Natural history of the critically endangered salamander *Pseudoeurycea robertsi*.

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Mexico is one of the most diverse countries that is losing a large amount of forest due to land use change, these data put Mexico in fourth place for global deforestation rate, therefore, Mexico occupies the first place in number of endangered species in the world with 665 endangered species. It is important to study amphibians because they are among the most threatened vertebrates on Earth and their populations are rapidly declining worldwide due primarily to the loss and degradation of their natural habitats.*Pseudoeurycea robertsi* is a micro-endemic and critically endangered Plethodontid salamander from the Nevado de Toluca Volcano and to date almost nothing is known about its natural history therefore, we survey fourteen sites of the Nevado de Toluca Volcano a mountain that is part of the Trans-Mexican Volcanic Belt, Mexico. We carry out the most exhaustive sampling scheme of this species throughout the Nevado de Toluca Volcano to know the number of individuals and the microhabitat features associated with the presence of *P. robertsi*. Likewise, we carry out a morphometric study and coloration measurements of *P. robertsi* individuals and we determine the potential distribution of *P. robertsi* and the other 3 species of plethodontids present in the NTV using ecological niche modeling and to determine the most important habitat features associated with the presence of salamander species, as well as to know the niche overlap among salamander species. This information will help raise conservation strategies for this micro-endemic and critically endangered salamander.
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Abstract

Mexico is one of the most diverse countries that is losing a large amount of forest due to land use change, these data put Mexico in fourth place for global deforestation rate, therefore, Mexico occupies the first place in number of endangered species in the world with 665 endangered species. It is important to study amphibians because they are among the most threatened vertebrates on Earth and their populations are rapidly declining worldwide due primarily to the loss and degradation of their natural habitats. *Pseudoeurycea robertsi* is a micro-endemic and critically
endangered Plethodontid salamander from the Nevado de Toluca Volcano and to date almost nothing is known about its natural history therefore, we survey fourteen sites of the Nevado de Toluca Volcano a mountain that is part of the Trans-Mexican Volcanic Belt, Mexico. We carry out the most exhaustive sampling scheme of this species throughout the Nevado de Toluca Volcano to know the number of individuals and the microhabitat features associated with the presence of P. robertsi. Likewise, we carry out a morphometric study and coloration measurements of P. robertsi individuals and we determine the potential distribution of P. robertsi and the other 3 species of pletodontids present in the NTV using ecological niche modeling and to determine the most important habitat features associated with the presence of salamander species, as well as to know the niche overlap among salamander species. This information will help raise conservation strategies for this micro-endemic and critically endangered salamander.

Key Words: endangered species; conservation; Plethodontidae; Abies forest; deforestation; environmental niche modelling; morphometric geometric; Cyt b; Nevado de Toluca Volcano; Trans-Mexican Volcanic Belt.

Introduction

Mexico is one of the most diverse countries that is losing a large amount of forest due to land use change (Masera et al., 1997). Most of the deforestation (80%) is taking place in the central and southern parts of Mexico (Masera et al., 1997; Herrera-Arroyo et al., 2013). These data put Mexico in fourth place for global deforestation rate (FAO, 2006; Ellis and Porter-Bolland, 2008; Heredia-Bobadilla et al., 2016, 2017) this generated that Mexico occupies the first place in number of endangered species in the world with 665 endangered species. Broken down, that includes 71 bird species, 96 mammals, 98 reptiles, 181 fish and 219 amphibians (ICUN, 2019). The Trans-Mexican Volcanic Belt (TMVB) is one of the biogeographic zones with the highest species richness and endemism across the country, and it is the most important region in terms of endemic amphibian and reptile species and the second most important in terms of species number (Flores-Villela and Canseco-Márquez, 2007). However, it is one of the most urbanized areas of the country and this fact is disrupting the natural connectivity of forest landscapes, 3.4% of the TMVB is highly fragmented by urban settlements and 44.7% by agricultural areas (Sunny et al., 2017).
creating small patches of habitat and reducing metapopulation dynamics between the populations of amphibians’ forest (Lowe et al., 2005; Honnay and Jacquemyn, 2007; Herrera-Arroyo et al., 2013).

