modal number of 17 costal grooves (*P. elongatus* = 18) and from *P. elongatus* and *P. stormi* by 2.5–3.5 intercostal folds between adpressed limbs (*P. elongatus* = 5–6; *P. stormi* = 4–5). The body of *P. asupak* is elongate with the tail slightly more than 80% of snout to vent length (mean TL/SVL for males: 0.83; females: 0.85) compared to 85–90% for *P. elongatus* and *P. stormi* (mean TL/SVL for *P. elongatus* males: 0.87; females: 0.91; and for *P. stormi* males: 0.90; females: 0.88). *Plethodon asupak* has relatively long fore- and hindlimbs. Males exhibit larger limb/SVL ratios compared to these measures in both *P. elongatus* and *P. stormi*: Ranges of FLB/SVL for males—*P. asupak*: 0.19–0.22; *P. elongatus*: 0.15–0.18; *P. stormi*: 0.16–0.19. Ranges of HLB/SVL for males—*P. asupak*: 0.22–0.25; *P. elongatus*: 0.17–0.22; *P. stormi*: 0.19–0.22.

Description of holotype.—An adult female (UMMZ 232026) with enlarged ova measuring 2–3 mm. Measurements before preservation (in mm): SVL, 57; head length (snout to posterior end of gular fold), 17.9; head width between angle of jaws, 10.2; interorbital distance, 2.4; internarial distance, 2.8; eye to naris distance, 2.2; forelimb length (measured from the anterior point of contact between the forelimb and body to the tip of the third digit), 12.5; hindlimb length (measured from the anterior point of contact between the hindlimb and the body to the tip of the third digit), 15.9; tail length, 62.8 (no evidence of regeneration). There are 17 costal grooves and 3 intercostal folds between adpressed limbs. There are a total of 60 premaxillary and maxillary teeth and a total of 15 vomerine teeth.

Coloration.—Color in life: Lateral portions of the body are chocolate brown, composed of brown and black pigmentation. The dorsal region of the head and body are distinguished from the lateral surfaces by brown and bronze pigmentation. The coloring of this dorsal stripe extends from the head to the tip of the tail. White and yellow flecks cover most parts of the body but are concentrated on the sides and limbs. The venter is mottled, with light gray patches distributed across a dark gray to purplish background. White flecking is also present on venter, particularly in gular region. The degree of mottling varies. The chin is gray with some mottling. Eyes are black with gold flecking on upper and lower surface (degree of gold varies).

Juveniles have two orange to reddish-brown stripes that extend from just posterior of the eyes toward the tail. The two stripes fuse into a single stripe just posterior to the vent region. Concentrated black pigment lines the margins of the dorsal stripes and is replaced by brown pigment along the lateral portions of the body. White to yellow flecks also appear across the entire body, concentrated on the lateral portions of the trunk, the dorsal surface of the head, and the limbs. Venter is dark gray to purple with white flecking.

Variation in the type series.—The type series consists of one adult female (MEP 0040, UMMZ 232026) with enlarged ova ca. 2–3 mm in diameter; a mature male (MEP0045, UMMZ 232003), a mature female with enlarged ova (MEP 0070, UMMZ 232004) and 2 unsexed juveniles (MEP 0038, UMMZ 232002 and MEP 0072, UMMZ 232005) (Appendix IV). All paratypes classified as mature adults resemble the type-specimen in coloration, presence of dorsal stripe, and gold flecking on eye. The degree of white flecks on dorsal and lateral regions of the head and body vary. The degree of mottling on the ventral side also varies among individuals. All mature adults have 17 costal grooves. Both mature females have 3 intercostal folds between adpressed limbs. The adult male has 2 intercostal folds between adpressed limbs. The two juveniles both exhibit the characteristic paired dorsal stripe, varied degrees of white

flecking, 16–17 costal grooves, and 2–3 intercostal folds.

