Phylogeography of *Pseudacris regilla* (Anura: Hylidae) in western North America, with a proposal for a new taxonomic rearrangement

Ernesto Recuero [a], Íñigo Martínez-Solano [a,b], Gabriela Parra-Olea [c,*], Mario García-París [a]

[a] *Museo Nacional de Ciencias Naturales, CSIC, José Gutiérrez Abascal, 2. 28006 Madrid, Spain*
[b] *Museum of Vertebrate Zoology, University of California, Berkeley, 3101 Valley Life Sciences Building, 94720 Berkeley, CA, USA*
[c] *Instituto de Biología, UNAM. AP 70-153, CP 04510, Ciudad Universitaria, México D.F., Mexico*

Abstract

The Baja California populations of *Pseudacris regilla*, a widespread species in Western North America ranging from British Columbia to southern Baja California, are characterized by extensive geographic fragmentation. We performed phylogeographic and historical demographic analyses on 609 bp of the cytochrome *b* mitochondrial gene of 110 individuals representing 28 populations to determine the relative influences of current and historical processes in shaping the present distribution of genetic diversity on the Baja California Peninsula. Haplotypes from this area were nested in a clade with three well-differentiated groups. Two of these groups are from Baja California Sur and another is from California and Baja California. The estimated date for the split of these groups, between 0.9–1 Ma, fits with previously proposed hypotheses of vicariance due to different transpeninsular seaways, although successive population fragmentation and expansion due to climatic oscillations during Pleistocene glaciations cannot be discarded. Historical demographic analyses detected signs of past population expansions, especially in the southernmost group. With respect to populations north of this region, two older clades were identified, one with haplotypes mainly distributed in central California, and the other corresponding to the northern half of the species range, in what apparently is a recurrent pattern in the Pacific coast of North America. Based on the concordance between mt-DNA and available allozyme data indicating that these species have a long independent evolutionary history, we propose to consider the three major clades as distinct species: *P. regilla*, *P. pacifica*, and *P. hypochondriaca*.
© 2005 Elsevier Inc. All rights reserved.

Keywords: Phylogeography; Historical demography; *Pseudacris regilla*; *Pseudacris pacifica*; *Pseudacris hypochondriaca*; mt-DNA; Midpeninsular seaway

1. Introduction

The Baja California Peninsula (BCP) is characterized by a number of historical peculiarities that have long attracted the interest of biologists. This narrow peninsula, over 1200 km long, is characterized by great ecological and geological complexity as well as a high biological diversity, and thus has been the subject of several biogeographic studies (Durham and Allison, 1960; Grismer, 1994a; Johnson and Ward, 2002; Murphy and Aguirre-León, 2002; Savage, 1960; Taylor and Regal, 1978; Wiggins, 1960; Wiggins, 1999).

For vertebrate taxa, three main biogeographical models have been postulated to explain the current distribution and diversity of Baja California's fauna. The first model, a late Quaternary dispersal related to climatic changes associated with glacial cycles (Orr, 1960; Savage, 1960) is based on the successive change of ecological conditions of the area. A second model postulates vicariant events during the Pliocene and Pleistocene due to different geographical barriers that isolated populations multiple times, the results of which are reflected in the diversity patterns found in different organisms that exhibit well-differentiated north vs. south intraspecific lineages

* Corresponding author.
E-mail address: gparra@ibiologia.unam.mx (G. Parra-Olea).

(Aguirre et al., 1999; Riddle et al., 2000a; Upton and Murphy, 1997). A third model would include, as a causal factor to explain current patterns, the late Miocene vicariant event associated with separation of the BCP from mainland Mexico, which seems to explain the patterns observed in several other species (Grismer, 1994a).

To test some of these hypotheses, Riddle et al. (2000b) analyzed mitochondrial DNA (mt-DNA) variation of several vertebrate species and found similar patterns in most of the analyzed taxa, supporting the existence of past barriers to gene flow, the most common pattern being two well-differentiated north-south clades, whose divergence presumably dated to the Pleistocene.

Unlike other vertebrate groups, the amphibian community of BCP presents a pattern that apparently fits with the existence of a peninsular effect (Busack and Hedges, 1984), with species richness decreasing from the north to the south of the peninsula, and only three species (*Scaphiopus couchii*, *Bufo punctatus*, and *Pseudacris regilla*) widely distributed along the entire peninsula. Interestingly, *B. punctatus*, the only amphibian species included in the analysis of Riddle et al. (2000b), does not display substantial mitochondrial
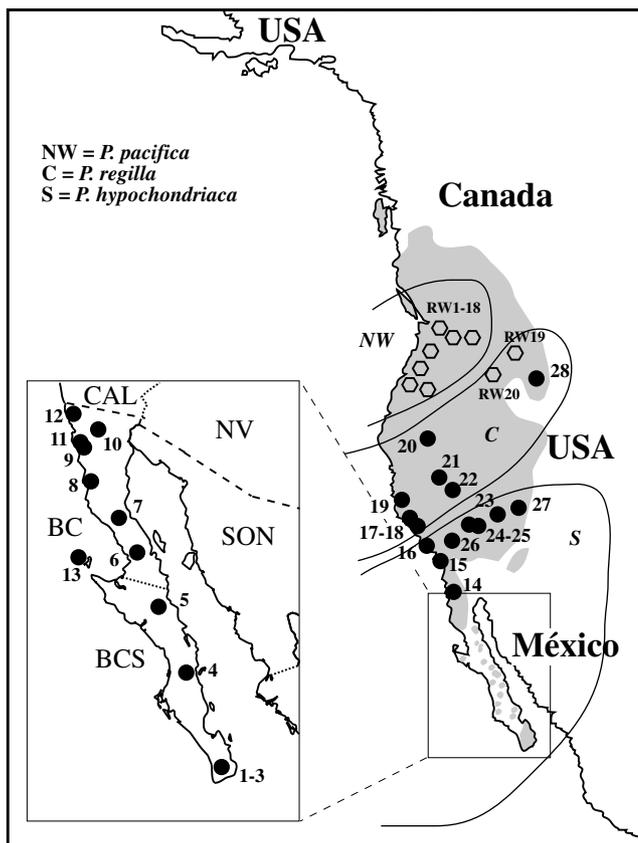

Fig. 1. Distribution of *P. regilla* in western North America, showing the new taxonomic proposal (the three groups delimited by solid lines), based on allozyme (Case et al., 1975) and mt-DNA data (Ripplinger and Wagner, 2004; this study). Populations sampled for mt-DNA are marked with hexagons (Ripplinger and Wagner, 2004) and black dots (this study, numbers refer to Table 1). Sampled populations in the Baja California Peninsula are highlighted. CAL = California, NV = Nevada, BC = Baja California, BCS = Baja California Sur, and SON = Sonora.

variation along the BCP, but presents a pattern conforming to the third hypothesis (Jaeger et al., 2005).

Pseudacris regilla is a widespread species present along the Pacific coast of North America from southern British Columbia to the southern tip of the BCP (Stebbins, 1985), being the most abundant and ubiquitous amphibian in western North America (Brattstrom and Warren, 1955; Matthews et al., 2001). Studies of mating call (Snyder and Jameson, 1965), morphology (Jameson et al., 1966), allozymes (Case et al., 1975), and mt-DNA (Ripplinger and Wagner, 2004) have revealed intraspecific variability, and several subspecies have been described, of which seven are currently recognized (Crother et al., 2000; Duellman, 1970). In BCP, *P. regilla* can be found in montane and mesic areas as well as in desert oases (Grismer, 2002a). According to the latest taxonomic revision, two subspecies are present in BCP: *P. r. hypochondriaca*, in the northern portion of the Peninsula; and *P. r. curta*, endemic to the area south to the Vizcaíno desert (Duellman, 1970) (Fig. 1).

In the present study, we used mt-DNA to determine the geographic patterns of genetic variation among the southern (BCP) populations of *P. regilla*. We performed phylogeographic and historical demographic analyses to determine such patterns and to postulate a solid hypothesis for the evolutionary history of this species in the BCP. Our results are discussed in the context of our current knowledge about general biogeographic patterns in the BCP, with implications for the taxonomy and conservation of the species in Baja California.