It is important to study amphibians because they are among the most threatened vertebrates on Earth (Catenazzi, 2015) and their populations are rapidly declining worldwide due primarily to the loss and degradation of their natural habitats (Pimm et al., 1995; Fisher and Shaffer, 1996; Davidson et al., 2001; Marsh and Trenham, 2001; Stuart et al., 2004; Mendelson et al., 2006; Wake and Vredenburg, 2008; Sodhi et al., 2008; Gamble et al., 2007; Ducatez and Shinev, 2017). Mexico is ranked fifth in worldwide amphibian diversity (Ochoa-Ochoa et al., 2009; Ordoñez-Ifarraguerrí et al., 2017), however, it is the second worldwide country in number of threatened amphibian species (Frias-Alvarez et al., 2010), with 43% of its 376-species threatened (Parra-Olea et al., 2014), the amphibians are threatened in part because of their low dispersion capacity and small home ranges (Blaustein et al., 1994; Beebee, 2005; Zeisset and Beebee, 2008; Hillman et al., 2014), also, they are highly sensitive to perturbations in both terrestrial and aquatic environments because of their dual life histories, highly specialized physiological adaptations and specific microhabitat requirements (Vitt et al., 1990; Wake, 1991; Blaustein, 1994; Blaustein et al., 1994a; Stebbins and Cohen, 1995). Salamanders are important as top-down controls of many invertebrate species and can also be a source of high energy prey for other predators (Davic and Welsh, 2004). In addition, salamanders can represent an important proportion of the vertebrate biomass in old growth forests (Davic and Welsh, 2004), and are thus vital to ecosystem function, also they provide an important indirect regulatory role in the processing of detritus-litter by ingestion of detritivore prey (Davic and Welsh, 2004).

Roberts’ false brook salamander, *Pseudoeurycea robertsi*, is a micro-endemic and critically endangered plethodontid genera/genus found in one of the TMVB volcanoes near Toluca in the state of Mexico: the ‘Nevado de Toluca’ volcano (NTV; SEMARNAT, 2010; IUCN SSC Amphibian Specialist Group, 2016; Sunny et al., 2019a). *P. robertsi* being one of the most threatened amphibians in Mexico with a vulnerability score of 18 (Wilson et al., 2013) has only been reported in one small area of 8km², within a narrow elevation distribution between 2,900 and 3,600 meters above sea level (masl; IUCN SSC Amphibian Specialist Group 2016). Other reports of *P. robertsi* populations presence within other locations has been confirmed by other studies (see Billie, 2009). Few studies have explored systematic and population genetics of this specie (Lynch...
et al., 1983; Parra-Olea, 2002; Bille, 2009; Van Rooij et al., 2011; González-Fernández et al., 2019; Sunny et al., 2019a), more research is needed on its natural history.

Therefore, the aims of this study were: (1) to carry out the most exhaustive sampling scheme of this species throughout the NVT to know the number of individuals and the microhabitat features associated with the presence of *P. robertsi*, (2) to carry out morphometric and coloration measurements of *P. robertsi* individuals, (3) to determine the potential distribution of *P. robertsi* and the other 3 species of pletodontids present in the NTV using ecological niche modeling and to determine the most important habitat features associated with the presence of salamanders species, as well as to know the niche overlap among salamanders species. This information will help raise conservation strategies for this micro-endemic and critically endangered salamander.

**Materials and methods**

**Population sampling and species identification**

A total of 150 individuals from 14 populations (Fig. 1) were sampled from mid-April to mid-October via visual encounter surveys (Crump and Scott, 1994) across the entire polygon of the NTV. Field permits and the approval of the ethics committee were given by the Universidad Autónoma del Estado de México and SEMARNAT (SGPA/DGVS/05701/16). During the survey, we walked back and forth across transects and looked for salamanders in tree stumps, inside fallen logs and under the bark, stones, fallen leaves, and moss. The sampling time was between 9 a.m. and 3 p.m., during which an area of approximately 10ha was covered in each sampling site. In order to sample salamanders, we took tail samples (2mm) for their DNA extraction (see Sunny et al., 2019a for a detailed description) and, subsequently, individuals were released back to the very site they were captured/capture site. The tail clipping sampling method is a low impact technique that does not affect the survival or growth of salamanders (Arntzen et al., 1999; Polich et al., 2013). The salamander tissues were preserved in 90% ethanol and then frozen at -20°C until processing the samples.

For identification in the field, we used the phenotypic characteristics mentioned by Bille, (2009) and later with molecular data to corroborate the correct identification of the species. In order to achieve the species molecular identification we extracted DNA from the salamanders’ tail tips with the Vivantis extraction kit, we specifically followed the manufacturer instructions. We used the
extracted DNA as a template for the mitochondrial *cytochrome b* gene (*cyt b*) amplification with the following primers: MVZ15 and MVZ16 (Moritz et al., 1992). Forward and reverse sequences of all individuals were aligned and edited manually using BIOEDIT 7.1.3 (Hall, 1999). The retrieved sequences of *cyt b* were blasted in GenBank (Morgulis et al., 2008) optimized for highly similar sequences (Megablast) to confirm they matched to *Pseudoeurycea robertsi* or *Pseudoeurycea leprosa*, considering the E value and the percentage of identity.