Etymology.—The word Asupak is the Shasta Indian name for Scott Bar, the area near the confluence of the Scott River and the Klamath River, and the type locality for *Plethodon asupak*. The Scott River and Valley area was first visited by various bands the Shasta Indians and eventually inhabited by the Karuk Tribe. The Karuk refer to indicator species in their understanding of nature and viewed salamanders as having the specific function of water purifier and as an omen of good luck (Hillman and Salter, 1997).

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APPENDIX I Population, sample identification number, GenBank accession numbers, indication as to whether individual used for morphological analysis, and University of Michigan Museum of Zoology catalog number for all specimens examined.

Population number	Sample ID	ATPase 6	Cytochrome b	Morphology	UMMZ Catalog Number	Species	mtDNA Clade	State	County	Locality
1	RAF0245	AY688208	AY688284			*Plethodon elongatus*	IV	OR	Josephine	Rainie Falls
2	GVC0244	AY688212	AY688279			*Plethodon elongatus*	IV	OR	Josephine	Graves Creek
3	FID0275	AY688204	AY688275			*Plethodon elongatus*	IV	OR	Josephine	Near Fiddler Gulch
4	POW0260	AY688209	AY688283			*Plethodon elongatus*	IV	OR	Josephine	Powell Creek
4	POW0266	AY688210				*Plethodon elongatus*	IV	OR	Josephine	Powell Creek
5	CAV0270	AY688203	AY688291			*Plethodon elongatus*	IV	OR	Josephine	Cave Creek
6	FRG0216	AY688196	AY688276			*Plethodon stormi*	I	OR	Jackson	Ferris Gulch
7	NMC0074	AY688157		x		*Plethodon stormi*	I	OR	Jackson	Nile Mile Creek
7	NMC0075	AY688158		x		*Plethodon stormi*	I	OR	Jackson	Nile Mile Creek
7	NMC0076	AY688159				*Plethodon stormi*	I	OR	Jackson	Nile Mile Creek
7	NMC0077	AY688160				*Plethodon stormi*	I	OR	Jackson	Nile Mile Creek
7	NMC0223	AY688202				*Plethodon stormi*	I	OR	Jackson	Nine Mile Creek

APPENDIX I
Continued.