2. Materials and methods

2.1. Sampling

We obtained tissue samples from 77 individuals, adults and tadpoles, from 12 populations on the BCP. The sampling was completed with 33 individuals from the Museum of Vertebrate Zoology tissue bank (University of California, Berkeley) corresponding to 16 additional populations from Baja California, California (Alta California), Nevada and Montana (Fig. 1; Table 1). *Pseudacris cadaverina*, the sister taxon to *P. regilla* (Moriarty and Cannatella, 2004), was used as outgroup.

2.2. Mitochondrial DNA amplification and sequencing

Total genomic DNA was extracted from ethanol-preserved tissues (muscle, liver, and tail fin from tadpoles) using a phenol–chloroform protocol (Sambrook et al., 1989), preceded by a digestion with proteinase K. Polymerase chain reaction (PCR) was used to amplify 609 bp of the mitochondrial cytochrome *b* gene (*cytb*), using the primers MVZ15 and MVZ18 (Moritz et al., 1992). PCRs were performed in a total volume of 25 μl, including 1 U *Taq* polymerase (Biotools, 5 U/ml), 1.0 μl of each primer (10 μmol/L), 0.4 mM dNTPs (10 nmol/L), 1.5 μl MgCl$_2$ (25 mmol/L), and 67 mM of a reaction buffer (Tris–HCl, pH 8.3, Biotools).

Table 1
Populations sampled in this study

ID	Locality	Latitude	Longitude	*n*	Haplotypes
1	México: Baja California: Sierra de la Laguna (1)	23° 31.711′ N	110° 01.591′ W	5	XIX, XX, XXI, XXII, XX
2	México: Baja California: Sierra de la Laguna (2)	23° 33.019′ N	109° 59.500′ W	3	XXIII, XXIV
3	México: Baja California: Sierra de la Laguna (3)	23° 32.783′ N	109° 58.464′ W	7	XIX, XXII, XXIII, XXV, XXVI
4	México: Baja California: Las Parras	25° 58.655′ N	111° 27.888′ W	9	I
5	México: Baja California: San Ignacio	27° 17.098′ N	112° 53.934′ W	15	II, III, IV, V, VI
6	México: Baja California Norte: La Ciénaga	28° 36.841′ N	114° 02.699′ W	5	VII, VIII, IX
7	México: Baja California Norte: Cataviña	29° 43.555′ N	114° 42.772′ W	7	VII, X
8	México: Baja California Norte: San Telmo	30° 58.554′ N	116° 05.882′ W	6	XI, XII, XIII, XIV
9	México: Baja California Norte: Ojos Negros	31° 52.795′ N	116° 21.609′ W	4	XIII, XV, XVI
10	México: Baja California Norte: Las Huertas	32° 00.089′ N	115° 57.034′ W	5	XI, XII, XIV, XVI
11	México: Baja California Norte: El Tigre	31° 57.150′ N	116° 44.048′ W	7	XI, XIII, XVII
12	México: Baja California Norte: El Descanso	32° 11.899′ N	116° 53.341′ W	7	XV, XVII, XVIII
13	México: Baja California Norte: Isla Cedros	28° 06.666′ N	115° 10.666′ W	2	XXVIII
14	USA: California: San Diego Co., Pala junction on Hwy. 76	33° 21.918′ N	117° 01.926′ W	1	XI
15	USA: California: Los Angeles Co., Santa Monica Mountains	34° 06.705′ N	118° 46.345′ W	1	XI
16	USA: California: Santa Barbara Co., Buellton	34° 36.656′ N	120° 11.951′ W	2	XV, XXIX
17	USA: California: San Luis Obispo Co., Los Padres Ntl. Forest	35° 17.626′ N	120° 19.327′ W	1	XXXIII
18	USA: California: San Luis Obispo Co., Santa Margarita	35° 25.487′ N	120° 34.052′ W	1	XI
19	USA: California: Monterey Co., McClusky Slough	36° 50.356′ N	121° 47.387′ W	2	XXXVII, XXXVIII
20	USA: California: Shasta Co., Shingleton	40° 28.077′ N	121° 53.082′ W	1	XI
21	USA: California: Alpine Co., Highland Lakes	38° 29.446′ N	119° 48.159′ W	2	XXXV, XXXVI
22	USA: California: Tuolumne Co., Kennedy Meadows	38° 19.634′ N	119° 39.418′ W	1	XXXIX
23	USA: California: Inyo Co., Surprise Canyon	36° 06.747′ N	117° 10.473′ W	4	XI
24	USA: California: Inyo Co., Little Lake	35° 56.218′ N	117° 54.343′ W	4	XXX, XXXII, XXXIII
25	USA: California: Inyo Co., Indian Joe Canyon	35° 49.772′ N	117° 23.620′ W	2	XI
26	USA: California: Kern Co., Bakersfield	35° 31.909′ N	118° 38.806′ W	1	XXX
27	USA: Nevada: Nye Co., Beatty	36° 54.500′ N	116° 45.500′ W	3	XXXI
28	USA: Montana: Missoula Co., Clark Fork River	46° 49.017′ N	113° 42.153′ W	2	XXXIV

Geographical coordinates, number of individuals sampled (*n*) and haplotypes found (see also Figs. 2 and 4).

PCRs consisted of 35 cycles with a denaturing temperature of 94 °C (1 min), annealing at 56 °C (1 min), and extension at 72 °C (1 min). Double-strand templates were cleaned using sodium acetate and ethanol to precipitate the PCR products and then re-suspended in 22 µl of ddH₂O. Sequencing reactions were performed for both strands and sequenced on an ABI PRISM 3700 DNA sequencer following the manufacturer's instructions.

2.3. Sequence alignment and phylogenetic analyses

All sequences were compiled using Sequence Navigator version 1.0.1 (Applied Biosystems) and aligned manually. Thirty-seven additional haplotypes obtained from Gen-Bank (Accession Nos. AY363181–AY363219, Ripplinger and Wagner, 2004), corresponding to 20 populations from Washington, Oregon, and Idaho were added to the final alignment for the phylogenetic analyses. Genetic divergence (*p*-uncorrected and maximum likelihood (ML)-corrected sequence divergence) in pairwise comparisons were calculated using the software PAUP*4.0b10 (Swofford, 2002). Mean sequence divergence between groups was calculated with MEGA2 (Kumar et al., 2001).

Phylogenetic analyses including all haplotypes were performed with PAUP. Maximum parsimony (MP) phylogenies were estimated using the heuristic search algorithm with TBR branch swapping and 10 random addition sequence replicates. Each base position was treated as an

unordered character with four alternative states. We used nonparametric bootstrapping (1000 pseudoreplicates) to assess the stability of internal branches in the resulting topologies (Felsenstein, 1985).

Data were analyzed with the software ModelTest 3.6 (Posada and Crandall, 1998) to determine the substitution model that best fit our data for subsequent maximum likelihood analyses (ML, Felsenstein, 1981) and to calculate the transition/transversion ratio. ML analyses were performed using the heuristic search algorithm in PAUP with model parameters estimated with Modeltest. We used nonparametric bootstrapping (100 pseudoreplicates) to assess the stability of internal branches.

Bayesian phylogenetic analyses were conducted with MrBayes 3.0 (Huelsenbeck and Ronquist, 2001). Analyses were initiated with random starting trees and run for 2,500,000 generations, sampling every 100 generations. Of the resulting 25,000 trees, 2000 were discarded as "burnin." Posterior clade probabilities were used to assess nodal support.

We tested the null hypothesis of clocklike rates in our sequence dataset with a likelihood ratio test (Felsenstein, 1981) as implemented in ModelTest 3.6.

2.4. Molecular diversity, genetic structure, and phylogeographic analyses

Estimates of mean nucleotide and haplotype diversities within the main mt-DNA lineages identified by the

previous analyses within *P. regilla* were calculated with DNASP 4.0 (Rozas and Rozas, 1999). We also used analysis of molecular variance (AMOVA, Excoffier et al., 1992) to characterize patterns of genetic variation at different hierarchical levels (individuals, populations, and the main mt-DNA lineages identified by phylogenetic analyses) as implemented by Arlequin v. 2000 (Schneider et al., 2000). Levels of significance of statistics characterizing variation at different hierarchical levels were assessed through 100,000 permutations.