Morphometric measurements and geometric morphometric

All *P. robertsi* individuals were photographed on a millimeter paper to obtain the following morphometric measurements: head length (HL), from the tip of the snout to the neck; head width (HW), across the widest point of the head; left eye diameter (LED), wide between eyes (WBE), median body width (MBW), across the trunk midway between the front and hind limb insertions, posterior femur length (PFL), both snout vent length (SVL), tail length (TLO), tail wide (TW) and total length (TL), from the posterior left limb insertion to the tip of the longest outstretched toe. To obtain these morphometric measurements, the pixels of each image were converted to millimeters using the software FIJI (Schindelin et al., 2012). For the geometric morphometric, the shape of the head was obtained using 11 landmarks throughout the contour of this structure, and for the eyes 3 landmarks were located as support points for the layout of fans that covered the entire head structure. For all the analyzed individuals of *P. robertsi* we used the software MAKEFAN6 (Sheets, 2003) and TPSDIG2 (Rohlf 2005). The configurations of the landmarks of each organism were adjusted by means of Procrustes using the COORDGEN6F (Sheets, 2003) software, in order to eliminate all information not related to the shape. With the software SEMILAND6 (Sheets, 2002), were aligned using the Procrustes superimposition method and the alignment protocol to eliminate the effect of location, orientation and size effect, and thus evaluate the shape variation (Moreno-Barajas et al., 2013). Using this data, we perform a Principal Components Analysis (PCA) using the PCAGEN6P (Sheets, 2002) software to identify the individuals that provide the most intraspecific variation, in addition, the algorithm allowed to visualize the variations of the configuration by means of vectors and deformation grids of each group, which were built applying the thin plate spline interpolation function (Bokstein, 1991). Finally, with the CVAGEN6 (Sheets, 2005) software, an Analysis of Canonical Variables (CVA)
was carried out with a reduction to 2 PC, to identify the possible segregation between groups, depending on the locality.

Coloration patterns

The photographic records were transformed into 8 bits’ images and RGB color space. We calculate the color frequency of the dorsal stripe of each individual using the 3D Color Inspector and Color Histogram plugins (Barthel, 2007) of the ImageJ 1.5 software, and finally we scaled the color channels and we made a PCA with the color data.

Microhabitat variables and environmental niche modelling

When we found a felled log, we captured the geographic coordinates, type of microhabitat, log species, if the log was naturally felled, its length and width and whether the log had salamanders, we calculated the fallen logs volume, number of individuals in each log and finally, to compare if there were differences between sites and characteristics of the microhabitat, different statistical tests were performed using the software Paleontological Statistics ‘‘PAST’’ 3.06 (Hammer, 2015). To calculated the percentage of forest cover and the percentage of Abies cover we used the ARCGIS 10.5 software and satellite images (SPOT 6/7) of very high resolution (1.5 m) for year 2015, we made a supervised classification considering six land cover types: Pinus forest, Abies forest, grasslands, agriculture, urban areas, and water sources. Likewise, to determine the most important habitat features associated with the presence of P. robertsi we perform an analysis of environmental niche modeling (ENM), we also made the ENM for the other species of Plethodontids found in the NTV (P. robertsi, P. leprosa, Aquilourycea cephalica and Isthmura bellii) in order to know if there is competition for environmental space and to know the overlapping index with the other Plethodontids. We considered the entire distribution of each species to make the ENM. The presence records were obtained from fieldwork and iNaturalist. We selected only the records from the last 10 years for the analysis. We defined a polygon (background) for each species which represents the accessibility area (Suárez-Atilano et al., 2017; Sunny et al., 2017; González-Fernández et al., 2018; Sunny et al. 2019b), considering the biogeographic regions of Mexico with geographical records or records near their borders (Sunny et al., 2017; González-
Fernández et al., 2018). We obtained bioclimatic variables from WorldClim (Hijmans et al., 2005); topographic and land cover variables were obtained from the National Institute of Statistics and Geography. We reclassified the land use map (series V, 1:250000, generated during the period 2011 to 2013; INEGI, 2013) in different exclusive classes that were converted to raster and transformed from categorical to continuous using a resample method that averages the value of the surrounding pixels to assign a new value to each pixel (González-Fernández et al., 2018; Sunny et al., 2019b). All layers were processed in a raster format, with 1 km resolution, using ARCGIS 10.5 and the packages raster (Hijmans, 2016) and rgdal (Bivand et al., 2017) for R software (version 3.4.0; R. Development Core Team, 2017). After a bibliographic review and Pearson correlation analysis to discard highly correlated variables ($R^2 > 0.7$, Dormann et al., 2013) we selected the following variables: natural grasslands percentage, induced grasslands percentage, percentage of arid vegetation, *Pinus* forest percentage, *Quercus* forest percentage, *Abies* forest percentage, cultivated forest, distance to water sources, agriculture percentage, cloud forest mountain percentage, aquatic vegetation, annual mean temperature, maximum temperature of the warmest month, minimum temperature of the coldest month, precipitation of the wettest month, precipitation of the driest month, elevation, aspect, slope (obtained from the elevation layer) and distance to urban settlements, for a better prediction of land use change. We used the maximum entropy modelling software (MAXENT; Phillips et al., 2006). We ran the model for each species in MAXENT with 10 replicates (Anderson et al., 2003; Chefaoui et al., 2005; Suárez-Atilano et al., 2017). We used linear and quadratic features because we had less than 80 records in most of the species (Merow et al., 2013). All analyses were performed using the logistic output for an easier interpretation and a convergence threshold of $1 \times 10^{-5}$ with 500 iterations (Pearson et al., 2007; Suarez-Atilano 2015; González-Fernández et al., 2018). We generated a continuous map of the potential distribution for each species because binary outputs can obscure important biological detail (Liu et al., 2013). To evaluate model performance, we applied the partial ROC analyses (Peterson et al., 2008; Osorio-Olvera et al., 2016). We used the software Niche ToolBox 0.4.1.5 for partial-ROC calculations in R (Osorio-Olvera et al., 2016).

