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

Armando Sunny [Corresp., Equal first author, 1] , **Carmen Caballero-Viñas** [2] , **Luis Duarte-deJesus** [1] , **Fabiola Ramírez-Corona** [3] , **Javier Manjarrez** [4] , **Giovanny González-Desales** [1] , **Xareni P. Pacheco** [1] , **Octavio Monroy-Vilchis** [1] , **Andrea González-Fernández** [Corresp. Equal first author, 4]

[1] Centro de Investigación en Ciencias Biológicas Aplicadas, Universidad Autónoma del Estado de México, Toluca, Estado de México, México

[2] Laboratorio de Sistemas Biosustentables, Universidad Autónoma del Estado de México, Toluca, Estado de México, México

[3] Departamento de Biología Evolutiva, Facultad de Ciencias, Universidad Nacional Autónoma de México, Ciudad de México, Ciudad de México, México

[4] Laboratorio de Biología Evolutiva, Facultad de Ciencias, Universidad Autónoma del Estado de México, Toluca, Estado de México, México

Corresponding Authors: Armando Sunny, Andrea González-Fernández
Email address: sunny.biologia@gmail.com, andreagofe@gmail.com

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.

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

2

3 Armando Sunny[a*], Carmen Caballero-Viñas[b], Luis Duarte-deJesus[a], Fabiola Ramírez-Corona[c],

4 Javier Manjarrez[d], Giovanny González-Desales[a], Xareni P. Pacheco[a], Octavio Monroy-Vilchis[a],

5 Andrea González-Fernández[d]

6

7 [a]Centro de Investigación en Ciencias Biológicas Aplicadas, Universidad Autónoma del Estado de

8 México, Instituto literario # 100, Colonia Centro, 50000. Toluca, Estado de México, México.

9

10 [b]Laboratorio de Sistemas Biosustentables, Facultad de Ciencias, Universidad Autónoma del

11 Estado de México, Instituto literario # 100, Colonia Centro, 50000. Toluca, Estado de México,

12 México.

13

14 [c]Taller de Sistemática y Biogeografía, Departamento de Biología Evolutiva, Facultad de Ciencias,

15 Universidad Nacional Autónoma de México, Avenida Universidad # 3000, Ciudad Universitaria,

16 04510. Ciudad de México, México.

17

18 [d]Laboratorio de Biología Evolutiva, Facultad de Ciencias, Universidad Autónoma del Estado de

19 México, Instituto literario # 100, Colonia Centro, 50000. Toluca, Estado de México, México.

20

21 *Corresponding author: E-mail, sunny.biologia@gmail.com, Tel.: +527223483375

22

23 **Running title: Natural history of *Pseudoeurycea robertsi*.**

24

25 **Abstract**

26 Mexico is one of the most diverse countries that is losing a large amount of forest due to land use

27 change, these data put Mexico in fourth place for global deforestation rate, therefore, Mexico

28 occupies the first place in number of endangered species in the world with 665 endangered species.

29 It is important to study amphibians because they are among the most threatened vertebrates on

30 Earth and their populations are rapidly declining worldwide due primarily to the loss and

31 degradation of their natural habitats. *Pseudoeurycea robertsi* is a micro-endemic and critically

32 endangered Plethodontid salamander from the Nevado de Toluca Volcano and to date almost
33 nothing is known about its natural history therefore, we survey fourteen sites of the Nevado de
34 Toluca Volcano a mountain that is part of the Trans-Mexican Volcanic Belt, Mexico. We carry
35 out the most exhaustive sampling scheme of this species throughout the Nevado de Toluca
36 Volcano to know the number of individuals and the microhabitat features associated with the
37 presence of *P. robertsi*. Likewise, we carry out a morphometric study and coloration measurements
38 of *P. robertsi* individuals and we determine the potential distribution of *P. robertsi* and the other
39 3 species of pletodontids present in the NTV using ecological niche modeling and to determine
40 the most important habitat features associated with the presence of salamander species, as well as
41 to know the niche overlap among salamander species. This information will help raise
42 conservation strategies for this micro-endemic and critically endangered salamander.

43 **Key Words:** endangered species; conservation; *Plethodontida*e; *Abies* forest; deforestation;
44 environmental niche modelling; morphometric geometric; *Cyt b*; Nevado de Toluca Volcano;
45 Trans-Mexican Volcanic Belt.

46

47 **Introduction**

48

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

62 creating small patches of habitat and reducing metapopulation dynamics between the populations

63 of amphibians' forest (Lowe et al., 2005; Honnay and Jacquemyn, 2007; Herrera-Arroyo et al.,

64 2013).

65   It is important to study amphibians because they are among the most threatened vertebrates

66 on Earth (Catenazzi, 2015) and their populations are rapidly declining worldwide due primarily to

67 the loss and degradation of their natural habitats (Pimm et al., 1995; Fisher and Shaffer, 1996;

68 Davidson et al., 2001; Marsh and Trenham, 2001; Stuart et al., 2004; Mendelson et al., 2006; Wake

69 and Vredenburg, 2008; Sodhi et al., 2008; Gamble et al., 2007; Ducatez and Shinev, 2017). Mexico

70 is ranked fifth in worldwide amphibian diversity (Ochoa-Ochoa et al., 2009; Ordoñez-Ifarraguerri

71 et al., 2017), however, it is the second worldwide country in number of threatened amphibian

72 species (Frías-Alvarez et al., 2010), with 43% of its 376-species threatened (Parra-Olea et al.,

73 2014), the amphibians are threatened in part because of their low dispersion capacity and small

74 home ranges (Blaustein et al., 1994; Beebee, 2005; Zeisset and Beebee, 2008; Hillman et al., 2014),

75 also, they are highly sensitive to perturbations in both terrestrial and aquatic environments because

76 of their dual life histories, highly specialized physiological adaptations and specific microhabitat

77 requirements (Vitt et al., 1990; Wake, 1991; Blaustein, 1994; Blaustein et al., 1994a; Stebbins and

78 Cohen, 1995). Salamanders are important as top-down controls of many invertebrate species and

79 can also be a source of high energy prey for other predators (Davic and Welsh, 2004). In addition,

80 salamanders can represent an important proportion of the vertebrate biomass in old growth forests

81 (Davic and Welsh, 2004), and are thus vital to ecosystem function, also they provide an important

82 indirect regulatory role in the processing of detritus-litter by ingestion of detritivore prey (Davic

83 and Welsh, 2004).

84   Roberts' false brook salamander, *Pseudoeurycea robertsi*, is a micro-endemic and

85 critically endangered plethodontid genera/genus found in one of the TMVB volcanoes near Toluca

86 in the state of Mexico: the 'Nevado de Toluca' volcano (NTV; SEMARNAT, 2010; IUCN SSC

87 Amphibian Specialist Group, 2016; Sunny et al., 2019a). *P. robertsi* being one of the most

88 threatened amphibians in Mexico with a vulnerability score of 18 (Wilson et al., 2013) has only

89 been reported in one small area of 8km^2, within a narrow elevation distribution between 2,900 and

90 3,600 meters above sea level (masl; IUCN SSC Amphibian Specialist Group 2016). Other reports

91 of *P. robertsi* populations presence within other locations has been confirmed by other studies (see

92 Billie, 2009). Few studies have explored systematic and population genetics of this specie (Lynch

93   et al., 1983; Parra-Olea, 2002; Bille, 2009; Van Rooij et al., 2011; González-Fernández et al.,

94   2019; Sunny et al., 2019a), more research is needed on its natural history.

95   Therefore, the aims of this study were: (1) to carry out the most exhaustive sampling

96   scheme of this species throughout the NVT to know the number of individuals and the microhabitat

97   features associated with the presence of *P. robertsi*, (2) to carry out morphometric and coloration

98   measurements of *P. robertsi* individuals, (3) to determine the potential distribution of *P. robertsi*

99   and the other 3 species of pletodontids present in the NTV using ecological niche modeling and to

100 determine the most important habitat features associated with the presence of salamanders species,

101 as well as to know the niche overlap among salamanders species. This information will help raise

102 conservation strategies for this micro-endemic and critically endangered salamander.

103

104 **Materials and methods**

105

106 Population sampling and species identification

107 A total of 150 individuals from 14 populations (Fig. 1) were sampled from mid-April to mid-

108 October via visual encounter surveys (Crump and Scott, 1994) across the entire polygon of the

109 NTV. Field permits and the approval of the ethics committee were given by the Universidad

110 Autónoma del Estado de México and SEMARNAT (SGPA/DGVS/05701/16). During the survey,

111 we walked back and forth across transects and looked for salamanders in tree stumps, inside fallen

112 logs and under the bark, stones, fallen leaves, and moss. The sampling time was between 9 a.m.

113 and 3 p.m., during which an area of approximately 10ha was covered in each sampling site. In

114 order to sample salamanders, we took tail samples (2mm) for their DNA extraction (see Sunny et

115 al., 2019a for a detailed description) and, subsequently, individuals were released back to the very

116 site they were captured/capture site. The tail clipping sampling method is a low impact technique

117 that does not affect the survival or growth of salamanders (Arntzen et al., 1999; Polich et al., 2013).

118 The salamander tissues were preserved in 90% ethanol and then frozen at -20°C until processing

119 the samples.

120 For identification in the field, we used the phenotypic characteristics mentioned by Bille, (2009)

121 and later with molecular data to corroborate the correct identification of the species. In order to

122 achieve the species molecular identification we extracted DNA from the salamanders' tail tips with

123 the Vivantis extraction kit, we specifically followed the manufacturer instructions. We used the

124   extracted DNA as a template for the mitochondrial *cytochrome b* gene (*cyt b*) amplification with

125   the following primers: MVZ15 and MVZ16 (Moritz et al., 1992). Forward and reverse sequences

126   of all individuals were aligned and edited manually using BIOEDIT 7.1.3 (Hall, 1999). The

127   retrieved sequences of *cyt b* were blasted in GenBank (Morgulis et al., 2008) optimized for highly

128   similar sequences (Megablast) to confirm they matched to *Pseudoeurycea robertsi* or

129   *Pseudoeurycea leprosa*, considering the E value and the percentage of identity.

130

131   Morphometric measurements and geometric morphometric

132

133   All *P. robertsi* individuals were photographed on a millimeter paper to obtain the following

134   morphometric measurements head length (HL), from the tip of the snout to the neck; head width

135   (HW), across the widest point of the head; left eye diameter (LED), wide between eyes (WBE),

136   median body width (MBW), across the trunk midway between the front and hind limb insertions,

137   posterior femur length (PFL), both snout vent length (SVL), tail length (TLO), tail wide (TW) and

138   total length (TL). from the posterior left limb insertion to the tip of the longest outstretched toe.

139   To obtain these morphometric measurements, the pixels of each image were converted to

140   millimeters using the software FIJI (Schindelin et al., 2012). For the geometric morphometric, the

141   shape of the head was obtained using 11 landmarks throughout the contour of this structure, and

142   for the eyes 3 landmarks were located as support points for the layout of fans that covered the

143   entire head structure. For all the analyzed individuals of *P. robertsi* we used the software

144   MAKEFAN6 (Sheets, 2003) and TPSDIG2 (Rohlf 2005). The configurations of the landmarks of

145   each organism were adjusted by means of Procrustes using the COORDGEN6F (Sheets, 2003)

146   software, in order to eliminate all information not related to the shape. With the software

147   SEMILAND6 (Sheets, 2002), were aligned using the Procrustes superimposition method and the

148   alignment protocol to eliminate the effect of location, orientation and size effect, and thus evaluate

149   the shape variation (Moreno-Barajas et al., 2013). Using this data, we perform a Principal

150   Components Analysis (PCA) using the PCAGEN6P (Sheets, 2002) software to identify the

151   individuals that provide the most intraspecific variation, in addition, the algorithm allowed to

152   visualize the variations of the configuration by means of vectors and deformation grids of each

153   group, which were built applying the thin plate spline interpolation function (Bokstein, 1991).

