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Falling apart and merging: diversification of slender salamanders (Plethodontidae: *Batrachoseps*) in the American West

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The plethodontid genus *Batrachoseps*, the slender salamanders, is the most diverse clade of salamanders in western North America, but it has posed taxonomic difficulties because it contains many morphologically cryptic species. A segment of the mitochondrial DNA gene cytochrome *b* was studied for 278 individuals densely sampled from throughout the range of all 18 described species and several undescribed species. Phylogenetic analyses of the mtDNA data identify six major clades, one corresponding to the subgenus *Plethopsis* and five within a monophyletic subgenus *Batrachoseps*. All major clades and most species within these clades display strong phylogeographic structuring. Comparisons of mtDNA and allozyme data show that several allozymically cohesive groups are not monophyletic with respect to mtDNA. We suggest that this phenomenon results from fragmentation of populations, divergence in allopatry, and then recontact and gradual merging of units caused predominantly by male-mediated gene flow. The mtDNA offers evidence that populations were once more isolated than they are now, while the patterns of allozyme variation reflect recent and current interactions among populations. The complex patterns of morphological, allozymic and mtDNA variation associated with the constantly changing geological landscape give insight into the nature of processes responsible for species formation in *Batrachoseps*. *Biological Journal of the Linnean Society*, 2002, **76**, 361–391.

ADDITIONAL KEYWORDS: California biogeography – mitochondrial DNA – secondary contact – speciation.

INTRODUCTION

Clade differentiation and divergence, and species formation, are central issues for understanding the phylogenetic history of lineages. Patterns of variation in molecular markers allow us to examine these and related issues in unprecedented detail. Not only are molecular data widely used to infer phylogenetic history, but they may also give insight into such biological factors as life history and population biology that play important roles in generating patterns of differentiation. Furthermore, the histories of markers with different transmission dynamics (i.e. maternally vs. paternally vs. biparentally transmitted markers) reflect differences in life histories of males and females, and can illuminate evolutionary dynamics associated with gene flow and interactions in contact zones. Traditional morphological approaches also remain important in investigations of diversification, as morphology often reflects adaptation at the organismal level. Comparisons of morphological data and molecular data derived from different kinds of markers can elucidate processes responsible for the overall patterns of divergence (Avise, 2000).

Because salamanders are so morphologically conservative, researchers turned to molecular markers for determining species borders rather early, and many salamander clades have been subjected to

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detailed analyses of genetic differentiation and geographical variation. The largest family, comprising the lungless salamanders (Plethodontidae), has been intensively studied (e.g. Highton *et al.*, 1989; Moritz *et al.*, 1992; Jackman & Wake, 1994; Highton, 1995; Tilley & Mahoney, 1996; Wake, 1997; Chippendale *et al*., 2000; García-París & Wake, 2000; García-París *et al*., 2000a, b; Highton & Peabody, 2000; Jockusch *et al.*, 2001; Mead *et al.*, 2001; Parra-Olea & Wake, 2001). Here we report the results of an extensive survey of variation in the mitochondrial gene cytochrome *b* in slender salamanders of the genus *Batrachoseps* (Plethodontidae), a group for which much allozyme data are also available (Yanev, 1978, 1980; Jockusch *et al.*, 1998, 2001). Discrepancies between theallozyme and mtDNA data sets highlight the importance of examining multiple data sets, and identify potentially fruitful areas for further study. In *Batrachoseps*, the patterns of allozyme variation appear to reflect recent and current patterns of interactions among populations, while the maternally transmitted mtDNA appears to reflect deeper history. We use these different data sets to infer the phylogenetic history of the genus, and to offer a perspective on how lineages of salamanders diversify and how species form.

The slender salamanders, genus *Batrachoseps*, are the most diverse clade of salamanders in western North America. All of these species are strictly terrestrial throughout life. They are secretive organisms, and all are at least subfossorial. Communal oviposition is documented in *B. gregarius*, and may also occur in other species (Jockusch & Mahoney, 1997). Although *Batrachoseps* occurs in highly diverse habitats (from rainforests to deserts), it is dependent on moisture and it is restricted to favourable microhabitats or to seasonally limited surface activity, especially in arid regions. Morphological variation in the group is relatively limited; as recently as 1954 only two species were recognised (Hendrickson, 1954). However, a combination of careful analysis of morphological variation (Brame & Murray, 1968), examination of patterns of genetic differentiation (Yanev, 1978, 1980; Jockusch, 1996; Jockusch *et al*., 1998, 2001; Wake & Jockusch, 2000) and discoveries of highly differentiated forms in unexpected places (Brame, 1970; Marlow *et al.*, 1979; Wake, 1996) has led to the recognition of additional species (Table 1). Currently, 18 species are recognized. *Batrachoseps* is the sister taxon of the supergenus *Bolitoglossa* (Jackman *et al*., 1997), the most diverse of the plethodontid clades (including all Neotropical salamanders), with over 200 species recognized. Thus, understanding the factors influencing diversification in *Batrachoseps* may also give insight into the impressive radiation of its sister group, which includes nearly 50% of known salamanders.

Monophyly of *Batrachoseps* is strongly supported by both morphological and molecular data (Jackman *et al*., 1997). Synapomorphies include the presence of only four digits on the hind feet, a large dorsal fontanelle in the skull and a projectile tongue attached to the jaw by an elongated genioglossus muscle (Wake, 1966; Lombard & Wake, 1986; Jackman *et al*., 1997). The basal split in *Batrachoseps* is between the subgenus (sg) *Plethopsis*, a small clade containing two described species, and the subgenus *Batrachoseps*. Monophyly of the two subgenera is well supported by molecular data (Jackman *et al*., 1997; Parra-Olea, 1999). *Plethopsis* is characterized by plesiomorphic osteology, while monophyly of sg *Batrachoseps* is supported by three morphological synapomorphies: fusion of the premaxillae in adults, loss of prefrontal bones and reduction or loss of the preorbital processes of the vomer (Wake, 1989; Jackman *et al*., 1997).

The relatively large and robust species of *Plethopsis* are morphologically well differentiated from each other. They are also well separated geographically and ecologically, with *B. wrighti* in the Cascade Range of Oregon and *B. campi* in the Inyo Mountains of southeastern California. An undescribed species of *Plethopsis* occurs in the Kern Plateau region and Scodie Mountains at the southern end of the Sierra Nevada, California (Fig. 1; D. B. Wake, K. P. Yanev & R. W. Hansen, unpubl.). Although relationships among these three species have not been resolved, their morphological and biochemical distinctiveness makes species boundaries clear, and the taxonomic status of members of this group is not controversial.

Most members of sg *Batrachoseps*, the attenuate clade, are specialized for a fossorial or semifossorial existence, and share a highly attenuate morphology with elongated body and tail and reduced limbs (Hendrickson, 1954; Jockusch, 1997). Many of the species are confusingly similar in morphology and some can be identified only by molecular characters. However, there are also species that stand out morphologically. For instance, *B. pacificus* and *B. stebbinsi* are larger than, and nearly as robust as, members of sg *Plethopsis* (Marlow *et al.*, 1979). Species in the attenuate clade are distributed in a mainly parapatric pattern, along both the Pacific Coast and the Sierra Nevada of California, with taxa extending from southcoastal Oregon into north-western Baja California, where *Batrachoseps* occurs along the coast, on offshore islands, and in the Sierra San Pedro Mártir. Of the 16 described species, eight have restricted distributions (Fig. 1): *B. simatus* in the Kern River Canyon at the southern end of the Sierra Nevada (Brame & Murray, 1968); *B. stebbinsi* in the Tehachapi Mountains (Brame & Murray, 1968); *B. gabrieli* in the eastern San Gabriel and western San Bernardino Mountains (Wake, 1996); *B. minor* and *B. incognitus* both primarily at higher

Table 1. Comparisons of recent taxonomies of the plethodontid salamander genus *Batrachoseps*

This paper	Yanev (1980)	Brame & Murray (1968)
Subgenus *Plethopsis*		
B. wrighti (Bishop, 1937)	*B. wrighti*	*B. wrighti*
B. campi Marlow *et al.*, 1979	*B.* sp. nov.	Unknown
B. sp. nov. Kern Plateau	Unknown	Unknown
Subgenus *Batrachoseps*		
attenuatus group		
B. attenuatus (Eschscholtz, 1833)	*B. attenuatus*	*B. attenuatus* (part)
relictus group		
B. relictus Brame & Murray, 1968	*B. pacificus relictus* (part)	*B. relictus* (part)
B. kawia Jockusch *et al.*, 1998	*B. p. relictus* (part)	*B. relictus* (part)
B. regius Jockusch *et al.*, 1998	*B. p. relictus* (part)	*B. relictus* (part)
B. diabolicus Jockusch *et al.*, 1998	*B. p. relictus* (part)	*B. relictus* (part)
pacificus group		
B. gavilanensis Jockusch *et al.*, 2001	*B. p.* ssp. nov. Gabilan Range	*B. attenuatus* (part)
B. luciae Jockusch *et al.*, 2001	*B. p.* ssp. nov. Santa Lucia Mts. (part)	*B. relictus* (part)
B. minor Jockusch *et al.*, 2001	*B. p.* ssp. nov. Santa Lucia Mts. (part)	*B. relictus* (part)
B. incognitus Jockusch *et al.*, 2001	*B. p.* ssp. nov. Santa Lucia Mts. (part)	*B. relictus* (part)
B. major Camp 1915	*B. p. major* and *B. aridus*	*B. major*
B. pacificus (Cope, 1865)	*B. p. pacificus*	*B. pacificus*
gabrieli group		
B. gabrieli Wake, 1996	Unknown	Unknown
nigriventris group		
B. nigriventris Cope 1869	*B. nigriventris* (part)	*B. attenuatus* (part) and *B. relictus* (part)
B. gregarius Jockusch *et al.*, 1998	*B. nigriventris* (part)	*B. attenuatus* (part)
B. simatus Brame & Murray, 1968	*B. simatus*	*B. simatus*
B. stebbinsi Brame & Murray, 1968	*B. stebbinsi*	*B. stebbinsi*

elevations in the central Coast Ranges (Jockusch *et al*., 2001); *B. pacificus* on the northern Channel Islands (Wake & Jockusch, 2000); and *B. kawia* and *B. regius* in eponymic river drainages in the Sierra Nevada (Jockusch *et al*., 1998). Eight attenuate species are more broadly distributed (Fig. 1): *B. attenuatus* from extreme south-western Oregon to Monterey Bay along the Pacific Coast and in the northern Sierra Nevada; *B. nigriventris* along the central California coast and into the Tehachapi Mountains; its close relative *B. gregarius* in the central and southern Sierra Nevada; *B. luciae* and *B. gavilanensis* in the central Coast Ranges centred in Monterey Co.; *B. major* in south-coastal California and northern Baja California, Mexico; *B. diabolicus* in the central Sierra Nevada; and *B. relictus* in the southern Sierra Nevada and Greenhorn Mountains (Yanev, 1980; Jockusch *et al*., 1998, 2001; Wake & Jockusch, 2000).

Yanev (1978, 1980) conducted the only previous comprehensive molecular study of relationships within the genus, surveying 19 allozyme loci in >100 populations from throughout the range. The deepest divergence in allozymes, as well as in mtDNA and morphology (Jackman *et al*., 1997), is between sg *Plethopsis* and sg *Batrachoseps*. Within sg *Batrachoseps*, *attenuatus*, *nigriventris* and *pacificus* groups were recognized by Yanev (1978, 1980) (see Fig. 2). Each group contained some populations of the widespread attenuate form that were formerly assigned to *B. attenuatus*, and this lack of correspondence with morphologically identified groups led to substantial taxonomic revision Yanev (1978, 1980) (see Table 1). Yanev's *attenuatus* group contained the single species *B. attenuatus*, with deepest allozyme divergence occurring between coastal populations and the single sampled Sierran population. The *nigriventris* group contained three named species, *B. nigriventris*, *B. stebbinsi*, and *B. simatus*. Yanev's *B. nigriventris* included attenuate forms from the central Coast Ranges, Santa Cruz Island, and the central and southern Sierra Nevada (Fig. 2). The deepest divergence within *B. nigriventris* was between coastal and Sierran populations, the

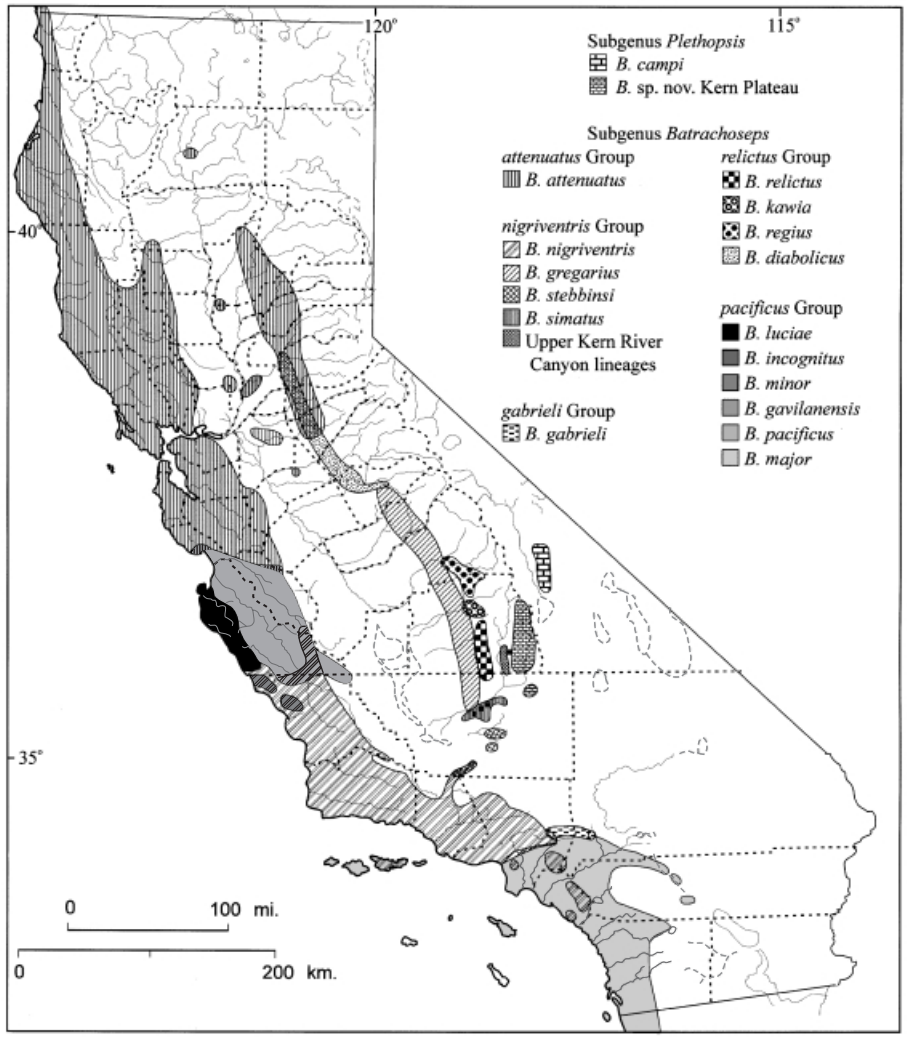


Figure 1. Distributions of the 18 described species and several lineages corresponding to undescribed species of *Batrachoseps* in California.

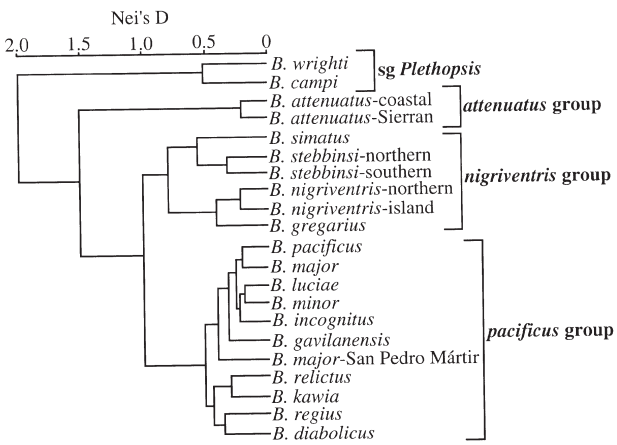


Figure 2. UPGMA phylogram of Nei's D (Nei, 1972) summarizing allozyme divergence among lineages of *Batrachoseps* (Yanev, 1978, 1980; unpublished).

latter of which have since been described as *B. gregarius* (Jockusch *et al*., 1998). *Batrachoseps stebbinsi* and *B. simatus*, which were first described by Brame & Murray (1968) on the basis of distinctive morphologies, were hypothesized to be sister taxa (Fig. 2). Yanev's *pacificus* group contained six highly differentiated units, which she called 'semispecies', in three geographical regions: the coast and islands of southern California, extending along the coast and Sierra San Pedro Mártir of Baja California, the central Coast Ranges, and the southern Sierra Nevada. Yanev (1978, 1980) treated her *pacificus* group taxa as subspecies (some unnamed) of a highly polytypic *B. pacificus*, but most of these have been elevated subsequently to full species, and two have been split into multiple species (Table 1; Jockusch *et al*., 1998, 2001; Wake & Jockusch, 2000).