Phylogeographic analyses were based on a nested cladistic analysis (NCA) of haplotype data. We constructed a haplotype network from mt-DNA sequences using the software TCS 1.18 (Clement et al., 2000), which follows the statistical parsimony algorithm described in Templeton et al. (1992). Then, a nested statistical design was used following the general guidelines provided by Templeton et al. (1995). Finally, we tested for the existence of geographical associations of the different clades by means of: (i) a categorical test, in which clades showing genetic and/or geographic variation are tested against their geographical location (permutational contingency analysis, see Templeton et al., 1995); and (ii) a second test that incorporates the information on geographical distances and relative positions among the sampled populations. These tests were performed with the GeoDis 2.2 software package (Posada et al., 2000, 1,000,000 permutations), and the evolutionary patterns were identified following the inference key (updated 14th July 2004) provided by these authors with GeoDis 2.2.

2.5. Historical demography

To explore the demographic histories of the main mt-DNA lineages within *P. regilla*, mismatch analysis of mt-DNA sequences within each group was performed with Arlequin v. 2000 (Schneider et al., 2000). This analysis compares the frequency distribution of pairwise differences between haplotypes with that expected under a model of population expansion. The fit of observed versus modeled distributions is assessed by a goodness-of-fit statistic (p), whose significance is tested using a bootstrap approach (1000 replicates). The frequency distribution is usually unimodal for lineages that have undergone recent population expansions and multimodal for lineages whose populations are either subdivided or in equilibrium.

A complementary approach to analyze the historical demography of the main lineages within sampled populations of *P. regilla* was based on the coalescent-based method of Kuhner et al. (1998). This method calculates maximum likelihood estimates of theta (θ_{ML}), where θ equals twice female effective population size (Ne) times mutation rate (μ), and an exponential growth parameter (g). Both parameters and their standard deviations were calculated using the software Fluctuate 1.4 (Kuhner et al., 1998). Each Markov chain Monte Carlo run consisted of 10 short chains (with sampling increments of 10; 1000 steps/chain) and 10 long chains (sampling increment: 10; 20,000 steps). A neighbor-joining tree based on uncorrected distances was used as a starting tree. We used several different starting values for g and performed different replicates with different seed numbers to check for convergence of results. We followed the criterion of Lessa et al. (2003) for the interpretation of results and assumed population growth if g was consistently higher than three times its standard deviation (SD).

Additionally, Fu's tests of neutrality (Fu, 1997) were performed for sequences within each of the previously identified mt-DNA lineages. Significant negative values of Fu's statistics can be interpreted (in the absence of selection, as it is assumed to be the case for mt-DNA) as a signature of population expansion. This statistics was calculated with Arlequin v. 2000 and its significance was assessed through 10,000 simulations.

3. Results

3.1. Phylogenetic analyses

No insertions or deletions were present in the sequences obtained. Fifty-nine variable positions were found among the 110 sequences of *cytb* in the samples of *P. regilla* analyzed, defining 39 different haplotypes (Fig. 2; and Table 1). These mutations involved nine non-synonymous substitutions. The TrN + I + G model of evolution was selected by AIC in ModelTest 3.6. The ML-estimated transition–transversion ratio was 20.6. All sequences were deposited in GenBank under Accession Nos. DQ195169–DQ195207.

The phylogenetic analyses recovered well-structured trees with three main groups (Figs. 2 and 3): a "northwestern" group present in Washington and Oregon; a "central" group, distributed from Central California through eastern Oregon and Idaho to western Montana; and a "southern" group, distributed in the southern half of California and in the BCP. The "southern" group includes the populations of *P. r. hypochondriaca* and those corresponding to *P. r. curta*, which are in turn separated into two clades corresponding to the populations from the Sierra de la Laguna ("Laguna" group) and populations from oases south to the Vizcaíno Desert (Las Parras and San Ignacio, "Oases" group). In general, bootstrap values are moderate to low in the "*hypochondriaca*" group, probably due to the overall low number of variable characters in the dataset (Figs. 2 and 3). ML-corrected sequence divergence values ranged from 0.17 to 1.63% between samples from Sierra de la Laguna, 0.17 to 0.69% between samples from Las Parras-San Ignacio, and 0.17 to 1.87% between samples within *P. r. hypochondriaca*. Within *P. r. curta*, haplotypes from La Laguna and Las Parras-San Ignacio differed by 1.26 to 2.99%. Differences between groups ranged from 1.26 to 3.98% between *P. r. curta* and *P. r. hypochondriaca*, and 3.96 to 8.50% between both subspecies and samples from the "central" group. Mean p-uncorrected distances between groups were 4.18% between the "*curta*" and "central" clades, 3.39% between "*hypochondriaca*" and "central," 1.91% between
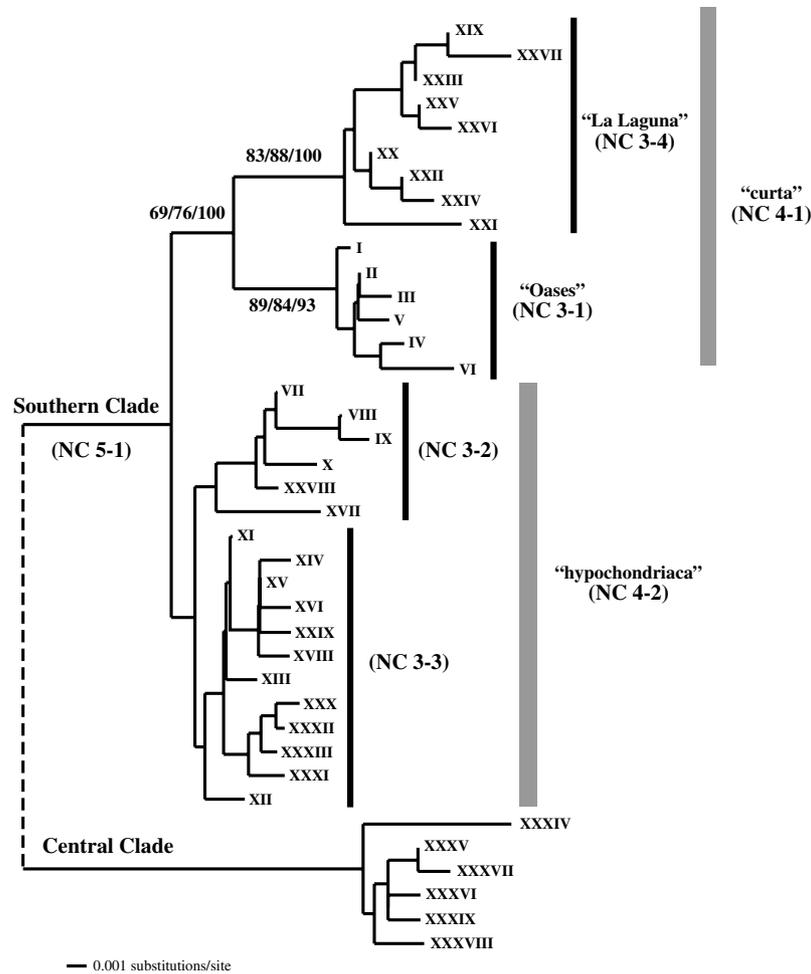
Fig. 2. Neighbor-joining tree based on maximum-likelihood corrected genetic distances depicting relationships between *cytb* haplotypes (Table 1) from the "Southern" and "Central" clades in this study. Bootstrap values (MP, ML, and % Bayesian posterior probabilities) at relevant nodes are shown. The main groups used in the nested clade analysis of sequence data are also shown (see also Fig. 4). Haplotype XI also occurs in some Central Clade populations.

"*hypochondriaca*" and "*curta*," and 1.71% within the "*curta*" clade. Distances between the "northwestern" group and the other two ranged from 5 to 6.5%.

The results of the likelihood ratio test showed no significant differences in the likelihood scores when comparing trees estimated with or without enforcing a molecular clock (with: $-\ln L = 1972.2613$; without, $-\ln L = 1929.5946$, ratio $= 85.333496$; df $= 77$; $p = 0.24133$). Thus, the null hypotheses of homogeneous evolutionary rates among sequences cannot be rejected.

Subsequent analyses were performed on the three main mt-DNA lineages recovered within "southern" populations: the "La Laguna" group (populations 1–3), the "oases" group (populations 4–5), and the "*hypochondriaca*" group (populations 6–18 and 22–26).