154   Finally, with the CVAGEN6 (Sheets, 2005) software, an Analysis of Canonical Variables (CVA)

155 was carried out with a reduction to 2 PC, to identify the possible segregation between groups,

156 depending on the locality.

157

158 Coloration patterns

159

160 The photographic records were transformed into 8 bits' images and RGB color space. We calculate

161 the color frequency of the dorsal stripe of each individual using the 3D Color Inspector and Color

162 Histogram plugins (Barthel, 2007) of the ImageJ 1.5 software, and finally we scaled the color

163 channels and we made a PCA with the color data.

164

165 Microhabitat variables and environmental niche modelling

166

167 When we found a felled log, we captured the geographic coordinates, type of microhabitat, log

168 species, if the log was naturally felled, its length and width and whether the log had salamanders,

169 we calculated the fallen logs volume, number of individuals in each log and finally, to compare if

170 there were differences between sites and characteristics of the microhabitat, different statistical

171 tests were performed using the software Paleontological Statistics ''PAST'' 3.06 (Hammer, 2015).

172 To calculated the percentage of forest cover and the percentage of *Abies* cover we used the

173 ARCGIS 10.5 software and satellite images (SPOT 6/7) of very high resolution (1.5 m) for year

174 2015, we made a supervised classification considering six land cover types: *Pinus* forest, *Abies*

175 forest, grasslands, agriculture, urban areas, and water sources. Likewise, to determine the most

176 important habitat features associated with the presence of *P. robertsi* we perform an analysis of

177 environmental niche modeling (ENM), we also made the ENM for the other species of

178 Plethodontids found in the NTV (*P. robertsi, P. leprosa, Aquilourycea cephalica* and *Isthmura*

179 *bellii*) in order to know if there is competition for environmental space and to know the overlapping

180 index with the other Plethodontids. We considered the entire distribution of each species to make

181 the ENM. The presence records were obtained from fieldwork and iNaturalist. We selected only

182 the records from the last 10 years for the analysis. We defined a polygon (background) for each

183 species which represents the accessibility area (Suárez-Atilano et al., 2017; Sunny et al., 2017;

184 González-Fernández et al., 2018; Sunny et al. 2019b), considering the biogeographic regions of

185 Mexico with geographical records or records near their borders (Sunny et al., 2017; González-

186 Fernández et al., 2018). We obtained bioclimatic variables from WorldClim (Hijmans et al., 2005);

187 topographic and land cover variables were obtained from the National Institute of Statistics and

188 Geography. We reclassified the land use map (series V, 1:250000, generated during the period

189 2011 to 2013; INEGI, 2013) in different exclusive classes that were converted to raster and

190 transformed from categorical to continuous using a resample method that averages the value of the

191 surrounding pixels to assign a new value to each pixel (González-Fernández et al., 2018; Sunny et

192 al., 2019b). All layers were processed in a raster format, with 1 km resolution, using ARCGIS 10.5

193 and the packages raster (Hijmans, 2016) and rgdal (Bivand et al., 2017) for R software (version

194 3.4.0; R. Development Core Team, 2017). After a bibliographic review and Pearson correlation

195 analysis to discard highly correlated variables ($R^2 > 0.7$, Dormann et al., 2013) we selected the

196 following variables: natural grasslands percentage, induced grasslands percentage, percentage of

197 arid vegetation, *Pinus* forest percentage, *Quercus* forest percentage, *Abies* forest percentage,

198 cultivated forest, distance to water sources, agriculture percentage, cloud forest mountain

199 percentage, aquatic vegetation, annual mean temperature, maximum temperature of the warmest

200 month, minimum temperature of the coldest month, precipitation of the wettest month,

201 precipitation of the driest month, elevation, aspect, slope (obtained from the elevation layer) and

202 distance to urban settlements, for a better prediction of land use change. We used the maximum

203 entropy modelling software (MAXENT; Phillips et al., 2006). We ran the model for each species

204 in MAXENT with 10 replicates (Anderson et al., 2003; Chefaoui et al., 2005; Suárez-Atilano et

205 al., 2017). We used linear and quadratic features because we had less than 80 records in most of

206 the species (Merow et al., 2013). All analyses were performed using the logistic output for an

207 easier interpretation and a convergence threshold of $1 X\times 10\text{-}5$ with 500 iterations (Pearson et al.,

208 2007; Suarez-Atilano 2015; González-Fernández et al., 2018). We generated a continuous map of

209 the potential distribution for each species because binary outputs can obscure important biological

210 detail (Liu et al., 2013). To evaluate model performance, we applied the partial ROC analyses

211 (Peterson et al., 2008; Osorio-Olvera et al., 2016). We used the software Niche ToolBox 0.4.1.5

212 for partial-ROC calculations in R (Osorio-Olvera et al., 2016).

213 We compute niche overlap from predictions of species distributions with the "D" similarity

214 statistic of Warren et al., (2008) in the software SDMTools (VanDerWal et al., 2014) for R and

215 we plot the niche overlap from predictions of species distributions in Niche ToolBox.

216

217   **Results**

218

219   Population sampling and species identification

220

221   It was quite complicated to differentiate between *P. robertsi* and *P. leprosa* in some cases, since

222   the coloration pattern can be confused as Bille, (2009) reported, so the analysis of the species

223   determination using the *cyt b* confirmed that in all populations 89% of all salamanders found were

224   *P. robertsi* and 11% were *P. leprosa* with an E value of 0.0 and an identity percentage of 98%

225   considering a sequence of 617 bp. Some sequences were deposited in the GenBank database with

226   the access numbers: MK357639-MK357709. Finally, we recorded 234 *P. robertsi* individuals, the

227   largest number of individuals reported for this species. The altitudinal range of our sampling

228   scheme goes from 2800-3460 MASL. At the moment, there is no information on the timing of

229   peak of *P. robertsi* abundance, the months we sampled comprise the beginning of the rainy and

230   dry seasons. In April (beginning of the rainy season) and in October (beginning of the dry season)

231   we did not record any individual. The maximum number of individuals were found at the end of

232   July and the beginning of August, and by the middle of September it was possible to find from 40

233   to 67% less salamanders. The number of individuals within each site in the total sampling scheme

234   varied from 0 to 53, Amanalco A had=53 individuals, Rancho Viejo=29, Meson Viejo=28,

235   Amanalco B=25, Las Lagrimas=24, Carretera=20, Palo Seco=18, Amanalco C=16, Santa

236   Cruz=11, Agua Bendita= 5, Raices= 4, San Juan de las Huertas=1, El Contadero=0 and Huacal

237   Viejo-Agua Bendita=0 (Fig. 2). The 99% of the salamanders were found under the bark of trees,

238   the rest was found under the leaf litter. In Agua Bendita we found together *P. robertsi*, *P. leprosa*

239   and *A. cephalica* in two fallen logs (Fig. 2), indicating that these salamanders can share the same

240   environmental niche. We found two individuals of *I. bellii* in Meson Viejo and one in Rancho

241   Viejo (Fig. 2). We found in August in Palo Seco a *P. robertsi* salamander attending an egg mass

242   of 32 white eggs with transparent parts, the eggs are slightly oval in shape and the clutch was found

243   under the bark of an *Abies* log.

244

245   Morphometric measurements and geometric morphometric

246

247 The average of the measurements of the juvenile salamanders were: HL=5.475, HW=4.150,

248 LED=1.075, WBE=1.800, MBW=4.025, PFL=1.750, SVL=18.543, TLO=14.758, TW=1.975,

249 TL=33.300. The average of the measurements of the adult salamanders were: HL=10.795,

250 HW=7.288, LED=2.218, WBE=2.712, MBW=7.243, PFL=3.936, SVL=45.414, TLO=43.736,

251 TW=3.814, TL=89.150. For the geometric morphometric analysis of the 234 individuals, only 139

252 individuals could be analyzed, of which 21 landmarks were placed on the head (Fig. 3A), of which

253 six were for the eye contour (Fig. 3A). The deformation grids of the landmarks found a greater

254 deformation in the central part of the head (Fig. 3B). The dispersion of the first two main

255 components in the PCA analysis showed a single group with a low-medium explained variation

256 (45%) between (Fig. 3C). The CVA showed that all individuals of *P. robertsi* from all study areas

257 are grouped into a single group (CV1 Eigenvalue= 0.658 and CV2 Eigenvalue=0.491, Axis1:

258 λ=0.1604, χ^2=232.431, df=110, p=9.01349 e-011; Axis 2: λ=0.2660, χ^2=168.182, df=90,

259 p=1.11354e-006).

260

261 Coloration patterns

262

263 The color of the dorsal stripes ranges from red to orange (R84, G35, B38 to R187 G124 B77; Fig.

264 4A), orange to yellow (R174 G135 B63 - R191 G178 B118; Fig. 4B) and brown to black (Brown=

265 R91 G86 B82 – R168 G152 B131 and Black= R28 G28 B28 – R68 G68 B68; Fig. 4C). We found

266 seven patterns of dorsal stripe coloration, two (Fig. 4a and Fig. 4b) that reported Bille (2009) and

267 5 new patterns (Fig. 4c to 4g), however we could not find the third pattern reported by Bille (2009).

268 The patterns that we found were the following: 1) well-defined, brick red dorsal stripe (Fig. 4a),

269 2) dense dorsal mottling (Fig. 4b), 3) the dorsal red line only present on the tail (Fig. 4c), 4) semi

270 well-defined, yellow dorsal stripe (Fig. 4d), 5) the dorsal yellow line only present on the tail (Fig.

271 4e), 6) almost patternless with few scattered spots (Fig. 4f) and 7) all black without spots or dorsal

272 line (Fig. 4g). All these patterns can also have different coloration gradients, with different shades

273 of yellow, brown or red.

274

275 Microhabitat variables and environmental niche modelling

276

277   A total of 873 fallen logs and stumps were sampled in all the study areas, the fallen logs volume

278   ranged from 1.3 to 19.6 m^3. The sites that only presented *Abies* forest were: El Contadero, Carretera

279   and San Juan de las Huertas (Fig. 5A), the sites that presented the most percentage of total forest

280   in 10 ha were Amanalco A, Amanalco B, Meson Viejo and Rancho Viejo. Likewise, Amanalco

281   A, Amanalco B, Meson Viejo and Rancho Viejo also had the highest percentage of *Abies* forest

282   cover (Fig. 5B). The sites that showed the most percentage of trees naturally fallen were Amanalco

283   A and Meson Viejo, while the sites that presented the most felled logs are Huacal Viejo-Agua

284   Bendita, El Contadero, Carretera, Santa Cruz and Palo Seco (Fig.5C), we found significant

285   differences between the length (F= 8.05 gl= 894 and p=2.08 E-15 Fig. 5D and Fig. 6A) and

286   diameter of trees per site (F= 3.359 gl= 894 and p=4.69E-05; Fig. 5D and Fig. 6B) and fallen logs

287   volume per site was greater in Las Lagrimas (Fig. 5E). The average length of the logs with

288   salamanders was 4.2 m and without salamanders was 2.7 m, finding significant differences (t=5.4,

289   gl= 789 and p= 0.0001), the average diameter of logs with salamanders was 35.7 cm and without

290   salamanders was 34.2 cm, no significant differences were found (t=1.02, gl= 789 and p= 0.31), of

291   the logs sampled 76% were *Abies* and 24% *Pinus*, the species of tree that presented more

292   salamanders was *Abies* with 77% and *Pinus* with 23%, we found salamanders in the 75% of *Abies*

293   fallen logs and 25% of *Pinus* fallen logs, finding significant differences (Chi^2=39.98, gl= 13 and

294   p= 0.0001), of these fallen logs 30% were naturally fallen and 70% were logs felled, finding

295   significant differences (Chi^2=817.73, gl= 13 and p= 0.0001).