Population number	Sample ID	ATPase 6	Cytochrome b	Morphology	UMMZ Catalog Number	Species	mtDNA Clade	State	County	Locality
8	GCR1239	AY688114				*Plethodon stormi*	I	OR	Jackson	Grouse Creek
9	CBC0235	AY688194	AY688267			*Plethodon stormi*	I	OR	Jackson	Carberry Creek
10	CHG0214	AY688195	AY688268			*Plethodon stormi*	I	OR	Jackson	China Gulch
11	GRA0236	AY688200	AY688277			*Plethodon stormi*	I	OR	Jackson	Gravel Pit
12	YJK0237	AY688207				*Plethodon stormi*	I	OR	Jackson	Yellow Jacket
13	EFI0020	AY688123	AY688272			*Plethodon stormi*	II	CA	Siskiyou	East Fork Indian
14	TCR0019	AY688122	AY688289			*Plethodon stormi*	II	CA	Siskiyou	Thompson Creek
15	HCT0107	AY688144	AY688280	x	232092	*Plethodon stormi*	I	CA	Siskiyou	Horse Camp Trail
16	ABR1211	AY688129				*Plethodon stormi*	I	CA	Siskiyou	Applegate Bridge
16	ABR0108	AY688130		x	232093	*Plethodon stormi*	I	CA	Siskiyou	Applegate Bridge
16	ABR0109	AY688131			232094	*Plethodon stormi*	I	CA	Siskiyou	Applegate Bridge
16	ABR0161			x	232099	*Plethodon stormi*	I	CA	Siskiyou	Applegate Bridge
16	ABR0162			x	232100	*Plethodon stormi*	I	CA	Siskiyou	Applegate Bridge
16	ABR0163			x	232101	*Plethodon stormi*	I	CA	Siskiyou	Applegate Bridge
17	HGS8010	AY688115				*Plethodon stormi*	I	CA	Siskiyou	Hutton Guard Station
18	JCR0224	AY688205	AY688302			*Plethodon stormi*	I	CA	Siskiyou	Joe Creek
19	ELI0217	AY688169	AY688274			*Plethodon stormi*	I	CA	Siskiyou	Eliot Creek
19	ELI0233	AY688206				*Plethodon stormi*	I	CA	Siskiyou	Eliot Creek
20	SDC0078	AY688192	AY688286		232078	*Plethodon stormi*	I	CA	Siskiyou	Seiad Creek
21	HCR0081	AY688132	AY688293	x	232081	*Plethodon stormi*	I	CA	Siskiyou	Horse Creek Northern
21	HCR0082	AY688133		x	232082	*Plethodon stormi*	I	CA	Siskiyou	Horse Creek Northern
21	HCR0083	AY688134		x	232083	*Plethodon stormi*	I	CA	Siskiyou	Horse Creek Northern
21	HCR0084	AY688135		x	232084	*Plethodon stormi*	I	CA	Siskiyou	Horse Creek Northern
21	HCR0085			x	232085	*Plethodon stormi*	I	CA	Siskiyou	Horse Creek Northern
22	HCR0079	AY688136		x	232079	*Plethodon stormi*	I	CA	Siskiyou	Horse Creek Southern
22	HCR0080			x	232080	*Plethodon stormi*	I	CA	Siskiyou	Horse Creek Southern
23	STC0001	AY688120				*Plethodon stormi*	II	CA	Siskiyou	Seattle Creek
23	STC0002	AY688121				*Plethodon stormi*	II	CA	Siskiyou	Seattle Creek
24	JMC0004	AY688118	AY688297			*Plethodon stormi*	II	CA	Siskiyou	Joe Miles Creek
24	JMC0005	AY688119				*Plethodon stormi*	II	CA	Siskiyou	Joe Miles Creek
24	JMC0091	AY688145		x	232087	*Plethodon stormi*	II	CA	Siskiyou	Joe Miles Creek
24	JMC0092	AY688182		x	232088	*Plethodon stormi*	II	CA	Siskiyou	Joe Miles Creek
24	JMC0093	AY688183			232089	*Plethodon stormi*	II	CA	Siskiyou	Joe Miles Creek
24	JMC0094	AY688184			232090	*Plethodon stormi*	II	CA	Siskiyou	Joe Miles Creek
25	EVM0051	AY688142		x	232077	*Plethodon stormi*	II	CA	Siskiyou	Evans Mountain
25	EVM0095	AY688143		x	232091	*Plethodon stormi*	II	CA	Siskiyou	Evans Mountain
25	EVM0119	AY688165		x	232095	*Plethodon stormi*	II	CA	Siskiyou	Evans Mountain
25	EVM0120	AY688179			232096	*Plethodon stormi*	II	CA	Siskiyou	Evans Mountain
25	EVM0121	AY688180	AY688301		232097	*Plethodon stormi*	II	CA	Siskiyou	Evans Mountain
25	EVM0122	AY688181			232098	*Plethodon stormi*	II	CA	Siskiyou	Evans Mountain
26	WGR0007	AY688116	AY688294			*Plethodon stormi*	II	CA	Siskiyou	West Grider Ridge
26	WGR0008	AY688117				*Plethodon stormi*	II	CA	Siskiyou	West Grider Ridge
27	WAG8013	AY688126				Scott River	III	CA	Siskiyou	Walker Gultch
27	WAG0014	AY688127	AY688290			Scott River	III	CA	Siskiyou	Walker Gultch
27	WAG0015	AY688128	AY688292			Scott River	III	CA	Siskiyou	Walker Gultch
28	MMC0012	AY688147	AY688299			Scott River	III	CA	Siskiyou	Muck-A-Muck Creek

APPENDIX I
Continued.

