A NEW SPECIES OF *PSEUDOEURYCEA* (CAUDATA: PLETHODONTIDAE) FROM NORTHERN OAXACA, MÉXICO

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We describe a new species of *Pseudoeurycea* from the northern-most high peak of the Sierra de Juárez, Oaxaca, México. This species belongs to the *P. juarezi* group, a monophyletic assemblage restricted to northern Oaxaca and comprising three species: *P. juarezi*, *P. saltator*, and *P. aurantia* sp. nov. *Pseudoeurycea aurantia* is the sister taxon to the clade formed by *P. juarezi* and *P. saltator*. The new species is diagnosed by a distinctive coloration and by divergent mitochondrial DNA sequences.

Key words: mitochondrial DNA, new species, phylogeny, salamander, systematics

INTRODUCTION

Pseudoeurycea is a large clade of neotropical salamanders that displays extensive morphological diversity and genetic differentiation (Parra-Olea & Wake, 2001; Parra-Olea, 2002). It ranges from northern México across the Isthmus of Tehuantepec into Guatemala and – with few exceptions – its species occur at elevations above 1200 m.

The systematics of *Pseudoeurycea* have changed recently, with several species newly described or in the process of description (Parra-Olea *et al.*, 2001; Wake & Campbell, 2001) and several more identified as new (Parra-Olea, 1999, 2002). A recent phylogenetic analysis (Parra-Olea, 2002) based on mtDNA showed the paraphyly of *Pseudoeurycea* with respect to *Lineatriton* and *Ixalotriton*. This paraphyly forced the transfer of *P. parva* to *Ixalotriton*, but the taxonomic problem involving *Lineatriton* is still unsolved (Parra-Olea & Wake, 2001).

The analyses of Parra-Olea (2002) found phylogenetic support for the recognition of three species groups within *Pseudoeurycea* (*P. bellii*, *P. gadovii* and *P. leprosa* species groups). Additionally, three taxa (*P. juarezi*, *P. saltator* and *P. unguidentis*) are not grouped with any of these and form part of the basal polytomy. *P. juarezi* and *P. saltator* are sister taxa, and their relationships to the rest of *Pseudoeurycea* are not resolved. *P. unguidentis* forms part of the basal polytomy on its own.

The group formed by *P. juarezi* and *P. saltator* (here termed the *P. juarezi* group), includes species characterized by morphology associated with semiarborial life, with long limbs and toes and slender bodies. The species inhabit terrestrial habits, under rocks, logs and under the bark of logs on the ground, or bromeliads. Their range is from high altitude, unforested habitats dominated by grasses to mid-elevation cloud forest in the Sierra de Juárez of northern Oaxaca, México.

The Sierra de Juárez, where all known species of the *P. juarezi* group occur, is a moderately high mountain chain (2900 m maximum altitude) which runs from the highlands of Cuicatlán Valley (Cerro Peña Verde) to the east of Ciudad de Oaxaca, with southern limits in the area of Cerro San Felipe and Cuajimoloyas. At Cerro San Felipe, the Sierra Aloapaneca intersects the Sierra de Juárez, and runs west and north of Cerro San Felipe. The vegetation changes according to altitudinal gradients from the nearly treeless summits of Cerro Pelón covered by low shrubs to the mesic cloud forest. The herpetofauna of the region, one of the richest in montane México (Casas-Andreu *et al.*, 1996) keeps providing many new species despite past intensive search. During the last eight years, six new salamanders (Hanken & Wake, 1994, 2001) and 10 anurans (Campbell & Duellman, 2000; Mendelson, 1997; Mendelson & Campbell, 1999; Toal, 1994; Toal & Mendelson, 1995; Ustach *et al.*, 2000) have been described for the Sierra de Juárez.