296

297   The MAXENT model performed better than expected by random, where the AUC showed high

298   average values across iterations (mean AUC ratios from 0.816 to 0.993 and p-values <0.001;

299   Table. 1). Also, Partial-ROC bootstrap tests showed significant ratio values of empirical AUC over

300   null expectations (mean AUC ratios ≥ 1.4 and p-values <0.001 in all cases; Table. 1). The most

301   important habitat features associated with the presence of *P. robertsi* according to the MAXENT

302   model were: *Abies* forest (38.8%) and maximum temperature of the warmest month (33.4%) for

303   *P. leprosa* were: maximum temperature of the warmest month (54.1%) and *Abies* forest (17.9%),

304   for *A. cephalica* were: maximum temperature of the warmest month (42.2%) and *Abies* forest

305   (21.6%), and for *I. bellii* was: minimum temperature of the coldest month (54.4%). *Abies* forest

306   cover percentage was positively correlated with probability of presence of *P. robertsi, P. leprosa*

307   and *A. cephalica*. For *I. bellii* probability of presence decreased with temperature lower than 5°C.

308   Probability of presence of *A. cephalica, P. leprosa* and *P. robertsi* decreased as maximum
309   temperature of the warmest month increased. It is important to note that the maximum temperature
310   of the warmest month and *Abies* forest cover percentage were the most important variable to
311   explain the potential distribution of three of the four species. Potential distribution maps for each
312   species are in Figs. 7A–7D. *P. robertsi* is the salamander species with the smallest distribution
313   (Fig. 7A), followed by *P. leprosa* (Fig. 6B), *A. cephalica* (Fig. 7C) and the salamander species that
314   has the widest distribution is *I. bellii* (Fig. 7D)*, however the four-salamander species are
315   distributed mainly in the mountains of the TMBV, *A. cephalica* is also distributed in the Sierra
316   Madre Oriental and *I. bellii*, in the Sierra Madre Oriental, in the Sierra Madre Occidental, the
317   Sierra Madre del Sur, the Cuenca del Balsas and the Costa Pacifica Mexicana. The greatest niche
318   overlap occurs between *P. robertsi and P. leprosa* (Fig. 8A), followed by *P. robertsi and A.*
319   *cephalica* (Fig. 8B) and finally between *P. robertsi and I. bellii* (Fig. 8C).

320

321   **Discussion**

322

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

331
332   Population sampling and species identification

333

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

337

338   The analysis of species identification through *cyt b*, showed that it is complicated to differentiate
339   between *P. robertsi* and *P. leprosa* by the pattern of coloration, size and morphometric

340 measurements since these characteristics are very variable (Bille 2009), however *P. robertsi* is

341 89% more abundant than *P. leprosa.* The elevation range observed for *P. robertsi* was slightly

342 larger than previously reported 2,800-3,600 m a.s.l. (Bille, 2009; IUCN SSC Amphibian Specialist

343 Group, 2016), within these altitudinal limits, we corroborate that this species is found in all the

344 *Abies* forests of the NVT since this forest is distributed in an altitudinal gradient of 2,400 to 3,500

345 m in the TMVB (Manzanilla-Quiñones et al., 2019). Bille, (2009) reported that at lower altitudes

346 it is more likely to find more individuals of *P. leprosa*, likewise, in this study, few *P. leprosa* and

347 *A. cephalica* individuals were found in all the altitudinal ranges, therefore it is more likely that *P.*

348 *robertsi* limits the distribution and abundance of *P. leprosa* by being a stronger competitor and

349 with higher levels of abundance and higher capacity for sexual encounter than *P. leprosa.*

350

351 Morphometric measurements and geometric morphometric

352

353 Our morphometric measurements are slightly smaller or similar to those reported for *P. leprosa*

354 and *A. cephalica* (Ramírez-Bautista et al., 2009) however it seems that tail is longer in *P. robertsi*

355 than in *P. leprosa* or *A. cephalica*. In contrast, *P. robertsi is* smaller than *I. bellii* (Ramírez-Bautista

356 et al., 2009). All individuals of *P. robertsi* from the eleven sampling sites presented similar

357 morphometric measurements and the geometric morphometric analysis showed that there are no

358 significant differences between the sampling sites, according to the landmarks used in the head.

359 These results are similar to Baken and Adams' (2019) as they found no differences between

360 salamander species using landmarks in the foot shape (, 2019). This means that salamanders

361 present little morphometric variation between individuals and even between species (Ramírez-

362 Bautista et al., 2009; Baken and Adams, 2019), suggesting that, although salamanders have limited

363 dispersal ability, small home ranges (Kleeberger and Werner, 1982; Ovaska, 1988; Gergits and

364 Jaeger, 1990; Gibbs, 1998a, b; Marvin, 1998) and are restricted to forest patches (Petranka et al.,

365 1993; Welsh and Droege, 2001), they can disperse enough to keep gene flow. It has been reported

366 that male salamanders have the ability to disperse through harsh habitats (Marsh et al., 2005)

367 especially when there are high levels of competition (Liebgold et al., 2011), this may favor a weak

368 population structure and similar phenotypes between the sampling sites in *P. robertsi* (Sunny et

369 al., 2019a).

370

371   Coloration patterns

372

373   In this study, we found two coloration patterns that were previously described by Bille (2009),

374   however we did not find the third pattern that he described, in which the salamander presents a

375   few spots on the body. Considering the patterns described by Bille, (2009) and those found in this

376   study, this species has a total of eight dorsal-tail stripe patterns. The great variety in dorsal-tail

377   stripe patterns and colorations suggests that this species has a great phenotypic variability and we

378   presume there may be more patterns with different types of combinations in addition to those

379   already described. Likewise, we found a great variation in the number, size and shape of the spots

380   and gradients of color. Bille, (2009) reported that the coloration patterns may be correlated with

381   the altitudinal range, however, we do not corroborate this hypothesis, we believed that it may be

382   correlated with the color of the log where they were found.

383

384   Environmental variables and environmental niche modelling

385   The largest number of individuals was found in well-preserved *Abies* forests (Amanalco A,

386   Amanalco B, Meson Viejo and Rancho Viejo), where they also found more natural fallen *Abies*

387   and *Pinus* logs. The largest number of individuals was found under the bark of *Abies* logs, because

388   in these trees the bark does not break into small pieces, generating an ecosystem that allows *P.*

389   *robertsi* to have shelter and food like: mollusks as slugs, insects and arachnids (Bille, 2009). In

390   low logging sites (Amanalco A and Meson Viejo), we found many fallen logs that fell down with

391   storms. These sites hold the maximum salamander individuals and logs were almost complete

392   (between 5 and 8 meters long approximately). In moderate logging sites, without clearings and cut

393   logs abandoned (between 1 and 3 meters long approximately), there were relatively high

394   individuals also. However, we found few salamanders in forests with many clearings (Huacal

395   Viejo-Agua Bendita, El Contadero, Carretera, Santa Cruz, Palo Seco and Raices), were most of

396   the fallen logs had been extracted or were small and thin. Therefore, we emphasize the need to

397   leave and actively restore the availability of dead wood in managed forests in order to preserve

398   these salamander species (Kouki, 2001; Parra-Olea et al., 2005; Monterrubio-Rico and Escalante-

399   Pliego, 2006; Ramírez-Bautista et al., 2009; Velo-Antón et al., 2013; Medina et al., 2018).

400   Likewise, fallen logs found in the grasslands, in the forest edges or in forest clearings were always

401   completely dry and tough while fallen logs found inside of the forest were all completely wet and

402 following a process of wood decomposition. We always found salamanders in wet logs with

403 intermediate levels of decomposition (field observations). This is consistent with the importance

404 of forest core areas reported in another study (González-Fernández et al., 2019) and is related with

405 the importance of humidity for this species as Plethodontids do not have lungs and rely on

406 cutaneous respiration (Petranka et al., 1993; deMaynadier and Hunter 1998).

407

408   ENM analysis show that the macro habitat preferred by *P. robertsi* is the *Abies* forest, as

409 well as *P. leprosa* and *A. cephalica*. However, *P. robertsi* is the most *Abies* forest specialist and

410 the species with the most restricted distribution whereas *P. leprosa* and *A. cephalica* are distributed

411 in all the mountains of the TMVB which contain the 91.1% of the *Abies* forests of the country

412 (Sunny et al., 2017). *I. bellii* has a much more extensive distribution in a great variety of vegetation

413 covers. Likewise, whereas *I. bellii* probability of presence decreased with temperature lower than

414 5°C, *A. cephalica, P. leprosa* and *P. robertsi* were limited by maximum temperatures, this can be

415 explained because these species are adapted to high elevations and cold environments. Therefore,

416 temperature increase caused by climate change will affect these three species more than those

417 adapted to lower elevations because species at the highest elevations are limited in their ability to

418 shift distribution upslope, thereby increasing the possibility of becoming extinct (Parra-Olea et al.,

419 2005; Velo-Antón et al., 2013), specially *P. robertsi* for having such a limited distribution. The

420 highest niche overlap occurred between *P. robertsi* and *P. leprosa* followed by *P. robertsi* and *A.*

421 *cephalica* and finally between *P. robertsi* and *I. bellii*.

422

423 Conclusions and conservation implications

424 Our results suggest that *Abies* forests are extremely important for *P. robertsi*, as well as for *P.*

425 *leprosa and A. cephalica* and that *Abies* forests with naturally fallen logs will hold more

426 salamander individuals. If these forests disappear or suffer grate reductions *P. robertsi* populations

427 can diminish or even become extinct. Thus, it is essential to protect the *Abies* forests of the NTV.

428 According to some authors *Abies* forest are in the 30.69% of the area in the NTV (16,164.18 ha;

429 Franco-Maass et al., 2006), or 6,847.8 ha according to Manzanilla-Quiñones et al., (2019).

430 Therefore, it is necessary to carry out a more precise study to delimit the area of the *Abies* forest

431 and prevent its deterioration by anthropogenic activities, such as pollution by garbage,

432 agrochemicals, fires and deforestation, to prevent the extinction of the populations of *P. robertsi*.

433   However, the Mexican government recently changed the protection status of the NTV from

434   National Park to a less restrictive category in terms of land use. This new category allows forest

435   harvesting practices with commercial proposes as well as the construction of ecotourism centers

436   in almost all *Abies* forest extension (Fig. 9)*,* which was well preserved under the National Park

437   category (Franco-Maass et al., 2006; Mastretta-Yanes et al., 2014; González-Fernández et al.,

438   2018). Thus, this change of category is compromising the habitat of the endemic and critically-

439   endangered *P. robertsi,* as well as other forest-dwelling animals (*Danaus plexippus*, *Aquiloeurycea*

440   *cephalica*, *Pseudoeurycea leprosa*, *Isthmura bellii*, *Sceloporus grammicus, S. spinosus, Aegolius*

441   *acadicus, Oculatus tolucae, Lynx rufus*, among others). Finally, we recommend to priorize in

442   conservation programs the areas of Amanalco, Meson Viejo and Rancho Viejo, since these areas

443   had higher levels of genetic diversity and number of individuals of *P. robertsi* populations (Sunny

444   et al., 2019a) likewise, these areas also have the most well conserved *Abies-Pinus* forests of the

445   NTV.

446

447   Acknowledgements

448

452

453   Compliance with ethical standards and conflict of interest

454

455   The authors declare that they have no conflict of interest. Our study received the field permits and

456   the approval of the ethics committee from Universidad Autónoma del Estado de México and

457   SEMARNAT (SGPA/DGVS/05701/16).

458

459   **References**

460

461   Anderson RP, Lew D, Peterson AT. 2003. Evaluating predictive models of species' distributions:

462   criteria for selecting optimal models. Ecological Modelling 162 (3):211–232.

463 Arntzen JW, Smithson A, Oldham RS. 1999. Marking and tissue sampling effects on body
464   condition and survival in the newt *Triturus cristatus*. Journal of Herpetology 33:567–576.