This survey of sequence variation in a mitochondrial gene, cytochrome *b*, was undertaken in order to further resolve the evolutionary history of *Batrachoseps*. Many lineages that were not available for Yanev's comprehensive survey have since become available for molecular study, including the recently discovered *B. gabrieli* (Wake, 1996), an undescribed species (sg *Plethopsis*) from the Kern Plateau (D. B. Wake, K. P. Yanev & R. W. Hansen, unpubl.), and several morphologically differentiated forms from the Kern River Canyon, a region already known to harbour at least two species (Brame & Murray, 1968). We found that the identification of major clades of *Batrachoseps* and deep level phylogeny was generally concordant between the allozyme and mtDNA data sets, but that there are many discrepancies within species groups (e.g. Jockusch *et al*., 1998; Wake & Jockusch, 2000). We propose an evolutionary scenario involving initial range expansion, followed by range fragmentation, divergence in allopatry, secondary contact, and gradual merging of units through male-mediated gene flow to explain these discrepancies. Data presented in this paper provide a robust hypothesis of the mtDNA phylogeny of *Batrachoseps* and illustrate the importance of examining multiple markers to determine species borders and infer evolutionary history.

MATERIAL AND METHODS

Taxon sampling

DNA of the mitochondrial gene cytochrome *b* was sequenced from 278 individuals. Of these, 132 individuals from 120 localities with 114 different haplotypes were included in the main phylogenetic analyses (Table 2; voucher specimens are listed in Appendix 1). Taxon sampling was designed to be comprehensive, both at and below the species level. Although not a panacea (e.g. Poe & Swofford, 1999), denser taxon sampling has been shown to improve the accuracy of both phylogenetic inference (e.g. Graybeal, 1998; Hillis, 1998) and of substitution model parameter estimation (Sullivan *et al.*, 1999; Pollock & Bruno, 2000). Dense taxon sampling may be especially important when characters are rapidly evolving (Hillis, 1996), as are third codon positions in cytochrome *b*. Multiple representatives were included from almost all groups that had been identified previously based on morphological or molecular data. For widespread groups, individuals were sampled from throughout their range, including the geographical extremes. Additionally, individuals from the vicinity of the type locality of all taxa previously described except *B. caudatus* (see Wake *et al.*, 1998), *B. relictus*, and *B. leucopus* were included to aid in taxonomic revision. *Batrachoseps relictus* has not been seen in the vicinity of its type locality in the lower Kern River Canyon since 1971, and no frozen tissue was available. The nearest extant population to the type locality is across the Kern River in the Greenhorn Mountains, Kern Co. (throughout the paper, all localities are in California, USA, unless otherwise specified) and two individuals from there were used. No material was available from the Coronados Islands, Baja California Norte, Mexico, the type locality of *B. leucopus* (currently a synonym of *B. major*), but individuals from southern San Diego Co., referred to *B. leucopus* by Dunn (1922), were included. No tissue was available for *Batrachoseps* from the Sierra San Pedro Mártir, Baja California Norte, Mexico, a population that Yanev (1978, 1980) treated as an undescribed subspecies in the *pacificus* complex. A subsequent allozyme survey placed it within *B. major* (Wake & Jockusch, 2000). As a result of preliminary analyses, sampling in several regions was expanded to localize geographical breaks in mtDNA lineages. Multiple individuals from 32 populations of *Batrachoseps* were sequenced to measure levels of intrapopulational variation and to test whether single individuals adequately represented each population for the purpose of phylogeny reconstruction.

DNA preparation, amplification, and sequencing

DNA was extracted from frozen tissue or from tissue preserved in Clark's solution using NaCl (Miller *et al.*, 1988) or Chelex (following the procedure of Moritz *et al*., 1992). Up to 784 base pairs of cytochrome *b* were amplified either as a single fragment using the primers MVZ 15 and MVZ 16 (Moritz *et al*., 1992) or as two overlapping fragments using primer pairs MVZ 15 to cytb2 (Kocher *et al*., 1989) and MVZ 25 (Moritz *et al*., 1992) to MVZ 16. Cytochrome *b* was selected for this analysis because its rapid evolutionary rate, especially at third codon positions, makes it generally useful for estimating relatively recent divergences (Brown *et al.*, 1979) and because its value in resolving the deepest relationships within *Batrachoseps* had already been demonstrated (Jackman *et al*., 1997). PCR was done under standard conditions, and the resulting products were cleaned and sequenced either using DNA Sequenase Kit version 2.0 (US Biochemical) and ^{35}S radiolabelled nucleotides, or the ABI Prism Dye-terminator cycle sequencing ready reaction kit (Perkin Elmer). Radiolabelled fragments were separated on a 6.0% polyacrylamide gel and scored manually. Dye-labelled fragments were separated on an ABI 377, and computer scored sequences were compared and edited using the program Sequence Navigator (Applied Biosystems). All sequences were aligned by eye prior to phylogenetic analysis.

Table 2. Localities of specimens included in the phylogenetic analysis of 114 haplotypes

ID no.	County	Exact locality	Latitude	Longitude
B. wrighti				
1	Marion (OR)	USFS 2231, 1.8 mi E jcn Hwy 46	44°47′00″N	121°58′00″W
2	Linn (OR)	Road up Green Mountain, 4.5 mi SE jcn Green Mountain Rd at Hammond Camp jcn	44°34′00″N	122°38′00″W
3	Lane (OR)	8 mi (air) south of Cougar Dam on Rd 1980 at Hardy Creek	43°59′00″N	122°15′00″W
B. campi				
4	Inyo	Barrel Springs, Inyo Mtns	36°53′29″N	118°04′23″W
5	Inyo	Hunter Canyon, Inyo Mtns, 650 m	36°41′59″N	117°51′10″W
6	Inyo	French Spring, 6 mi NE (air) Lone Pine, Inyo Mtns, 1750 m	36°40′29″N	117°59′42″W
B. sp. nov. Kern Plateau				
7	Kern	Scodie Mtns, vic. McIver's Spring, 2000 m	35°37′19″N	118°04′20″W
8	Inyo	Ninemile Canyon, 30 mi S Olancha, 1650 m	35°51′08″N	117°59′55″W
9	Tulare	2.1 mi SSE Sherman Pass Rd on USFS 22S19, Kern Plateau, 2750 m	35°57′35″N	118°21′05″W
B. attenuatus				
10	Del Norte	Along Hunter Creek Rd, 0.4 mi E jcn Hwy 101, 3.5 mi N Klamath	41°34′00″N	124°03′30″W
11	Tehama	Thomes-Newville Reservoir site, 0.5 mi S Williams Butte	39°51′50″N	122°34′45″W
12	Mendocino	along Hwy 20 W of McGuire Hill, 12.5 mi E Hwy 1	39°21′37″N	123°37′41″W
13	Mendocino	Hwy 253 (Booneville-Ukiah Rd), 4 mi E Booneville	39°01′20″N	123°19′00″W
14	Sonoma	0.2 mi N Wohler Rd Bridge over Russian River on E side river	38°30′15″N	122°52′30″W
15	Sonoma	0.3 mi N Wohler Rd Bridge over Russian River on W side river	38°30′45″N	122°53′30″W
16	Sonoma	Bodega Marine lab	38°19′00″N	123°04′20″W
17	Sonoma	1.5 mi NE 101 on Hwy 128	38°42′54″N	122°53′20″W
18	Marin	N end Glen Trail, Point Reyes National Seashore	38°00′00″N	122°48′50″W
19	Marin	1 mi SE Inverness	38°05′30″N	122°50′30″W
20	Sonoma	Pythian Rd, 1 mi NE Hwy 12 at Oakmont	38°26′00″N	122°36′00″W
21	Yolo	Hwy 128, 8 mi SW Winters	38°30′45″N	122°06′00″W
22	Yolo	Along Putah Creek, 0.75 mi E of Old Davis Rd	38°32′20″N	121°44′40″W
23	Calaveras	3.2 mi W West Point	38°23′20″N	120°34′30″W
24	Marin	Gerstle Park and adjoining land, San Rafael (N = 3)	37°57′54″N	122°31′55″W
25	Alameda	University of California campus, Berkeley	37°51′24″N	122°15′45″W
26	San Francisco	Alemany near Mission St. overpass, San Francisco	37°43′40″N	122°25′45″W
27	Santa Clara	Arroyo Mocho	37°27′40″N	121°31′00″W
28	Santa Cruz	Swanton Rd, 0.1 mi SE Hwy 1	37°05′05″N	122°16′00″W
29	Santa Clara	Soda Springs Rd, 0.4 mi E Alma Bridge Rd	37°11′00″N	121°58′30″W
30	Santa Cruz	Hwy 9, 0.7 mi N jcn Hwy 1	36°59′30″N	122°02′00″W
31	Santa Cruz	Branciforte Rd, 0.1 mi S jcn Glen Canyon Rd	36°59′40″N	122°00′40″W
32	Santa Clara	Summit Rd, 2.9 mi NW Mt. Madonna Rd (N = 2)	37°02′15″N	121°44′45″W
33	Merced	Pacheco Pass	37°04′05″N	121°12′50″W
B. nigriventris				
34	Monterey	Redwood Gulch at Hwy 1	35°50′15″N	121°23′30″W
35	Monterey	Ranchita Canyon Rd, 4.4 mi SW Cholame Valley Rd	35°51′30″N	120°29′30″W
36	San Luis Obispo	Santa Rita-Old Creek Rd, 3.4 mi SW Vineyard Dr in Templeton	35°31′20″N	120°46′00″W
37	San Luis Obispo	Santa Rita-Old Creek Rd, 3.6 mi SW Vineyard Dr in Templeton	35°31′23″N	120°46′09″W
38	San Luis Obispo	Stony Creek Campground between Pine Ridge and Garcia Mtns, 23 mi NE Arroyo Grande via Hausna Rd	35°12′40″N	120°15′30″W
39	Santa Barbara	Tepusquet Rd, 2.4 mi S jcn Hwy 166	34°59′30″N	120°11′30″W
40	Santa Barbara	Upper Zaca Station Rd, vic. Zaca lake, just inside National Forest boundary	34°46′30″N	120°02′00″W
41	Santa Barbara	Winchester Canyon Rd, 1.0 mi N US Hwy 101	34°26′20″N	119°54′20″W
42	Kern	Mt. Pinos, *c.* 0.2 mi W McGill campground	34°49′45″N	119°06′40″W

Table 2. *Continued*

ID no.	County	Exact locality	Latitude	Longitude
43	Santa Barbara	800 m W Stanton Ranch, Central Valley, Santa Cruz Island	33°59′46″N	119°43′21″W
44	Ventura	Squaw Flat Rd at Maple Creek, 3.1 mi N Oak Flat Ranger Station	34°29′00″N	118°54′00″W
45	Los Angeles	Bouquet Canyon Rd, 0.3 mi E jcn Spunky Canyon Rd	34°35′00″N	118°21′45″W
46	Los Angeles	Hondo Canyon, 1 km from jcn Topanga Canyon Blvd.	34°05′30″N	118°37′20″W
47	Los Angeles	Coldbrook Camp, Upper Slope	34°17′30″N	117°50′15″W
48	Los Angeles	Coldbrook Camp, 16.2 mi above Sierra Madre Blvd.	34°18′00″N	117°50′00″W
49	Los Angeles	Soldier Creek, Falling Springs Resort	34°18′15″N	117°50′10″W
50	Los Angeles	Upper campus, California State Polytechnic University, Pomona	34°03′40″N	117°49′00″W
51	Orange	S side Aliso Creek, 200 m NE Hwy 1, S Laguna Beach	33°31′00″N	117°45′00″W
B. gregarius				
52	Madera	Sugar Pine, Sierra National Forest	37°26′30″N	119°38′45″W
53	Fresno	Unnamed meadow 2 mi SW of Dinkey Mtn	37°00′00″N	119°08′00″W
54	Tulare	Road along S Fork Kaweah River, 4.2 mi SE Hwy 198	36°23′30″N	118°52′15″W
55	Tulare	N side Middle Fork Tule River, 1.8 mi W Wishon Drive	36°09′14″N	118°44′13″W
56	Tulare	Rd along Arrastre Creek (Old Stage Rd), 1.4 mi SE White River	35°48′00″N	118°52′00″W
57	Kern	Rancheria Rd, 2.8 mi N Kern River bridge	35°27′40″N	118°49′30″W
B. simatus				
58	Kern	Cottonwood Creek at Hwy 178	35°25′20″N	118°49′15″W
59	Kern	Dougherty Creek, Kern River Canyon	35°28′00″N	118°43′00″W
60	Kern	Breckenridge Mtn vic. Squirrel Meadow, 2000 m (N = 2)	35°28′00″N	118°34′45″W
61	Kern	W side of Clear Creek just above confluence with Kern River, 750 m	35°34′30″N	118°31′45″W
62	Kern	Erskine Creek Canyon, Piute Mtns, 4.2 mi from mouth of canyon, 1200 m	35°35′30″N	118°26′30″W
Undescribed species of *Batrachoseps* from the upper Kern River Canyon				
63	Kern	Unnamed Canyon along Cannell Trail, *c*. 0.5 mi (air) E Kern River, *c*. 0.6 mi (air) N Camp Owens, 900 m	35°47′40″N	118°26′20″W
64	Tulare	1 mi (air) ENE Hospital Flat campground, 1050 m	35°50′00″N	118°26′20″W
65	Tulare	Corral Creek Campground	35°51′00″N	118°27′00″W
66	Tulare	E side Kern River along Mtn Rd 99, 0.8 mi N Brin Cyn jcn with Kern River (N = 2)	35°57′20″N	118°28′55″W
67	Tulare	Upper Kern River Canyon at bridge to Johnsondale (N = 2)	35°58′15″N	118°29′00″W
B. stebbinsi				
68	Kern	Bear Trap Canyon, 4.6 mi E mouth of canyon	34°54′30″N	118°42′10″W
69	Kern	Caliente Creek Canyon	35°18′30″N	118°34′30″W
B. gavilanensis				
70	Santa Cruz	Rodeo Gulch Rd, 0.2 mi S jcn Mtn View Rd	37°03′10″N	121°58′00″W
71	Santa Cruz	Hazel Dell Rd, 0.7 mi W jcn Mt. Madonna Rd	37°00′00″N	121°44′45″W
72	Monterey	Zmudowsky Beach State Park	36°50′30″N	121°48′15″W
73	Monterey	Moss Landing, vic. Marine Laboratory	36°48′20″N	121°47′00″W
74	Monterey	Aquajito and Monhollan Rds, S Del Monte Golf Course	36°34′40″N	121°52′15″W
75	San Benito	Willow Creek Rd, 2.9 mi NW jcn Old Hernandez Rd	36°32′20″N	121°06′00″W
76	San Benito	Coalinga Rd at San Benito/Fresno Co. line	36°17′30″N	120°40′50″W
77	Monterey	West Border Fort Hunter Liggett, Nacimiento Fergusson Rd	35°58′43″N	121°20′51″W
78	Monterey	Fort Hunter Liggett, 0.7 mi E western reservation boundary, 4.0 mi W Stony Creek	35°58′30″N	121°20′30″W
79	Kern	Cottonwood Canyon on Hwy 41, *c*. 1 mi E county line	35°46′30″N	120°10′30″W