A visit to the Sierra Peña Verde – the northern-most high peak of the Sierra de Juárez in the highlands above the Cuicatlán Valley – uncovered a morphologically distinct new salamander that resembles species of the *P. juarezi* group. The analysis of mitochondrial DNA (mtDNA) partial sequences of the Cytochrome *b* (Cyt *b*) and 16S genes confirmed its close relationship with members of the *P. juarezi* group, but it is not the sister taxon of any of the known species. We present here a new phylogenetic hypothesis for the group together with the description of this new taxon. We provide some ecological data and describe the eggs of the new species.

MATERIALS AND METHODS

MORPHOLOGICAL DESCRIPTION

The descriptions follow the format used by Lynch & Wake (1989) for other species in the genus *Pseudoeurycea*, and include the same basic characters and measurements. Larger measurements were taken using dial calipers (to the nearest 0.1 mm), but measurements of feet, toes and some head dimensions

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(e.g. additional measurements of the holotype), as well as tooth counts were taken using a stereoscopic micro-scope equipped with an eyepiece graticule. All measurements are in mm. The distance from the tip of the snout to the posterior end of the vent is treated as standard length (SL). Colour notes are based on field notes taken from living specimens, and on preserved specimens. Osteological descriptions are based on two cleared and bone-cartilage differentially stained specimens (IBH 13797, 13798).

Material examined: Nine specimens from 4 km W Peña Verde, Oaxaca, México, 2805 m elevation, 17° 50.20' N, 96° 47.05' W, (EBUAP2052-2058; · IBH13793-13794). Material for coloration and mtDNA comparisons consisted of specimens of *P. juarezi*, and *P. saltator* recently collected (Parra-Olea *et al.*, 1999, Parra-Olea, unpubl. data). Comparative measurements between *P. juarezi* and *P. saltator* were taken from Lynch & Wake (1989). Institutional abbreviations: EBUAP: Escuela de Biología, Universidad Autónoma de Puebla, México; IBH: Colección Nacional de Anfibios y Reptiles, Instituto de Biología, UNAM, México.

MTDNA SEQUENCES

We obtained partial sequences of 16S and Cyt *b* for the single specimen from which tissue was available (IBH13793). Whole genomic DNA was extracted from small amounts of ethanol-preserved tissues, using the Quiagen DNA extraction kit. We sequenced 554 base pairs of the large 16S subunit ribosomal mtDNA gene corresponding to positions 2510-3059 in the human mitochondrial genome (Anderson *et al.*, 1981), and 570 base pairs of the Cytochrome *b* gene, expanding from codon 7 of the *Xenopus* Cyt *b* gene (Roe *et al.*, 1985). Amplification was done via the polymerase chain reaction (PCR) (Saiki *et al.*, 1988), using the primers "MVZ15", "MVZ18" (Moritz *et al.*, 1992) for Cyt *b*, and the primers "16Sar" and "16Sbr" (Palumbi *et al.*, 1991) for 16S. PCR reactions consisted of 38 cycles with a denaturing temperature of 92°C (1 min), annealing at 48-50°C (1 min), and extension at 72°C (1 min) in a Techne PHC-1 thermocycler. PCR reactions were run in a total volume of 25 μl, using 0.5 pmol of each primer. Double strand templates were cleaned using a QIAquick PCR purification kit (QIAgen). We used 1 μl of PCR product as the template for cycle sequencing reactions in a 10 μl total volume with the Perkin-Elmer Ready Reaction Kit to incorporate dye-labeled dideoxy terminators. Thermal cycling was performed using standard conditions. Cycle sequencing products were purified using ethanol precipitation and run in an ABI 310 capillary sequencer.

The sequences were compiled using Sequence Navigator™ version 1.0.1 (Applied Biosystems), and aligned to the previously published data set for species of *Pseudoeurycea* (Parra-Olea, 2002). Pairwise comparisons of corrected sequence divergence (Kimura 2-parameter; Kimura, 1980), were obtained using the computer program PAUP*4.0b8a (Swofford, 2002).