465 Arroyo–Rodríguez V, Saldaña–Vázquez RA, Fahrig L, Santos BA. 2017. Does forest
466   fragmentation cause an increase in forest temperature? Ecological Research 32:81–88.

467 Baken EK, Adams DC. 2019. Macroevolution of arboreality in salamanders. Ecology and
468   Evolution.

469 Barthel KU. 2007. Color Inspector 3D. http://rsb.info.nih.gov/ij/plugins/color-inspector.html,
470   Accessed: 20019.

471 Beebee TJC. 2005. Conservation genetics of amphibians. Heredity 95:423–427.

472 Bille T. 2009. Field observations on the salamanders (Caudata: Ambystomatidae, Plethodontidae)
473   of Nevado de Toluca, Mexico. Raices 1(2):5.

474 Bivand R, Keitt T, Rowlingson B. 2017. rgdal: bindings for the geospatial data abstraction library.

475 Blaustein AR, Wake DB, Sousa WP. 1994. Amphibian declines: judging stability, persistence, and
476   susceptibility of populations to local and global extinctions. Conservation Biology 8(1):
477   60–71.

478 Blaustein AR. 1994. Amphibian declines: judging, stability, persistence, and susceptibility of
479   populations to local and global extinctions. Conservation Biology 8: 60–71.

480 Bookstein FL. 1991. Morphometrics Tools for Landmarks Data: Geometry and Biology.
481   Cambridge University Press, Cambridge.

482 Catenazzi A. 2015. State of the world's amphibians. Annu Rev Environ Resour 40:91–119.

483 Chefaoui RM, Hortal J, Lobo JM. 2005. Potential distribution modelling, niche characterization
484   and conservation status assessment using GIS tools: a case study of Iberian Copris species.
485   Biological Conservation 122 (2): 327–338.

486 Crump ML, Scott NJ Jr. 1994. Visual encounter Surveys. In: Heyer WR, Donnelly MA,
487   McDiarmid RW, Hayek LC, Foster MC (eds) Measuring and monitoring biological
488   diversity: standard methods for amphibians. Smithsonian Institution Press, Washington, pp
489   84–92.

490 Davic RD, Welsh Jr HH. 2004. On the ecological roles of salamanders. Annual Review of Ecology,
491   Evolution, and Systematics 35: 405–434.

492 Davidson C, Shaffer HB, Jennings MR. 2001. Declines of the California red–legged frog: climate,
493   UV–B, habitat, and pesticides hypotheses. Ecological Applications 11:464–479.

494   deMaynadier PG, Hunter ML. 1998. Effects of silvicultural edges on the distribution and
495   abundance of amphibians in Maine. Conservation Biology 12:340–352.

496   Dormann CF, Elith J, Bacher S, Buchmann C, Carl G, Carré G, Marquéz JRG, Gruber B,
497   Lafourcade B, Leitão PJ, Münkemüller T. 2013. Collinearity: a review of methods to deal
498   with it and a simulation study evaluating their performance. Ecography 36 (1): 27–46.

499   Ducatez S, Shine R. 2017. Drivers of extinction risk in terrestrial vertebrates. Conservation Letters
500   10:186–194.

501   Ellis EA, Porter–Bolland L. 2008. Is community–based forest management more effective than
502   protected areas?: A comparison of land use/land cover change in two neighboring study
503   areas of the Central Yucatan Peninsula, Mexico. Forest Ecology and Management
504   256:1971–1983.

505   Fahrig L. 2017. Ecological responses to habitat fragmentation per se. Annual Review of Ecology,
506   Evolution, and Systematics 48:1–23.

507   FAO Statistics database. 2006. WebCite at http://faostat.fao.org/ on 10 June 2016.

508   Fisher, RN, Shaffer HB. 1996. The decline of amphibians in California's Great Central Valley.
509   Conservation Biology 10:1387–1397.

510   Flores–Villela O, Canseco–Márquez L. 2007. Riqueza de la herpetofauna. Universidad Nacional
511   Autónoma de México, México, DF: 407–420.

512   Franco–Maass S, Regil–García HH, González–Esquivel C, Nava–Bernal G. 2006. Cambio de uso
513   del suelo y vegetación en el Parque Nacional Nevado de Toluca, México, en el periodo
514   1972–2000. Investicacion Geografica 61:38–57.

515   Franco–Maass, S, Regil García, HH, Ordóñez–Díaz JAB. 2006. Dinámica de perturbación–
516   recuperación de las zonas forestales en el Parque Nacional Nevado de Toluca. Madera y
517   Bosques 12(1): 17–28.

518   Frías–Alvarez P, Zúniga–Vega JJ, Flores–Villela O. 2010. A general assessment of the
519   conservation status and decline trends of Mexican amphibians. Biodiversity and
520   Conservation 19(13):3699–3742.

521   Gamble LR, McGarigal K, Compton BW. 2007. Fidelity and dispersal in the pond–breeding
522   amphibian, *Ambystoma opacum*: implications for spatio–temporal population dynamics
523   and conservation. Biological Conservation 139:247–257.

524   Gergits WF, Jaeger RG. 1990. Field observations of the behavior of the red–backed salamander
525   (*Plethodon cinereus*): courtship and agonistic interactions. Journal of Herpetology 24(1):
526   93–95.

527   Gibbs JP. 1998a. Amphibian movements in response to forest edges, roads, and streambeds in
528   southern New England. Journal of Wildlife Management 1:584–589.

529   Gibbs JP. 1998b. Distribution of woodland amphibians along a forest fragmentation
530   gradient. Landscape Ecology 13(4):263–268.

531   González–Fernández A, Manjarrez J, García–Vázquez U, D'Addario M, Sunny A. 2018. Present
532   and future ecological niche modelling of garter snake species from the Trans–Mexican
533   Volcanic Belt. PeerJ 6, e4618.

534   González-Fernández A, Arroyo-Rodríguez V, Ramírez-Corona F, Manjarrez J, Aguilera-
535   Hernández A, Sunny A. 2019. Local and landscape drivers of the number of individuals
536   and genetic diversity of a microendemic and critically endangered salamander. Landscape
537   Ecology 1-12.

538   Hall TA. 1999. BioEdit: a user–friendly biological sequence alignment editor and analysis
539   program for Windows 95/98/NT. Nucleic Acids Symposium Series (Vol. 41, No. 41, pp. 95–98).
540   [London]: Information Retrieval Ltd., c1979–c2000.

541   Hammer Ø. 2015. PAST: Paleontological Statistics software package for education and data
542   analysis. Palaeontol Electron 4(1). http://folk.uio.no/ohammer/past/.

543   Helzer CJ, Jelinski DE. 1999. The relative importance of patch area and perimeter–area ratio to
544   grassland breeding birds. Ecological Applications 9:1448–1458.

545   Heredia–Bobadilla RL, Monroy–Vilchis O, Zarco–González MM, Martínez–Gómez D, Mendoza–
546   Martínez GD, Sunny A. 2017. Genetic variability and structure of an isolated population
547   of *Ambystoma altamirani*, a mole salamander that lives in the mountains of one of the
548   largest urban areas in the world. Journal of Genetics 96(6): 873.

549   Heredia–Bobadilla, RL, Monroy–Vilchis O, Zarco–González MM, Martínez–Gómez D,
550   Mendoza–Martínez GD Sunny A. 2016. Genetic structure and diversity in an isolated
551   population of an endemic mole salamander (*Ambystoma rivulare* Taylor, 1940) of central
552   Mexico. Genetica 144:689–698.

553   Herrera–Arroyo ML, Sork VL, González–Rodríguez A, Rocha–Ramírez V, Vega E, Oyama K.
554   2013. Seed–mediated connectivity among fragmented populations of *Quercus castanea*
555   (Fagaceae) in a Mexican landscape. American Journal of Botany 100: 1663–1671.

556   Hijmans RJ, Cameron SE, Parra JL, Jones PG, Jarvis A. 2005. Very high resolution interpolated
557   climate surfaces for global land areas. International Journal of Climatology 25:1965–1978

558   Hijmans RJ. 2016. RASTER (accessed 4 June 2017)

559   Hillman SS, Drewes RC, Hedrick MS, Hancok TV. 2014. Physiological vagility: correlations with
560   dispersal and population genetic structure of amphibians. Physiological and Biochemical
561   Zoology 87: 105–112.

562   Honnay O, Jacquemyn H. 2007. Susceptibility of common and rare plant species to the genetic
563   consequences of habitat fragmentation. Conservation Biology 21: 823 – 831.

564   INEGI, National Institute for Statistics and Geography. 2013. Conjunto nacional de uso de suelo
565   y vegetación a escala 1:250,000 Serie V. México: DGG–INEGI.

566   IUCN 2019. The IUCN Red List of Threatened Species. Version 2019–1.
567   https://www.iucnredlist.org

568   IUCN SSC Amphibian Specialist Group. 2016. *Pseudoeurycea robertsi*. The IUCN Red List of
569   Threatened   Species   2016:
570   e.T59393A53983925. http://dx.doi.org/10.2305/IUCN.UK.2016–
571   3.RLTS.T59393A53983925.en. Downloaded on 03 May 2018.

572   Kapos V, Ganade G, Matsui E, Victoria RL. 1993. ∂13C as an indicator of edge effects in tropical
573   rainforest reserves. Journal of Ecology 81:425–432.

574   Kapos, V. 1989. Effects of isolation on the water status of forest patches in the Brazilian Amazon.
575   Journal of Tropical Ecology 5(2): 173–185.

576   Kleeberger SR, Werner JK. 1982. Home range and homing behavior of *Plethodon cinereus* in
577   northern Michigan. Copeia 409–415.

578   Kouki J, Löfman S, Martikainen P, Rouvinen S, Uotila A. 2001. Forest fragmentation in
579   Fennoscandia: linking habitat requirements of wood–associated threatened species to
580   landscape and habitat changes. Scandinavian Journal of Forest Research 16:27–37.

581   Liebgold EB, Brodie ED, Cabe PR. 2011. Female philopatry and male-biased dispersal in a
582   direct-developing salamander, *Plethodon cinereus*. Molecular Ecology 20(2):249–257.

583 Liu C, White M, Newell G. 2013. Selecting thresholds for the prediction of species occurrence
584   with presence-only data. Journal of Biogeography 40 (4):778–789.

585 Lowe AJ, Boshier D, Ward M, Bacles CFE, Navarro C. 2005. Genetic resource impacts of habitat
586   loss and degradation; reconciling empirical evidence and predicted theory for Neotropical
587   trees. Heredity 95:255 – 273.

588 Lynch JF, Wake DB, Yang SY. 1983. Genic and morphological differentiation in Mexican
589   Pseudoeurycea (Caudata: Plethodontidae), with a description of a new species. Copeia
590   884–894.

591 Manzanilla–Quiñones U, Martínez–Adriano CA, Aguirre–Calderón ÓA. 2019. Historical and
592   current spatial modeling of the sacred fir (*Abies religiosa* [Kunth] Schltdl. and Cham.) in
593   the Trans–Mexican Volcanic Belt. Revista Chapingo Serie Ciencias Forestales y del
594   Ambiente 25 (2):201–217.

595 Marsh DM, Milam GS, Gorham NP, Beckman NG. 2005. Forest roads as partial barriers to
596   terrestrial salamander movement. Conservation Biology 19(6):2004–2008.

597 Marsh DM, Trenham PC. 2001. Metapopulation dynamics and amphibian conservation.
598   Conservation Biology 15:40–49.

599 Marvin GA. 1998. Territorial behavior of the plethodontid salamander *Plethodon kentucki*:
600   influence of habitat structure and population density. Oecologia 114(1):133–144.

601 Masera OR, Ordoñez MJ, Dirzo R. 1997. Carbon emissions from Mexican forests: Current
602   situation and long–term scenarios. Climatic Change 35:265–295.

603 Mastretta–Yanes A, Cao R, Nicasio–Arzeta S, Quadri P, Escalante–Espinosa T, Arredondo L,
604   Piñero D. 2014. ¿Será exitosa la estrategia del cambio de categoría para mantener la
605   biodiversidad del Nevado de Toluca? Oikos 12:7–17.