Table 2. *Continued*

ID no.	County	Exact locality	Latitude	Longitude
B. luciae				
80	Monterey	Bottcher's Gap, end of Palo Colorado Rd, 8.2 mi SE Hwy 1	36°21′10″N	121°48′30″W
81	Monterey	Coast Rd at S jcn Hwy 1 near Big Sur (N = 2)	36°17′20″N	121°50′40″W
82	Monterey	Arroyo Seco, vic. campground, Los Padres National Forest	36°14′00″N	121°29′00″W
83	Monterey	Kirk Creek at Hwy 1	35°59′25″N	121°29′42″W
84	Monterey	Ponderosa Campground, Nacimiento Fergusson Rd	36°00′30″N	121°24′00″W
B. incognitus				
85	Monterey	Hwy 1, 0.4 mi N San Luis Obispo Co. line	35°48′00″N	121°20′45″W
86	San Luis Obispo	Pine Mountain, San Simeon Creek Rd, 14.2 mi from jcn Hwy 1 (N = 2)	35°41′30″N	121°05′30″W
B. minor				
87	San Luis Obispo	Santa Rita-Old Creek Rd, 5.3 mi SW jcn Vineyard Dr	35°31′30″N	120°48′00″W
B. pacificus				
88	Santa Barbara	Santa Rosa Island, Lobo Creek, 2.5 mi NW ranch	34°00′20″N	120°05′15″W
89	Ventura	Middle Anacapa Island	34°00′20″N	119°23′45″W
90	Ventura	East Anacapa Island, adjacent to heliport	34°01′00″N	119°22′00″W
B. major				
91	Los Angeles	Exposition Blvd., Los Angeles	34°02′30″N	118°18′00″W
92	San Bernardino	City Creek Forest Service Station, off Hwy 330, 750 m	34°11′10″N	117°10′45″W
93	Riverside	Cabazon shaft, 1.5 mi SSW Cabazon	33°54′00″N	116°48′00″W
94	Riverside	150–180 m SW Snow Creek Village, 400 m	33°52′18″N	116°41′15″W
95	Riverside	Motte Rimrock Reserve	33°48′00″N	117°15′00″W
96	Riverside	Hidden Palms Canyon Ecological Reserve, 550 m (*B. m. aridus*)	33°38′00″N	116°23′00″W
97	Los Angeles	Santa Catalina Island (N = 2)	33°21′00″N	118°25′00″W
98	San Diego	Juliett Area, Camp Pendleton	33°13′22″N	117°21′24″W
99	San Diego	Talega Canyon Rd, 0.9 mi E of Christianitos Rd at Camp Talego, Camp Pendleton	33°27′02″N	117°33′52″W
100	San Diego	Route S6, 4.6 mi S jcn Route S7	33°18′00″N	116°53′30″W
101	San Diego	*c.* 1 mi SW entrance to Mt Gower Co. Park (N = 2)	33°01′50″N	116°46′15″W
102	San Diego	Torrey Pines State Reserve (N = 2)	32°56′00″N	117°15′27″W
103	San Diego	Hwy 94 at jcn Jamacha Rd	32°44′20″N	116°57′00″W
104	San Diego	Marron Valley	32°37′38″N	116°46′37″W
105	Mexico	Islas de Todos Santos, W Ensenada, Baja California, Mexico (N = 2)	31°47′00″N	116°48′00″W
B. gabrieli				
106	Los Angeles	Rockbound Canyon, above Hwy 39, 1150 m	34°18′02″N	117°49′57″W
107	San Bernardino	Waterman Canyon, *c.* 1 km S Waterman Canyon Station, W Hwy 18	34°12′30″N	117°17′26″W
B. diabolicus				
108	Placer	Hwy 49 at N Fork American River, west side, vic. Auburn	38°54′50″N	121°02′15″W
109	El Dorado	Hwy 49 at N Fork American River, east side, vic. Auburn	38°54′50″N	121°02′20″W
110	El Dorado	Hwy 49, 1.1 mi E bridge over Middle Fork American River	38°54′40″N	121°01′30″W
111	Calaveras	Hwy 12 at jcn Hwy 49, 1.5 mi NW San Andreas	38°12′20″N	120°41′45″W
112	Calaveras	Ponderosa Way, 4.0 mi (air) S Mountain Ranch	38°10′50″N	120°32′20″W
113	Calaveras	Vallecitos-Columbia Rd, 4.9 mi S jcn Hwy 4 at Vallecitos	38°02′45″N	120°27′45″W
114	Mariposa	Hwy 49 at Bagby, Hell Hollow	37°36′30″N	120°08′15″W
B. regius				
115	Fresno	Black Rock Rd, ~1.0–1.5 mi N bridge over N Fork of Kings River, Sierra National Forest	36°52′45″N	119°07′00″W
116	Tulare	Along Alder Creek above Ash Mountain Station, Sequoia National Park	36°29′34″N	118°49′32″W

Table 2. *Continued*

ID no.	County	Exact locality	Latitude	Longitude
B. kawia				
117	Tulare	South Fork Rd at Park Boundary, Sequoia National Park, 1050 m	36°21′30″N	118°46′30″W
B. relictus				
118	Tulare	Sherman Pass Rd, 5.9 mi E of Cherry Hill Rd, Kern Plateau, 2400 m	35°58′40″N	118°23′00″W
119	Tulare	Road to Sugarloaf Village, 0.6 mi SE Sugarloaf Village, Sequoia National Forest	35°49′15″N	118°38′25″W
120	Kern	Tiger Flat Rd (USFS 23S16), 0.55 mi N jcn Hwy 155 at Greenhorn Summit	35°44′30″N	118°33′20″W

Sample sizes with $N > 1$ are indicated in parentheses. ID no. is the population number used in Figures 3 and 4. Localities are in California, USA, unless otherwise indicated. Abbreviations: Hwy, Highway, jcn, junction, Mtn, Mountain, Rd, Road, vic., vicinity.

Phylogenetic analysis

Identical haplotypes were merged into a single haplotype using the search and merge function in MacClade 3.0 (Maddison & Maddison, 1992) with the option to merge taxa as long as resolution of missing or ambiguous character states could make the haplotypes identical. Base composition, stationarity of base composition, pairwise divergences and number of variable and informative sites were calculated in PAUP*4.0b6(Altivec) (Swofford, 2001). We used the Kimura-2 parameter (K2P; Kimura, 1980) distance as an index of divergence because it is widely reported for salamander data. Because monophyly of each of the subgenera is well established (Jackman *et al*., 1997; Parra-Olea, 1999), they were used as reciprocal outgroups in all analyses, with *Plethopsis* formally specified as a monophyletic outgroup to root the trees. Extensive analysis of the 233-haplotype data set was impractical because of the computer time required. Therefore, a preliminary analysis used neighbour-joining with K2P distances to identify clades of closely related haplotypes. A pruned data set was constructed that contained a single representative of each clade in which all members differed by <1.5%. This level was chosen because it reduced the data set to a size at which all major methods of phylogenetic analysis could be used, and also because it corresponds to a level below which multiple hits are not expected (Templeton *et al.*, 1992). Within these shallowly diverged clades, the haplotype for which the data were most complete was selected to represent the clade.

Multiple methods of phylogenetic inference were used in analyses of the pruned data set because each method is known to fail under some evolutionarily plausible conditions (Swofford *et al*., 1996). Because each method fails under different conditions, more confidence can be placed in clades found in multiple methods of analysis, a result supported by simulations (Kim, 1993). Accordingly, maximum parsimony (MP; Hennig, 1966), maximum likelihood (ML; Felsenstein, 1981) and weighted least squares (LS; Fitch & Margoliash, 1967) methods were used to infer the phylogeny of slender salamanders. All analyses were conducted in PAUP*4.0b6 or b8 (Swofford, 2001).

The most parsimonious trees were sought using two weighting schemes. In one, all characters and character state transitions were weighted equally; in the other, all second codon position changes and first codon position transversions were weighted 10 fold relative to third codon position transitions, while first codon position transitions and third codon position transversions were weighted five fold relative to third codon position transitions. Rate classes were determined empirically by examining the degree of saturation within each codon position and type of change, and categories showing the highest rates of change were given the lowest weights. These weights are relatively high and thus indicate which nodes are most robust to changes in weighting scheme. Because initial parsimony searches spent long periods of time in large islands of non-parsimonious trees, subsequent searches were constrained to save a maximum of two trees per replicate (PAUP commands: nchuck = 2, chucklen = 1). All searches were heuristic. Starting trees were obtained by random addition (100 replicates for each weighting scheme) and subjected to TBR branch swapping. The minimal length trees found in each search were then used as the starting trees for a heuristic search with TBR branch swapping, with no constraint on the number of trees saved, and the strict

consensus of all most parsimonious trees was calculated.

For maximum likelihood analysis, one of the trees resulting from the equally weighted MP analysis was used to compare models of sequence evolution using the program Modeltest vs. 3.04 (Posada & Crandall, 1998), and the preferred model was used in a single round of ML analysis using neighbour-joining to obtain the starting tree and TBR branch swapping. To reduce computation time, parameters were estimated on the Modeltest tree and fixed in the ML analysis.

Because changes in base composition can result in inaccurate phylogenetic inference under most methods, we used the weighted least squares (LS) method with LogDet distances, which are relatively insensitive to variation in base composition (Swofford *et al*., 1996). Starting trees were obtained by neighbour-joining and by 100 replicates of random addition, then subjected to TBR branch swapping. Negative branch lengths were prohibited, as recommended by Swofford *et al*. (1996). Analyses were done both assuming that all sites evolved at the same rate and by setting Pinvar = 0.4707 (the maximum likelihood estimate calculated under the HKY + I model on one of the trees obtained in the ML search; this is essentially identical to the ML estimate calculated under the GTR + I model) with additional PAUP commands: RemoveFreq = Proportional and EstFreq = Constant.

Support for nodes was estimated using bootstrapping (Felsenstein, 1985) for both the MP and LS analyses. One thousand (equal-weights MP) or 100 (weighted MP, LS) bootstrap pseudoreplicates were analysed using a heuristic search strategy. Starting trees were obtained by random addition (MP) or neighbour-joining (LS), then subjected to TBR branch swapping. Ten (equal-weights MP) or three (weighted MP) random addition replicates were done per bootstrap pseudoreplicate. A maximum of 100 trees (equal-weights MP) or 25 trees (weighted MP) was saved in each random addition replicate to reduce search times. The reduction in search effort relative to the initial searches is not expected to bias the bootstrap values of nodes that are well supported (DeBry & Olmstead, 2000). For LS analyses, use of starting trees obtained by random addition found the same best tree as did use of the starting tree obtained by neighbour-joining, so the latter was used because it requires less search time. No limit was placed on the number of trees saved in LS analyses. Support for nodes in parsimony trees was also estimated using the decay index (Bremer, 1988). Decay indices were calculated for all nodes in the strict consensus of most parsimonious trees found under each weighting scheme using constrained searches to find the shortest tree not compatible with each node of interest (heuristic searches, starting tree determined by random addition with 100 random addition replicates per constraint tree, TBR branch swapping, nchuck = 2, chucklen = 1). The constraint trees and file of PAUP commands were produced automatically in MacClade 4.0 (Maddison & Maddison, 2001).

Support for nodes in the ML tree was estimated using Bayesian analysis as implemented in MrBayes (Huelsenbeck & Ronquist, 2001). The model matched the model used in the ML analysis, and all prior probabilities were set to their default (uniform or dirichlet) distributions. Eight runs were initiated from random starting trees, and allowed to proceed for between 236000 and 415250 generations. Trees were sampled every 10 generations. The first 5000 trees from each run were discarded, and posterior probabilities were calculated as the frequency of each clade in the remaining 215600 trees.

RESULTS

MOLECULAR EVOLUTION

The cytochrome *b* sequences showed numerous features typical of vertebrate cytochrome *b*. No insertions or deletions were observed, and there was substantial base composition bias, particularly against guanines in the third codon position (Table 3). The χ^2-test of base composition homogeneity is consistent with no difference in base frequencies across taxa when the test is

Table 3. Base composition and variability of cytochrome *b* sequences

					All taxa		Only sg *Batrachoseps*		
	A (%)	C (%)	G (%)	T (%)	Variable sites (*N*)	Informative sites (*N*)	Variable sites (*N*)	Informative sites (*N*)	Total sites (*N*)
First codon positions	26.5	18.9	24.3	30.3	91	67	82	56	261
Second codon positions	21.4	24.9	14.2	40.0	25	11	18	5	261
Third codon positions	38.1	26.7	4.1	31.1	257	247	251	239	262
All sites	28.7	23.5	14.2	33.7	374	325	352	300	784

done on all data. However, when the test is done on data from each codon position separately, stationarity of base frequencies at third codon positions is rejected ($\chi^2 = 470.6$, d.f. = 339, $P < 0.00001$). This difference can be attributed to differences between the subgenera, as P-values are > 0.9 when the test is done on each subgenus individually. The frequency of C and G at third codon positions is substantially higher in sg *Plethopsis (*32.1% C, 9.1% G) than in sg *Batrachoseps (*26.3% C, 3.7% G).

Change at third codon positions was most extensive, with variation occurring at 98% of third codon positions, but at only 35% of first codon positions and 10% of second codon positions (Table 3). In addition, almost all variation at third codon positions was phylogenetically informative (96% of variable sites), but substantially less variation at first and second codon position sites was phylogenetically informative (74% and 44%, respectively), indicating that many of the non-silent changes are concentrated at the very tips of the tree. The high level of change at third positions did not result only from inclusion of a divergent outgroup, as 96% of third position sites are variable within sg *Batrachoseps* (Table 3).

Haplotype distributions and pruning of the data set

We identified 233 unique haplotypes from the 278 individuals sampled. Eighteen haplotypes occurred at two or more localities. Generally, these localities were geographically close (<25 km), but in five cases, identical haplotypes were more widely separated. Multiple putatively conspecific individuals (84 total, 2–10 per locality) were sampled from 32 localities. In general, differentiation within localities was extremely limited; these 84 individuals had only 57 different haplotypes, and in almost all cases, haplotypes from a single locality differed by at most a few mutational steps. At four localities (*B. attenuatus* from locality 24, B. *major* from locality 102, and two localities not included in the pruned data set), differentiation was sufficiently great (K2P of 2.2–2.9%) to place haplotypes in different, albeit closely related, terminal clades. At two localities (64 and 101), haplotypes displayed substantial differentiation (7.9–8.1%), and belonged to different geographically contiguous clades. Only one of the haplotypes from locality 64 was included in the extensive phylogenetic analysis; the other belongs to the shallowly differentiated clade represented by locality 66. These data suggest that sampling a single individual per species per locality is generally sufficient, except in areas where clade borders overlap.

Analyses were conducted on a pruned data set generated by keeping single representatives of clades in which all haplotypes were very similar. The 119 removed haplotypes fell into 57 clades (2–10 individuals each, average = 3.1) with maximum differentiation of 1.5% identified in a neighbour-joining tree of K2P distances. For three of these clades, bootstrap support was <50% (because of the presence of other very similar haplotypes that were not included in the clade). The average bootstrap support for the 54 clades in which it exceeded 50% was 89%. Two haplotypes were restored because they represented biogeographically or taxonomically interesting groups: *B. major* from Catalina Island (locality 97), the type locality of *B. catalinae*, which is currently treated as a subjective junior synonym of *B. major* (Wake & Jockusch, 2000), and *B. relictus* from the Kern Plateau (118), the only individual of this species known from that geographical region. Two additional haplotypes were excluded even though their initial phylogenetic position was not fully resolved because they were within 1.5% of included haplotypes, resulting in a total of 114 haplotypes representing 132 individuals that were used in the more extensive analyses. The included sequences were on average 90% complete.

Although the direct comparison between the 2-state HKY + I + Γ and the 6-state GTR + I + Γ models of sequence evolution was significant, the addition of only one more parameter did not significantly improve the model fit. Thus, Modeltest (Posada & Crandall, 1998) selected the HKY + I +Γ as the preferred model for ML analyses, with the following parameters estimated on the test tree and fixed in the subsequent analysis (given in PAUP command language): Base = (0.3740 0.2308 0.1096); Nst = 2; Tratio = 7.4747; Rates = gamma; Shape = 1.0111; Pinvar = 0.4767.

Identification of and relationships among and within major lineages

All analyses, with one exception (see below), concordantly identify six major clades within the genus *Batrachoseps*, all of which had high support (Fig. 3; throughout, support levels are indicated as range of bootstrap support in MP and LS analyses/posterior probability from Bayesian analysis): sg *Plethopsis* (100%/0.99) and five major lineages within a monophyletic sg *Batrachoseps*: (i) *B. attenuatus* (100%/1.00); (ii) *B. gabrieli* (100%/1.00); (iii) the *B. nigriventris* group (98–100%/1.00), containing *B. nigriventris*, *B. gregarius*, *B. stebbinsi*, *B. simatus*, and three unnamed lineages from the Kern River Canyon, Kern and Tulare counties; (iv) the *B. relictus* group (91–100%/0.96), containing *B. relictus*, *B. kawia*, *B. regius*, and *B. diabolicus*; and (v) the *B. pacificus* group (<50–80%/0.90), containing *B. pacificus*, *B. major major*, and *B. m. aridus* from southern California, as well as *B. gavilanensis*, *B. luciae*, *B. minor*, and *B. incognitus* from central coastal California. In LS

analyses, the *pacificus* group is not monophyletic. Instead, *B. gavilanensis* appears as the sister to *B. gabrieli*, although the two are connected by an extremely short internode. Monophyly of the *pacificus* group is found using identical methods of LS analysis when all 233 haplotypes are included.