Phylogenetic inference was based primarily on maximum parsimony analyses (MP: Swofford, 2002). MP phylogenies were estimated using the heuristic search algorithm for each tree-building methodology. We used 10 repeated randomized input orders for taxa in all MP analysis to minimize the effect of entry sequence on the topology of the resulting cladograms. MP analyses were conducted without the steepest descent option, and with accelerated character transformation (ACCTRAN) optimization, tree bisection-reconnection (TBR) branch swapping, and zero-length branches collapsed to yield polytomies. We used nonparametric bootstrapping (1000 pseudoreplicates) and decay indices to assess the stability of internal branches in the resulting topologies (Felsenstein, 1985; Felsenstein & Kishino, 1993). Each base position was treated as an unordered character with four alternative states. Gaps were treated as missing data.

DESCRIPTION OF NEW SPECIES

PSEUDOEURYCEA AURANTIA, NEW SPECIES
PEÑA VERDE SALAMANDER
SALAMANDRA DE PEÑA VERDE

Holotype. EBUAP2051, an adult male collected 4 km W Peña Verde, Oaxaca, México; 2805 m elevation; 17° 50.20' N, 96° 47.05' W, on 7 May 2000 by Luis Canseco Márquez (Fig. 1a).

Paratypes. All from Peña Verde, Oaxaca, México: IBH13793-13794 (two specimens), and EBUAP2052-2058 (seven specimens), same data as the holotype.

Diagnosis. We include this species in the genus *Pseudoeurycea* based on the following osteological characters: distal tarsal five separated from tarsal four, smaller than four, and not articulated with central; premaxillary single; middle digits markedly larger than the outer; and presence of sublingual fold. It is distinguished from all other *Pseudoeurycea* by its orange coloration. This species is closely related to members of the *P. juarezi* group based on morphology and mtDNA (Figs. 1, 4). It is distinguished from *P. juarezi* in having fewer maxillary and premaxillary teeth (63-81 vs. 74-100 for *P. juarezi*), and a shorter tail (relative tail length=0.88 vs. 0.96 in *P. juarezi*); and from *P. saltator* in having a larger size (maximum SL = 51 mm vs. 48 in *P. saltator*), and a shorter tail (relative tail length =0.88 vs. 1.05 in *P. saltator*).

Description. A medium-sized salamander; SL in two adult males 43-43.4 (mean=43.2) and in eight females 38.5-51.7 (mean=44.6), with relatively robust habitus; head relatively broad, (15-16% SL in both males, and females); snout broadly rounded, more truncate in males than in females; neck region ill-defined, only slightly narrower than head; eyes moderate in size, only slightly protuberant. Adult males present mental gland well developed. Parotoid glands not evident. Costal folds 13, counting one each in axilla and groin. Limbs are rela-
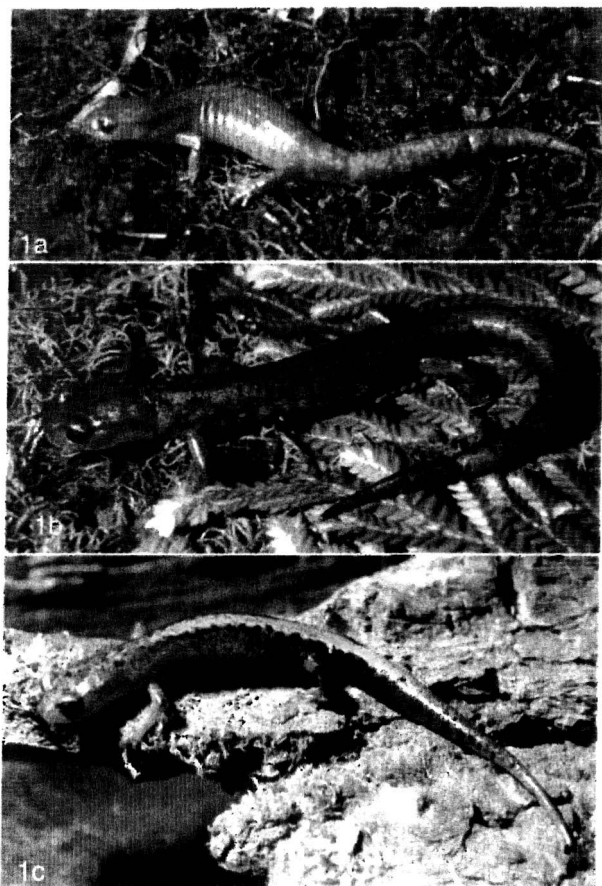
FIG. 1. Live specimens of members of the *P. juarezi* group –
a, *P. aurantia*, EBUAP 2052 (paratype), b, *P. juarezi* (Cerro
Pelón, Oaxaca, México); c, *P. saltator* (La Esperanza,
Oaxaca, México).