We compute niche overlap from predictions of species distributions with the “D” similarity statistic of Warren et al., (2008) in the software SDMTools (VanDerWal et al., 2014) for R and we plot the niche overlap from predictions of species distributions in Niche ToolBox.
Results

Population sampling and species identification

It was quite complicated to differentiate between *P. robertsi* and *P. leprosa* in some cases, since the coloration pattern can be confused as Bille, (2009) reported, so the analysis of the species determination using the *cyt b* confirmed that in all populations 89% of all salamanders found were *P. robertsi* and 11% were *P. leprosa* with an E value of 0.0 and an identity percentage of 98% considering a sequence of 617 bp. Some sequences were deposited in the GenBank database with the access numbers: MK357639-MK357709. Finally, we recorded 234 *P. robertsi* individuals, the largest number of individuals reported for this species. The altitudinal range of our sampling scheme goes from 2800-3460 MASL. At the moment, there is no information on the timing of peak of *P. robertsi* abundance, the months we sampled comprise the beginning of the rainy and dry seasons. In April (beginning of the rainy season) and in October (beginning of the dry season) we did not record any individual. The maximum number of individuals were found at the end of July and the beginning of August, and by the middle of September it was possible to find from 40 to 67% less salamanders. The number of individuals within each site in the total sampling scheme varied from 0 to 53, Amanalco A had=53 individuals, Rancho Viejo=29, Meson Viejo=28, Amanalco B=25, Las Lagrimas=24, Carretera=20, Palo Seco=18, Amanalco C=16, Santa Cruz=11, Agua Bendita= 5, Raices= 4, San Juan de las Huertas=1, El Contadero=0 and Huacal Viejo-Agua Bendita=0 (Fig. 2). The 99% of the salamanders were found under the bark of trees, the rest was found under the leaf litter. In Agua Bendita we found together *P. robertsi*, *P. leprosa* and *A. cephalica* in two fallen logs (Fig. 2), indicating that these salamanders can share the same environmental niche. We found two individuals of *I. bellii* in Meson Viejo and one in Rancho Viejo (Fig. 2). We found in August in Palo Seco a *P. robertsi* salamander attending an egg mass of 32 white eggs with transparent parts, the eggs are slightly oval in shape and the clutch was found under the bark of an *Abies* log.

Morphometric measurements and geometric morphometric
The average of the measurements of the juvenile salamanders were: HL=5.475, HW=4.150, LED=1.075, WBE=1.800, MBW=4.025, PFL=1.750, SVL=18.543, TLO=14.758, TW=1.975, TL=33.300. The average of the measurements of the adult salamanders were: HL=10.795, HW=7.288, LED=2.218, WBE=2.712, MBW=7.243, PFL=3.936, SVL=45.414, TLO=43.736, TW=3.814, TL=89.150. For the geometric morphometric analysis of the 234 individuals, only 139 individuals could be analyzed, of which 21 landmarks were placed on the head (Fig. 3A), of which six were for the eye contour (Fig. 3A). The deformation grids of the landmarks found a greater deformation in the central part of the head (Fig. 3B). The dispersion of the first two main components in the PCA analysis showed a single group with a low-medium explained variation (45%) between (Fig. 3C). The CVA showed that all individuals of *P. robertsi* from all study areas are grouped into a single group (CV1 Eigenvalue= 0.658 and CV2 Eigenvalue=0.491, Axis1: \( \lambda=0.1604, \chi^2=232.431, df=110, p=9.01349 \times 10^{-11} \); Axis 2: \( \lambda=0.2660, \chi^2=168.182, df=90, p=1.11354e-006 \)).