All methods of analysis also concordantly identify the deepest branching patterns. The basal split can be placed between sg *Plethopsis* (our outgroup) and sg *Batrachoseps* in all analyses, as expected based on previous results (e.g. Jockusch, 1996; Jackman *et al*., 1997). Within sg *Batrachoseps*, the basal split is always between *B. attenuatus* and the remainder of the subgenus, with moderate to high (57–87%/0.84) support. The *gabrieli* and *pacificus* groups also form a clade in all analyses (51–86%/0.90 support). The relationship between the *nigriventris*, *relictus* and *pacificus* + *gabrieli* clades is less well resolved. All three possible resolutions are found, and support for the favoured resolution is always low (Fig. 3). On average, the subgenera are 25.4% diverged, while the range of average divergences between major clades within the subgenus *Batrachoseps* is from 14.0% (between the *pacificus* and *gabrieli* groups) to 19.2% (between the *attenuatus* and *relictus* groups). Numerous well supported clades with substantial geographical structuring occur within each of the major groups; these are discussed by group below.

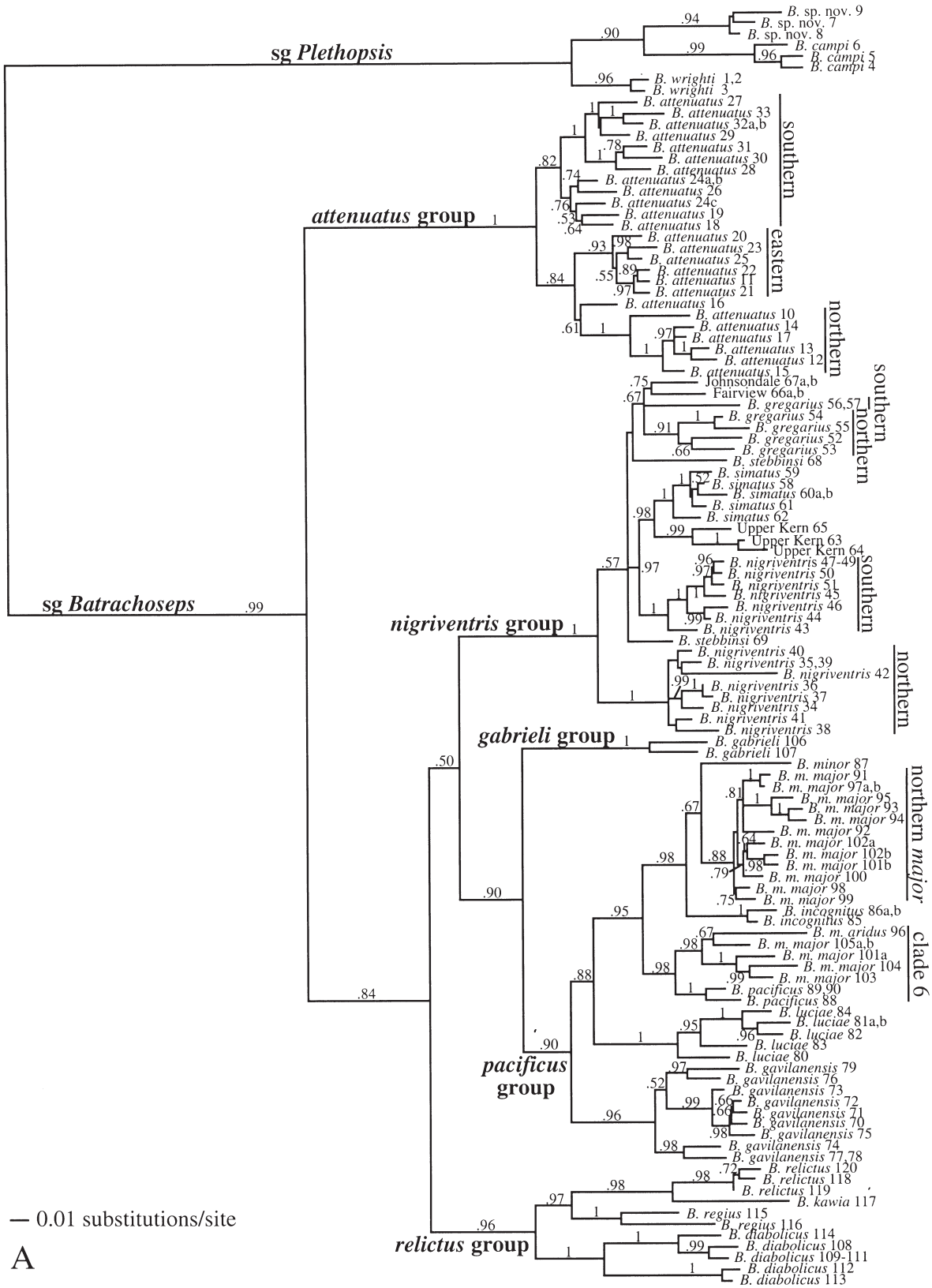
The subgenus Plethopsis

Of the 11 haplotypes identified in sg *Plethopsis*, eight were retained in the comprehensive analyses. The levels of divergence among species (average K2P of 11.4–11.8%) are similar to the deepest levels of divergence found within some of the major clades in sg *Batrachoseps*, and monophyly of each of the three species receives high support (89–100%/0.94–0.99; Fig. 3). The maximum divergences detected within species of sg *Plethopsis* are generally low in comparison to those found in the sister group (1.8% in *B. wrighti*, 4.2% in *B. campi* and 3.7% in the Kern Plateau species). Although sampling was less dense than in sg *Batrachoseps*, these lower divergences are probably not artifacts of inadequate sampling, at least in *B. campi* and the Kern Plateau species, where the most geographically and allozymically divergent populations were included. Relationships among the three species of *Plethopsis* are not concordantly resolved, with equal-weights MP favouring a sister group relationship between the Kern Plateau species and *B. wrighti* (65% support), but other methods favouring a sister group relationship between *B. campi* and the Kern Plateau species (53–67%/0.90 support; Fig. 3). These last two species, separated from each other by the dry Owens Valley, are the only *Batrachoseps* to occur east of the crest of the Sierra Nevada.

The attenuatus group

The *attenuatus* group contains 51 haplotypes in the complete data set, of which 25 remained after pruning. All analyses identify three major lineages within *B. attenuatus* (Figs 3,4A): a southern clade (72–100%/0.82 support, maximum divergence 7.1%), extending from the northern edge of Monterey Bay to Marin Co., north of San Francisco Bay (localities 18, 19, 24 and 26–33); a northern clade (94–100%/1.00 support, maximum divergence 6.0%), extending from Sonoma Co. to Oregon (localities 10, 12–15 and 17); and an eastern clade (83–94%/0.93 support, maximum divergence 4.4%), found in the foothills of the Sierra Nevada and from Tehama County to east of San Francisco Bay along the eastern borders of the Coast Ranges (localities 11, 20–23 and 25). One haplotype, from Bodega Bay (locality 16) on the Pacific Coast in Sonoma Co., was not consistently placed into any of these clades; it may be the sister lineage to all other *attenuatus* (weighted MP), to the northern clade (equal-weights MP, ML), or to the eastern clade (LS). The southern clade differs by an average of 9.6% from the northern clade and 8.1% from the eastern clade. The northern and eastern clades differ from each other by an average of 7.6%. The northern and eastern

Figure 3. Results of phylogenetic analyses on 114 *Batrachoseps* haplotypes. Numbers after taxon names identify haplotype locality as in Table 2. A. Single most likely tree (ln likelihood = –11831.19) found in likelihood analysis under HKY + I + G model with parameters as described in the text. Numbers on branches or adjacent to nodes are posterior probabilities that were ≥0.50. B. Strict consensus of 352 trees of length 2340 (all characters included) identified in maximum parsimony analyses using equal weights. These trees have a consistency index of 0.245 and a retention index of 0.760. Numbers above branches are bootstrap values > 50%, and numbers below branches are decay indices. C. Major lineages found in weighted MP analysis, based on strict consensus of 864 trees found initially and additional MP trees found in decay index searches; these trees are of length 5632 with a consistency index of 0.335 and a retention index of 0.820. Numbers before slash are bootstrap values > 50%, and numbers after slash are decay indices. D. Single best tree (length = 57.519) found in least squares analysis using LogDet distances and including rate variation. The single best tree (length 34.105) found without rate variation is identical to this one except in the branches marked with an asterisk (*). Numbers to the left and right of the slash are bootstrap support from analyses with and without rate variation, respectively. Only bootstrap values >50% are shown.


sg Plethopsis
sg Batrachoseps
attenuatus group
nigriventris group
gabrieli group
pacificus group
relictus group
B. sp. nov. 9
B. sp. nov. 7
B. sp. nov. 8
B. campi 6
B. campi 5
B. campi 4
B. wrighti 1,2
B. wrighti 3
B. attenuatus 27
B. attenuatus 33
B. attenuatus 32a,b
B. attenuatus 29
B. attenuatus 31
B. attenuatus 30
B. attenuatus 28
B. attenuatus 24a,b
B. attenuatus 26
B. attenuatus 24c
B. attenuatus 19
B. attenuatus 18
B. attenuatus 20
B. attenuatus 23
B. attenuatus 25
B. attenuatus 22
B. attenuatus 11
B. attenuatus 21
B. attenuatus 16
B. attenuatus 10
B. attenuatus 14
B. attenuatus 17
B. attenuatus 13
B. attenuatus 12
B. attenuatus 15
Johnsondale 67a,b
Fairview 66a,b
B. gregarius 56,57
B. gregarius 54
B. gregarius 55
B. gregarius 52
B. gregarius 53
B. stebbinsi 68
B. simatus 59
B. simatus 58
B. simatus 60a,b
B. simatus 61
B. simatus 62
Upper Kern 65
Upper Kern 63
Upper Kern 64
B. nigriventris 47-49
B. nigriventris 50
B. nigriventris 51
B. nigriventris 45
B. nigriventris 46
B. nigriventris 44
B. nigriventris 43
B. stebbinsi 69
B. nigriventris 40
B. nigriventris 35,39
B. nigriventris 42
B. nigriventris 36
B. nigriventris 37
B. nigriventris 34
B. nigriventris 41
B. nigriventris 38
B. gabrieli 106
B. gabrieli 107
B. minor 87
B. m. major 91
B. m. major 97a,b
B. m. major 95
B. m. major 93
B. m. major 94
B. m. major 92
B. m. major 102a
B. m. major 102b
B. m. major 101b
B. m. major 100
B. m. major 98
B. m. major 99
B. incognitus 86a,b
B. incognitus 85
B. m. gridus 96
B. m. major 105a,b
B. m. major 101a
B. m. major 104
B. m. major 103
B. pacificus 89,90
B. pacificus 88
B. luciae 84
B. luciae 81a,b
B. luciae 82
B. luciae 83
B. luciae 80
B. gavilanensis 79
B. gavilanensis 76
B. gavilanensis 73
B. gavilanensis 72
B. gavilanensis 71
B. gavilanensis 70
B. gavilanensis 75
B. gavilanensis 74
B. gavilanensis 77,78
B. relictus 120
B. relictus 118
B. relictus 119
B. kawia 117
B. regius 115
B. regius 116
B. diabolicus 114
B. diabolicus 108
B. diabolicus 109-111
B. diabolicus 112
B. diabolicus 113
southern
eastern
northern
southern
northern
southern
northern
northern major
clade 6
— 0.01 substitutions/site
A