3.2. Molecular diversity

Values of nucleotide and haplotype diversity are presented in Table 2. In general, the values of haplotypic diversity observed are high (0.78–0.90) and similar between the main mt-DNA lineages, although it was always highest in

the *hypochondriaca* group, where up to 18 haplotypes were observed, and lowest in the "oases" group (six haplotypes).

Results from AMOVA indicate that most of the observed variation among mt-DNA lineages is related to differences between groups (68.03% of the total variance observed). Lower values were observed for variance related to differences among populations within groups (15.50%) and within populations (16.47%). All hierarchical components of genetic variation were highly significant ($p < 0.0001$).

3.3. Phylogeography

Haplotypes from the "central" and "northwestern" groups fall outside the 95% confidence limit for the maximum parsimony connection of haplotypes, which was fixed at 10 mutational steps. The remaining 33 haplotypes were arranged in a nested design within a single five-step clade (Fig. 4). The three main mt-DNA groups recovered in the phylogenetic analyses of haplotypes ("Laguna," "Oases," and "*hypochondriaca*") corresponded to three-step clades 3–4, 3–1, and 3–2 + 3–3, respectively.
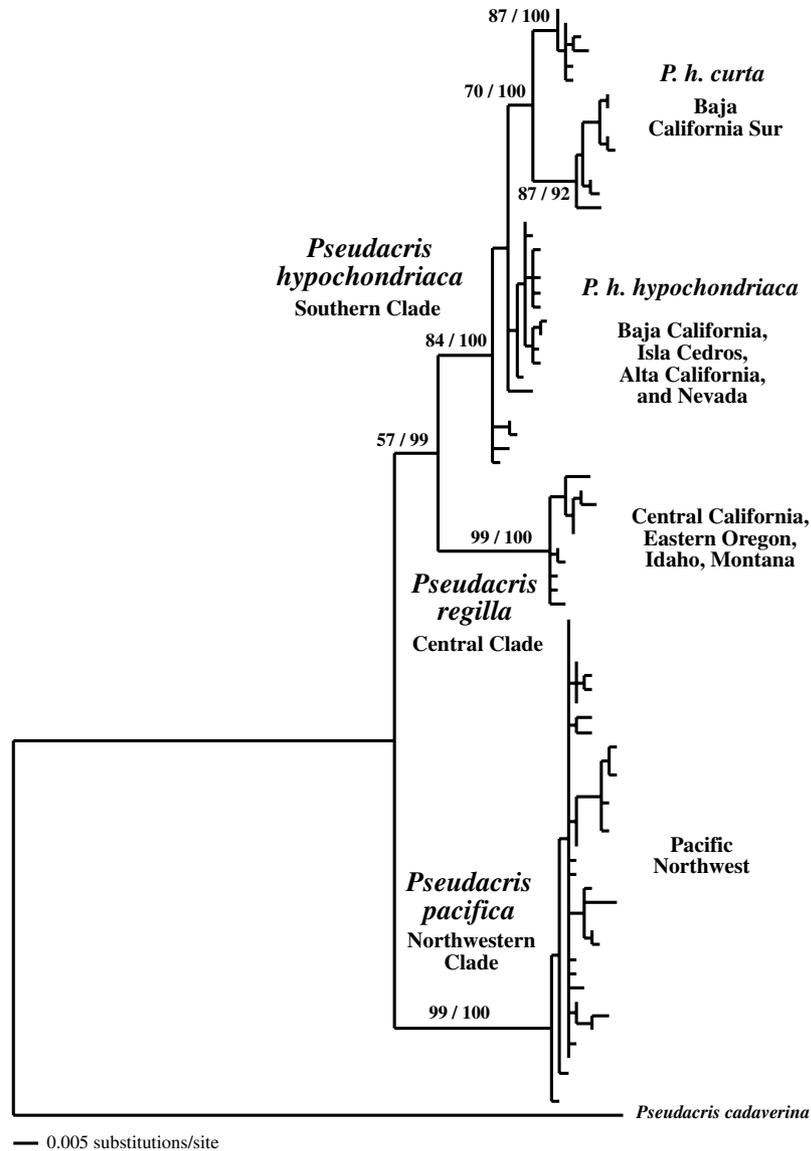
Fig. 3. Maximum likelihood tree (−ln L = 1937.4627) of *cytb* sequences (Ripplinger and Wagner, 2004, this study) showing the new proposal of taxonomic rearrangement for populations formerly included within of *P. regilla*. Bootstrap values (MP and % Bayesian posterior probabilities) at relevant nodes are shown.

Table 2
Molecular diversity (number of haplotypes, nucleotide and haplotype diversity), Fu's *Fs* statistic and maximum likelihood historical demographic parameters: θ (1/sites∗generation) and g (1/μ∗generations) in the main mt-DNA lineages within *P. regilla* identified in the present study

mt-DNA lineage	N	No. of haplotypes	Nucleotide diversity (SD)	Haplotype diversity (SD)	Fu's Fs	g (SD)	θ (SD)
hypochondriaca	64	18	0.006 (0.003)	0.904 (0.025)	−4.56 ns	319.681 (150.158)	0.015 (0.002)
Oases	24	6	0.003 (0.002)	0.790 (0.051)	−0.31 ns	248.484 (334.163)	0.003 (0.001)
Laguna	15	9	0.005 (0.003)	0.886 (0.069)	−2.87*	857.048 (229.564)	0.027 (0.010)

Standard deviations (SD) in parentheses N = sample size.
  * significant.

We found significant geographical association between clades and their geographical locations at all nesting levels (Table 3). Hypotheses derived from the interpretation of Dc and Dn values according to Templeton's (2004) updated inference key are also shown in Table 3. For clade 1–1, including haplotype I (characterizing all individuals from the population of Las Parras, see Table 1), and the haplotypes II, IV, and VI (found only in the population of San Ignacio), the analysis could not discriminate between long distance movements and the combined effects of gradual movement during a past range expansion and subsequent fragmentation. The same inference was produced for clades 3–2 (haplotypes from southernmost populations of the *hypochondriaca* group—including Isla Cedros—vs. haplotypes from some northern populations of *hypochondriaca*) and clade 4–2 (haplotypes nested within clade 3–2 vs.
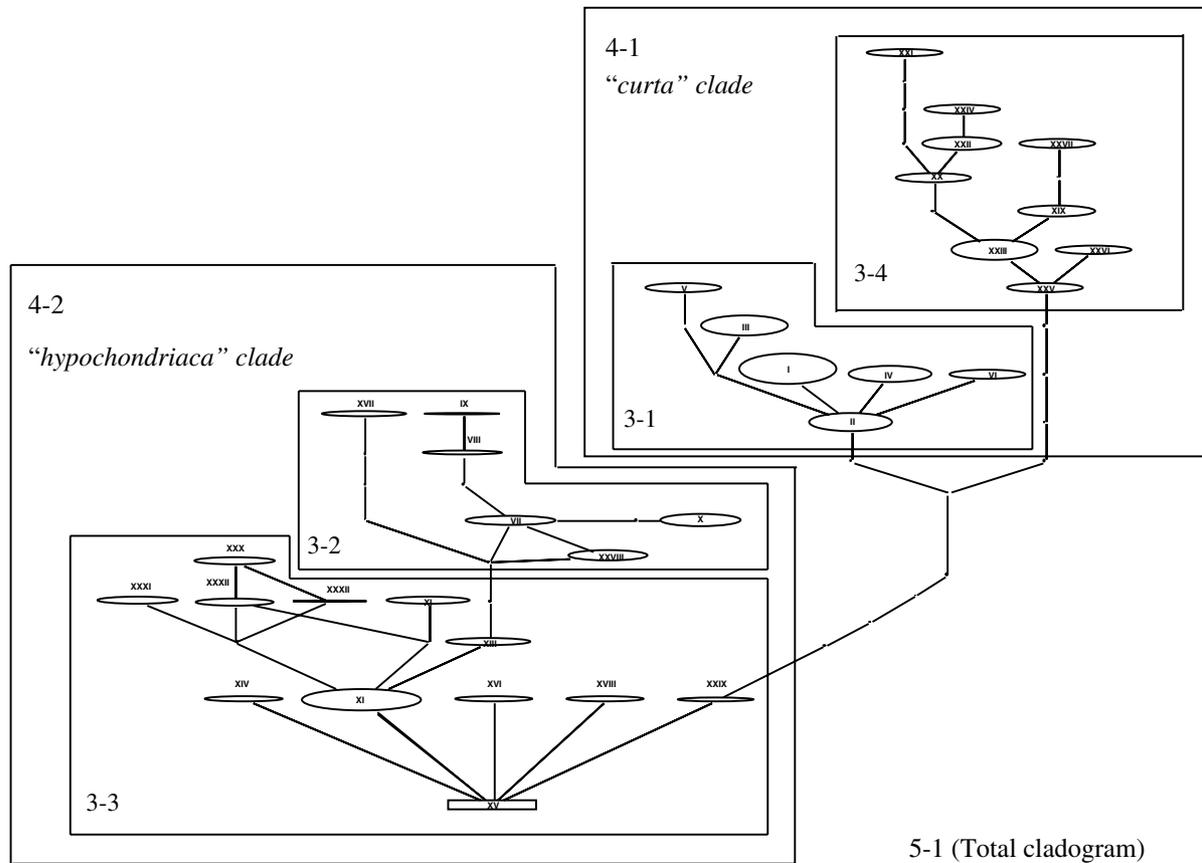
Fig. 4. Nested design for the statistical parsimony haplotype network of *cytb* sequences from *P. regilla* in this study (see haplotype designation in Table 1).