606 Medina JP, Sánchez–Jasso JM, Sealy SG, Salgado–Miranda C, Soriano–Vargas E. 2018. Highest
607   Elevational Records for Northern Saw–whet Owls (*Aegolius acadicus*). Journal of Raptor
608   Research 52(1):94–100.

609 Mendelson JR, Lips KR, Gagliardo RW, Brodie ED. 2006. Confronting amphibian declines and
610   extinctions. Science 313:48.

611 Merow C, Smith MJ, Silander Jr JA. 2013. A practical guide to MaxEnt for modeling species'
612   distributions: what it does, and why inputs and settings matter. Ecography 36 (10):1058–
613   1069.

614   Monterrubio–Rico TC, Escalante–Pliego P. 2006. Richness, distribution and conservation status
615   of cavity nesting birds in Mexico. Biological Conservation 128(1):67–78.

616   Moreno–Barajas R, Rodríguez–Romero F, Velázquez–Rodríguez A, Aragón–Martínez A. 2013.
617   Variación geográfica en *Phrynosoma orbiculare* (Sauria: Phrynosomatidae): análisis de las
618   subespecies. Acta Zoológica Mexicana 29(1):129–143.

619   Morgulis A, Coulouris G, Raytselis Y, Madden TL, Agarwala R, Schäffer AA. 2008. Database
620   indexing for production MegaBLAST searches. Bioinformatics 24(16):1757–1764.

621   Moritz C, Schneider CJ, Wake DB. 1992. Evolutionary relationships within the *Ensatina*
622   *eschscholtzii* complex confirm the ring species interpretation. Systematic Biology
623   41(3):273–291.

624   Nowakowski AJ, Watling JI, Thompson ME, Brusch GA, Catenazzi A, Whitfield SM, Kurz DJ,
625   Suárez–Mayorga A, Aponte–Gutiérrez A, Donnelly MA, Todd BD. 2018. Thermal biology
626   mediates responses of amphibians and reptiles to habitat modification. Ecology Letters
627   21:345–355.

628   Ochoa–Ochoa L, Urbina–Cardona JN, Vázquez LB, Flores–Villela O, Bezaury–Creel J. 2009. The
629   effects of governmental protected areas and social initiatives for land protection on the
630   conservation of Mexican amphibians. PLoS One 4(9):e6878.

631   Ordoñez–Ifarraguerri A, Siliceo–Cantero HH, Suazo–Ortuño I, Alvarado–Díaz J. 2017. Does a
632   Frog Change its Diet along a Successional Forest Gradient? The Case of the Shovel–Nosed
633   Treefrog (*Diaglena spatulata*) in a Tropical Dry Forest in Western Mexico. Journal of
634   Herpetology 51(3):411–416.

635   Osorio–Olvera L, Barve V, Barve N, Soberón J. 2016. Nichetoolbox: from getting biodiversity
636   data to evaluating species distribution models in a friendly GUI environment, R package
637   version 0.2. 0.0.

638   Ovaska K. 1988. Spacing and movements of the salamander *Plethodon vehiculum*. Herpetologica
639   377–386.

640   Pandey D. 1993. Forest resources assessment 1990: tropical countries. FAO.

641   Parra–Olea G, Flores–Villela O, Mendoza–Almeralla C. 2014. Biodiversidad de anfibios en
642   México. Revista Mexicana de Biodiversidad 85:460–466.

643   Parra–Olea G, Martínez–Meyer E, De León GPP. 2005. Forecasting Climate Change Effects on
644   Salamander Distribution in the Highlands of Central Mexico. Biotropica 37(2):202–208.

645   Parra–Olea G. 2002. Molecular phylogenetic relationships of neotropical salamanders of the genus
646   Pseudoeurycea. Molecular Phylogenetics and Evolution 22(2):234–246.

647   Pearson RG. 2007. Species distribution modelling for conservation educators and practitioners.
648   Bulletin of the American Museum of Natural History 3:54–89.

649   Peterson AT, Papes M, Soberón J. 2008. Rethinking receiver operating characteristic analysis
650   applications in ecological niche modelling. Ecological Modelling 213:63–72.

651   Petranka JW, Eldridge ME, Haley KE. 1993. Effects of timber harvesting on southern Appalachian
652   salamanders. Conservation Biology 7:363–377.

653   Pfeifer M, Lefebvre V, Peres CA, Wearn O, Marsh C, Banks–Leite C, Butchart S, Arroyo–
654   Rodríguez V, Barlow J, Cerezo A, Cisneros L, D'Cruze N, Faria D, Hadley A, Klingbeil
655   B, Kormann U, Lens L, Rangel GM, Morante–Filho JC, Olivier P, Peters S, Pidgeon A,
656   Ribeiro D, Scherber C, Schneider–Maunoury L, Struebig M, Urbina–Cardona N, Watling
657   JI, Willig M, Wood E, Ewers R. 2017. Creation of forest edges has a global impact on
658   forest vertebrates. Nature 551:187–191.

659   Phillips SJ, Anderson RP, Schapire RE. 2006. Maximum entropy modeling of species geographic
660   distributions. Ecological Modelling 190:231–259

661   Pimm SL, Russell GJ, Gittleman JL, Brooks TM. 1995. The future of biodiversity. Science
662   269:347–350.

663   Polich RL, Searcy CA, Shaffer E. 2013. Effects of tail clipping on survivorship and growth of
664   larval salamanders. Journal of Wildlife Management 77:1420–1425.

665   R Development Core Team. 2017. R: a language and environment for statistical computing.
666   Vienna: R Foundation for Statistical Computing. (accessed 1 May 2017)

667   Ramírez–Bautista A, Hernández–Salinas U, García–Vázquez UO, Leyte–Manrique A, Canseco–
668   Márquez L. 2009. Herpetofauna del Valle de México: diversidad y conservación.
669   Universidad Autónoma del Estado de Hidalgo, 213.

670   Rohlf FJ. 2005. tpsDig2 software. State Univ of New York, Stony Brook, NY.

671   Schindelin J, Arganda–Carreras I, Frise E et al. 2012. "Fiji: an open–source platform for
672   biological–image analysis", Nature methods 9(7):676–682,

673   SEMARNAT. 2010. Norma Oficial Mexicana NOM–059–SEMARNAT–2010, Protección
674   ambiental–Especies nativas de México de flora y fauna silvestres–Categorías de riesgo y

675   especificaciones para su inclusión, exclusión o cambio. Lista de especies en riesgo. Diario

676   Oficial de la Federación, 10 diciembre 2010, México.

677   Sheets HD. 2002. Semiland6 software. Canisius College, Buffalo, NY.

678   Sheets HD. 2003. IMP–Integrated Morphometrics Package. Buffalo: Department of Physics,

679   Canisius College.

680   Sheets HD. 2005. CVAGen6, Canonical Variates Analysis program for the analysis of shape,

681   based on partial warp scores.

682   Sheets HD. 2005. CVAGen6, Canonical Variates Analysis program for the analysis of shape,

683   based on partial warp scores.

684   Sodhi NS, Bickford D, Diesmos AC, Lee TM, Koh LP, Brook BW, Sekercioglu CH, Bradshaw

685   CJA. 2008. Measuring the Meltdown: Drivers of Global Amphibian Extinction and

686   Decline. PLoS ONE 3: e1636

687   Stebbins RC, Cohen NW. 1995. Declining amphibians. A Natural History of Amphibians.

688   Princeton University Press, Princeton, NJ, USA, 210–251.

689   Stuart SN, Chanson JS, Cox NA, Young BE, Rodrigues ASL, Fischman DL, Waller RW. 2004.

690   Status and trends of amphibian declines and extinctions worldwide. Science 306:1783–

691   1786.

692   Suárez–Atilano M, Rojas-Soto O, Parra JL, Vázquez-Domínguez E. 2017. The role of the

693   environment on the genetic divergence between two Boa imperator lineages. Journal of

694   Biogeography 44 (9): 2045–2056.

695   Suárez–Atilano M, Rojas–Soto O, Parra JL, Vázquez–Domínguez E. 2017. The role of

696   environment on the genetic divergence between two *Boa imperator* lineages. Journal of

697   Biogeography 44(9):2045–2056

698   Suarez–Atilano M. 2015. Filogeografía de *Boa constrictor* (Serpents: Boidae) en México y Centro

699   América. PhD Thesis, Instituto de Ecología, UNAM, México. Available:

700   bibliotecacentral.unam.mx. Accessed. 25 November 2015.

701   Sunny A, Duarte–deJesus L, Aguilera–Hernández A, Ramírez–Corona F, Suárez–Atilano M,

702   Percino–Daniel R, Manjarrez J, Monroy-Vilchis O, González-Fernández A. 2019a.

703   Genetic diversity and demography of the critically endangered Roberts' false brook

704   salamander (*Pseudoeurycea robertsi*) in Central Mexico. Genetica 1–16.

705   Sunny A, Gandarilla-Aizpuro FJ, Monroy-Vilchis O, Zarco-Gonzalez MM. 2019b. Potential
706   distribution and habitat connectivity of *Crotalus triseriatus* in Central Mexico. Herpetozoa
707   32:139–148.

708   Sunny A, González–Fernández A, D'Addario M. 2017. Potential distribution of the endemic
709   imbricate alligator lizard (*Barisia imbricata imbricata*) in highlands of central Mexico.
710   Amphibia-Reptilia 38(2): 225–231.

711   Temple S. 1986. Predicting impacts of habitat fragmentation on forest birds: a comparison of two
712   models. In: Verner J, Morrison M, Ralph CJ (eds) Wildlife 2000: modeling habitat
713   relationships of terrestrial vertebrates. University of Wisconsin Press, Madison, Wisconsin,
714   USA, pp 301–304.

715   Tscharntke T, Tylianakis JM, Rand TA, Didham RK, Fahrig L, Batary P, Bengtsson J, Clough Y,
716   Crist TO, Dormann CF, Ewers RM, 2012. Landscape moderation of biodiversity patterns
717   and processes-eight hypotheses. Biological Reviews 87(3):661–685.

718   Van Rooij P, Martel A, Nerz J, Voitel S, Van Immerseel F, Haesebrouck F, Pasmans F. 2011.
719   Detection of *Batrachochytrium dendrobatidis* in Mexican bolitoglossine salamanders
720   using an optimal sampling protocol. EcoHealth 8(2):237–243.

721   VanDerWal J, Falconi L, Januchowski S, Shoo L, Storlie C, VanDerWal MJ. 2014. Package
722   'SDMTools'. R package.

723   VanDerWal J, Falconi L, Januchowski S, Shoo L, Storlie C. 2014. SDMTools: Species
724   Distribution Modelling Tools: Tools for processing data associated with species
725   distribution modelling exercises. R package version 1:1–221.

726   Velo–Antón G, Parra JL, Parra–Olea G, Zamudio KR. 2013. Tracking climate change in a
727   dispersal–limited species: reduced spatial and genetic connectivity in a montane
728   salamander. Molecular Ecology 22:3261–3278.

729   Vitt LJ, Caldwell JP, Wilbur HM, Smith DC. 1990. Amphibians as harbingers of decay.
730   BioScience 40(6):418–418.

731   Wake DB, Vredenburg VT. 2008. Are we in the midst of the sixth mass extinction? A view from
732   the world of amphibians. Proceedings of the National Academy of Sciences of the United
733   States of America. 105:11466–11473.

734   Wake DB. 1991. Declining amphibian populations. Science 253(5022):860–861.

735 Warren DL, Glor RE, Turelli M, Funk D. 2009. Environmental niche equivalency versus
736   conservatism: quantitative approaches to niche evolution. Evolution 62:2868–2883;
737   Erratum: Evolution 65:1215

738 Warren DL, Glor RE, Turelli M. 2008. Environmental niche equivalency versus conservatism:
739   quantitative approaches to niche evolution. Evolution: International Journal of Organic
740   Evolution 6211:2868–2883.