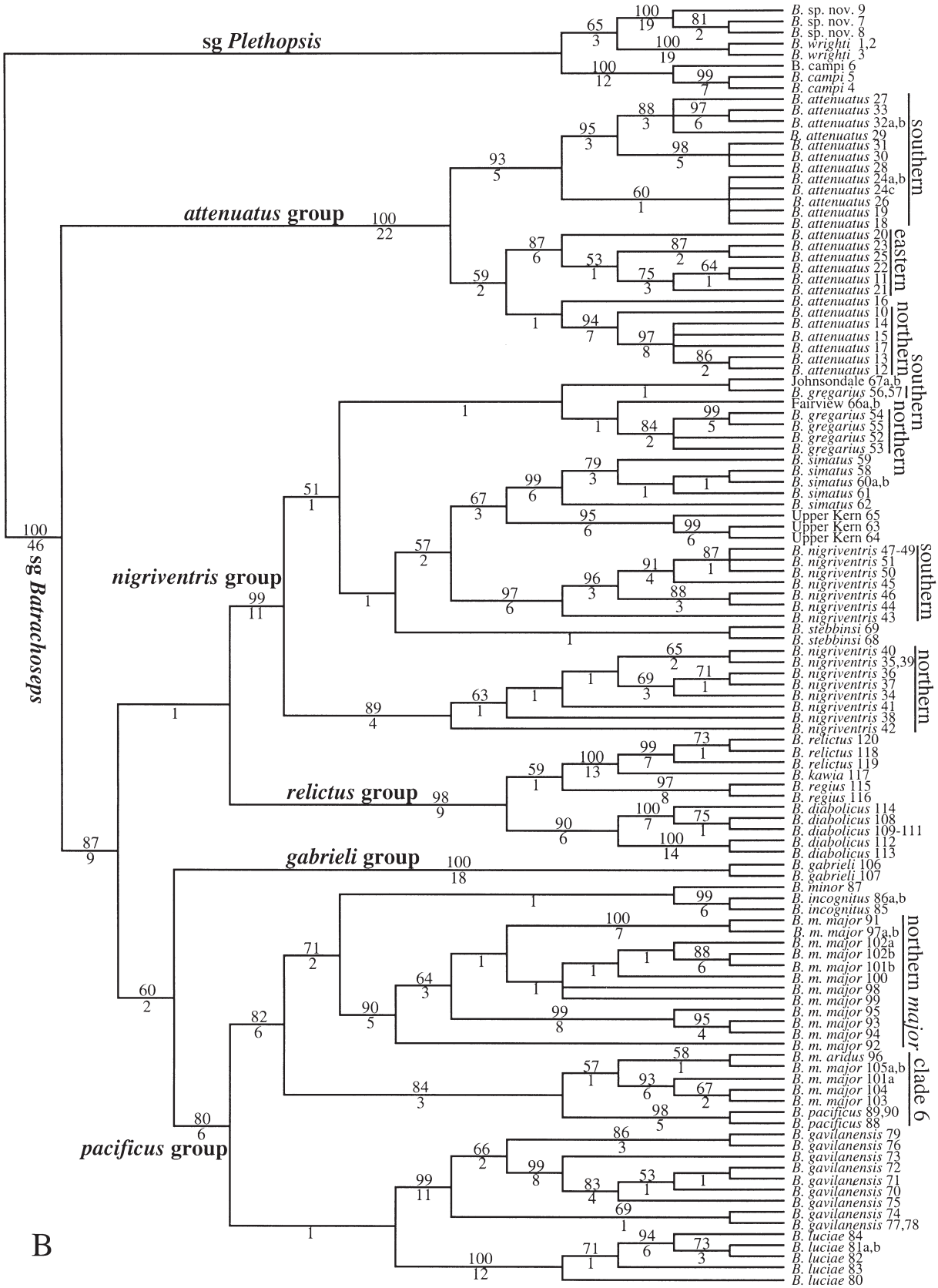


Figure 3. *Continued*

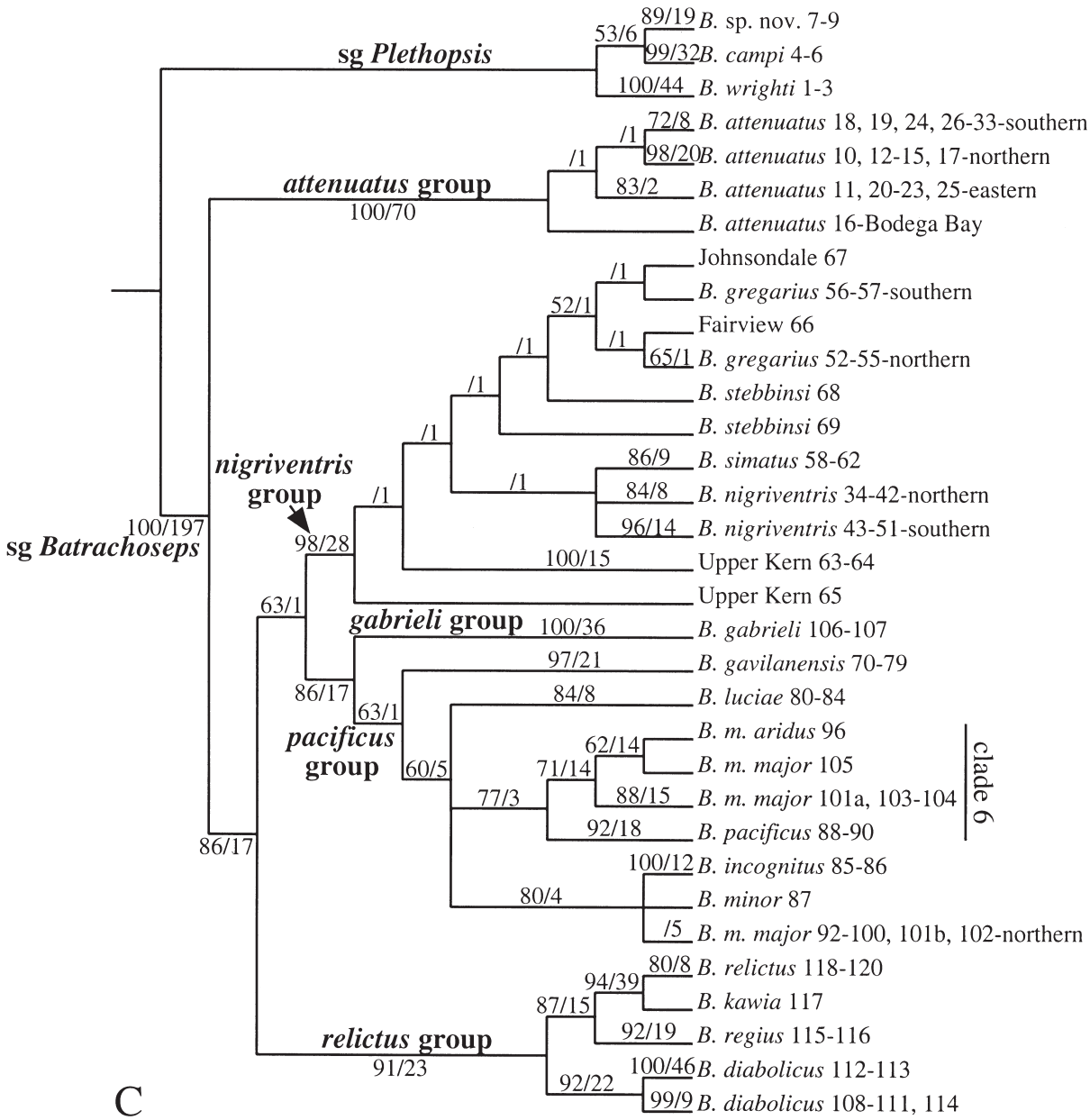


Figure 3. *Continued*

clades together with the Bodega Bay haplotype form a monophyletic group in all analyses except weighted MP, although this relationship is not strongly supported (59–65%/0.84). Substantial phylogeographic structure is found within each of these groups, and many of the lower level relationships are strongly supported (Fig. 3). *Batrachoseps attenuatus* appears to be continuously distributed throughout Marin and Sonoma counties, the region in which all three clades have been found (e.g. localities 14–20 and 24; Fig. 4A).

In general, the 1.5% rule eliminated only haplotypes from the geographical vicinity of the retained haplotype, but there were two significant exceptions. The Sierran populations of *B. attenuatus* are relatively undifferentiated (maximum divergence of 1.1% across ≈ 150 km) and the included individual (23) is deeply nested within the eastern clade as the sister to a haplotype from the eastern edge of San Francisco Bay (locality 25; 86–93%/0.98 support). The northernmost populations of the northern clade of *B. attenuatus*, ranging from Humboldt Co. north to Oregon, are also very similar (maximum divergence of 1.3% over >100 km). The single representative included (10) differs by an average of 5.6% from its well supported

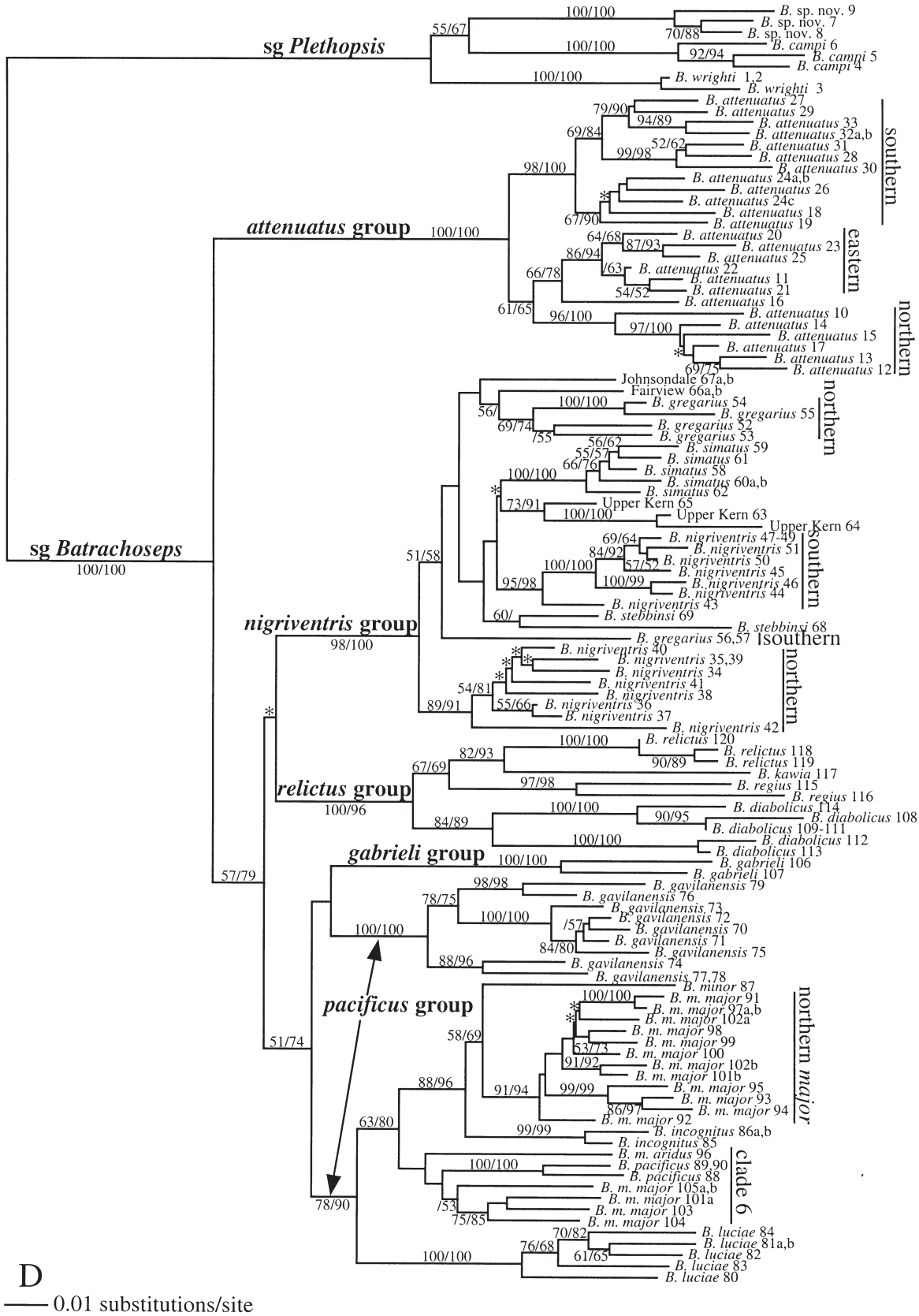


Figure 3. *Continued*

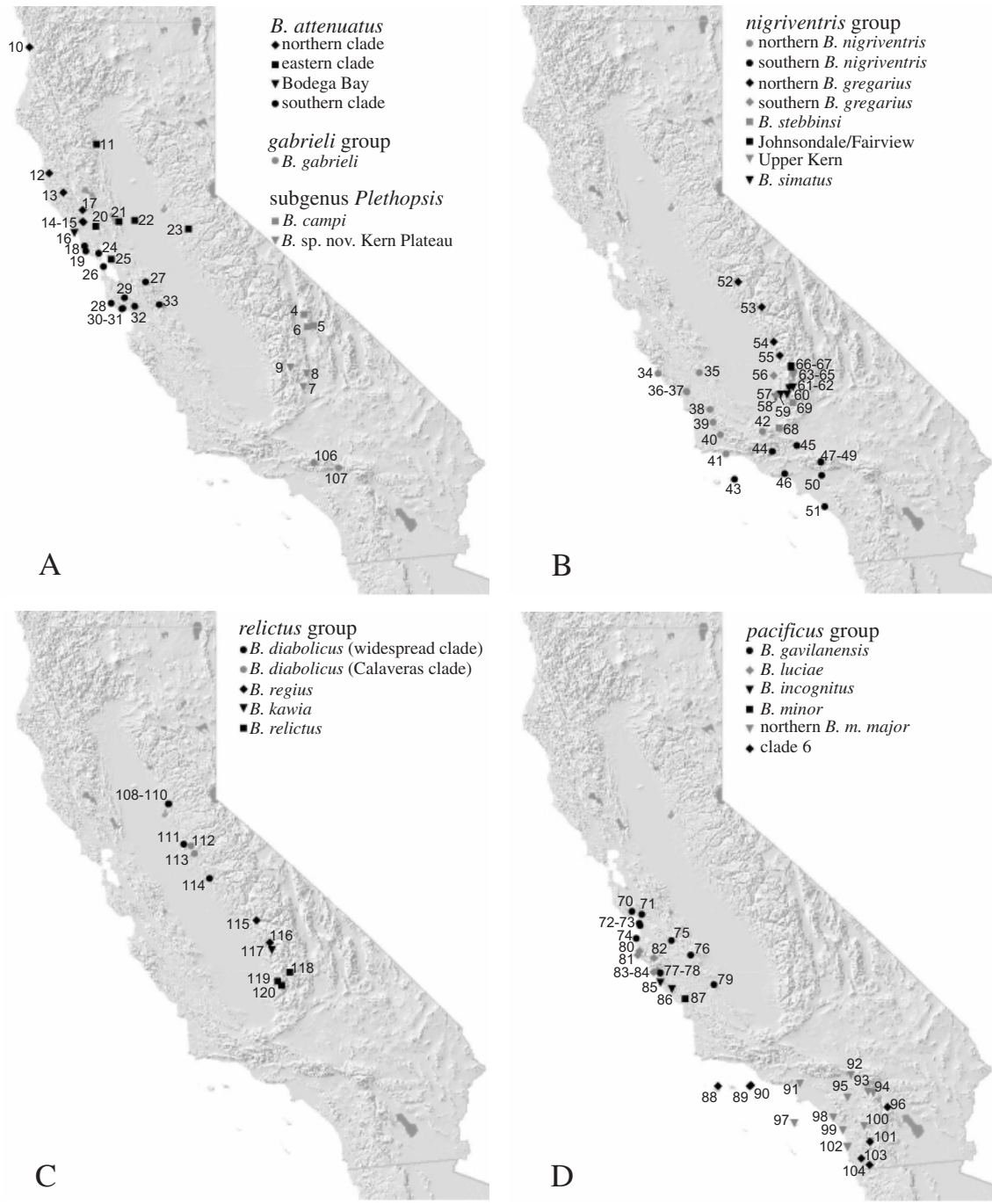


Figure 4. Distributions of haplotypes included in the comprehensive phylogenetic analyses, identified by group and by lineages within each major group. Numbers identify localities as in Table 2. A. *B. attenuatus*, *B. gabrieli* and California species of *Plethopsis*. B. the *nigriventris* group. C. the *relictus* group. D. the *pacificus* group.

(91–100%/1.00) sister group, which contains all other members of the northern clade.

The nigriventris group

The *nigriventris* group contains all representatives of the named species *B. nigriventris*, *B. gregarius*, *B. simatus*, and *B. stebbinsi*, as well as mtDNA lineages representing undescribed taxa in the Kern River Canyon region. Of the 51 haplotypes assignable to the *nigriventris* group, 32 were sufficiently differentiated to be included in the pruned data set. Grouping these haplotypes into the largest clades that were consis-

tently identified and well supported across analyses resulted in the identification of 10 lineages (Figs 3,4B), five of which are represented by a single haplotype. The 10 lineages correspond to (i) northern *B. nigriventris* (84–91%/1.00 support; localities 34–42, extending from northern San Luis Obispo and southern Monterey counties as far south as Santa Barbara and western Ventura counties); (ii) southern *B. nigriventris* (95–98%/1.00 support, localities 43–51 in eastern Ventura, Los Angeles and Orange counties, and including the sample from Santa Cruz Island); (iii) northern *B. gregarius* (65–84%/0.91 support, localities 52–55 extending along the foothills of the Sierra Nevada from just south of Yosemite National Park at least to the Tule River, Tulare Co.); (iv) southern *B. gregarius* (localities 56–57 farther south in Tulare and Kern counties); two lineages within *B. stebbinsi* – (v) northern (locality 69) and (vi) southern Kern Co. (locality 68); (vii) *B. simatus* (86–100%/1.00 support, localities 58–62 in the lower Kern River Canyon); and three additional lineages in the upper Kern River Canyon referred to as (viii) Upper Kern (56–95%/0.99 support; localities 63–65), (ix) Fairview (locality 66), and (x) Johnsondale (locality 67). All multisample lineages except Upper Kern are monophyletic in all analyses. Weighted MP splits the Upper Kern haplotypes into two clades (average divergence 4.0%), which form sequential branches at the base of the *nigriventris* group. Maximum differentiation within the lineages represented by multiple haplotypes ranged from 3.0% in *B. simatus* to 7.3% in northern *B. nigriventris*. Relationships among the 10 *nigriventris* group lineages are not concordantly resolved in different analyses. No clade containing multiple lineages occurred in all five analyses or received >70% bootstrap support in any analysis (although several relationships had high posterior probabilities). Three higher level clades occurred in four of the five analyses, including a basal split between northern *B. nigriventris* and the remainder of the *nigriventris* group (Fig. 3; in all analyses except weighted MP) and a clade containing *B. simatus*, Upper Kern, and southern *B. nigriventris* (in all analyses except weighted MP; posterior probability of 0.97).

Of the four named species in the *nigriventris* group, only the monophyly of *B. simatus* was consistently supported by the cytochrome *b* data (Fig. 3). Monophyly of *B. stebbinsi* was found only in the LS and equal-weights MP analyses. The two sampled populations, which differ by 5.7%, are morphologically similar and distinguished from most other members of the *nigriventris* group by their robust morphology. In weighted MP and ML, the southern *stebbinsi* haplotype was the sister group to *B. gregarius* + Johnsondale + Fairview, while the position of the northern *stebbinsi* haplotype varied. *Batrachoseps gregarius* and *B. nigriventris* each contain two well supported clades of mtDNA haplotypes. The two *gregarius* lineages differ by an average of 8.1% and the two *nigriventris* lineages by an average of 8.2%. Monophyly of the two *gregarius* lineages is never recovered. A monophyletic *B. nigriventris* was included among the best trees only in the weighted MP analysis. It is noteworthy that the southern *B. nigriventris* clade contains the Santa Cruz Island population (locality 43), which differs by an average of 3.8% from mainland representatives of this clade. Populations of northern *B. nigriventris* occur on the mainland immediately to the north (6.1% divergence between 41 and 43), while southern *B. nigriventris* occupies the mainland to the east of Santa Cruz Island (Fig. 4B).

The differentiation detected within the Kern River Canyon, at the southern end of the Sierra Nevada, is particularly striking. Habitat for salamanders in this region is restricted to more mesic spots along the sides of the generally arid canyon, and four distinct lineages in the *nigriventris* group occur along the ≈ 100 km of river where salamanders have been found (Figs 3 and 4B). Minimum average sequence divergence among these four lineages is 5.0% between the geographically close Johnsondale and Fairview lineages; maximum average divergence is 8.1% between the Upper Kern and Johnsondale lineages. The Fairview lineage was previously suggested to differ from *B. simatus* based on morphology (Brame & Murray, 1968; average sequence divergence 6.7%), and the Johnsondale lineage was unknown prior to this study, but is also morphologically differentiated (somewhat larger and more robust than the Fairview animals). The four Kern Canyon lineages never form a monophyletic group, although pairs of these lineages cluster in some analyses (Fig. 3). The Johnsondale and Fairview lineages approach each other within a few hundred meters and are monophyletic in ML analyses (posterior probability of 0.75), but otherwise paraphyletic with respect to one (LS) or both (MP) lineages of *B. gregarius*. Lower (*B. simatus*) and Upper Kern populations form a monophyletic group (average divergence 6.6%) in analyses using equal-weights MP, ML (posterior probability of 0.98), and LS with rate variation. Representatives of the Upper Kern and Fairview lineages (average divergence 7.2%) were found in sympatry at locality 64.