### Coloration patterns

The color of the dorsal stripes ranges from red to orange (R84, G35, B38 to R187 G124 B77; Fig. 4A), orange to yellow (R174 G135 B63 - R191 G178 B118; Fig. 4B) and brown to black (Brown= R91 G86 B82 – R168 G152 B131 and Black= R28 G28 B28 – R68 G68 B68; Fig. 4C). We found seven patterns of dorsal stripe coloration, two (Fig. 4a and Fig. 4b) that reported Bille (2009) and 5 new patterns (Fig. 4c to 4g), however we could not find the third pattern reported by Bille (2009).

The patterns that we found were the following: 1) well-defined, brick red dorsal stripe (Fig. 4a), 2) dense dorsal mottling (Fig. 4b), 3) the dorsal red line only present on the tail (Fig. 4c), 4) semi well-defined, yellow dorsal stripe (Fig. 4d), 5) the dorsal yellow line only present on the tail (Fig. 4e), 6) almost patternless with few scattered spots (Fig. 4f) and 7) all black without spots or dorsal line (Fig. 4g). All these patterns can also have different coloration gradients, with different shades of yellow, brown or red.

### Microhabitat variables and environmental niche modelling
A total of 873 fallen logs and stumps were sampled in all the study areas, the fallen logs volume ranged from 1.3 to 19.6 m$^3$. The sites that only presented *Abies* forest were: El Contadero, Carretera and San Juan de las Huertas (Fig. 5A), the sites that presented the most percentage of total forest in 10 ha were Amanalco A, Amanalco B, Meson Viejo and Rancho Viejo. Likewise, Amanalco A, Amanalco B, Meson Viejo and Rancho Viejo also had the highest percentage of *Abies* forest cover (Fig. 5B). The sites that showed the most percentage of trees naturally fallen were Amanalco A and Meson Viejo, while the sites that presented the most felled logs are Huacal Viejo-Agua Bendita, El Contadero, Carretera, Santa Cruz and Palo Seco (Fig.5C), we found significant differences between the length (F= 8.05 gl= 894 and p=2.08 E-15 Fig. 5D and Fig. 6A) and diameter of trees per site (F= 3.359 gl= 894 and p=4.69E-05; Fig. 5D and Fig. 6B) and fallen logs volume per site was greater in Las Lagrimas (Fig. 5E). The average length of the logs with salamanders was 4.2 m and without salamanders was 2.7 m, finding significant differences (t=5.4, gl= 789 and p= 0.0001), the average diameter of logs with salamanders was 35.7 cm and without salamanders was 34.2 cm, no significant differences were found (t=1.02, gl= 789 and p= 0.31), of the logs sampled 76% were *Abies* and 24% *Pinus*, the species of tree that presented more salamanders was *Abies* with 77% and *Pinus* with 23%, we found salamanders in the 75% of *Abies* fallen logs and 25% of *Pinus* fallen logs, finding significant differences (Chi$^2$=39.98, gl= 13 and p= 0.0001), of these fallen logs 30% were naturally fallen and 70% were logs felled, finding significant differences (Chi$^2$=817.73, gl= 13 and p= 0.0001).