Population number	Sample ID	ATPase 6	Cytochrome b	Morphology	UMMZ Catalog Number	Species	mtDNA Clade	State	County	Locality
28	MMC0013	AY688148				Scott River	III	CA	Siskiyou	Muck-A-Muck Creek
28	MMC0049	AY688150		x	232026	Scott River	III	CA	Siskiyou	Muck-A-Muck Creek
28	MMC0050	AY688154		x	232027	Scott River	III	CA	Siskiyou	Muck-A-Muck Creek
28	MMC0053			x	232008	Scott River	III	CA	Siskiyou	Muck-A-Muck Creek
28	MMC0054			x	232009	Scott River	III	CA	Siskiyou	Muck-A-Muck Creek
28	MMC0055			x	232010	Scott River	III	CA	Siskiyou	Muck-A-Muck Creek
28	MMC0056			x	232011	Scott River	III	CA	Siskiyou	Muck-A-Muck Creek
28	MMC0057				232002	Scott River	III	CA	Siskiyou	Muck-A-Muck Creek
28	MMC0058			x	232012	Scott River	III	CA	Siskiyou	Muck-A-Muck Creek
28	MMC0059			x	232001	Scott River	III	CA	Siskiyou	Muck-A-Muck Creek
28	MMC0060	AY688149			232014	Scott River	III	CA	Siskiyou	Muck-A-Muck Creek
28	MMC0062	AY688151			232015	Scott River	III	CA	Siskiyou	Muck-A-Muck Creek
28	MMC0063	AY688155	AY688300		232016	Scott River	III	CA	Siskiyou	Muck-A-Muck Creek
28	MMC0064	AY688156		x	232003	Scott River	III	CA	Siskiyou	Muck-A-Muck Creek
28	MMC0065	AY688152			232017	Scott River	III	CA	Siskiyou	Muck-A-Muck Creek
28	MMC0067			x	232019	Scott River	III	CA	Siskiyou	Muck-A-Muck Creek
28	MMC0068	AY688153		x	232020	Scott River	III	CA	Siskiyou	Muck-A-Muck Creek
28	MMC0072			x	232024	Scott River	III	CA	Siskiyou	Muck-A-Muck Creek
29	SFM0016	AY688124	AY688287			Scott River	III	CA	Siskiyou	Mill Creek
29	MCR0087	AY688185		x	232006	Scott River	III	CA	Siskiyou	Mill Creek
29	MCR0088	AY688201		x	232004	Scott River	III	CA	Siskiyou	Mill Creek
29	MCR0089	AY688146	AY688296	x	232007	Scott River	III	CA	Siskiyou	Mill Creek
29	MCR0090	AY688186			232005	Scott River	III	CA	Siskiyou	Mill Creek
30	OTG0099	AY688161		x	232032	*Plethodon elongatus*	IV	CA	Siskiyou	Ottley Gulch
30	OTG0100	AY688164	AY688282	x	232033	*Plethodon elongatus*	IV	CA	Siskiyou	Ottley Gulch
30	OTG0127	AY688187			232036	*Plethodon elongatus*	IV	CA	Siskiyou	Ottley Gulch
30	OTG0128	AY688188		x	232037	*Plethodon elongatus*	IV	CA	Siskiyou	Ottley Gulch
30	OTG0130	AY688189			232039	*Plethodon elongatus*	IV	CA	Siskiyou	Ottley Gulch
31	GRI0147	AY688190	AY688278			*Plethodon elongatus*	IV	CA	Siskiyou	Grider Creek
32	CLC0101	AY688137			232034	*Plethodon elongatus*	IV	CA	Siskiyou	Clear Creek
32	CLC0102	AY688138	AY688269	x	232035	*Plethodon elongatus*	IV	CA	Siskiyou	Clear Creek
32	CLC0146	AY688166			232055	*Plethodon elongatus*	IV	CA	Siskiyou	Clear Creek
33	ELC0097	AY688140	AY688273	x	232030	*Plethodon elongatus*	IV	CA	Siskiyou	Elk Creek
33	ELC0098	AY688141		x	232031	*Plethodon elongatus*	IV	CA	Siskiyou	Elk Creek
33	ELC0131	AY688172		x	232040	*Plethodon elongatus*	IV	CA	Siskiyou	Elk Creek
33	ELC0132			x	232041	*Plethodon elongatus*	IV	CA	Siskiyou	Elk Creek
33	ELC0133	AY688173		x	232042	*Plethodon elongatus*	IV	CA	Siskiyou	Elk Creek
33	ELC0134	AY688174		x	232043	*Plethodon elongatus*	IV	CA	Siskiyou	Elk Creek
33	ELC0135	AY688175		x	232044	*Plethodon elongatus*	IV	CA	Siskiyou	Elk Creek
33	ELC0136	AY688176		x	232045	*Plethodon elongatus*	IV	CA	Siskiyou	Elk Creek
33	ELC0137	AY688177		x	232046	*Plethodon elongatus*	IV	CA	Siskiyou	Elk Creek