Surprisingly, the *B. simatus* (Lower Kern) clade (86–100%/1.00 support) contains mtDNA haplotypes from two populations that are morphologically distinct and had never been placed in *B. simatus*. A population from Breckenridge Mountain (locality 60), high above the canyon (*c.* 1500 m) but geographically close (<15 km), differs dramatically from *B. simatus* in morphology and osteology as well as in allozymes (D.

Wake, personal observation), yet it is nested within *B. simatus*, which renders its taxonomic status uncertain. The Cottonwood Creek population (locality 58) occurs outside of the mouth of the Kern Canyon, on the floor of the San Joaquin Valley, which has a dramatically different climate (much hotter and drier in summer) than in the deep and shaded canyon. Members of this population are slender and attenuate relative to individuals from inside the canyon, and were thought previously to be assignable to *B. gregarius*.

The relictus group

The *relictus* group includes four named species from the Sierra Nevada: *B. diabolicus*, *B. relictus*, *B. regius* and *B. kawia*; monophyly of each of the three represented by multiple haplotypes is well supported (80–100%/0.98–1.00 support; Fig. 3). Until their description as species (Jockusch *et al*., 1998), all of these were included within one of the 'semispecies' of *B. pacificus*, but the group is clearly distinct from the *pacificus* group. Of the 14 haplotypes sampled, 11 were retained in the pruned data set. Relationships among the four taxa are concordantly resolved in all analyses. The basal split is between *B. diabolicus* and the other taxa (59–87%/0.97 support for monophyly of the latter), with an average divergence of 13.0%, the deepest observed within any major clade. *Batrachoseps kawia* and *B. relictus*, which differ by an average of 8.4%, form a clade (82–100%/0.98) to which *B. regius* is sister (average divergence is 11.6%). Although *B. regius* has a small range, it nonetheless includes two divergent (7.2%) haplotypes, one from the type locality (115) in the Kings River drainage and one from a newly discovered population (116) in the Kaweah River drainage in Sequoia National Park. Differentiation within *B. relictus* is low (maximum divergence 2.2%); the single known individual of this species from the Kern Plateau (locality 120, on the east side of the Kern River) was only 1.2–2.2% diverged from individuals from the Greenhorn Mountains (localities 118–119, on the west side of the river), and may be nested within them (Fig. 3). Within *B. diabolicus*, differentiation is extensive. The basal split (average divergence 9.6%) separates a haplotype clade (localities 112–113; 100%/1.00 support) confined to Calaveras Co. from a clade (99–100%/1.00 support) that ranges from the Merced River, Mariposa Co., north through Calaveras Co. to the American River, Placer Co. (localities 108–111 and 114; Fig. 4C). Differentiation in the first clade of *B. diabolicus* is limited (1.6% maximum). Differentiation in the second clade reaches 4.8%; this clade has a widespread haplotype (locatlities 109–111) in the northern part of its range (Fig. 4C), indicating that it may have undergone a recent range expansion.

The pacificus group

The *pacificus* group was the most intensively sampled with 99 haplotypes obtained from 126 individuals representing 96 populations; 36 haplotypes were included in the pruned data set. Of the five major clades, the *pacificus* group had the weakest support for monophyly (<50–80%/0.90), and in the LS analyses, it was rendered paraphyletic with respect to the *gabrieli* group (bootstrap support <50% for the *B. gabrieli-B. gavilanensis* clade; Fig. 3D). The *pacificus* group contains six lineages, four of which correspond to recently described species from the central Coast Ranges (Jockusch *et al*., 2001; Fig. 4D): *B. gavilanensis* (maximum divergence 7.8%; 97–100%/0.96 support), *B. luciae* (maximum divergence 7.3%; 84–100%/1.00 support), *B. incognitus* (maximum divergence 2.0%; 99–100%/1.00 support) and *B. minor* (only one specimen examined). The other two lineages are restricted to southern California (Fig. 4D). One of these clades corresponds to the 'northern *major*' of Wake & Jockusch (2000) (maximum divergence 5.1%; 90–94%/0.88 support except in weighted MP, <50% support). This clade is geographically cohesive and includes northern and western populations of *B. m. major*. These populations range from the southern slopes of the Santa Monica, San Gabriel and San Bernardino mountains into San Diego Co., extending almost to the Mexican border very near the coast (localities 92–102). The sixth *pacificus* group lineage, which we refer to as clade 6 (8.2% maximum sequence divergence; <50–84%/0.98 support), contains populations from areas peripheral to but bordering on the range of northern *major*. The most widespread of the units within clade 6 is what Wake & Jockusch (2000) called 'southern *major*' (*B. m. major* from central and southern inland San Diego Co., and approaching the coast near the city of San Diego, localities 101, 103–104). Clade 6 also includes *B. m. major* from Mexico (locality 105), *B. m. aridus* (locality 96) and *B. pacificus* (localities 88–90).

Different phylogenetic analyses are largely concordant in their depiction of relationships among the six lineages of the *pacificus* group (Fig. 3). Northern *major* always forms a clade with *B. minor* and *B. incognitus* (5.7–9.7% sequence divergence; 71–96%/0.98 support), although relationships among these three taxa are unresolved. *Batrachoseps minor* is sister either to its geographical neighbour *B. incognitus* or to *B. major*. These three lineages cluster with clade 6 (9.0% average sequence divergence; 63–82%/0.95 support for the larger clade) in all analyses except weighted MP, in which it is equally parsimonious for them to cluster instead with *B. luciae*. The *pacificus* group, exclusive of *B. gavilanensis* also forms a relatively well supported (60–90%/0.88) clade in all analyses except equal-weights MP, in which *B. luciae* and *B. gavila-*

nensis cluster (<50% support). On average, *B. gavilanensis* and the remaining taxa are 12.3% diverged. Phylogeography and differentiation of the central coastal populations were discussed in detail by Jockusch *et al*. (2001), and are not addressed here.

Clade 6 contains populations that are well differentiated from one another morphologically and are geographically widely separated (Fig. 4D; see also Wake & Jockusch, 2000). *Batrachoseps m. aridus* is a morphologically and ecologically distinctive form that is allopatric with respect to other *Batrachoseps* and is reported only from two desert canyons on the eastern edge of the range of northern *major*. *Batrachoseps pacificus* is restricted to the northern Channel Islands, at the western edge of the range of northern *major*. Southern *major* occurs to the south and inland of northern *major*, but is morphologically very similar to it. *Batrachoseps m. major* from the Todos Santos Islands (population 105), off the north-west coast of Baja California, Mexico, also belongs to clade 6. In clade 6, *B. pacificus* was always sister to a clade containing southern *B. m. major* from San Diego Co. (average divergence 6.1%) and the population from Todos Santos Island (average divergence 5.9%). These latter two (which do not differ much in morphology) differ by an average of 5.3%. The placement of *B. m. aridus* varied; it is sister to the Todos Santos *major* in MP and ML analyses, but basal in the clade in LS analyses. Divergence of *B. m. aridus* to southern *major* averages 7.5%, and to *pacificus* it averages 7.3%. Relationships in clade 6 generally received <70% bootstrap support, but some posterior probabilities are high (Fig. 3).

The non-monophyly of *B. major* mtDNA haplotypes led us to sample extensively within its range. We identified 46 northern *major* haplotypes, of which 12 (11 selected by the 1.5% rule, plus the Catalina Island sample) were included in phylogenetic analyses. Many of the internal branches in northern *major* are very short, and the inferred relationships among included haplotypes differed in different methods of analysis (Fig. 3). In all treatments, the Catalina Island (locality 97) haplotype is sister to a haplotype from the city of Los Angeles (locality 91; 100%/1.00 support), from which it differed by <1.0% (see also Wake & Jockusch, 2000). Fifteen Mexican and southern *major* haplotypes were found, of which four were included in the phylogenetic analyses. Haplotypes belonging to northern and southern *major* lineages, which differ by an average of 9.0%, were found in sympatry in northern San Diego County (at locality 101), and in near sympatry in the city of San Diego (not included in full analysis).

The gabrieli group

Seven *B. gabrieli* haplotypes were identified. These fell into two shallowly diverged (<1.5%) clades, which differed from each other by an average of 5.2%. Monophyly of the two *B. gabrieli* haplotypes included in extensive analyses was always strongly supported (100%/1.00). In analyses including all seven haplotypes, specimens from the type locality in the San Gabriel Mountains of southern California cluster with a haplotype from ≈ 1 km away (locality 106). The other clade is more broadly distributed to the east, and it ranges from the southern slopes of Mt. Baldy in the eastern San Gabriel Mountains to the southern slopes of the western San Bernardino Mountains (locality 107).

DISCUSSION

Strong phylogeographic structuring and extensive differentiation of cytochrome *b* are found in *Batrachoseps*. The mtDNA and allozyme data concordantly identify the subgenera *Plethopsis* and *Batrachoseps* as sister taxa (Yanev, 1978, 1980; Jackman *et al*., 1997), and these data are consistent with morphological analyses (Wake, 1989; Jackman *et al*., 1997). The contrast in diversity of the two main clades is notable; sg *Plethopsis* contains only three species (two described) while sg *Batrachoseps* contains 16 described species and several undescribed species. Furthermore, the deepest genetic divisions in sg *Plethopsis* are substantially lower than those in sg *Batrachoseps*. Despite this, the California species of sg *Plethopsis* contrast greatly with *B. (Plethopsis) wrighti* in both morphology and ecology. The California species have a more robust, generalized morphology and are found in arid to desertic habitats, while *B. wrighti* is largely restricted to wet, heavily forested areas of the northern Cascade Mountains of Oregon. The California species of *Plethopsis* also differ in morphology and ecology. Within sg *Batrachoseps*, five major mtDNA clades are identified, whereas the allozyme study recognized only three major groups (Yanev, 1978, 1980). Boundaries of the *attenuatus* and *nigriventris* groups are identical in the two data sets, and in both, *B. attenuatus* is inferred to be sister to the rest of the subgenus. The third allozymically identified group, Yanev's (1980) *B. pacificus* superspecies, is divided at its basal node into the *pacificus* and *relictus* groups in the mtDNA trees. The mtDNA trees do not support the clustering of the *relictus* and *pacificus* groups to the exclusion of the *nigriventris* group. The fifth mtDNA clade resulted from the discovery of *B. gabrieli* (Wake, 1996), which mtDNA data identify as sister to the *pacificus* group.

Sequential outgroups for the genus *Batrachoseps* are the supergenus *Bolitoglossa* and the supergenus *Hydromantes* (Jackman *et al*., 1997). The cladogenetic events separating these taxa are ancient (possibly as early as the late Mesozoic) and the morphological

divergence is substantial, making reconstruction of ancestral traits for *Batrachoseps* difficult. Within the genus as a whole, derived osteological traits are found only in sg *Batrachoseps* (Jackman *et al*., 1997). The undescribed species of *Plethopsis* resembles generalized members of the genera *Pseudoeurycea* (supergenus *Bolitoglossa*) and *Hydromantes* in general morphology; it has a robust habitus with a broad head and short tail. Perhaps such a morphology is ancestral for *Batrachoseps*. However, the presence of only four toes on the hind feet is a character that is unexpected in lineages that have always been robust, as elsewhere in plethodontids, and more generally in salamanders, a reduction in the number of digits is associated with short limbs and elongate bodies. Whatever the ancestral form, homoplasy of morphology is certain to have occurred within the genus because of the relatively slender habitus of *B. wrighti* (within *Plethopsis*, otherwise robust) and the relatively robust habitus of *B. pacificus* and *B. stebbinsi* (within sg *Batrachoseps*, otherwise attenuate).

A striking feature of the mtDNA data is the large number of contact zones identified or inferred between members of the same major clade. Some of these mtDNA contact zones occur in areas that are relatively uniform with respect to allozyme differentiation. This shows that significant discordance exists with respect to population relationships depicted in trees based on the mtDNA and allozyme data (compare Figs 2 and 3; see below and also Jockusch *et al*., 1998, 2001; Wake & Jockusch, 2000). Discordance between mtDNA, which is transmitted exclusively by females, and allozymes, which are encoded by biparentally transmitted markers, can result from incorrect phylogeny inference or from a variety of biological and genealogical processes, including differential lineage sorting, introgression of mtDNA and male-mediated gene flow (Maddison, 1997). Examples of all of these have been well documented in natural populations. *Batrachoseps* stands out for the number of lineages that appear to be affected. Widespread discrepancies between population relationships inferred using the two data sets indicate that some conserved feature(s) of the biology of *Batrachoseps* probably contributes to this pattern. We suggest that the discordance results from a combination of three factors: (i) low vagility causing a general tendency towards genetic fragmentation; (ii) greater philopatry in females than in males, resulting in gene flow being predominantly male-mediated; and (iii) very slow divergence in mate recognition systems.

These three factors can be integrated with biogeographic information to suggest a general scenario for diversification in salamanders. Allopatric divergence is the rule in salamanders (e.g. Good & Wake, 1992; Highton, 1999; Highton & Peabody, 2000), and genetic fragmentation probably begins as soon as a species disperses over a substantial geographical range, especially in areas such as California, which has been subjected to dramatic changes in climate and topography over the tens of millions of years that *Batrachoseps* has been evolving. As species fragment, mtDNA differences between geographically separated units accumulate rapidly because of the high rate of evolution of mtDNA and the greater philopatry and hence isolation of females. Because of the dynamic landscape and cyclical climatic changes (notably major shifts in precipitation), fragments that have begun to diverge and have evolved fixed differences in haplotypes come back into contact. The fate of populations in secondary contact depends on their ability to interbreed and the fitness of hybrid offspring. If mate recognition systems generally diverge slowly, then many genetically distinct populations will merge. Because of male-biased dispersal, merger of nuclear genes will proceed faster than that of mtDNA haplotypes, so, for a time, the mtDNA haplotype patterns will reveal a deeper level of phylogenetic history. Some of the fragments will behave as species when they meet, either because they have undergone significant ecological and morphological evolution or because large amounts of time have passed, leading to an accumulation of differences in mate recognition systems sufficient to prevent interbreeding. The classic ring species complex, *Ensatina*, which has a range similar to that of *Batrachoseps* in California, illustrates many stages in this process (Jackman & Wake, 1994; Wake, 1997; Wake & Schneider, 1998). The salamandrid *Taricha* is another California salamander that shows evidence of fragmentation and differentiation (Tan & Wake, 1995), and additional sampling may disclose secondary contacts in this group as well. A similar scenario to explain discordance between allozyme and mtDNA markers was recently proposed for *Desmognathus orestes* in the southern Appalachians (Mead *et al*., 2001). Below we discuss how the elements of this scenario apply to the divergence of slender salamanders, the most speciose salamander group in the American West.

Genetic fragmentation

Patterns of deep genetic divergence across small geographical distances are relatively common in plethodontids living in geographically old areas (e.g. *Bolitoglossa subpalmata* group, García-París *et al*., 2000a; *Bolitoglossa mexicana*, García-París *et al*., 2000b; *Desmognathus ochrophaeus* complex, Tilley & Mahoney, 1996; *Ensatina*, Wake, 1997; *Eurycea*, Chippendale *et al*., 2000; *Oedipina*, *Nototriton*, and *Cryptotriton*, García-París & Wake, 2000; *Plethodon*, Highton & Peabody, 2000), and *Batrachoseps* fits this pattern (Jockusch *et al*., 2001).

Genetic fragmentation in *Batrachoseps* has been accentuated by the great age of the clade and the low vagility of these salamanders. Fossil trackways are known from Miocene deposits in the Sierra Nevada (Peabody, 1959), and allozyme data suggest early Tertiary dates for the divergence of the major clades within sg *Batrachoseps* (Yanev, 1980). Low vagility is the prelude to the pattern seen in *Batrachoseps* of isolation by distance over relatively small distances (Wake & Jockusch, 2000; Jockusch *et al*., 2001). The maximum observed movements in mark–recapture studies of *B. attenuatus* and *B. major* are of the order of 5 m over a period of months or years (Hendrickson, 1954; Cunningham, 1960; Maiorana, 1974). The effects of low vagility are compounded by the geologically complex history of California, which has involved substantial geological fragmentation and differential movement of parts of the landscape (Atwater, 1989). Major plate tectonic movements and changes in sea level have affected *Batrachoseps* more than most other vertebrates studied in California to date (e.g. Jackman & Wake, 1994; Tan & Wake, 1995; Rodríguez-Robles *et al.*, 1999; Macey *et al*., 2001; Maldonado *et al.*, 2001; Rodríguez-Robles *et al.*, 2001), most probably because it is both older and more sedentary.