tively long; digits typically meet when limbs are pressed
to the side of trunk in males, or separated by no more
than one costal interspace; in females, appressed limbs
fail to meet by one to one and one half costal
interspaces. Hands and feet relatively well developed,
digits relatively long and slender for *Pseudoeurycea*,
without subterminal pads; fifth toe well developed but

FIG. 2. Geographic distribution of species of the *P. juarezi*
group in the Sierra de Juárez, Oaxaca, México. Shaded areas,
> 2000 m.

much shorter than the fourth. Digits in order of decreas-
ing length: fingers 3-2-4-1; toes 3-4-2-5-1. Tail
relatively stout, and short – 72-100% SL; Maxillary
teeth 66 (mean=66) in males, 55–67 (mean=62.5) in fe-
males; premaxillary teeth 2-4 (mean=3) and enlarged in
adult males, 8–19 (mean=13.5) and smaller in females;
vomerine teeth in long rows, 17-20 (mean=18.5) in
males, 17–29 (mean=21.5) in females.

Coloration in life. The ground colour is reddish-
brown dorsally grading to pale yellow ventrally. A
broad and conspicuous orange mid-dorsal stripe extends
from the scapular region to the tip of the tail. In the head
region the dorsal stripe is broken into darker flecks.
Bright yellow spots are present all over the dorsum and
are more concentrated on the tail. A dark line runs from
the nostril, posteriorly to the neck region. Some speci-
mens present a few dark spots along the sides of the
body and tail. The limbs generally are the same reddish-
brown as the dorsum. The underside of the body and tail
is uniform pale yellow with no spots. There is a bright
yellow stripe beneath the lip area. The iris is coppery
gold.

Coloration in alcohol. The coloration in alcohol con-
trasts with that of the living animals, only in the degree
of brightness. The dorsum is dark brown, with a dark
orange stripe running from the scapular region to the tip
of the tail. The venter is pale yellow.

Measurements of holotype (in mm). Head width 6.8;
head depth 3.6; eyelid length 3.0; eyelid width 2.1; ante-
rior rim of orbit to snout 2.4; interorbital distance 2.7;
distance between corners of eyes 5.6; snout to forelimb
15.1; nostril diameter 0.2; distance between external
nares 2.4; projection of snout beyond mandible 0.7;
snout to gular fold 11.2; width across shoulders 6.3;
snout to posterior angle of vent 45.0; snout to anterior
angle of vent 41.6; axilla to groin 23.2; tail length 47.0;
tail depth at base 4.7; tail width at base 5.3; forelimb
length 10.0; width of hand 3.0; hind limb length 12.7;
width of foot 4.7; length of longest (third) toe 1.8; length
of fifth toe 1.0. Numbers of teeth: premaxillary 4; max-
illary 29/37; vomerine 9/8.