APPENDIX I
Continued.

Population number	Sample ID	ATPase 6	Cytochrome b	Morphology	UMMZ Catalog Number	Species	mtDNA Clade	State	County	Locality
33	ELC0138	AY688178		x	232047	*Plethodon elongatus*	IV	CA	Siskiyou	Elk Creek
33	ELC0139			x	232048	*Plethodon elongatus*	IV	CA	Siskiyou	Elk Creek
33	ELC0140			x	232049	*Plethodon elongatus*	IV	CA	Siskiyou	Elk Creek
33	ELC0141			x	232050	*Plethodon elongatus*	IV	CA	Siskiyou	Elk Creek
34	SDV0110	AY688193	AY688298			*Plethodon elongatus*	IV	CA	Siskiyou	Seiad Valley
35	IRA0022	AY688211	AY688281			*Plethodon elongatus*	IV	CA	Siskiyou	Independence RA
36	DLC0117	AY688139	AY688270			*Plethodon elongatus*	IV	CA	Siskiyou	Dillon Creek
37	DOC0052			x	232028	*Plethodon elongatus*	IV	CA	Siskiyou	Dobbins Creek
37	DOC0096	AY688167			232029	*Plethodon elongatus*	IV	CA	Siskiyou	Dobbins Creek
37	DOC0148	AY688168	AY688271	x	232056	*Plethodon elongatus*	IV	CA	Siskiyou	Dobbins Creek
37	DOC0149			x	232057	*Plethodon elongatus*	IV	CA	Siskiyou	Dobbins Creek
37	DOC0150	AY688170		x	232058	*Plethodon elongatus*	IV	CA	Siskiyou	Dobbins Creek
37	DOC0151			x	232059	*Plethodon elongatus*	IV	CA	Siskiyou	Dobbins Creek
37	DOC0152	AY688171			232060	*Plethodon elongatus*	IV	CA	Siskiyou	Dobbins Creek
37	DOC0153	AY688197			232061	*Plethodon elongatus*	IV	CA	Siskiyou	Dobbins Creek
37	DOC0154	AY688198			232062	*Plethodon elongatus*	IV	CA	Siskiyou	Dobbins Creek
37	DOC0155	AY688199			232063	*Plethodon elongatus*	IV	CA	Siskiyou	Dobbins Creek
38	SBC0115	AY688162				*Plethodon elongatus*	IV	CA	Siskiyou	Sandy Bar Creek
39	SMB0116	AY688163	AY688288			*Plethodon elongatus*	IV	CA	Siskiyou	Somes Bar
40	SAW0205	AY688191	AY688285			*Plethodon elongatus*	IV	CA	Siskiyou	Sawyers Bar
41	DEE0283		AY688295			*Plethodon elongatus*	IV	CA	Siskiyou	DeadMan East
		AY688125				*Plethodon dunni*		OR	Douglas	South of Canyonville
		AY688213	AY688303			*Plethodon vandykeii*		WA	Lewis	Willapa Hills

APPENDIX II Tests for differences among species (*Plethodon elongatus*, *P. stormi*, and Scott River populations) and homogeneity of variance.

