

# Distribution, habitat suitability, conservation state and natural history of endangered salamander *Bolitoglossa pandi*

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**Background.** Pandi's mushroom-tongue salamander *Bolitoglossa pandi* is one of the most threatened amphibians in South America, as well as a spotlight species on conservation Colombian agenda. Few studies have provided relevant information that helps Colombian government to formulate lines of action for its conservation. Given this dearth of information, its threat assessments have been based on very limited information.

**Methods.** We conducted surveys for salamanders in four municipalities of Cundinamarca-Colombia, using two approaches: visual encounter surveys (Guaduas and Villeta) and the basic sampling protocol for single-species occupancy modeling (Supatá and Venecia). A multivariate approach was employed to explore correlation between habitat structure and natural history traits, abundance, as well as detection / non-detection of *B. pandi*. We evaluated *B. pandi* activity pattern through kernel density curves for each sampling occasion and explored salamander abundance variability during their activity period by performing a nested ANOVA.

**Results.** We report the discovery of two new populations of *B. pandi* which represent the most northwestern records known for the distribution of this species. A significant correlation between body length, body mass and habitat structure were observed. Multivariate analyses indicated that leaf litter depth, environmental mean temperature, percent vegetation cover, and altitude were the habitat variables that explain 60.32% of *B. pandi* abundance variability, as well as being the main drivers of its optimal habitat. *B. pandi* exhibit an activity pattern characterized by two main activity peaks, in which niche partitioning was observed. We found a healthy, stable, highly dense, with high replacement rate between body size classes and constant recruitment rate population of *B. pandi* (>1300 individuals).

**Discussion.** Given the high habitat specificity of *B. pandi*, it is highly vulnerable to local changes on its habitat variables. Thus, we recommend that *B. pandi* is retained as Endangered (EN) on the IUCN Red List based on the IUCN Criterion B, given its restricted extent of occurrence (ca. 2,500 km<sup>2</sup>), as well as the ongoing habitat loss within its range due to agriculture, cattle ranching, logging, and urban development that constantly reduce its suitable habitat.

1 **Distribution, habitat suitability, conservation state and natural history of**  
2 **endangered salamander *Bolitoglossa pandi***

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26

## 27 Abstract

28

29 **Background.** Pandi's mushroom-tongue salamander *Bolitoglossa pandi* is one of the  
30 most threatened amphibians in South America, as well as a spotlight species on the  
31 Colombian conservation agenda. Few studies have provided relevant information that  
32 helps the Colombian government to formulate lines of action for its conservation; so,  
33 given this dearth of information, its threat assessments have been based on very limited  
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36 Cundinamarca–Colombia, using two approaches: visual encounter surveys (Guaduas  
37 and Villeta) and the basic sampling protocol for single-species occupancy modeling  
38 (Supatá and Venecia). A multivariate approach was employed to explore the correlation  
39 between habitat structure and natural history traits, abundance, as well as detection /  
40 non-detection of *B. pandi*. We evaluated the *B. pandi* activity pattern through kernel  
41 density curves for each sampling occasion and explored the salamander abundance  
42 variability during their activity period by performing a nested ANOVA.

43 **Results.** We report the discovery of two new populations of *B. pandi*, which represent  
44 the most northwestern records known for the distribution of this species. A significant  
45 correlation between body length, body mass, and habitat structure as was observed.  
46 Multivariate analyses indicated that leaf litter depth, environmental mean temperature,  
47 percent vegetation cover, and altitude were the habitat variables that explain the  
48 60.32% of the *B. pandi* abundance variability, as well as the main drivers of its optimal  
49 habitat. *B. pandi* exhibits an activity pattern characterized by two main activity peaks, in  
50 which niche partitioning was observed. We found a healthy, stable, highly dense, with  
51 high replacement rate between body size classes and constant recruitment rate  
52 population of *B. pandi* (>1300 individuals).

53 **Discussion.** Given the high habitat specificity of *B. pandi*, the species is highly  
54 vulnerable to local changes on its habitat variables. Thus, we recommend that *B. pandi*  
55 is retained as Endangered (EN) on the IUCN Red List based on the IUCN Criterion B,  
56 given its restricted extent of occurrence (ca. 2,500 km<sup>2</sup>), as well as the ongoing habitat  
57 loss within its range due to agriculture, cattle ranching, logging, and urban development  
58 that constantly reduce its suitable habitat.

59

## 60 Introduction

61 Colombia is home to more than 850 species of amphibians, and much of this diversity is  
62 concentrated in the Andean Forest (Acosta-Galvis, 2019). Geographic distributions of  
63 fauna in this biome are often restricted, and also endemism is common (Lynch &  
64 Suárez-Mayorga, 2002). Andean forests are the most threatened habitats in the country  
65 due to the drastic transformation of native vegetation by urban growth, mining,  
66 agriculture, and cattle ranching (Etter, Andrade, Saavedra, & Cortés, 2018). As a

67 consequence, and given that the majority of threatened Colombian amphibian species  
68 are concentrated between 1800-3600 a.s.l., habitat loss is their shared major threat.