The MAXENT model performed better than expected by random, where the AUC showed high average values across iterations (mean AUC ratios from 0.816 to 0.993 and p-values <0.001; Table. 1). Also, Partial-ROC bootstrap tests showed significant ratio values of empirical AUC over null expectations (mean AUC ratios $\geq$ 1.4 and p-values <0.001 in all cases; Table. 1). The most important habitat features associated with the presence of *P. robertsi* according to the MAXENT model were: *Abies* forest (38.8%) and maximum temperature of the warmest month (33.4%) for *P. leprosa* were: maximum temperature of the warmest month (54.1%) and *Abies* forest (17.9%), for *A. cephalica* were: maximum temperature of the warmest month (42.2%) and *Abies* forest (21.6%), and for *I. bellii* was: minimum temperature of the coldest month (54.4%). *Abies* forest cover percentage was positively correlated with probability of presence of *P. robertsi*, *P. leprosa* and *A. cephalica*. For *I. bellii* probability of presence decreased with temperature lower than 5°C.
Probability of presence of *A. cephalica*, *P. leprosa* and *P. robertsi* decreased as maximum temperature of the warmest month increased. It is important to note that the maximum temperature of the warmest month and *Abies* forest cover percentage were the most important variable to explain the potential distribution of three of the four species. Potential distribution maps for each species are in Figs. 7A–7D. *P. robertsi* is the salamander species with the smallest distribution (Fig. 7A), followed by *P. leprosa* (Fig. 6B), *A. cephalica* (Fig. 7C) and the salamander species that has the widest distribution is *I. bellii* (Fig. 7D), however the four-salamander species are distributed mainly in the mountains of the TMBV, *A. cephalica* is also distributed in the Sierra Madre Oriental and *I. bellii*, in the Sierra Madre Oriental, in the Sierra Madre Occidental, the Sierra Madre del Sur, the Cuenca del Balsas and the Costa Pacifica Mexicana. The greatest niche overlap occurs between *P. robertsi* and *P. leprosa* (Fig. 8A), followed by *P. robertsi* and *A. cephalica* (Fig. 8B) and finally between *P. robertsi* and *I. bellii* (Fig. 8C).

**Discussion**

Our study reports the first comprehensive evaluation of the natural history of *P. robertsi*, one of the most threatened and extremely endemic salamanders in Mexico. This represents the most extensive dataset recorded in terms of number of samples (N=150), morphological measurements, coloration patterns, localities (11), micro habitat features and macro habitat analyses. Historically there are just one basic ecological study on this species and have considered a significant lesser number of individuals, localities and a very basic habitat information (Bille, 2009). Furthermore, our study is the most complete study of the natural history of this species. This information will help to make informed management decisions focused on the conservation of this micro-endemic species.

Population sampling and species identification

We found that *P. robertsi* was present in all well preserved *Abies* forest of the NTV, presenting a much wider distribution than previously thought (Bille, 2009; IUCN SSC Amphibian Specialist Group, 2016), extending its range of distribution to the entire NVT where the *Abies* forest occurs.

The analysis of species identification through *cyt b*, showed that it is complicated to differentiate between *P. robertsi* and *P. leprosa* by the pattern of coloration, size and morphometric
measurements since these characteristics are very variable (Bille 2009), however *P. robertsi* is 89% more abundant than *P. leprosa*. The elevation range observed for *P. robertsi* was slightly larger than previously reported 2,800-3,600 m a.s.l. (Bille, 2009; IUCN SSC Amphibian Specialist Group, 2016), within these altitudinal limits, we corroborate that this species is found in all the Abies forests of the NVT since this forest is distributed in an altitudinal gradient of 2,400 to 3,500 m in the TMVB (Manzanilla-Quiñones et al., 2019). Bille, (2009) reported that at lower altitudes it is more likely to find more individuals of *P. leprosa*, likewise, in this study, few *P. leprosa* and *A. cephalica* individuals were found in all the altitudinal ranges, therefore it is more likely that *P. robertsi* limits the distribution and abundance of *P. leprosa* by being a stronger competitor and with higher levels of abundance and higher capacity for sexual encounter than *P. leprosa*.

Morphometric measurements and geometric morphometric

Our morphometric measurements are slightly smaller or similar to those reported for *P. leprosa* and *A. cephalica* (Ramírez-Bautista et al., 2009) however it seems that tail is longer in *P. robertsi* than in *P. leprosa* or *A. cephalica*. In contrast, *P. robertsi* is smaller than *I. bellii* (Ramirez-Bautista et al., 2009). All individuals of *P. robertsi* from the eleven sampling sites presented similar morphometric measurements and the geometric morphometric analysis showed that there are no significant differences between the sampling sites, according to the landmarks used in the head. These results are similar to Baken and Adams’ (2019) as they found no differences between salamander species using landmarks in the foot shape (, 2019). This means that salamanders present little morphometric variation between individuals and even between species (Ramírez-Bautista et al., 2009; Baken and Adams, 2019), suggesting that, although salamanders have limited dispersal ability, small home ranges (Kleeberger and Werner, 1982; Ovaska, 1988; Gergits and Jaeger, 1990; Gibbs, 1998a, b; Marvin, 1998) and are restricted to forest patches (Petranka et al., 1993; Welsh and Droge, 2001), they can disperse enough to keep gene flow. It has been reported that male salamanders have the ability to disperse through harsh habitats (Marsh et al., 2005) especially when there are high levels of competition (Liebgold et al., 2011), this may favor a weak population structure and similar phenotypes between the sampling sites in *P. robertsi* (Sunny et al., 2019a).
In this study, we found two coloration patterns that were previously described by Bille (2009), however we did not find the third pattern that he described, in which the salamander presents a few spots on the body. Considering the patterns described by Bille, (2009) and those found in this study, this species has a total of eight dorsal-tail stripe patterns. The great variety in dorsal-tail stripe patterns and colorations suggests that this species has a great phenotypic variability and we presume there may be more patterns with different types of combinations in addition to those already described. Likewise, we found a great variation in the number, size and shape of the spots and gradients of color. Bille, (2009) reported that the coloration patterns may be correlated with the altitudinal range, however, we do not corroborate this hypothesis, we believed that it may be correlated with the color of the log where they were found.