	ANOVA among species		Levene's test for homogeneity of variance	
Variable	Males	Females	Males	Females
Snout–vent length				
F =	4.99	9.44	1.15	3.18
P =	0.018	0.0006	0.33	0.053
Head length				
F =	4.15	9.2	0.99	0.51
P =	0.03	0.0007	0.39	0.60
Head width				
F =	7.26	18.81	0.6	0.56
P =	0.0045	<0.0001	0.48	0.57
Interocular distance				
F =	10.80	11.15	2.37	1.07
P =	0.0007	0.0002	0.12	0.35
Internarial distance				
F =	11.24	22.74	0.29	1.99
P =	0.0006	<0.0001	0.75	0.15
Eye–nostril distance				
F =	3.19	7.20	1.36	1.39
P =	0.063	0.0027	0.28	0.26
Forelimb length				
F =	16.09	23.01	0.64	2.76
P =	<0.001	<0.0001	0.53	0.78
Hindlimb length				
F =	12.38	22.06	1.53	2.83
P =	0.0004	<0.0001	0.24	0.07

APPENDIX II
Continued.

Variable	ANOVA among species		Levene's test for homogeneity of variance	
	Males	Females	Males	Females
Max-premax. teeth				
F =	1.54	3.23	0.47	0.54
P =	0.2422	0.058	0.63	0.59
Vomerine teeth				
F =	1.39	1.34	1.80	0.37
P =	0.2767	0.2825	0.19	0.69
Costal grooves				
F =	10.45	5.66	0.62	0.99
P =	0.0009	0.008	0.54	0.38
Intercostal folds				
F =	91.81	9.64	0.31	0.63
P =	<0.0001	0.0006	0.73	0.54

APPENDIX III Test for sexual dimorphism using ANCOVA.

	ANCOVA between sexes within species with SVL as covariate				Least-square adjusted means For ANCOVA					
					P. elongatus		*P. stormi*		Scott River	
Variable	*P. elongatus*	*P. stormi*	Scott River		M	F	M	F	M	F
Head length				Adj.mean	11.66	11.44	13.65	13.62	14.65	14.91
F =	102.6	67.92	0.65	SE	0.2	0.16	0.29	0.15	0.93	0.9
P =	<0.0001	<0.0001	0.59	t-test	0.39		0.91		0.11	
				Slope	NS		NS		NS	
				Intercept	NS		NS		NS	
									X	
Head width				Adj.mean	6.81	6.52	8.12	8.05	9.23	9.13
F =	197.03	27.44	19.45	SE	0.07	0.06	0.29	0.15	0.19	0.19
P =	<0.0001	<0.0001	0.0001	t-test	0.008		0.83		0.6	
	^Sex × SVL			Slope	0.0005		NS		NS	
				Intercept	X		NS		NS	
Interocular distance				Adj.mean	2.1	1.95	2.52	2.39	2.99	2.69
F =	9.11	1.63	2.03	SE	0.09	0.07	0.23	0.12	0.11	0.11
P =	0.0006	0.2245	0.168	t-test	0.22		0.64		0.09	
				Slope	NS		NS		NS	
				Intercept	NS		NS		NS	
Internarial distance				Adj.mean	1.92	1.97	2.31	2.37	3.02	2.85
F =	19.29	8.31	4.08	SE	0.07	0.06	0.13	0.06	0.09	0.09
P =	<0.0001	0.0017	0.035	t-test	0.58		0.71		0.2	
				Slope	NS		NS		NS	
				Intercept	NS		NS		NS	
Eye–nostril distance				Adj.mean	1.43	1.45	1.67	1.76	1.87	1.96
F =	6.30	23.73	1.00	SE	0.11	0.09	0.07	0.03	0.12	0.12
P =	0.0038	<0.0001	0.43	t-test	0.87		0.28		0.58	
				Slope	NS		NS		NS	
				Intercept	NS		NS		NS	
									X	
Forelimb length				Adj.mean	8.51	8.7	10.63	10.89	12.96	12.67
F =	74.22	23.55	13.06	SE	0.16	0.13	0.36	0.18	0.21	0.2
P =	<0.0001	<0.0001	0.0006	t-test	0.38		0.54		0.33	
				Slope	NS		NS		NS	
				Intercept	NS		NS		NS	
Hindlimb length				Adj.mean	10.02	10.29	13.12	13	14.95	15.02
F =	78.78	39.11	20.86	SE	0.22	0.17	0.33	0.17	0.25	0.24
P =	<0.0001	<0.0001	<0.0001	t-test	0.53		0.77		0.85	
				Slope	NS		NS		NS	
				Intercept	NS		NS		NS	