741 Welsh HH, Droege S. 2001. A case for using plethodontid salamanders for monitoring biodiversity
742   and ecosystem integrity of North American forests. Conservation Biology 15(3):558–569.

743 Zeisset I, Beebee TJC. 2008. Amphibian phylogeography: a model for understanding historical
744   aspects of species distributions. Heredity 101:109–119.

745 Zhang Z, Schwartz S, Wagner L, Miller W. 2000. A greedy algorithm for aligning DNA sequences.
746   Journal of Computational Biology 7(1–2):203–14.

Figure 1

Nevado de Toluca Volcano map and sampled sites.

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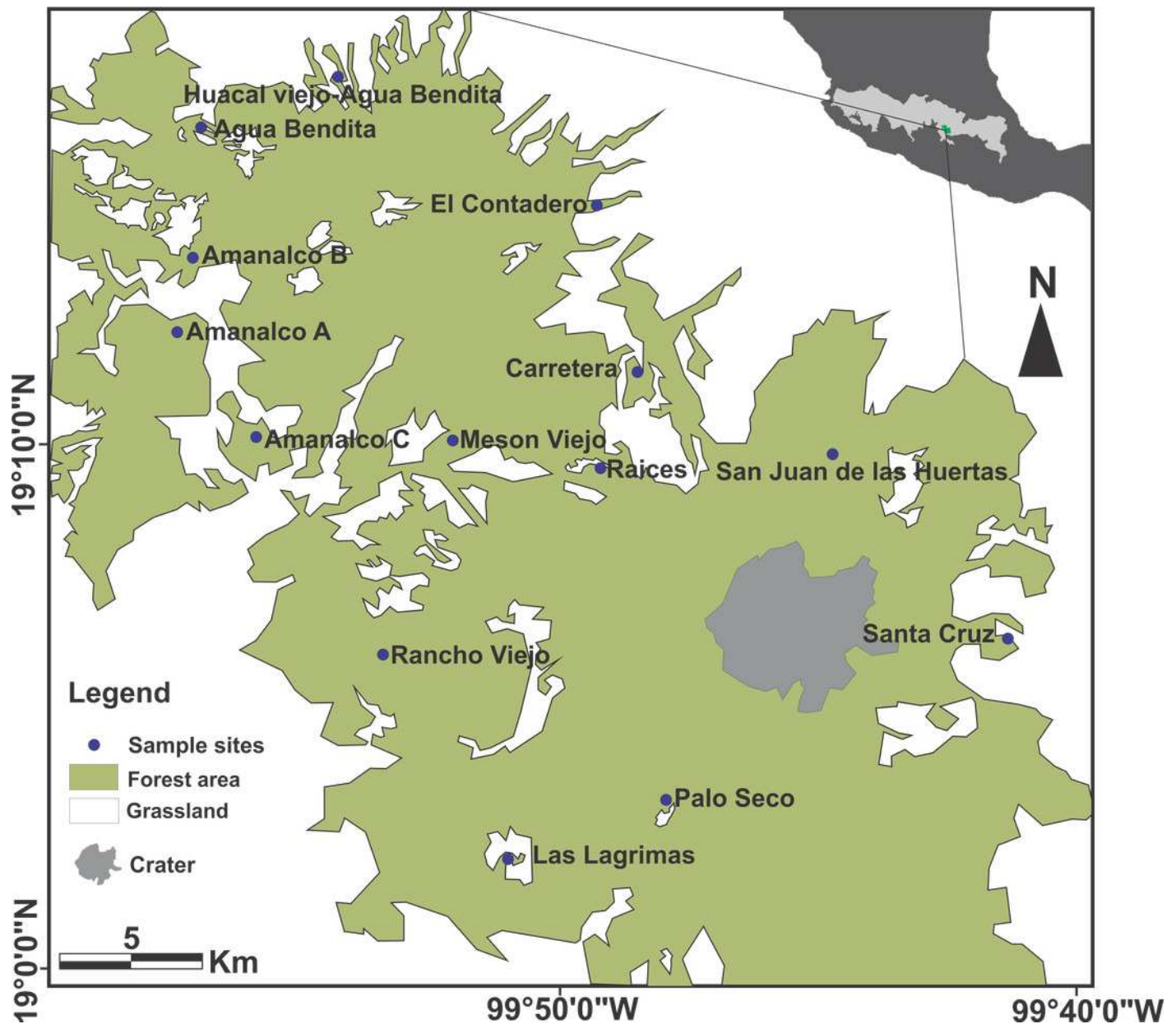
Figure 2

Population sampling

Number of individuals sampled per site.

Number of species per site
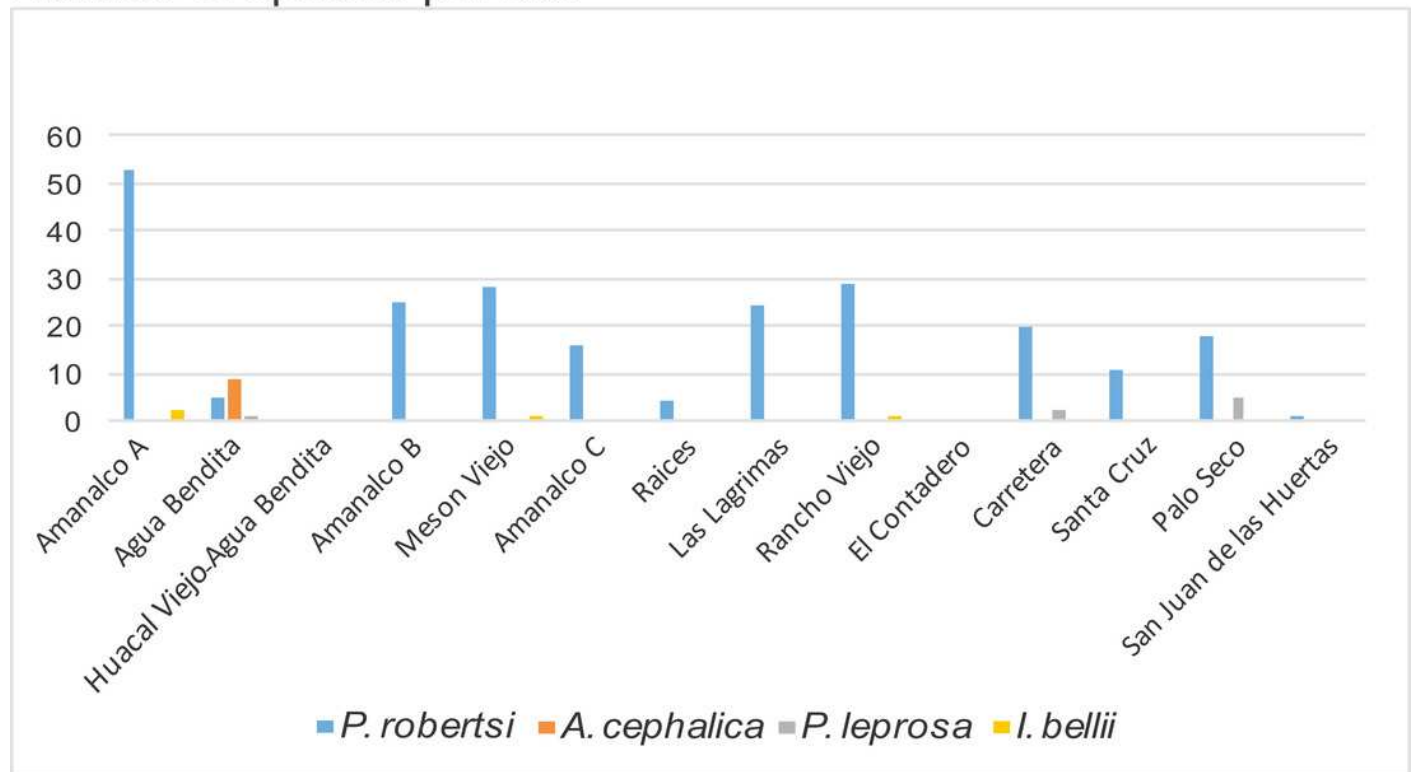
Figure 3

Morphometric measurements and geometric morphometric

A) Location of landmarks in the head of *Pseudoeurycea robersi.* 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.
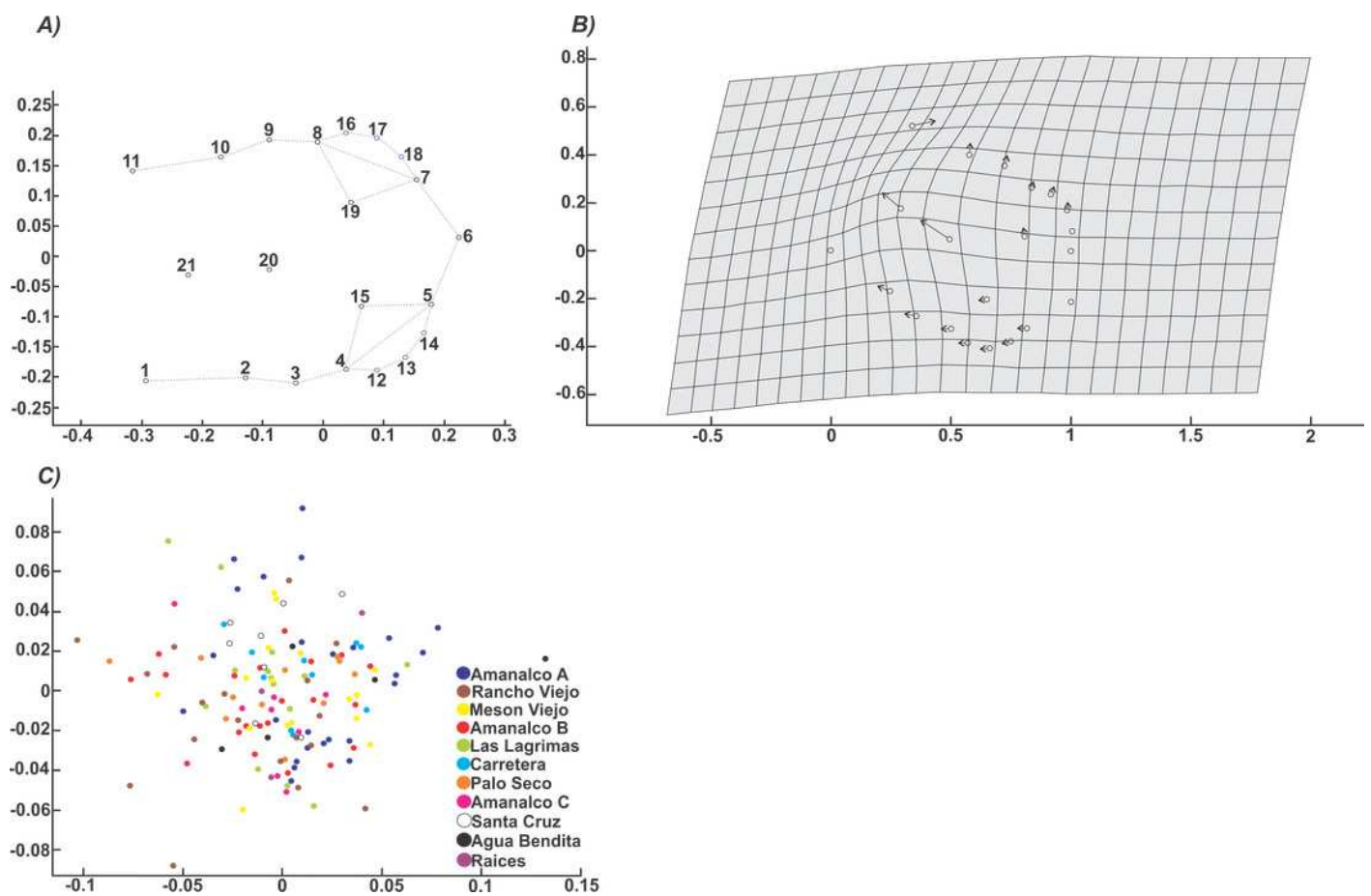
Figure 4