Environmental variables and environmental niche modelling

The largest number of individuals was found in well-preserved Abies forests (Amanalco A, Amanalco B, Meson Viejo and Rancho Viejo), where they also found more natural fallen Abies and Pinus logs. The largest number of individuals was found under the bark of Abies logs, because in these trees the bark does not break into small pieces, generating an ecosystem that allows P. robertsi to have shelter and food like: mollusks as slugs, insects and arachnids (Bille, 2009). In low logging sites (Amanalco A and Meson Viejo), we found many fallen logs that fell down with storms. These sites hold the maximum salamander individuals and logs were almost complete (between 5 and 8 meters long approximately). In moderate logging sites, without clearings and cut logs abandoned (between 1 and 3 meters long approximately), there were relatively high individuals also. However, we found few salamanders in forests with many clearings (Huacal Viejo-Agua Bendita, El Contadero, Carretera, Santa Cruz, Palo Seco and Raices), were most of the fallen logs had been extracted or were small and thin. Therefore, we emphasize the need to leave and actively restore the availability of dead wood in managed forests in order to preserve these salamander species (Kouki, 2001; Parra-Olea et al., 2005; Monterrubio-Rico and Escalante-Pliego, 2006; Ramírez-Bautista et al., 2009; Velo-Antón et al., 2013; Medina et al., 2018). Likewise, fallen logs found in the grasslands, in the forest edges or in forest clearings were always completely dry and tough while fallen logs found inside of the forest were all completely wet and
following a process of wood decomposition. We always found salamanders in wet logs with intermediate levels of decomposition (field observations). This is consistent with the importance of forest core areas reported in another study (González-Fernández et al., 2019) and is related with the importance of humidity for this species as Plethodontids do not have lungs and rely on cutaneous respiration (Petranka et al., 1993; deMaynadier and Hunter 1998).

ENM analysis show that the macro habitat preferred by *P. robertsi* is the *Abies* forest, as well as *P. leprosa* and *A. cephalica*. However, *P. robertsi* is the most *Abies* forest specialist and the species with the most restricted distribution whereas *P. leprosa* and *A. cephalica* are distributed in all the mountains of the TMVB which contain the 91.1% of the *Abies* forests of the country (Sunny et al., 2017). *I. bellii* has a much more extensive distribution in a great variety of vegetation covers. Likewise, whereas *I. bellii* probability of presence decreased with temperature lower than 5°C, *A. cephalica*, *P. leprosa* and *P. robertsi* were limited by maximum temperatures, this can be explained because these species are adapted to high elevations and cold environments. Therefore, temperature increase caused by climate change will affect these three species more than those adapted to lower elevations because species at the highest elevations are limited in their ability to shift distribution upslope, thereby increasing the possibility of becoming extinct (Parra-Olea et al., 2005; Velo-Antón et al., 2013), specially *P. robertsi* for having such a limited distribution. The highest niche overlap occurred between *P. robertsi* and *P. leprosa* followed by *P. robertsi* and *A. cephalica* and finally between *P. robertsi* and *I. bellii*.