Secondary contact and sex-biased dispersal

When range shifts mediated by geological, climatic and other environmental changes lead to geographical contact between lineages belonging to the same major clade of *Batrachoseps*, the lineages frequently are insufficiently isolated reproductively to maintain their independence, so gene flow and genetic admixture result in phylogenetic reticulation. The mtDNA data confirm that contact between well differentiated lineages within the major species groups is common. Twelve mtDNA contact zones, with levels of sequence divergence ranging from 5.0 to 12.4%, have been identified within the five major clades of sg *Batrachoseps*. Only two within-group contact zones were previously identified using morphological or allozyme data, between *B. nigriventris* and *B. stebbinsi*, which co-occur in the Tehachapi Mountains in southern Kern Co. (Wake & Jockusch, 2000), and between *B. luciae* and *B. gavilanensis* along ≈ 80 km in Monterey Co. (Yanev, 1978; Jockusch *et al*., 2001). These two contact zones are also apparent in the mtDNA data, as are 10 additional intragroup contact zones. Sympatry between differentiated mtDNA lineages belonging to the same major clade has been pinpointed in two instances, between two *nigriventris* group lineages (Fairview and Upper Kern; Fig. 4B) in the upper Kern River Canyon and between two lineages of *B. major* (northern and southern) in inland northern San Diego Co. (Fig. 4D). Differentiated lineages closely approach each other in eight more instances in areas that generally do not correspond to present day barriers to slender salamander dispersal, and contacts almost certainly will be found with more intensive surveys. The three major clades of *attenuatus* are expected to meet in Marin and/or Sonoma Co. (Fig. 4A). Three additional contact zones are expected in the *nigriventris* group (Fig. 4B): between northern and southern *B. nigriventris* (in the Santa Ynez or Santa Monica Mountains of Santa Barbara and Ventura counties; see also Wake & Jockusch, 2000); between northern and southern *B. gregarius* lineages in southern Tulare Co. (see also Jockusch *et al*., 1998); and between the Johnsondale and Fairview lineages, which occur within a few hundred meters of each other in the upper Kern Canyon, Tulare Co. Two *relictus* group contact zones are expected: one between *B. regius* and *B. kawia* in the vicinity of Sequoia National Park and one between divergent clades of *B. diabolicus*, which occur within 15 km of each other in Calaveras Co. (Fig. 4C). Two *pacificus* group members, *B. luciae* and *B. incognitus*, occur within 25 km of each other in southcoastal Monterey Co. (Fig. 4D). Finally, the two *gabrieli* clades are separated by <15 km in the San Gabriel Mountains.

Allozyme data are available from populations spanning six of 12 within-group mtDNA contact zones in *Batrachoseps* (Yanev, 1978, 1980; Jockusch *et al*., 1998, 2001; Wake & Jockusch, 2000). In only one case, contact between western *B. stebbinsi* and northern *B. nigriventris*, which differ by 10.6%, are the lineages completely isolated in both allozyme and mtDNA analyses. In the other five cases, some admixture occurs, or is inferred to have taken place in the past, as evidenced by nuclear gene flow across the mtDNA contact zone. Although sex-specific patterns of dispersal have not been studied in *Batrachoseps*, in the plethodontid *Ensatina eschscholtzii* males move more frequently than females over short distances and also move the longest absolute distances (Staub *et al.*, 1995). These sex-based differences in movement are sufficient to produce a pattern of male-biased gene flow (Jackman & Wake, 1994). The molecular patterns recorded for *Batrachoseps* are consistent with this hypothesis. Similar patterns of deep differentiation in mtDNA combined with less differentiation in biparentally transmitted markers or markers transmitted only through males, have been documented in species in which behavioural data also confirm that females are more philopatric (Melnick & Hoelzer, 1992).

Male-mediated gene flow, rather than introgression of mtDNA, is supported as the cause of discordance between data sets by the concordant identification of lineages in three cases, and by the geographical distribution of mtDNA haplotype clades in one. In *B.*

luciae and *B. gavilanensis*, lineages are concordantly identified and the relationships are concordantly inferred (Figs 2 and 3), but patterns of allelic variation suggest limited gene flow between the two (Jockusch *et al*., 2001). In the contact zones between northern and southern *B. nigriventris* and between *B. luciae* and *B. incognitus*, lineages are also concordantly identified, but relationships hypothesized from analyses of allozyme and mtDNA data sets differ (Fig. 2 vs. Fig. 3). In each case, allozyme data indicate that geographically neighbouring taxa are more closely related to each other than to geographically distant taxa, which have more closely related mtDNA haplotypes. In two cases, northern and southern *gregarius* and northern and southern *major*, the clustering of populations into lineages is not concordant between the allozyme and mtDNA data. The *gregarius* patterns are compatible with either male-mediated gene flow or introgression of mtDNA. In the case of *major*, in which no break occurs in the allozymes despite the existence of two deeply differentiated (9.0%) non-sister mtDNA clades, the distribution of these and related mtDNA lineages supports the hypothesis of male-mediated gene flow (Wake & Jockusch, 2000).

Evolution of reproductive isolation

Contact zones occur not only within species groups, but also between them. At least 10 pairs of lineages, involving representatives of all major clades in sg *Batrachoseps* (Campbell, 1931; Lowe & Zweifel, 1951; Brame & Murray, 1968; Yanev, 1978, 1980; Jockusch *et al*., 1998, 2001; Wake & Jockusch, 2000), are involved in what we infer to be secondary contacts. When contact occurs between lineages belonging to different major mtDNA clades, sympatry is found, which can range from geographically narrow (e.g. *B. attenuatus* and *B. gavilanensis* in Santa Cruz Co., *B. nigriventris* and *B. gavilanensis* in south-eastern Monterey Co.; Fig. 1), to more extensive (e.g. *B. nigriventris* and *B. major* in southern California), without evidence of gene flow. We do not know of a single instance of hybridization across major groups in *Batrachoseps*. This indicates that reproductive isolation has evolved. Although the five major groups in sg *Batrachoseps* are completely isolated at present, there may have been some reticulation among them in the past. Such reticulation could result in non-concordance of allozyme and mtDNA phylogenies at deeper levels within sg *Batrachoseps*. A possible example is the sister group relationship between the *relictus* and *pacificus* groups, to the exclusion of the *B. nigriventris* group, in the allozyme data, a relationship not supported by the mtDNA data. However, we note that the cytochrome *b* data do not support any relationships at this level strongly, so testing hypotheses of ancient reticulation requires additional data.

Comparisons of within- and between-group contact zones suggest that the outcome of contact between divergent taxa is correlated with degree of genetic differentiation. With the exception of the *B. nigriventris–B. stebbinsi* contact zone, where the taxa are strongly morphologically differentiated, gene flow occurs between units that differ by 8.1–12.4%, although the units that differ by 11.1% or more (e.g. *B. luciae* and *B. gavilanensis*) may be maintaining their integrity despite low levels of ongoing or recent gene flow. MtDNA divergence between the sympatric but reproductively isolated major clades is at least 14.0%. Because the threshold level at which divergence in mtDNA is accompanied by loss of gene flow appears to be high, loss of reproductive compatibility must generally be very slow to evolve in *Batrachoseps*. Unfortunately, nothing is known about mate recognition in *Batrachoseps* and courtship is undescribed. In other plethodontid salamanders, complex courtship behaviour and pheromonal cues are both involved in mating (Arnold, 1977; Houck & Reagan, 1990). Behavioural studies of diverse plethodontids suggest that some degree of sexual isolation is the norm among allozymically differentiated allopatric populations (reviewed in Arnold *et al.*, 1993). Interpopulational variation in pheromone composition has also been documented (Rollman *et al.*, 2000). In the *Desmognathus ochrophaeus* complex, sexual isolation increases with increasing geographical distances (Tilley *et al.*, 1990). These data all support a model of gradual divergence in mate recognition systems in allopatry, but are not sufficient to predict the outcome of contact between differentiated populations in the field, which may depend both on the degree of sexual isolation and the fitness of hybrid individuals.

It is unclear what role morphological divergence between lineages plays in the evolution of reproductive isolation in *Batrachoseps*. In general we take morphological divergence as an indicator of ecological differences as well, but evidence of ecological divergence is relatively weak. In two instances where morphologically larger and more robust species co-occur with smaller, more slender species, the larger (*B. stebbinsi* and *B. gabrieli*) are associated with rocks and talus while the smaller (*B. nigriventris* is sympatric with both) is found under small surface cover (Wake, 1996). However, no obvious ecological differences separate the larger and more robust *B. major* from the smaller and more slender *B. nigriventris*, although *B. major* does occur in hotter, drier lowland sites for the most part. The two within-group contact zones in which the evidence for limitations on gene flow is strongest are between morphologically differentiated forms (*B. stebbinsi* and northern *B. nigriventris* and

B. luciae and *B. gavilanensis*). However, gene flow might occur even when there are substantial phenotypic differences. For instance we interpreted the close allozymic similarity of *B. m. aridus* and northern *B. m. major*, two forms that are highly distinctive both morphologically and ecologically, as being the result of exchange of genes in the recent past (Wake & Jockusch, 2000). Southern *B. gregarius* is the most attenuate *Batrachoseps* known, and yet it appears to be connected by gene flow to the morphologically more typical northern *B. gregarius*.

Most cases of extensive gene flow across deep mtDNA boundaries occur between morphologically more similar forms (e.g. within *B. nigriventris* and *B. major*; it is possible that this similarity is a result of gene flow). However, morphological similarity does not guarantee resumption of gene flow when genetically differentiated units come into geographical contact, as several of the between-group contact zones (notably involving *B. attenuatus*, *B. gavilanensis*, and *B. nigriventris*) are between species that are so similar morphologically that individuals in the zone of sympatry cannot be reliably distinguished without molecular studies (Yanev, 1978). At some point, genetic divergence is sufficient to guarantee that species coming into secondary contact will no longer be capable of exchanging genes, regardless of the degree of morphological similarity. We might expect rare hybrids in such instances, but we have not found them. Evidence from this study and from our published and unpublished allozyme studies suggests that no genetic exchange takes place beyond an mtDNA divergence of ≈ 14% (data herein) or a Nei genetic distance of ≈ 0.4 (Jockusch *et al*., 2001).

MtDNA and species status

If the apparent conflicts between mtDNA and allozyme data are caused by male-mediated gene flow, then current patterns of interactions among populations are best inferred from allozyme data, while the mtDNA patterns reflect vestiges of a deeper history, as coalescence times for mtDNA will be greatly retarded by female philopatry (Hoelzer, 1997). Consequently, allozyme data will be more informative for delineation of species, and mtDNA data may be positively misleading about species status. One consequence of this complex reticulate history is the possibility of species with para- or even polyphyletic mtDNA, which can result when male-mediated gene flow reconnects lineages that are not sister lineages or when a new species originates from a part of a widespread taxon otherwise held together by gene flow. Non-monophyly of mtDNA is found in *B. nigriventris*, *B. gregarius* and *B. major*. The mtDNA of southern *B. nigriventris* is more closely related to that of *B. stebbinsi*, *B. gregarius*, *B. simatus* and the undescribed Kern River Canyon lineages than it is to the mtDNA of northern *B. nigriventris* (Fig. 3). *Batrachoseps gregarius* is rendered paraphyletic in most analyses by the exclusion of morphologically differentiated taxa in the Upper Kern River Canyon. The mtDNA of southern *B. m. major* is more closely related to that of *B. m. aridus* and *B. pacificus* than to that of northern *B. m. major*, which in turn has mtDNA more closely related to that of *B. minor* and *B. incognitus* (Fig. 3). *Batrachoseps pacificus*, *B. minor* and *B. incognitus* are morphologically differentiated and geographically separated from *B. major* (Figs 1 and 4), providing evidence that they are on independent evolutionary trajectories and thus merit recognition as distinct species under the evolutionary species concept. Furthermore, the species in which mtDNA is not monophyletic do not appear to contain independent evolutionary units, as allozyme data suggest that merger of populations belonging to different mtDNA lineages may be underway (Jockusch *et al*., 1998, 2001; Wake & Jockusch, 2000). These examples highlight the importance of relying on multiple markers reflecting more than the maternal history of populations when making taxonomic decisions, as well as the inadequacy of depicting species relationships in a single non-reticulating tree and the importance of sampling multiple individuals per species when inferring species level relationships.

Although mtDNA data should not be the sole basis for taxonomic decisions, they do raise the possibility that several of the currently recognized species of *Batrachoseps* are composed of multiple species. In particular, the taxonomic status of divergent, putatively conspecific mtDNA lineages that occur in close geographical proximity requires further investigation in those cases where allozyme data are not available. This geographical apposition of differentiated haplotypes is striking in *B. diabolicus* in Calaveras Co. (localities 111–113; Fig. 4C), as well as in *B. gabrieli* (localities 106–107; Fig. 4A), where the two identified lineages also show subtle differences in colouration. In both of these cases, the geographical proximity involves one clade that is relatively widespread and shallowly differentiated, suggesting that that clade may have spread recently into the present contact zone. The appropriate taxonomic status of the individual from population 106, which is most closely related to topotypic *B. regius*, is also unresolved. The two haplotypes that we assign to *B. regius* are greatly diverged (7.2%), although they are separated by <50 km. Finally, determination of the number of species in the Kern River region is uncertain. The haplotypes found there belong to at least two and possibly as many as four different clades, one of which is itself deeply differentiated. All populations from the lower Kern River Canyon are assignable to *B. simatus*,

but the populations in the upper Kern River Canyon, which are currently not assigned to any species, may belong to as many as three undescribed species. We do not recommend any taxonomic revision at this point, as data from biparentally transmitted markers might indicate that these lineages are not as isolated as their mtDNA suggests.

Historical biogeography

The genus *Batrachoseps* is old, and reconstruction of its historical biogeography requires detailed consideration of the geological history of the west coastal region of the North American continent over the last half of the Tertiary as well as all of the Quaternary. Such an analysis is beyond the scope of this paper. However, broad patterns of distribution will be considered briefly. Both Yanev (1980) and Hendrickson (1986) have argued that *Batrachoseps* has been in the American West for a long time. There is strong evidence that *Batrachoseps* is the sister taxon of the large supergenus *Bolitoglossa*, which includes all tropical plethodontids (Jackman *et al*., 1997), and it is reasonable to conclude that vicariant events associated with the extended San Andreas Fault system, which is intermittently continuous between northern California and central-western Mexico, may have been responsible for the original separation of these major clades (Hendrickson, 1986; Wake, 1987). Weak support for this hypothesis comes from unsubstantiated accounts of *Batrachoseps* in Jalisco, Mexico (Gadow, 1905), and near La Paz, Baja California Sur, Mexico (Lockington, 1880). Although voucher specimens for both records exist, extensive field work has failed to uncover any additional specimens. Verification of these records would provide evidence that *Batrachoseps* originated in the south, as these populations might represent remnants of an ancestral stock stranded in fragments of the earth's crust that have remained far to the south of present-day distributional limits. Given the very deep molecular divergences within both *Batrachoseps* and the supergenus *Bolitoglossa*, the separation is likely to have been very early, possibly in the late Mesozoic (Miocene fossil trackways from the Sierra Nevada are clearly modern *Batrachoseps*; Peabody, 1959).

The first event in the history of the genus was divergence of sg *Plethopsis* from sg *Batrachoseps*. Today there is a clear geographical separation between the northern and inland *Plethopsis* and the more coastal and southern sg *Batrachoseps*. Possibly, the uplift of the Sierra Nevada system, which was taking place throughout the Tertiary, was a factor in the vicariant divergence of these clades. *Plethopsis* displays what appears to be a relict distribution and includes three species that live in the most extreme environments: *B. wrighti* in the most northern and coldest region; *B. campi* in the hot, extremely arid and inhospitable Inyo Mountains and borders of Saline Valley; and an as yet undescribed species at the highest elevations within the genus, on the harsh, mainly arid Kern Plateau and borders of Owens Valley. No member of sg *Batrachoseps* occurs in such peripheral habitats (one population of *B. relictus* is known from the uplands immediately east of the Kern River), and only *B. major aridus* approaches the extreme ecological conditions encountered by *Plethopsis*.

Within sg *Batrachoseps*, the deepest split is between the most northern lineage, *B. attenuatus*, and its more southern sister group, which contains four major clades. The *attenuatus* lineage had probably penetrated far to the north by the early Miocene (for discussion of dating see Yanev, 1980; Jockusch *et al*., 2001). Subsequently, the *relictus* lineage became established in the Sierra Nevada, and the *pacificus* and *gabrieli* lineages split. The *pacificus* group has fragmented in the lowlands, while the *gabrieli* group now has an extremely restricted distribution in the eastern San Gabriel and western San Bernardino mountains of southern California. It occurs just south of the ancient San Andreas Fault zone, which may have been a factor in its isolation and differentiation. The *nigriventris* lineage has differentiated in the southern inland area, especially in the southern Sierra Nevada, the Tehachapi Mountains, and adjoining areas.