69         Among threatened Colombian amphibians, salamanders of the *Bolitoglossa*  
70 genus are one of the spotlight amphibian groups on Colombian conservation agenda.  
71 This genus is the most diverse and geographically widespread lineages of  
72 Plethodontidae salamanders inhabiting the Neotropics. Currently, *Bolitoglossa*  
73 comprises 131 species, 24 of which reside in Colombia across several types of tropical  
74 habitats (Wake, 2017; Acosta-Galvis, 2019). Many species of *Bolitoglossa* exhibit  
75 patterns of restricted geographic ranges [i.e. *Bolitoglossa capitana* (Brame & Wake,  
76 1963b), *B. hypacra* (Brame Jr & Wake, 1962), *B. hiemalis* (Lynch, 2001), among  
77 others]. However, the high morphological crypsis and an incomplete understanding of  
78 the morphological variability among Andean *Bolitoglossa* species make suitable  
79 taxonomic decisions about their distributional ranges hard to achieve (Acosta-Galvis &  
80 Gutiérrez-Lamus, 2012).

81         The ecology and life history of *Bolitoglossa* salamanders are poorly understood  
82 compared to their Nearctic congeners; so less than 11.5% of *Bolitoglossa* species have  
83 been investigated with published research on their diet, reproduction, foraging activities,  
84 thermal ecology, ecological interactions, microhabitat use, or habitat preferences  
85 (Anderson & Mathis, 2006; Bruce, 1997; Cadenas, Pérez-Sánchez, Villa, & De  
86 Ascensão, 2009; Cruz, Galindo, & Bernal, 2016; del Río-Gracia, Serrano-Cardozo, &  
87 Ramírez-Pinilla, 2014; Houck, 1977; Jimenez, 1994; Ortega, Monares-Riaño, &  
88 Ramírez-Pinilla, 2009; Salgado-Aráuz, 2005; Sarmiento et al., 2011). Given this dearth  
89 of information, the conservation status of many of these species is Data Deficient (DD)  
90 or has been based on limited information.

91         According to the IUCN Red List of Threatened Species, *Bolitoglossa pandi*  
92 (Brame & Wake, 1963b) is Endangered B1ab (iii), based on its restricted geographical  
93 range; it was originally described based on a single specimen (holotype ZSZMH 2858,  
94 an adult female with SVL 50.4 mm, collected in 1913 by Wilhelm Frietsche) from the  
95 municipality of Pandi, Cundinamarca (exact locality unknown), in the cloud forests on  
96 the western slope of the Cordillera Oriental of Colombia. Subsequently, Hanken and  
97 Wake (1982) reported a second specimen 75km north (airline) from the type locality, in  
98 a bromeliad inside the primary cloud forest, near the municipality of Albán  
99 (Cundinamarca), at 2400 m a.s.l.

100         Acosta-Galvis and Rueda-Almonacid (2004) reported a third specimen (an adult  
101 female with snout-vent length (SVL) 44.7 mm, collected by Franz Kaston, ICN 45500)  
102 from nearby the type locality and described the associated habitat as relict wet areas  
103 covered by lush trees and shrubs. Acosta-Galvis and Gutiérrez-Lamus, (2012) included  
104 a new record for the Supatá region (adult male with SVL 37.63 mm, MUJ 7921)  
105 representing the northernmost record of the species. Hence, the known distribution of *B.*  
106 *pandi* includes four localities from the sub-Andean forests between 1300-2400 m a.s.l.,

107 throughout the western slopes of the Cordillera Oriental. Since its first extinction risk  
108 assessment was performed 14 years ago, few studies have provided relevant  
109 information for the formulation of strategies and an action plan for its conservation (del  
110 Río-García et al., 2014).

111 The aims in this study are: (1) to describe the geographic range extension of *B.*  
112 *pandi* along the western slopes of the Cordillera Oriental; (2) to explore the relationship  
113 between habitat structure and natural history traits of this species; (3) to describe its  
114 activity pattern and population structure; (4) to expand the knowledge of the variability of  
115 certain morphological characters and life-history traits of this poorly known species; (5)  
116 and to provide a conservation state reassessment of *B. pandi*.

117

## 118 **Materials & Methods**

### 119 **Ethics statement**

120 Sex was not determined on living salamanders due to the high risk of injury to the  
121 animal. Fieldwork was done under the scientific research non-commercial purpose  
122 permit of collection of wild specimens of biological diversity issued by the National  
123 University of Colombia (Research Project 38615), and the Colombian National  
124 Environmental Licensing Authority (ANLA) by resolution No. 0255 of 14 March 2014.