Conclusions and conservation implications

Our results suggest that *Abies* forests are extremely important for *P. robertsi*, as well as for *P. leprosa* and *A. cephalica* and that *Abies* forests with naturally fallen logs will hold more salamander individuals. If these forests disappear or suffer grate reductions *P. robertsi* populations can diminish or even become extinct. Thus, it is essential to protect the *Abies* forests of the NTV. According to some authors *Abies* forest are in the 30.69% of the area in the NTV (16,164.18 ha; Franco-Maass et al., 2006), or 6,847.8 ha according to Manzanilla-Quiñones et al., (2019). Therefore, it is necessary to carry out a more precise study to delimit the area of the *Abies* forest and prevent its deterioration by anthropogenic activities, such as pollution by garbage, agrochemicals, fires and deforestation, to prevent the extinction of the populations of *P. robertsi*.
However, the Mexican government recently changed the protection status of the NTV from National Park to a less restrictive category in terms of land use. This new category allows forest harvesting practices with commercial proposes as well as the construction of ecotourism centers in almost all *Abies* forest extension (Fig. 9), which was well preserved under the National Park category (Franco-Maass et al., 2006; Mastretta-Yanes et al., 2014; González-Fernández et al., 2018). Thus, this change of category is compromising the habitat of the endemic and critically-endangered *P. robertsi*, as well as other forest-dwelling animals (*Danaus plexippus*, *Aquiloeurycea cephalica*, *Pseudoeurycea leprosa*, *Isthmura bellii*, *Sceloporus grammicus*, *S. spinosus*, *Aegolius acadicus*, *Oculatus tolucae*, *Lynx rufus*, among others). Finally, we recommend to prioritize in conservation programs the areas of Amanalco, Meson Viejo and Rancho Viejo, since these areas had higher levels of genetic diversity and number of individuals of *P. robertsi* populations (Sunny et al., 2019a) likewise, these areas also have the most well conserved *Abies-Pinus* forests of the NTV.

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Compliance with ethical standards and conflict of interest

The authors declare that they have no conflict of interest. Our study received the field permits and the approval of the ethics committee from Universidad Autónoma del Estado de México and SEMARNAT (SGPA/DGVS/05701/16).

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Sheets HD. 2005. CVAGen6, Canonical Variates Analysis program for the analysis of shape, based on partial warp scores.


Figure 1

Nevado de Toluca Volcano map and sampled sites.

Nevado de Toluca Volcano indicating *Pseudoeurycea robertsi* sampled sites.
Figure 2

Population sampling

Number of individuals sampled per site.

Number of species per site

![Bar chart showing number of species per site.](chart.png)
Figure 3

Morphometric measurements and geometric morphometric

A) Location of landmarks in the head of *Pseudoeurycea robensi*. B) The deformation grid of the landmarks found we found a greater deformation in the central part of the head. C) The PCA analysis showed a single group with a low-medium explained variation.
Coloration patterns

Color pattern of the dorsal stripes. A) Dorsal stripes color ranges from red to orange. B) Dorsal stripes color ranges from orange to yellow. C) Dorsal stripes color ranges from brown to black. The dorsal stripe patterns range from: a) Well-defined, brick red dorsal stripe. b) Dense dorsal mottling. c) The dorsal red line only present on the tail. (d) Semi well-defined, yellow dorsal stripe. e) The dorsal yellow line only present on the tail. f) Almost patternless with few scattered spots. g) All black without spots or dorsal line.
Figure 5

Microhabitat variables

A) Percentage of three species per site. B) Percentage of total forest and *Abies* forest in 10 ha per site. C) Percentage of trees naturally fallen and logs felled per site. D) Average length and diameter of trees per site. E) Falled logs volume per site.
Figure 6

Mean and whisker plots of the ANOVA analysis

Mean and whisker plots of the ANOVA analysis between the A) average length and and B) diameter of trees per site.
Figure 7

Environmental niche modelling

A) *P. robertsi*  

B) *P. leprosa*

C) *A. cephalica*  

D) *I. bellii*

Legend:
- High probability
- Low probability
Figure 8

Environmental niche overlap between the salamander species

Environmental niche overlap between the four-salamander species. A) Environmental niche overlap between *Pseudoeurycea robersi* and *Pseudoeurycea leprosa*. B) Environmental niche overlap between *Pseudoeurycea robersi* and *Aquiloeurycea cephalica*. C) Environmental niche overlap between *Pseudoeurycea robersi* and *Isthmura bellii*. 
Figure 9

Nevado de Toluca Volcano with the commercial logging allowed areas

Nevado de Toluca Volcano area showing the two types of forest in dark and light green, in red the commercial logging allowed areas and in the blue dots are the sampling sites.
Table 1 (on next page)

Potential distribution model performance

Potential distribution AUC and Partial-ROC values.
<table>
<thead>
<tr>
<th>Species</th>
<th>AUC</th>
<th>Partial-ROC</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. robertsi</td>
<td>0.993</td>
<td>1.970</td>
</tr>
<tr>
<td>P. leprosa</td>
<td>0.964</td>
<td>1.846</td>
</tr>
<tr>
<td>A. cephalica</td>
<td>0.888</td>
<td>1.430</td>
</tr>
<tr>
<td>I. bellii</td>
<td>0.816</td>
<td>1.403</td>
</tr>
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