Variation. About 75% of females reach a larger size
than males and have a more robust body. The basic col-
our pattern is the same, but some individuals display
black spots along the sides of the body and tail. The dark
line that runs from the nostril to the neck area across the
eye is less evident in adult females.

Distribution. Pseudoeurycea aurantia has been col-
lected only at the type locality – Peña Verde, Oaxaca,
México, at 2805 m (17° 50.20' N, 96° 47.05' W) (Fig. 2).

Natural history. This species occurs in cloud forest
where it is found under rocks, the bark of logs, and in or
under decaying wood. The coexisting species of sala-
manders at Peña Verde include an undescribed species
of *Pseudoeurycea* (D. Wake, pers. comm.), *Thorius
papaloae* and *Cryptotriton adelos* (Canseco-Márquez,
in press).

One female (EBUAP2057), collected on 7 May,
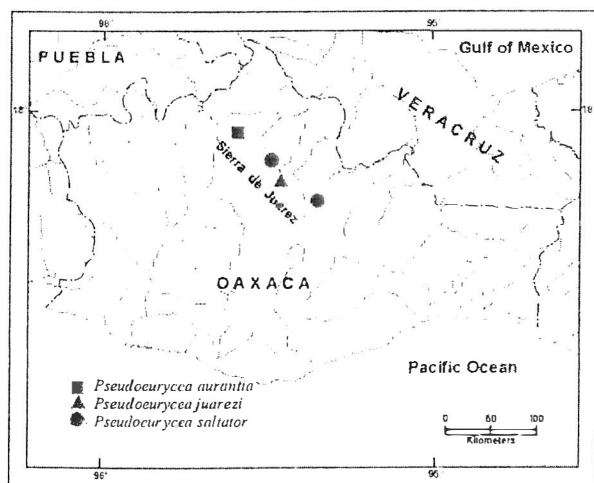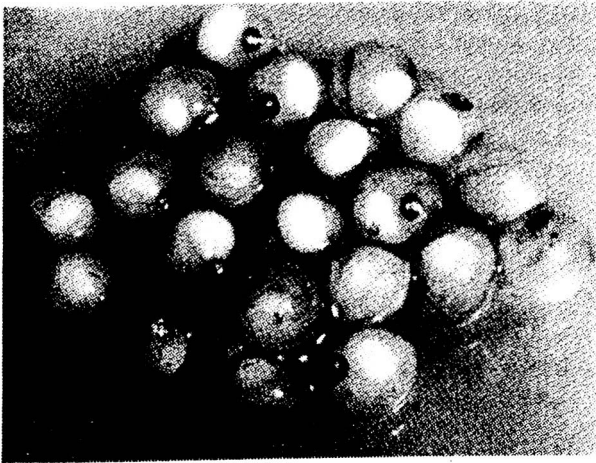2000, 49.3 SL, was guarding a clutch of 22 eggs under

FIG. 3. Egg clutch of *P. aurantia*, at stage 31 of Marks and Collazo (1998). Egg diameter, including surrounding capsules ranges from 4.2 to 5.2 mm (mean=4.89) after preservation.