Table 3
Results of the nested cladistic analysis of haplotypes of *P. regilla*

Haplotype group	Chi-square statistic	Inference chain	Inferred event
Clade 1–1	18.00 ($p < 0.0001$)	1–2–3–5–15–21	Insufficient genetic evidence to discriminate between long distance movements and the combined effects of gradual movement during a past range expansion and fragmentation (Las Parras, S. Ignacio)
Clade 1–18	8.00 (ns)	1–19	Allopatric fragmentation (Isla Cedros, La Ciénaga-Cataviña)
Clade 2–3	9.30 (ns)	1–2–3–4	Restricted gene flow with isolation by distance (Isla Cedros, La Ciénaga, Cataviña)
Clade 2–4	21.61 ($p < 0.01$)	1–2–11–17–4	Restricted gene flow with isolation by distance (San Telmo-Las Huertas, Nevada-S. California)
Clade 3–1	4.80 (ns)	1–2–3–4	Restricted gene flow with isolation by distance (S. Ignacio, S. Ignacio-Las Parras)
Clade 3–2	18.00 ($p < 0.001$)	1–19–20–2–11–12–13–21	Insufficient genetic evidence to discriminate between long distance movements and the combined effects of gradual movement during a past range expansion and fragmentation (La Ciénaga-Cataviña-Isla Cedros, El Tigre-El Descanso)
Clade 4–1	32.57 ($p < 0.0001$)	1–19	Allopatric fragmentation (La Laguna, Las Parras-S. Ignacio)
Clade 4–2	51.27 ($p < 0.0001$)	1–2–11–12–13–21	Insufficient genetic evidence to discriminate between long distance movements and the combined effects of gradual movement during a past range expansion and fragmentation (La Ciénaga-Cataviña-Isla Cedros-El Tigre-El Descanso, Nevada-S. California, BCN)
Total cladogram	104,00 ($p < 0.0001$)	1–19	Allopatric fragmentation (*curta*, *hypochondriaca*)

For each clade showing geographic and/or genetic variation, results of the categorical χ^2 test, and inference chain and inferred events after the geographical distances tests are presented.

haplotypes from all other populations of *hypochondriaca*). Allopatric fragmentation was inferred for clades 1–18 (including haplotype XXVIII, exclusive of individuals from Isla Cedros, vs. haplotypes from inland southernmost populations of *hypochondriaca*), 4–1 (haplotypes found in La Laguna vs. those found in Las Parras and San Ignacio), and the total cladogram (haplotypes from *P. r. curta* vs. *P. r. hypochondriaca*). Finally, restricted gene flow with isolation by distance was inferred for clades 2–3 (haplotypes nested within 1–18 vs. haplotypes found in the population of La Ciénaga vs. haplotypes found in Cataviña), 2–4 (haplotypes from San Telmo and Las Huertas vs. haplotypes from
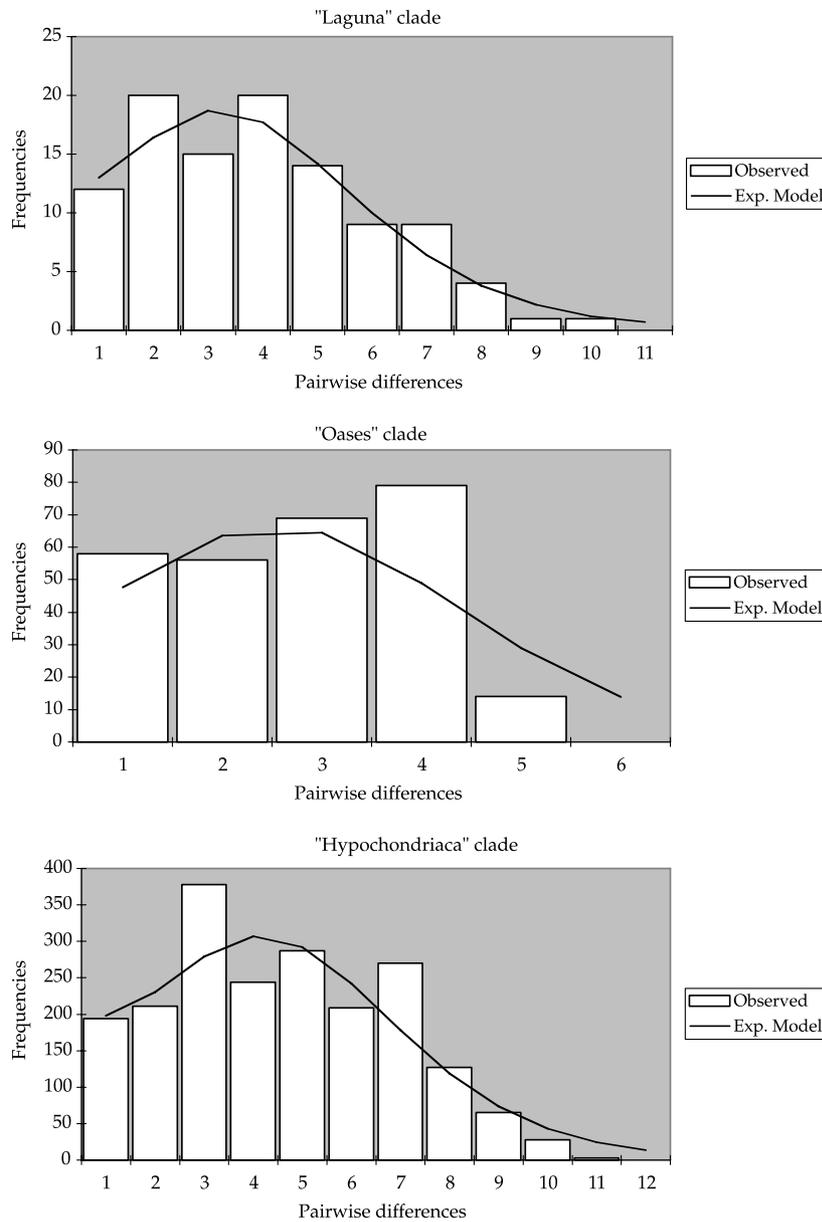
Fig. 5. Mismatch distributions of *cytb* sequences in the three mt-DNA lineages identified in this study. Solid bars represent the observed frequencies of pairwise differences between haplotypes; open bars represent those expected under the model of population expansion.

Nevada and southern California), and 3–1 (haplotypes III and V, exclusive from S. Ignacio, vs. haplotypes nested within 1–1).

3.4. Historical demography

The mismatch distributions for the three main mt-DNA lineages identified are presented in Fig. 5. The population expansion model was not rejected in either case ($p = 0.20$, 0.54, and 0.93, respectively).

Female effective population size (θ) and growth (g) were estimated by ML for the three "southern" lineages. The results are presented in Table 2. The values of θ obtained in different simulations were always higher in the "Laguna" group, being on average more than two times the values

observed for the "*hypochondriaca*" group and almost 10 times higher than those from the "Oases" clade, suggesting differences in ancestral population sizes in these lineages. ML estimates of g for each of these groups showed the same trends, being highest in the "Laguna" group and lowest in the "Oases" group. The only lineage that consistently showed high and positive values of g and where $g > 3$ SD (g) was the "Laguna" group. The "*hypochondriaca*" group always had positive values of g, but different runs produced inconclusive results on the basis of the criterion employed ($1 < g > 4$ SD (g) in different simulations). Finally, the "Oases" group did not show evidence of demographic expansion, with g values associated with high standard errors.