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.
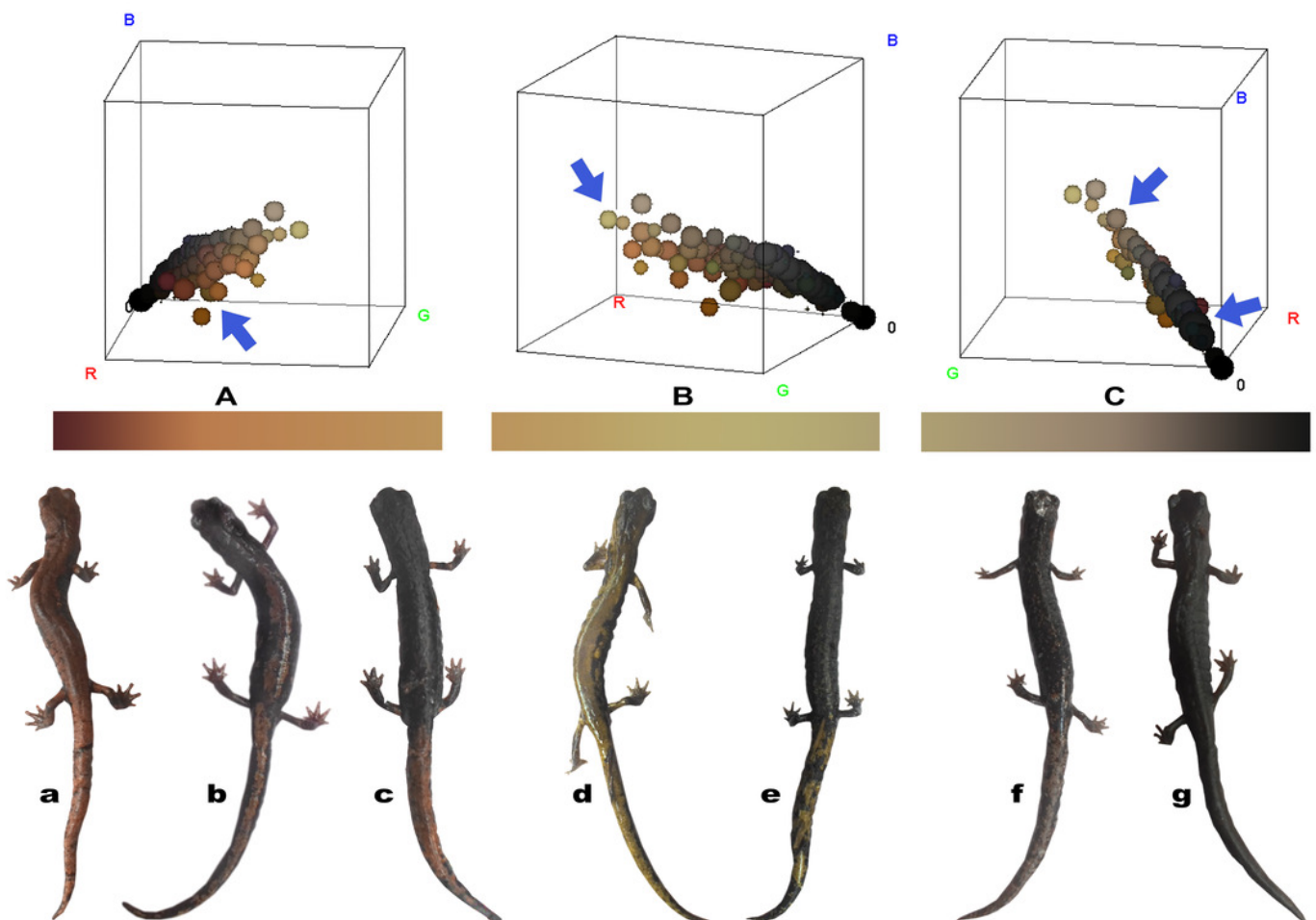
Figure 5

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.
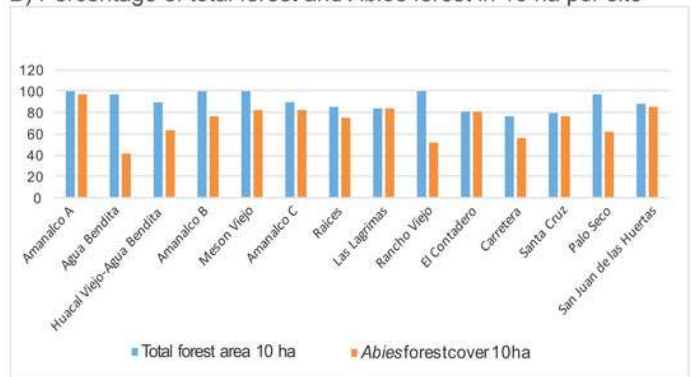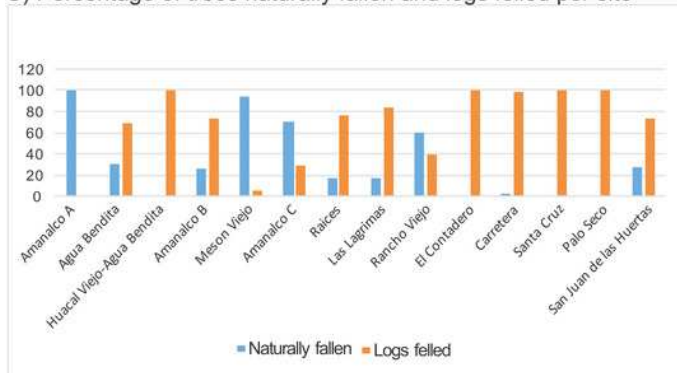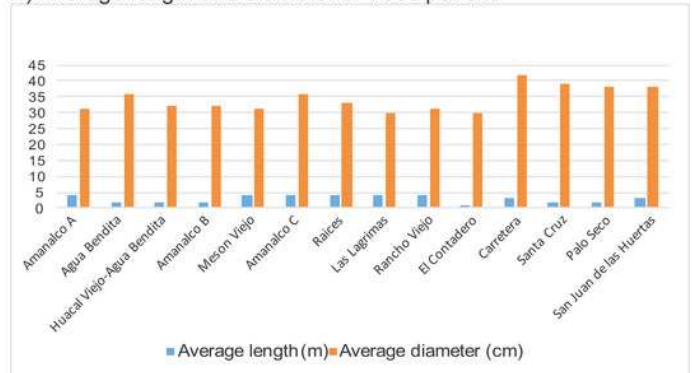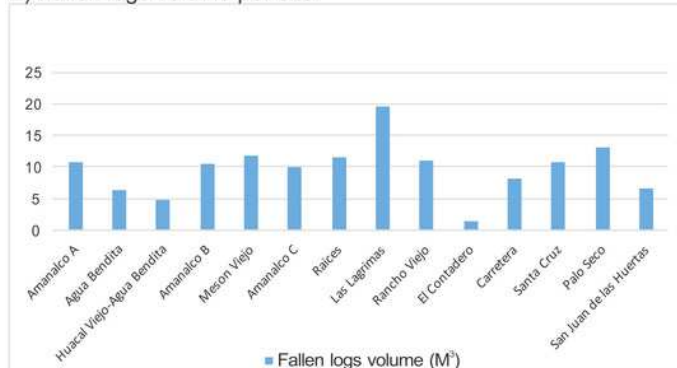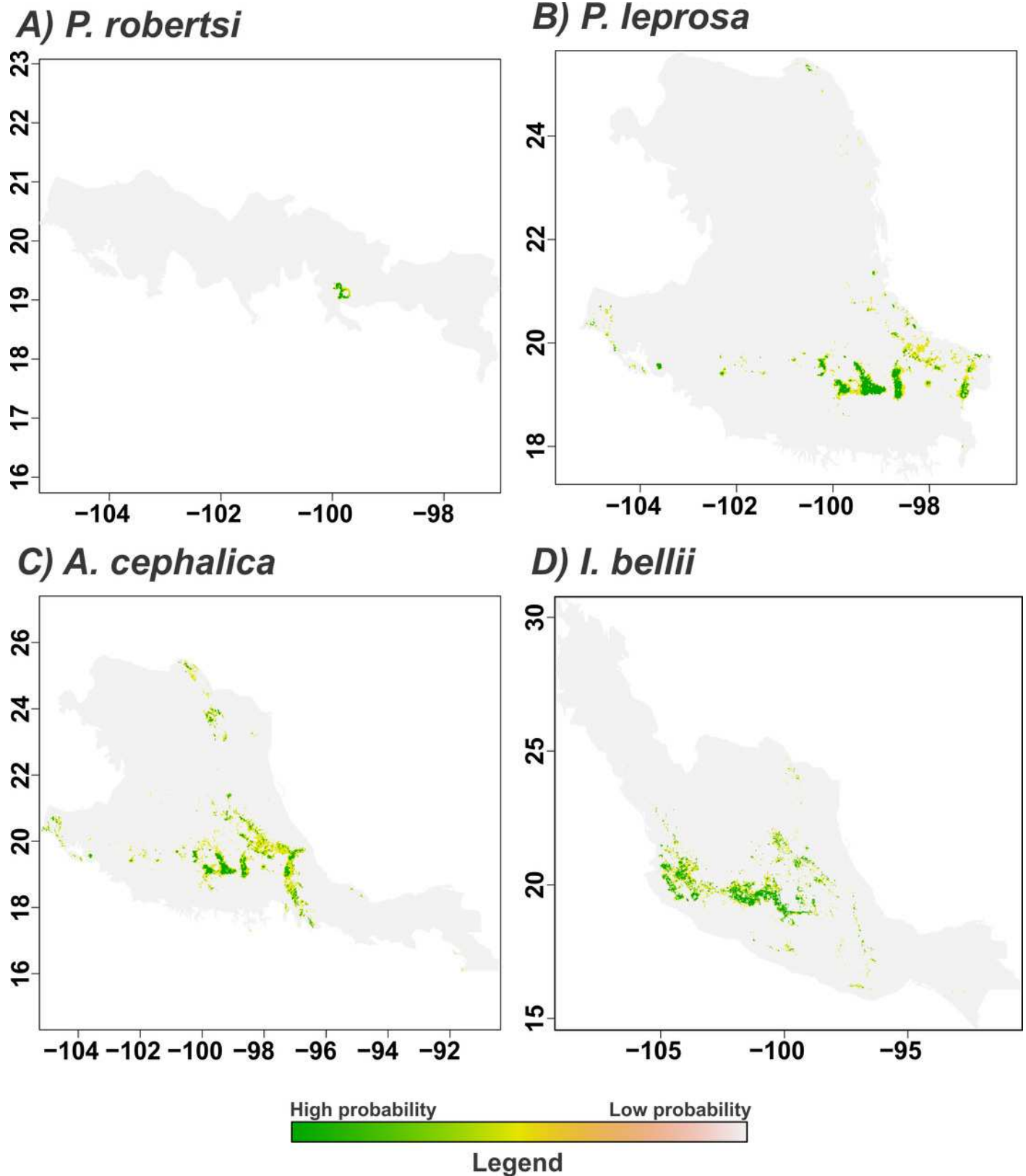
Figure 6