the bark of a log. The eggs were attached in a bead like fashion. The eggs were preserved in 10% neutral buffered formalin. Egg diameter, including surrounding capsules ranges from 4.2 to 5.2 mm (mean=4.89) after preservation. All embryos were preserved at approximately the same stage of development (Fig. 3), which corresponds to stage 31 of Marks & Collazo (1998). Dorsal pigmentation is extensive and extends onto proximal portions of each limb. The retina is black in all
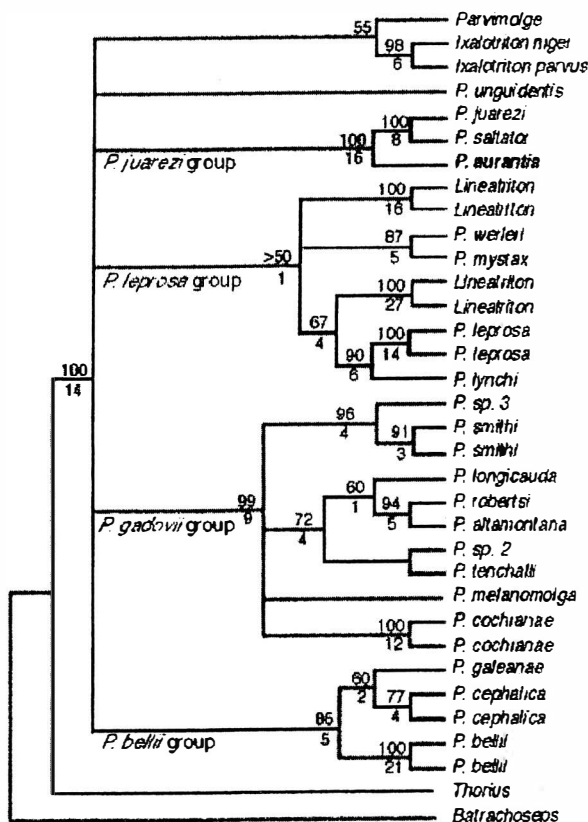

FIG. 4. Maximum Parsimony strict consensus tree of the 16S and Cyt *b* combined data set (1092 bp, TL=1467 steps, CI=0.406, RI=0.564). Numbers above branches are nonparametric bootstrap values (1000 replicates), numbers below branches are decay index values.

of the embryos. The lens is white. Rudiments of all five digits are visible on the hind limbs and rudiments of all four digits are visible in the forelimbs. Gills are tribranchiate.

Etymology. The specific epithet is derived from the Latin *aurantium*, and is used as an adjective in reference to the distinctive orange coloration of this species.

Molecular characters. Parra-Olea (2002) recently reported the results of an extensive molecular analysis of phylogenetic relationships of members of the genus *Pseudoeurycea* (*sensu lato*). Three species groups were recognized (*P. bellii, P. gadovii* and *P. leprosa*). The relationships of three additional species (*P. juarezi, P. saltator* and *P. unguidentis*) were uncertain. The samples used by Parra-Olea (2002) and in the present study under the designation of *P. unguidentis* were collected in an area relatively distant from the type locality of the species (Cerro San Felipe, Oaxaca, México) and based on preliminary morphological data they might represent a different species (D. B. Wake, pers. comm.).

We have now added sequences of 16S and Cyt *b* for the new species, *P. aurantia*, to the data set. The smallest divergences of *P. aurantia* from any other species are for *P. juarezi* (3.9% Cyt *b*, 0.3% 16S) and *P. saltator* (3.8% Cyt *b*, 0.3% 16S). Divergences between *P. aurantia* and sequences from all other *Pseudoeurycea* species groups are large, including *P. aurantia* to *P. gadovii* group (10.7–14.6% Cyt *b*, 3.8–4.8% 16S); *P. aurantia* to *P. bellii* group (15.0–16.6% Cyt *b*, 5.4–6.4% 16S); *P. aurantia* to *P. leprosa* group (11.7–17.0% Cyt *b*, 3.5–4.9%16S).

A maximum parsimony analysis produced nine equally parsimonious trees (L=1467 steps, CI=0.406, RI=0.564, 318 characters were parsimony informative). The strict consensus topology (Fig. 4) does not differ considerably from the published topology for the genus *Pseudoeurycea* (Parra-Olea, 2002). There is a high level of support for recognition of the *P. gadovii* (decay 9, bs 99%) and *P. bellii* (decay 5, bs 86%) species groups, and low support for the *P. leprosa* group (bs <50%). *Pseudoeurycea aurantia* is a sister taxon to the clade formed by *P. juarezi* and *P. saltator* (*P. juarezi* group, decay 16, bs 100%). The *P. juarezi* clade forms part of the basal polytomy.