The results of Fu's neutrality tests were non significant for the "Oases" (Fu's $Fs = -0.31$) and "*hypochondriaca*"

groups ($Fs = -4.56$), whereas they were significant for the "Laguna" group ($Fs = -2.87$, $p < 0.05$) (Table 2).

4. Discussion

4.1. Evolutionary history and taxonomy of P. regilla

The results of this study show three main haplotype clades ("northwestern," "central," and "southern") congruent with allozyme-based phenogram of Case et al. (1975) (see their Fig. 1). Case et al.'s (1975) allozyme analysis revealed three major groups within *P. regilla*, characterized by high genetic distances (mean D_{Nei} values ranging from 0.18 to 0.21). Our "southern" (*hypochondriaca* + *curta*) clade would correspond to the southern California-Baja California group in Case et al. (1975), while our "central" clade (including central California, Idaho, eastern Oregon and Montana) would correspond to their central California group. Finally, our "northwestern" group, with populations from Washington and Oregon corresponds with the third group in Case et al. (1975), which included populations from Oregon. Levels of genetic diversification within the "northwestern" clade are, however, much lower than those observed in southern populations. Thus, there is concordance between nuclear (allozymes) and mt-DNA, although the latter suggests substructuring within the southern California–Baja California group. Unfortunately, the analysis of Case et al. (1975) lacked samples from the southernmost population in BCP (Los Cabos region, La Laguna), and thus more samples would be needed to confirm this pattern.

Among the haplotypes analyzed from populations on the BCP, there is a clear division between southern and northern populations. This pattern has been observed in several vertebrates in the region (see Riddle et al., 2000b), leading to the resurrection of an old biogeographical hypothesis consisting of vicariance due to a midpeninsular seaway (Johnson, 1924; Nelsen, 1921). This seaway was thought to exist approximately 1–1.6 Ma (Riddle et al., 2000b; Upton and Murphy, 1997), although there is no geological evidence for a seaway at any time in the past.

To elucidate the possible events that caused the observed patterns, it is important to determine the age of the split, but the application of molecular clocks is not always straightforward. For *cytb* in amphibians, published sequence divergence rates range from 0.8% (Tan and Wake, 1995) to 3.6% (Babik et al., 2004). Ripplinger and Wagner (2004) used a divergence rate for *P. regilla* of 2%, which lies within the mentioned range for amphibians and is consistent with data for other vertebrates (Wilson et al., 1985). According to this calibration, the split between "*hypochondriaca*" and "*curta*" occurred around 1 Ma, and subsequently, populations of "*curta*" fragmented about 0.9 Ma. This estimation would be in close agreement with the postulated midpeninsular seaway, 1–1.6 Ma. However, to explain the phylogeographic structure observed within the "*curta*" clade would also require another barrier to dispersal located in the area of the Isthmus of La Paz. Aguirre

et al. (1999) studied patterns of genetic structure in *Urosaurus* and suggested this additional seaway. These authors dated both seaways around 1.5 Ma. Assuming that there was a sea-level increase that flooded part of the central Peninsula (see Haq et al., 1988), it would not be unlikely that it happened somewhere else, especially in the area of the Isthmus, where maximum elevation is about 10 m above sea level (Grismer et al., 2002b; see also Murphy and Aguirre-León, 2002). However, at present, there is no geological evidence for the existence of two or more transpeninsular seaways in Baja California in the Pleistocene and the inferred locations vary over a wide latitudinal range of about 650 km depending on the taxonomic group examined.

Alternatively to the midpeninsular seaway hypothesis, the observed pattern might be associated with glacial events during the Pleistocene. In that period, environmental conditions in BCP were more mesic than at present (Upton and Murphy, 1997). Based on these glacial events, Savage (1960) proposed a general biogeographical scenario for the BC herpetofauna involving a succession of fragmentations of the distributional ranges of mesophilic species related to aridification processes during interglacial periods, followed by range expansions during glacial maxima. Our data also fit this model, which is similar to those proposed for other peninsulas affected by Pleistocene glaciations (see for example Taberlet et al., 1998).

The deep divergence between the three main clades suggests some vicariant events dating at least from the Pliocene that affected the Pacific coast of North America. Ripplinger and Wagner (2004) related the differentiation of their "Coastal" and "Inland" clades with the orogeny of the Cascade Mountains, which began approximately 4 Ma ago and drastically changed the environmental conditions of the area. Apparently, this event has caused a genetic division in other taxa from this region (Carstens et al., 2004; Good, 1989; Howard et al., 1993; Nielson et al., 2001; Steele et al., 2005) and together with the uplift of other mountain chains on the Pacific Coast has produced repetitive patterns of genetic diversification across multiple taxa (Calsbeek et al., 2003; Shaffer et al., 2004).

4.2. Evolutionary history of P. regilla in Baja California

The highest level in our nested cladistic analysis consists of two main four-step clades, one restricted to southern BCP and the other distributed in Baja California, California, and western Nevada. These clades, according to Templeton (2004) inference key, formed after allopatric fragmentation of the ancestral stock for both groups. As stated above, fragmentation between *hypochondriaca* and *curta* would have occurred around 1 Ma ago, while fragmentation of *curta* into the "Laguna" and "Oases" groups occurred around 0.9 Ma. Unfortunately, we lack samples from lowlands in the Cape region that would provide additional information on the events that affected the "*curta*" clade, and to discriminate the possible alternative evolutionary scenarios.

Samples within the "*hypochondriaca*" group were clustered into two third-level clades. One of them included the populations from the southern part of the distribution, associated mainly with arid regions (Grismer, 1994b), and also the population from Isla Cedros. Between these two groups, the inference key offers two alternative hypotheses to explain the observed pattern: long distance movements or a gradual range expansion followed by fragmentation. The limited dispersal abilities of this species, and of amphibians in general (Smith and Green, 2005), make unlikely the existence of long distance movements, and thus we favor the hypothesis of range expansion and subsequent fragmentation of the populations due to recent desertification. We also favor the same hypothesis to explain the pattern observed in the clade containing the populations from Las Parras and San Ignacio (the "Oases" group), which at present are also surrounded by unsuitable habitat (Grismer, 1994b).

Allopatric fragmentation was inferred for the clade including the haplotype found in individuals from Isla Cedros and those from La Ciénaga and Cataviña. Isla Cedros was once a prolongation of the Vizcaíno Peninsula (Grismer et al., 1994), but has been isolated from the mainland during the last 9000–15,000 years (Murphy et al., 1995; Wilcox, 1978). Other species have relict populations on this island (Grismer and Mellink, 1994; Grismer et al., 1994) and some of them were formerly described as endemic taxa (Grismer, 1988; Grismer et al., 1994; Montanucci, 2004; Murphy et al., 1995).

There are signs of demographic growth in the three main mt-DNA lineages, but the sign is strongest in populations from the Sierra de La Laguna. The "*hypochondriaca*" group is characterized by relative demographic stability, but there are also some restrictions to gene flow, especially apparent in populations from arid areas between the southernmost populations within this group and also between these and populations from more mesic habitats north to El Rosario.

Within the "Oases" clade, populations are prone to isolation due to the arid conditions of the surrounding area, and frogs are confined to areas where water is available (Grismer, 2002a). The results of the nested cladistic analysis for this clade indicate restrictions to gene flow between sampled populations.

We found some differences between the results of the different tests used to infer historical demographic trends in the three main groups detected. In general, Fu's tests and ML estimates of demographic parameters appear more conservative than mismatch distributions. In any case, congruence between different tests with regard to the historical demography of the "Laguna" group points to this region as an important refugial area for *P. regilla* in Baja California. Further studies are, however, required to confirm other refugia along the BCP. The analysis of more variable nuclear markers, such as microsatellites, would provide very useful information to ascertain present demographic trends and to contrast them to the historical events inferred from mt-DNA analyses.