APPENDIX III
Continued.

	ANCOVA between sexes within species with SVL as covariate				Least-square adjusted means For ANCOVA					
					P. elongatus		*P. stormi*		Scott River	
Variable	*P. elongatus*	*P. stormi*	Scott River		M	F	M	F	M	F
Max.-premax.teeth				Adj.mean	46.03	49.9	47.2	54.6	47.21	60.4
F =	2.33	3.61	5.09	SE	1.68	1.67	4.38	2.32	2.72	2.65
P =	0.1357	0.0429	0.019	*t*-test	0.14		0.16		0.005	
				Slope	NS		NS		NS	
				Intercept	NS		NS		0.023	
							X			
Vomerine teeth				Adj.mean	13.87	11.68	13.07	13.02	13	13.22
F =	2.58	0.89	0.016	SE	0.83	0.83	1.24	0.66	0.9	0.79
P =	0.1118	0.4721	0.9212	*t*-test	0.092		0.97		0.86	
				Slope	NS		NS		NS	
				Intercept	0.032		NS		NS	
							X		X	
Costal grooves				Adj.mean	17.7	17.7	17.54	17.1	16.53	16.7
F =	0.79	0.93	0.97	SE	0.2	0.16	0.35	0.17	0.22	0.21
P =	0.5155	0.4513	0.44	*t*-test	0.95		0.3		0.49	
				Slope	NS		NS		NS	
				Intercept	NS		NS		NS	
							X		X	
Intercostal folds				Adj.mean	5.76	5.55	5.48	4.82	2.68	3.5
F =	3.82	11.69	7.92	SE	0.18	0.14	0.33	0.16	0.23	0.22
P =	0.0268	0.0003	0.0043	*t*-test	0.36		0.09		0.026	
	^ Sex × SVL			Slope	NS		0.028		NS	
				Intercept	NS		X		0.026	

^ Sex × SVL indicates there is no significant linear model (no significant relationship with SVL).
X Indicates a significant difference in slope and no comparison is made.

APPENDIX IV Morphological measurements (mm) for specimens examined and included in type series.

Field tag	MEP 0040 Holotype	MEP 0038	MEP 0045	MEP 0070	MEP 0072
Sample ID	MMC0049	MMC0057	MMC0064	MCR0088	MCR0090
UMMZ	232026	232002	232003	232004	232005
Locality	Muck-A-Muck Siskiyou Co., CA	Muck-A-Muck Siskiyou Co., CA	Muck-A-Muck Siskiyou Co., CA	Mill Creek Siskiyou Co., CA	Mill Creek Siskiyou Co., CA
Sex	F	J	M	F	J
SVL	70.7	31.3	54.2	66.3	41.5
TL	62.8	26.8	46.5	53.6	30.1
HL	17.9	8.5	13.8	15.2	10.7
HW	10.2	5.3	8.4	8.7	6.5
IO	2.4	1.6	2.8	3.1	2
IN	2.8	1.5	2.6	2.9	1.7
EN	2.2	0.9	2	1.9	1.6
FLB	12.5	7.3	12	12.5	8.6
HLB	15.9	8	13.4	15.6	9.6
PMM	60	49	52		
VM	8, 7	5, 5	7, 8		
COS	17	16	17	17	17
INCOS	3	2	2	3	2