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.
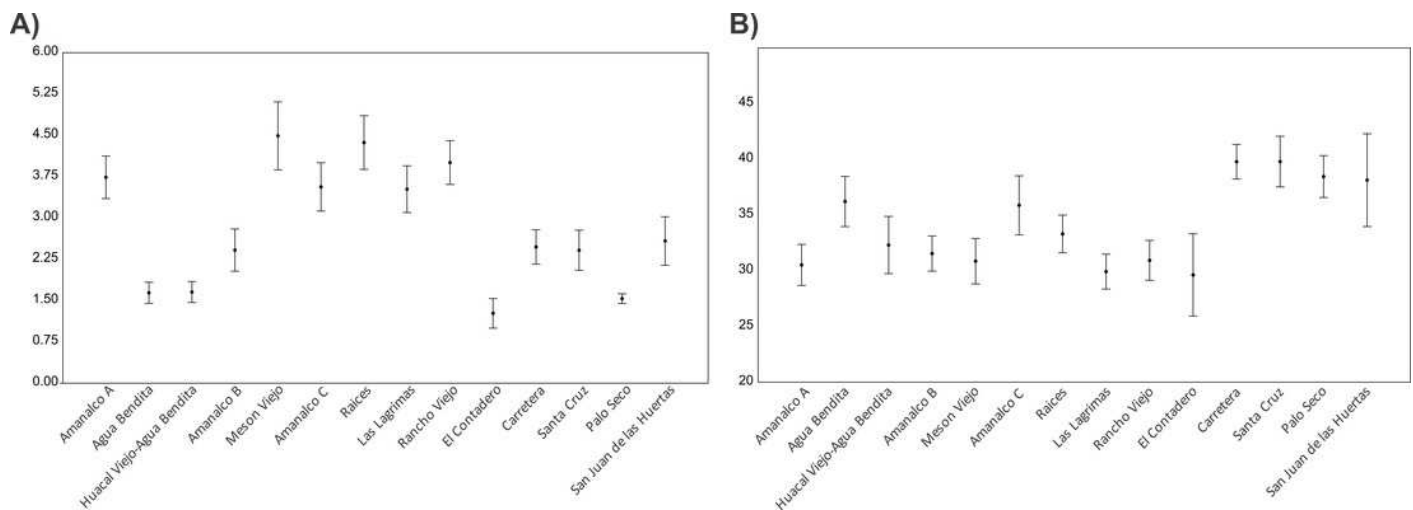
Figure 7

Environmental niche modelling

Potential distribution maps for each salamander species. A). *Pseudoeurycea robersi*. B) *Pseudoeurycea leprosa*. C) *Aquiloeurycea cephalica*. D) and *Isthmura bellii.*
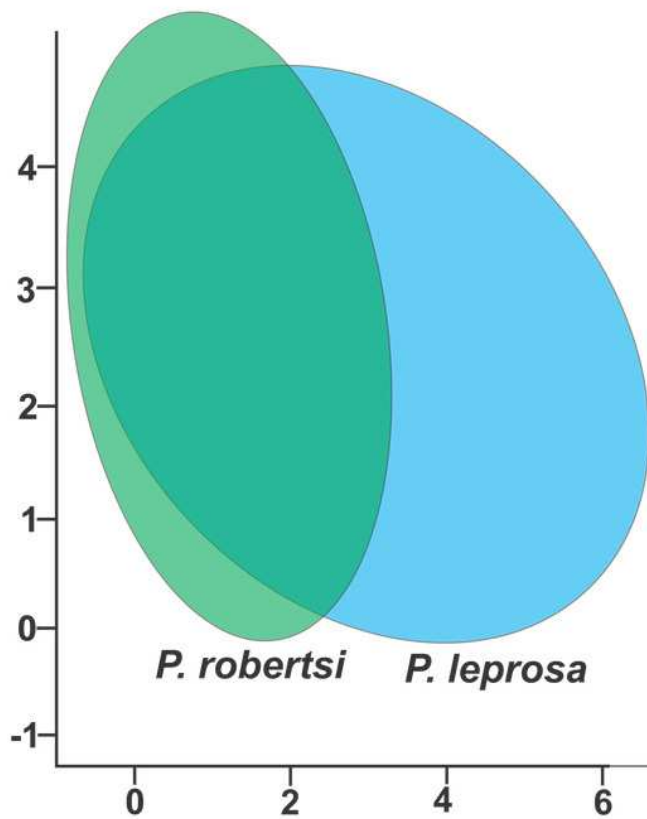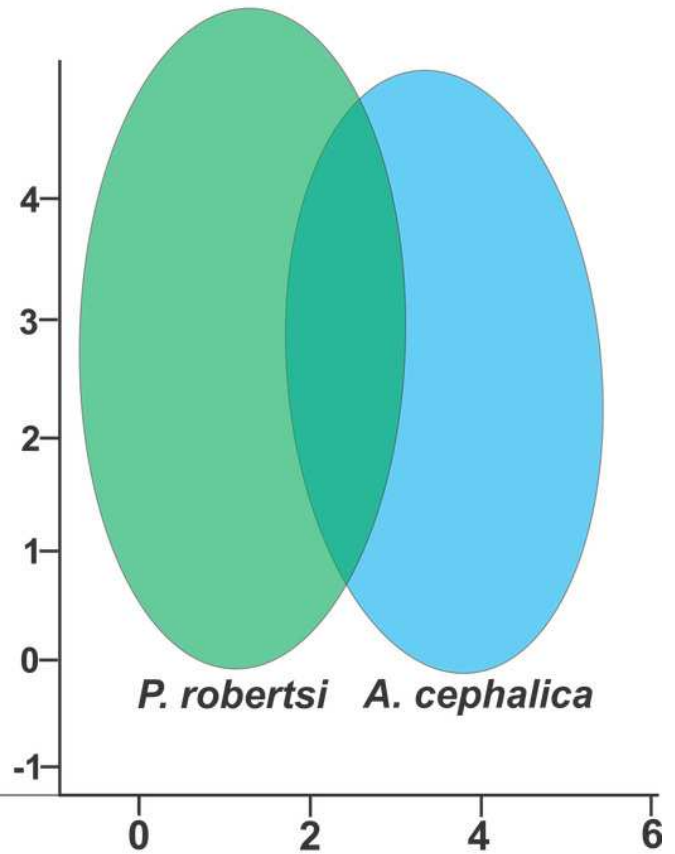
A) *P. robertsi*

B) *P. leprosa*

C) *A. cephalica*

D) *I. bellii*

High probability   Low probability

Legend

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*.
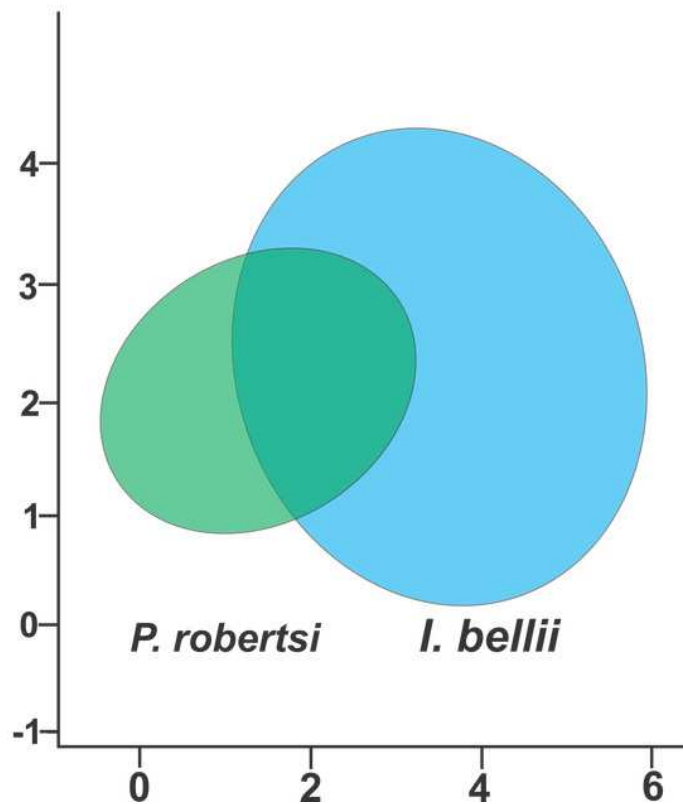
A) *P. robertsi, P. leprosa*

B) *P. robertsi, A. cephalica*
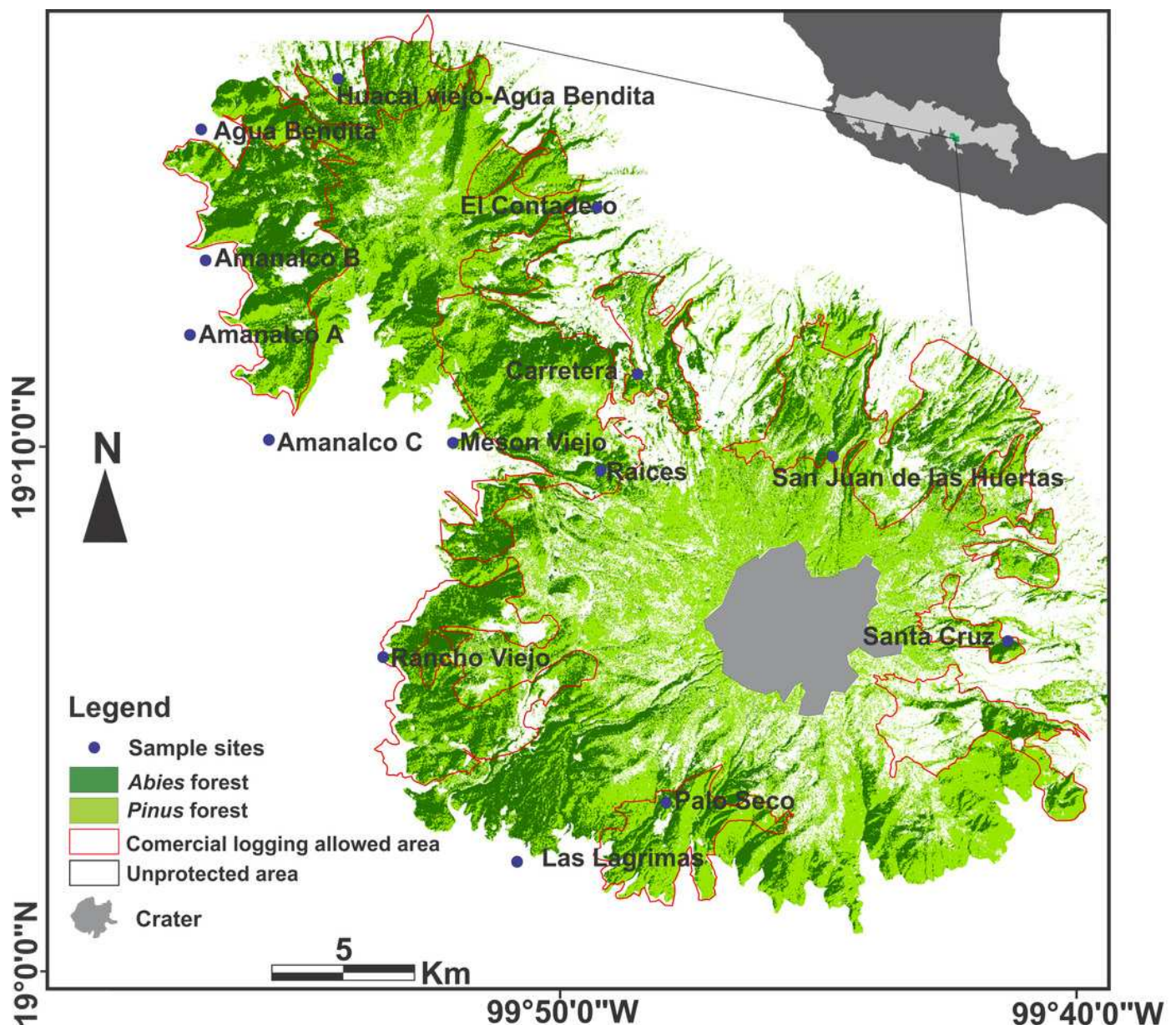

C) *P. robertsi, I. bellii*

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.

1

	AUC	Partial-ROC
P. robertsi	0.993	1.970
P. leprosa	0.964	1.846
A. cephalica	0.888	1.430
I. bellii	0.816	1.403

2