4.3. Taxonomic implications

According to the results of the phylogeographic analyses, the "southern" clade includes two population groups that appear to constitute well-differentiated, independent evolutionary lineages currently in allopatry. Genetic divergence between "*curta*" and "*hypochondriaca*" suggests an older split of the two groups than was suggested by Jameson et al. (1966) in their taxonomic revision of the species, where they explained the significant morphometric differences between populations from different regions throughout the species range on the basis of the climatic oscillations in the last 11,000 years.

The "central" clade is represented in our sample by populations corresponding to *P. r. palouse* from Montana, *P. r. sierrae* from the Sierra Nevada of California, and *P. r. regilla* from Central California. The samples from Idaho and Eastern Oregon included by Ripplinger and Wagner (2004) in their "inland clade" are also included in this clade. All other samples used by these authors and included in their "coastal clade" form a basal highly differentiated "northwestern" clade, which is basically concordant with the range of *P. r. pacifica*.

From the allozyme data published by Case et al. (1975), Highton (2000) rejected the idea of *P. regilla* representing a single species. The information provided by the work of Case et al. (1975) is not complete due to sampling constraints. However, both allozyme and mt-DNA are congruent, suggesting a division of *P. regilla* into three independent groups that deserve taxonomic recognition at the species level. *P. regilla* (Baird and Girard, 1852) corresponds to the populations ranging from Central California to Montana. Populations from the northwest should be regarded as *Pseudacris pacifica* (Jameson et al., 1966) *stat. nov*. Southern populations, from Nevada and southern California to the Cape region in Baja California, would take the name *Pseudacris hypochondriaca* (Hallowell, 1854) *stat. nov.*, with two different subspecies, *P. h. hypochondriaca* from the Vizcaíno desert to the north and *P. h. curta* (Cope, 1867) distributed south of the Vizcaíno Desert to the southern tip of BCP. Additional studies on variation in nuclear markers will be helpful to determine the precise distribution of the three lineages and to delimit possible contact zones.

4.4. Conservation implications

Our results depict two well-differentiated lineages among populations of *P. h. curta* in southern Baja California. At present, both groups face very different situations from a conservation perspective. On the one hand, *P. h. curta* from the Sierra La Laguna apparently presents large, well-preserved populations distributed over a large (over 110,000 ha) and strongly protected area (Reserve of the Biosphere "Sierra de la Laguna"). On the other hand, populations of *P. h. curta* from the "Oases" clade are characterized by extensive isolation, and as a consequence they are very vulnerable to disturbance, such as the introduction of

exotic species. The presence of introduced *Rana catesbeiana* is displacing native *P. hypochondriaca* in the oasis of San Ignacio (Grismer and McGuire, 1993; personal observations). Conservation measures to preserve the oases as well as other breeding sites, such as traditional water reservoirs for cattle will help to preserve populations of *P. hypochondriaca* and other species in the region.

Acknowledgments

References

Aguirre, G.L., Morafka, D.J., Murphy, R.W., 1999. The peninsular archipelago of Baja California: a thousand kilometers of tree lizard genetics. Herpetologica 55, 369–381.

Babik, W., Branicki, W., Sandera, M., Litvinchuk, S., Borkin, L., Irwin, J.T., 2004. Mitochondrial phylogeography of the moor frog, *Rana arvalis*. Mol. Ecol. 13, 1469–1480.

Brattstrom, B.H., Warren, J.W., 1955. Observations on the ecology and behavior of the Pacific treefrog, *Hyla regilla*. Copeia 1955, 181–191.

Busack, S.D., Hedges, S.B., 1984. Is the peninsular effect a red herring? Am. Nat. 123, 266–275.

Calsbeek, R., Thompson, J.N., Richardson, J., 2003. Patterns of molecular evolution and diversification in a biodiversity hotspot: the California Floristic Province. Mol. Ecol. 12, 1021–1029.

Carstens, B.C., Stevenson, A.L., Degenhardt, J.D., Sullivan, J., 2004. Testing nested phylogenetic and phylogeographic hypotheses in the *Plethodon vandykei* species group. Syst. Biol. 53, 781–792.

Case, S.M., Haneline, P.G., Smith, M.F., 1975. Protein variation in several species of Hyla. Syst. Zool. 24, 281–295.

Clement, M.D., Posada, D., Crandall, K.A., 2000. TCS: a computer program to estimate gene genealogies. Mol. Ecol. 9, 1657–1660.

Crother, B.I., Boundy, J., Campbell, J.A., de Queiroz, K., Frost, D.R., Highton, R., Iverson, J.B., Meylan, P.A., Reeder, T.W., Seidel, M.E., Sites Jr., J.W., Taggart, T.W., Tilley, S.G., Wake, D.B., 2000. Scientific and standard English names of amphibians and reptiles of North America North of Mexico, with comments regarding confidence in our understanding. Herp. Circ. 29, iv + 1D82.

Duellman, W.E., 1970. The hylid frogs of Middle America. Monogr. Mus. Nat. Hist. Univ. Kansas 1, 1–753.

Durham, J.W., Allison, E.C., 1960. The geologic history of Baja California and its marine faunas. Syst. Zool. 9, 47–91.

Excoffier, L., Smouse, P.E., Quattro, J.M., 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA data. Genetics 131, 479–491.

Felsenstein, J., 1981. Evolutionary trees from DNA sequences: a maximum likelihood approach. J. Mol. Evol. 17, 368–376.

Felsenstein, J., 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39, 783–791.

Fu, Y.X., 1997. Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. Genetics 147, 915–925.

Good, D.A., 1989. Hybridization and cryptic species in *Dicamptodon*. Evolution 43, 728–744.

Grismer, L.L., 1988. Geographic variation, taxonomy and biogeography of the anguid genus *Elgaria* (Reptilia: Squamata) in Baja California, mexico. Herpetologica 44, 139–154.

Grismer, L.L., 1994a. The origin and evolution of the peninsular herpetofauna of Baja California, Mexico. Herpetol. Nat. Hist. 2, 51–106.

Grismer, L.L., 1994b. Ecogeography of the peninsular herpetofauna of Baja California, México and its utility in historical biogeography, pp. 89–125. In: Brown, P.R., Wright J.W. (Eds.), Herpetology of the North American Deserts. Proceedings of a Symposium. Southwest. Herpetol. Soc., Spec. Publ. 5, pp. 1–311.

Grismer, L.L., 2002a. Amphibians and Reptiles of Baja California, Including its Pacific Islands and the Islands of the Sea of Cortes. University of California Press, Berkeley, California.

Grismer, L.L., 2002b. A re-evaluation of the evidence for a mid-peninsular seaway in Baja California: a reply to Riddle et al. Herp. Rev. 33, 15–16.

Grismer, L.L., McGuire, J.A., 1993. The oases of central Baja California, México. Part I.A preliminary account of the relictual mesophilic herpetofauna and the status of the oases. Bull. Southern Calif. Acad. Sci. 92, 2–24.

Grismer, L.L., Mellink, E., 1994. The addition of *Sceloporus occidentalis* to the herpetofauna of Isla de Cedros, Baja California, Mexico and its historical and taxonomic implications. J. Herpetol. 28, 120–126.

Grismer, L.L., McGuire, J.A., Hollingsworth, B.D., 1994. A report on the herpetofauna of the Vizcaino Peninsula, Baja California, Mexico with a discussion of its biogeographic and taxonomic implications. Bull. Southern Calif. Acad. Sci. 93, 45–80.

Haq, B.U., Hardenbol, J., Vail, P.R., 1988. Mesozoic and Cenozoic chronostratigraphy and cycles of sea-level change. Soc. Econ. Paleontol. Mineral. Spec. Publ. 42, Tulsa, OK, p. 71108.

Highton, R., 2000. Detecting cryptic species using allozyme data. In: Bruce, R.C., Jaeger, R.G., Houck, L.D. (Eds.), The Biology of Plethodontid Salamanders. Kluwer Academic/Plenum Publishers, New York, NY, pp. 215–241.

Howard, J.H., Seeb, L.W., Wallace, R., 1993. Genetic variation and population divergence in the *Plethodon vandykei* species group. Herpetologica 49, 238–247.

Huelsenbeck, J.P., Ronquist, F., 2001. MrBayes: Bayesian inference of phylogeny. Bioinformatics 17, 754–755.

Jaeger, J.R., Riddle, B.R., Bradford, D.F., 2005. Cryptic Neogene vicariance and Quaternary dispersal of the red-spotted toad (*Bufo punctatus*): insights on the evolution of North American warm desert biotas. Mol. Ecol. 14, 3033–3048.