DISCUSSION

The salamander fauna of the Sierra de Juárez in Oaxaca, México is highly diversified, including 22 described species of the genera *Pseudoeurycea, Thorius, Lineatriton, Chiropterotriton* and *Cryptotriton*. Systematic studies of the salamander fauna of the region started during the 1930's and 40's with descriptions of *P. smithi, P. cochranae* and *P. unguidentis* (Taylor, 1939, 1941, 1943) and species of *Thorius* (Taylor, 1940). Several more species of salamander have been described recently (Hanken & Wake, 1994, 2001; Papenfuss & Wake, 1987; Wake & Campbell, 2001), and there are still a few others yet undescribed (Wake, pers. com.). This is one of the regions of México most frequented by herpetologists, but *P. aurantia* occurs in a

remote area of Sierra de Juárez (Fig. 2). Peña Verde is the northern-most peak, and is of difficult access even now, probably the reason this species remained unknown despite field work in the general area. This species seems to be another montane specialist with restricted distribution, as is true of closely related species.

Based on morphology and mtDNA *P. aurantia* is related to the other two members of the *P. juarezi* group: *P. juarezi* and *P. saltator*, both of which also occur in the Sierra de Juárez. These three species share a general common morphology (Fig. 1) and their differences include size (*P. saltator* is the smallest of the three) number of teeth and tail length. *Pseudoeurycea juarezi* and *P. saltator* live in the same general area and differ in the altitudinal segregation of their habitats. *Pseudoeurycea saltator* is an arboreal dweller of tropical mesic cloud forest found at mid-elevations (1500-1800 m). *P. aurantia* is the most distant geographically, and occurs at elevations similar to *P. juarezi* (2200-2900 m); both occupy terrestrial habitats in the pine forest and barren alpine lands at higher elevations.

The addition of mtDNA sequences of *P. aurantia* to the previously published data set for *Pseudoeurycea*, did not alter the resulting topology (Parra-Olea, 2002). There is strong support for the *P. gadovii*, and *P. bellii* groups, and lower support for the *P. leprosa* group. There is strong bootstrap support for the new *P. juarezi* group that now includes *P. aurantia*. The basal relationships are unresolved, so the affinities of the *P. juarezi* group to the other clades of *Pseudoeurycea* and allied genera are undefined. The geographic and genetic distances between *P. juarezi* and *P. saltator* are smaller than those from *P. aurantia* to either, but morphological differentiation is larger between *P. saltator* and *P. juarezi*. Compared with *P. juarezi*, *P. saltator* is smaller in size, has a longer tail, more vomerine teeth, and a unique coloration (Lynch & Wake, 1989).

Speciation within the *P. juarezi* group happened in a limited geographic area relative to the general pattern found in *Pseudoeurycea*. This group likely represents another example of the influence of sharp ecological zonation across elevational gradients for species formation in tropical salamanders (García-París *et al.*, 2000). In these situations combined localized ecological gradients trigger selection which acts to generate phenotypic divergence and diversity, overcoming the potential homogenizing effects of gene flow, which in combination with high geological complexity promote species formation.

The Sierra de Juárez must be regarded as an important biodiversity centre not only for the high number of taxa inhabiting the mountain chain but also as a focus of species formation from ancient to relatively recent times. The small mtDNA divergence found between *P. saltator* and *P. juarezi* suggests that speciation between them occurred recently, possibly during the Pleistocene. This recent divergence will probably result in non-congruent single gene phylogenies given the short time

provided for gene coalescence and the possibility of introgression along the ecotones between these geographically close taxa. The recency of the lineage split among species of the *P. juarezi* group is an unusual case within tropical salamanders, which generally display large mtDNA divergences among species (Parra-Olea, 2002). Salamanders of the *P. juarezi* species group represent an ideal study case for species formation in tropical regions since different stages of the speciation process are represented by different taxa within the group in a reduced geographic area.

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