Species of *Batrachoseps* are largely parapatrically distributed, and areas of sympatry are typically characterized by such unfavourable ecological conditions that populations are low density and widely scattered. Such conditions apply to the geographical overlap of *B. attenuatus* and *B. diabolicus*, *B. attenuatus* and *B. gavilanensis*, and *B. gavilanensis* and *B. nigriventris* (Fig. 1). Thus, we postulate that when well marked species (such as those belonging to different major lineages) meet, they replace one another geographically, possibly because of ecological interactions that favour residents. This preemptive occupancy of space is perhaps a general phenomenon associated with a largely subterranean existence. For example, closely related fossorial mammals typically show such patterns of parapatry, with only narrow, local zones of sympatry (J. L. Patton, personal communication).

Diversification in *B. attenuatus* has occurred primarily in the northern Coast Ranges. The northern, southern and eastern clades of *B. attenuatus* approach each other in the vicinity of the Russian River (Fig. 4A), and at present no major geographical barriers separate them. However, the Russian River was larger and may have posed a more formidable barrier in the past. *Ensatina* also shows great differentiation in this region, where the subspecies *oregonensis* and

xanthoptica come into secondary contact (Wake, 1997). At its southern limit just north of Monterey Bay, *B. attenuatus* occurs in sympatry with *B. gavilanensis*, a *pacificus* group lineage. This is also the southern limit of a number of other amphibian taxa: *Aneides flavipunctatus*; *Ensatina eschscholtzii xanthoptica*; *Dicamptodon ensatus*; *Taricha granulosa*; and *Ambystoma macrodactylum croceum* (Wake, 1997). This region may well have been physically separated from more southern areas by a long-persistent seaway (Yanev, 1980; Jockusch *et al*., 2001). Penetrance of *B. attenuatus* into the Sierra Nevada was evidently recent because the haplotypes from the Sierra Nevada closely resemble those from populations immediately east of San Francisco Bay and are deeply nested within the eastern clade of *B. attenuatus*. They could have crossed the Central Valley as part of a Pleistocene expansion, and today isolated populations persist on the valley floor (Fig. 1). A similar pattern of dispersal was postulated for another terrestrial plethodontid salamander, *Ensatina eschscholtzii xanthoptica* (Stebbins, 1949), and is supported by genetic evidence (Wake & Yanev, 1986; Moritz *et al*., 1992). At its southern extreme in the Sierra Nevada, *B. attenuatus* overlaps with a *relictus* group lineage, *B. diabolicus*.

The *relictus* lineage is restricted to the western Sierra Nevada and associated foothills. It is generally associated with relatively mesic montane areas (with the exception of some populations of *B. diabolicus*), and our phylogenetic analysis suggests vicariant separation from north to south. It occurs at high elevations only in the central Sierra Nevada, south of the areas of maximal Pleistocene glaciation, which may well have delimited its distribution to the north. At the southern end of its range, *B. relictus* has crossed the Kern River in two places. Populations in the lower Kern River Canyon are apparently extinct. However, there is an extant population on the Kern Plateau, east of the Kern River, which is closely related to *B. relictus* from the Greenhorn Mountains on the west side of the river. The Kern River is an apparent barrier to several salamanders, including *B. simatus*. The lower Kern River Canyon is also where the orange colour of *Ensatina eschscholtzii platensis* changes rather abruptly to the lemon-yellow of *Ensatina eschscholtzii croceater*, and this is a tectonically active area that may well have experienced geographical isolating events in the past. At present, the Kern River Canyon may be an important barrier because the region is so inhospitable for salamanders rather than because of difficulties in crossing the river.

The lineages with the most complicated distributional patterns are the *nigriventris* and *pacificus* groups. The *pacificus* group consists of an array of mainly parapatric lineages that extend from north of Monterey Bay to the southernmost extent of the range in the Sierra San Pedro Mártir, in northern Baja California, Mexico, but with a gap in the south Coast Ranges occupied by *B. nigriventris*. A scenario consistent with both crustal movements and phylogeny can be made for much of the diversification of the *pacificus* lineage (for detailed analysis see Jockusch *et al*., 2001). Despite the primarily northern distribution of lineages, the *pacificus* group probably originated in the south and then individual sublineages moved north on crustal fragments following land movement along the San Andreas fault.

The distribution of the *nigriventris* group is in many respects the most puzzling. Like *B. attenuatus*, the *nigriventris* group has both coastal and Sierran representatives, although the strong bias of sublineages is for a Sierran origination. It is mainly associated with the mountains at the southern end of the Sierra Nevada, a complex geological region that includes the Tehachapi Mountains. This region is characterized by geological instability, and the large San Andreas and Garlock Fault systems cross each other, contributing to its dynamic geological history. Great faults also extend up the Kern River Canyon and nearby areas, adding further to the unsettled conditions. This is the region in which the *nigriventris* group is maximally differentiated, possibly because this is inhospitable habitat for a salamander that requires that its skin remain moist at all times. The Kern Canyon region is extraordinary in the high number of distinct mitochondrial clades represented, and it may have been both a staging ground for evolution of the group as well as a 'museum' that preserves representatives of three of the six main lineages of the genus.

Batrachoseps is distributed on many of the islands off the coast of southern California and northern Baja California. It is found on all of the northern Channel Islands, as well as on Santa Catalina, at least three of the Islas Los Coronados, and one of the Islas Todos Santos. All of these islands are currently isolated from the mainland, with separations ranging from 6 km (Todos Santos) to 45 km (Santa Rosa, in the northern Channel Islands). East Anacapa is the closest of the northern Channel Islands to the mainland at ≈ 20 km. Distances have certainly varied with the marked differences in sea level that have taken place from Pliocene to the present, and the 20 km distance may have been as little as 6 km in the late Pleistocene. No evidence of a connection of the northern Channel Islands to the mainland is known to us, and continuity of marine sediments is evidence that the islands have never had an areal connection to land (Junger & Johnson, 1980; Vedder & Howell, 1980). The islands were all connected to each other at various times in the Pleistocene, including as recently as 17 000–18 000 years ago (Vedder & Howell, 1980). The north-

ern Channel Islands are largely of volcanic origin, and large permanent islands have been available in the region for only 2–3 million years (Sorlein, 1994). At least some species of *Batrachoseps* are relatively saline tolerant for amphibians (Licht *et al.*, 1975), and it is likely that salamanders reached the islands by rafting (Yanev, 1980).

The California Channel Islands in general are not known for much endemism, but there are some exceptions, for example among land snails; distinctive forms of grey foxes and jays have also evolved on the northern islands. One morphologically and genetically distinct salamander, *B. pacificus*, is endemic to the northern islands (see Yanev, 1980; for a detailed biogeographic analysis); its closest extant mainland relatives are located >100 km to the south. It is substantially diverged from the geographically closest mainland populations, which are assigned to northern *B. m. major*. The Pleistocene connections among the northern Channel Islands probably account for the fact that one haplotype of *B. pacificus* occurs on both San Miguel and Santa Cruz. However, the populations of *B. pacificus* on the different islands are allozymically distinct (Wake & Jockusch, 2000; and unpublished data), suggesting that there is more to be learned concerning the history of these salamanders. On Santa Cruz, *B. pacificus* and *B. nigriventris* occur in sympatry essentially all over the island, and it is curious that *B. nigriventris* is restricted to only a single island. The *B. nigriventris* haplotype is sister to mainland populations of southern *B. nigriventris*, located south-east of the island, and is only distantly related to the northern *B. nigriventris* lineage, which occupies the mainland directly north of the island.

The population of *B. m. major* from South Todos Santos off northern Baja California, Mexico, is highly divergent from its mainland relatives; it is most closely related to, but distinct in mtDNA and allozymes, from *B. m. aridus*, southern *B. m. major* and *B. pacificus* (see also Wake & Jockusch, 2000). However, the geographically closest mainland populations, from Ensenada, were not available for study. Santa Catalina appears to be the most recently settled island; its population, once considered to be a distinct species (Dunn, 1922), is very similar to northern *B. m. major* from the adjacent mainland in both morphology and mtDNA. The only island population that we did not study is that from the Coronados Islands, also named as a distinct species by Dunn (1922) but now considered to be *B. m. major*.

All of California has been subjected to dramatic geological changes during the Cenozoic, and vicariant events associated with geological changes have doubtless had major effects on differentiation of *Batrachoseps*. The present coastline has been assembled from parts of Earth's crust that have moved partly independently for long distances along the San Andreas Fault system, and the assembly corresponds, to a large degree, to what we understand of the differentiation of and relationships among *Batrachoseps* lineages (Jockusch *et al.*, 2001). Less is known about the geological history of the southern Sierra Nevada region, but our findings suggest that geological events have also played important roles in the diversification of *Batrachoseps* in that area.

CONCLUSION

Salamanders of the genus *Batrachoseps* have undergone extensive diversification in California, but the small amount of morphological differentiation has masked the history of this clade, which is slowly being uncovered through the use of molecular markers. The complex geological and climatic history of California in combination with the biology of these highly sedentary organisms is responsible for much of the diversification, which has involved fragmentation and *in situ* differentiation. Frequently the differentiated segments have experienced recontact. Either sympatry, typically geographically limited, between species-level taxa then occurs, or the lineages merge, showing that they have not evolved full reproductive isolation. The sex-limited mitochondrial markers are signatures of the deeper history, whereas the biparentally inherited allozymic markers demonstrate that merger is occurring. Half a century ago these common salamanders were thought to constitute but a single, somewhat variable species in California, but a combination of discoveries in the field of previously unknown, morphologically distinct taxa and in the laboratory of molecularly distinctive forms has led to the understanding that the sg *Batrachoseps* is a complex of five well supported clades, three of which contain four or more species. While our cytochrome *b* data do not fully resolve the relationships among these five major clades, they do provide robust support for many relationships within these clades, and identify many regions in which deeply differentiated clades are in geographical proximity.

Morphologically conservative organisms pose a special challenge for evolutionists and systematists. Sometimes morphologically uniform taxa are also genetically uniform, but especially in ancient lineages such as salamanders morphological uniformity often masks profound genetic differentiation. We have found instances (e.g. *B. stebbinsi* and *B. major aridus*) of morphologically divergent taxa nested high in this generally morphologically conservative clade, indicating that morphology can diverge rapidly. More often, however, molecular data reveal astonishingly large (e.g. numerous fixed differences at allozyme loci and >15% divergence in mtDNA) degrees of divergence

with virtually no detectable morphological change (as in the comparison of *B. attenuatus* and *B. gavilanensis*). This rich mosaic provides insight into the interplay of diverse processes during phylogenesis.

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APPENDIX 1

TISSUE SAMPLES USED IN DNA SEQUENCING AND VOUCHER SPECIMENS

For each sequenced specimen identified here with the population number shown in Table 2, vouchers are listed as '(tissue sample, specimen voucher)'. All collections are at the Musem of Vertebrate Zoology (MVZ), University of California, Berkeley.

Abbreviations used are: s, salamander frozen tissue collection; Y, Yanev frozen tissue collection; DBW, alcohol tissue and specimen collections of David Wake; sks, Carnoy collection of salamander tissues of Stanley Sessions; DSMP (Duncan S. M. Parks) and locality names, alcohol tissue collection.

1 (s-12949, MVZ224903); 2 (s-13229, MVZ224899); 3 (s-11847, MVZ220713); 4 (s-5667, MVZ172644); 5 (Y-4404, MVZ150422); 6 (s-9987, MVZ227119); 7 (s-6530, MVZ158278); 8 (DBW4856, MVZ222944); 9 (s-11883, MVZ219121); 10 (Y-3767, MVZ151638); 11 (s-7334, MVZ158894); 12 (DBW5292, MVZ226670); 13 (Y-4768, MVZ152018); 14 (s-12672, MVZ221030); 15 (s-12675, MVZ221033); 16 (Y-4622, MVZ152904); 17 (Y-2162, MVZ152998); 18 (DBW5206, MVZ225931); 19 (Y-376, MVZ151846); 20 (s-10099, MVZ205426); 21 (Y-2455, MVZ152818); 22 (s-12843, MVZ222688); 23 (s-10223, MVZ205357); 24a (s-13231, MVZ224433); 24c (s-13232, MVZ224442); 24b (s-13367, MVZ224441); 25 (s-13234, MVZ224355); 26 (s-13233, MVZ224481); 27 (Y-643, MVZ152670); 28 (s-12002, MVZ220535); 29 (s-13441, MVZ230762); 30 (Y-2364, MVZ156969); 31 (DSMP 92, MVZ230745); 32a (DSMP 70, MVZ230742); 32b (DSMP 72, MVZ230744); 33 (Y-4886, MVZ156999); 34 (DBW5186, MVZ225996); 35 (s-12974, MVZ224918); 36 (s-9618, MVZ195540); 37 (DBW5916, DBW5916); 38 (DBW5936, DBW5936); 39 (s-12766, MVZ224643); 40 (Y-4812, MVZ154037); 41 (Y-3161, MVZ154029); 42 (s-11874, MVZ219146); 43 (s-13138, MVZ222760); 44 (S-12350, MVZ220496); 45 (DBW5329, MVZ226699); 46 (DBW5187, DBW5187); 47 (s-13168, MVZ222695); 48 (s-9655, MVZ191665); 49 (s-10398, MVZ206251); 50 (s-13254, MVZ225706); 51 (s-13078, MVZ222715); 52 (s-12711, MVZ224516); 53 (s-11871, MVZ219157); 54 (Y-3122, MVZ157350); 55 (DBW5941, DBW5941); 56 (Y-3038, MVZ154077); 57 (s-3275, MVZ167636); 58 (s-12075, MVZ220494); 59 (s-11743, MVZ218033); 60a (s-5225, MVZ163661); 60b (s-5227, MVZ163663); 61 (s-12321, MVZ220498); 62 (s-6951, MVZ158457); 63 (DBW5236, MVZ226712); 64 (DBW5225, MVZ226708); 65 (s-12042, no voucher); 66a (s-12331, MVZ220477); 66b (s-12332, MVZ220478); 67a (s-12868, MVZ224858); 67b (s-12869, MVZ224859); 68 (s-5142, MVZ169121); 69 (sks1372, MVZ167794); 70 (DBW4748, DBW4748); 71 (DSMP 81, MVZ230747); 72 (Y-1012, MVZ154836); 73 (s-12886, MVZ224703); 74 (DBW5931, DBW5931); 75 (Y-3276, MVZ155622); 76 (Y-3492, MVZ155499); 77 (DBW5151, DBW5151); 78 (Y-3382, MVZ154891); 79 (DBW5893, DBW5893); 80 (s-3147, MVZ167488); 81a (s-3160, MVZ167501); 81b (s-3162, MVZ167503); 82 (s-12588, MVZ224756); 83 (DBW5185, DBW5185); 84 (DBW5147, DBW5147); 85 (s-12871, MVZ224773); 86a (s-12838, MVZ224790); 86b (s-12874, MVZ224786); 87 (s-12753, MVZ224919); 88 (Y-5131, MVZ232901); 89 (DBW5333, MVZ226702); 90 (s-5826, MVZ172660); 91 (Y-4102, MVZ154320); 92 (DBW5451, DBW5451); 93 (s-3472, MVZ167880); 94 (DBW5444, DBW5444); 95 (Motte, no voucher); 96 (s-12744B, MVZ222553); 97a (s-3801, MVZ168561); 97b (Y-4286, MVZ154341); 98 (DBW4847, MVZ222914); 99 (DBW4845, MVZ222912); 100 (Y-5027, MVZ155748); 101a (s-13343, MVZ225685); 101b (s-13347, MVZ225689); 102a (Torrey3, no voucher); 102b (Torrey1, no voucher); 103 (Y-5087, MVZ155736); 104 (Marron, no voucher); 105a (Y-2394, MVZ156427); 105b (Y-2414, MVZ156447); 106 (s-13391, MVZ223571); 107 (DBW5442, MVZ228306); 108 (s-13074, MVZ224848); 109 (s-13116, MVZ224840); 110 (s-5920, MVZ158260); 111 (s-5750, MVZ172281); 112 (s-10450, MVZ227150); 113 (Y-5096, MVZ156543); 114 (s-12875, MVZ224836); 115 (s-12876, MVZ224799); 116 (DBW5865, DBW5865); 117 (DBW5854); 118 (s-6589, MVZ158244); 119 (s-12872, MVZ224835); 120 (s-9094, MVZ190983).