Jameson, D.L., Mackey, J.P., Richmond, R.C., 1966. The systematics of the Pacific tree frog, *Hyla regilla*. Proc. Calif. Acad. Sci. 33, 551–620.

Johnson, I., 1924. Expedition of the California Academy of Sciences to the Gulf of California in 1921. The botany (vascular plants). Proc. Calif. Acad. Sci. 12, 951–1218.

Johnson, R.A., Ward, P.S., 2002. Biogeography and endemism of ants (Hymenoptera: Formicidae) in Baja California, Mexico: a first overview. J. Biogeogr. 29, 1009–1026.

Kuhner, M.K., Yamato, J., Felsenstein, J., 1998. Maximum likelihood estimation of population growth rates based on coalescent. Genetics 149, 429–434.

Kumar, S., Tamura, K., Jakobsen, I.B., Nei, M., 2001. MEGA2: Molecular Evolutionary Genetics Software. Arizona State University, Tempe, Arizona, USA.

Lessa, E.P., Cook, J.A., Patton, J.L., 2003. Genetic footprints of demographic expansion in North America, but not in Amazonia, during the Late Quaternary. Proc. Natl. Acad. Sci. USA 100, 10331–10334.

Matthews, K.R., Pope, K.L., Preisler, H.K., Knapp, R.A., 2001. Effects of nonnative trout on Pacific treefrogs (*Hyla regilla*) in the Sierra Nevada. Copeia 2001, 1130–1137.

Montanucci, R.R., 2004. Geographic variation in *Phrynosoma coronatum* (Lacertilia, Phrynosomatidae): further evidence for a peninsular archipelago. Herpetologica 60, 117–139.

Moriarty, E.C., Cannatella, D.C., 2004. Phylogenetic relationships of the North American chorus frogs (*Pseudacris*: Hylidae). Mol. Phylogenet. Evol. 30, 409–420.

Moritz, C., Schneider, C.J., Wake, D.B., 1992. Evolutionary relationships within the *Ensatina eschscholtzii* complex confirm the ring species interpretation. Syst. Biol. 41, 273–291.

Murphy, R.W., Aguirre-León, G., 2002. The nonavian reptiles. Origins and evolution. In: Case, T.D., Cody, M.L., Ezcurra, E. (Eds.), A New Island Biogeography of the Sea of Cortez. Oxford University Press, NY, pp. 181–220.

Murphy, R.W., Kovac, V., Haddrath, O., Allen, G.S., Fishbein, A., Mandrak, N.E., 1995. mtDNA gene sequence, allozyme, and morphological uniformity among red diamond rattlesnakes, *Crotalus ruber* and *Crotalus exsul*. Can. J. Zool. 73, 270–281.

Nelsen, E.E., 1921. Lower California and its natural resources. Natl. Acad. Sci. 16, 1–194.

Nielson, M., Lohman, K., Sullivan, J., 2001. Phylogeography of the tailed frog (*Ascaphus truei*): implications for the biogeography of the Pacific Northwest. Evolution 55, 147–160.

Orr, R.T., 1960. An analysis of the recent land mammals. Syst. Zool. 9, 171–179.

Posada, D., Crandall, K.A., 1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14, 817–818.

Posada, D., Crandall, K.A., Templeton, A.R., 2000. GeoDis: a program for the cladistic nested analysis of the geographical distribution of genetic haplotypes. Mol. Ecol. 9, 487–488.

Riddle, B.R., Hafner, D.J., Alexander, L.F., 2000a. Comparative phylogeography of Baileys' Pocket Mouse (*Chaetodipus bailey*) and the *Peromyscus eremicus* species group: historical vicariance of the Baja California peninsular desert. Mol. Phylogenet. Evol. 17, 161–172.

Riddle, B.R., Hafner, D.J., Alexander, L.F., Jaeger, J.R., 2000b. Cryptic vicariance in the historical assembly of a Baja California Peninsular Desert biota. Proc. Natl. Acad. Sci. USA 97, 14438–14443.

Ripplinger, J.I., Wagner, R.S., 2004. Phylogeography of northern populations of the Pacific treefrog, *Pseudacris regilla*. Northwestern Nat. 85, 118–125.

Rozas, J., Rozas, R., 1999. DNASP, version 3: an integrated program for molecular population genetics and molecular evolution analysis. Bioinformatics 15, 174–175.

Sambrook, J., Fritsch, E.F., Maniatis, T., 1989. Molecular Cloning: A Laboratory Manual, second ed. Cold Spring Harbor Lab Press, New York.

Savage, J.M., 1960. Evolution of a peninsular herpetofauna. Syst. Zool. 9, 184–212.

Schneider, S., Roessli, D., Excoffier, L., 2000. Arlequin ver. 2000: A Software for Population Genetics Data Analysis. Genetics and Biometry Laboratory, University of Geneva, Switzerland.

Shaffer, H.B., Fellers, G.M., Voss, S.R., Oliver, J.C., Pauly, G.B., 2004. Species boundaries, phylogeography, and conservation genetics of the red-legged frog (*Rana aurora/draytonii*) complex. Mol. Ecol. 13, 2667–2677.

Smith, M.A., Green, D.M., 2005. Dispersal and the metapopulation paradigm in amphibian ecology and conservation: are all amphibian populations metapopulations? Ecography 28, 110–128.

Snyder, W.F., Jameson, D.L., 1965. Multivariate geographic variation of mating call in populations of the Pacific tree frog (*Hyla regilla*). Copeia 1965, 129–142.

Stebbins, R.C., 1985. Western Reptiles and Amphibians. Houghton Mifflin, New York.

Steele, C.A., Carstens, B.C., Storfer, A., Sullivan, J., 2005. Testing hypotheses of speciation timing in *Dicamptodon copei* and *Dicamptodon aterrimus* (Caudata: Dicamptodontidae). Mol. Phylogenet. Evol. 36, 90–100.

Swofford, D., 2002. PAUP*: Phylogenetic analysis using parsimony (* and other Methods), Version 4. Sinauer Associates, Sunderland, MA.

Taberlet, P., Fumagalli, L., Wust-Saucy, A.G., Cosson, J.F., 1998. Comparative phylogeography and postglacial colonization routes in Europe. Mol. Ecol. 7, 453–464.

Tan, A.M., Wake, D.B., 1995. MtDNA phylogeography of the California newt, *Taricha torosa* (Caudata, Salamandridae). Mol. Phylogenet. Evol. 4, 383–394.

Taylor, R.J., Regal, P.J., 1978. The peninsular effect on species diversity and the biogeography of Baja California. Am. Nat. 112, 583–593.

Templeton, A.R., 2004. Statistical phylogeography: methods of evaluating and minimizing inference errors. Mol. Ecol. 13, 789–809.

Templeton, A.R., Crandall, K.A., Sing, C.F., 1992. A cladistic analysis of phenotypic association with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III. Cladogram estimation. Genetics 132, 619–633.

Templeton, A.R., Routman, E., Phillips, C.A., 1995. Separating population structure from population history: a cladistic analysis of the geographical distribution of mitochondrial DNA haplotypes in the tiger salamander, *Ambystoma tigrinum*. Genetics 140, 767–782.

Upton, D.E., Murphy, R.W., 1997. Phylogeny of the side-blotched lizards (Phrynosomatidae: *Uta*) based on mtDNA sequences: support for a mid-peninsular seaway in Baja California. Mol. Phylogenet. Evol. 8, 104–113.

Wiggins, I.L., 1960. The origin and relationships of the land flora. Syst. Zool. 9, 148–165.

Wiggins, D.A., 1999. The peninsular effect on species diversity: a reassessment of the avifauna of Baja California. Ecography 22, 542–547.

Wilcox, B.A., 1978. Supersaturated island fauns: a species-age relationship for lizards on post-Pleistocene land-bridge islands. Science 199, 996–998.

Wilson, A.C., Cann, R.L., Carr, S.M., George, M., Gyllensten, U.B., Helm-Bychowski, K.M., Higuchi, R.G., Palumbi, S.R., Prager, E.M., Sage, R.D., Stoneking, M., 1985. Mitochondrial DNA and two perspectives on evolutionary genetics. Biol. J. Linn. Soc. 26, 375–400.