125 This study was conducted following the Colombian animal welfare law and the collection  
126 of wild specimens of the biological diversity acts (Ley 1774, 2016; Decreto 1376, 2013),  
127 as well as considering the Universal Declaration on Animal Welfare (UDAW) endorsed  
128 by Colombia in 2007.

129

### 130 **Study area**

131 We searched for salamanders at nine localities in four municipalities of Cundinamarca  
132 located on the western slope of the Cordillera Oriental of Colombia: Guaduas, Supatá,  
133 Villeta, and Venecia (Fig. 1). Searches at each locality were carried out within an  
134 altitudinal gradient ranging from 1638 to 2315 m a.s.l (Table 1). The sampled area  
135 includes sub-Andean and Andean forests, as well as areas transformed by urban  
136 growth, agriculture, and cattle ranching. The sampling area is characterized by bimodal  
137 climate [high dry season (2<sup>nd</sup> half of Dec, Jan, Feb, 1<sup>st</sup> half of Mar); high rainy season  
138 (2<sup>nd</sup> half of Mar, Apr, May, Jun); low dry season (Jul, Aug, 1<sup>st</sup> half of Sept), low rainy  
139 season (2<sup>nd</sup> half of Sept, Oct, Nov, 1<sup>st</sup> half of Dec)]. We monitored the environmental  
140 temperature (ET) and relative humidity (RH) at sampling sites using thermo-  
141 hygrometers model EBI 20-TH1 Ebro®.

142

### 143 **Sampling and data collection**

144 We conducted surveys for salamanders using two approaches. First, during the rainy  
145 season in April–May 2013, we performed visual encounter surveys (VES; Crump &  
146 Scott, 1994) in three localities associated with cloud forests throughout an altitudinal  
147 gradient (1648–2002 m a.s.l) at the municipalities of Guaduas and Villeta (Table 1). Two  
148 researchers surveyed day and night for five consecutive days investing a total of 100 h  
149 of sampling effort.

150 Second, we employed the basic sampling protocol described by Mackenzie et al.,  
151 (2003) for single-species occupancy modeling in six localities, three in the municipality  
152 of Supatá and three in Venecia. We randomly selected a total of 296 quadrats (5 m x 5  
153 m), which were located throughout an altitudinal gradient at each sampling locality  
154 (1600–2300 m a.s.l), grouping the following vegetation covers: relicts of the Andean  
155 forest, restored the Andean riparian forest, grove pastures, pastures and roadsides  
156 (Table 1). During three sampling occasions (September–October 2017, March 2018,  
157 and July 2018), each quadrat was surveyed day and night for five consecutive days by  
158 ten researchers, resulting in a total of 2100 h of sampling effort. During each survey, the  
159 detection / non-detection of *B. pandi* specimens were recorded. When a salamander  
160 was present, we measured its perch height using a measuring tape ( $\pm 0.1$  cm). Once  
161 salamanders were caught, we measured their weight (Mass g) with a Pesola®  
162 dynamometer of 50g ( $\pm 0.1$  g) and took photographs to measure their body size [SVL =  
163 snout-vent length (mm), TL = Tail length (mm)] using the software Image–J version 1.52  
164 (Schneider, Rasband, & Eliceiri, 2012). The sex was not determined on living  
165 salamanders due to the high risk of injury to the animal. All specimens of *B. pandi* were  
166 subsequently released near the quadrat in which they were sighted.

167 Based on 17 specimens collected, euthanized using lidocaine 2%, and fixed in  
168 10% formalin (Chen & Combs, 2001), we described the morphological variability of *B.*  
169 *pandi*. We made a small incision in the groin region to identify the sex and sexual  
170 maturity through macroscopic observation of the gonads. All the morphological  
171 terminology employed follows several contributions (Brame Jr & Wake 1962, 1963;  
172 Acosta-Galvis & Restrepo 2001; Lynch 2001; Acosta-Galvis & Hoyos 2006; Acosta-  
173 Galvis & Gutierrez-Lamus 2012; Brcko, Hoogmoed, & Neckel-Oliveira, 2013; Bingham,  
174 Papenfuss, Lindstrand, & Wake, 2018). All these specimens were deposited in the  
175 amphibian collection at Instituto de Investigación de Recursos Biológicos Alexander von  
176 Humboldt, (IAvH-Am), as well as in the amphibian collection at the Instituto de Ciencias  
177 Naturales de la Universidad Nacional de Colombia (ICN).

178

### 179 **Habitat structure data collection**

180 We used the Point Intercept Method described by Elzinga, Salzer, and Willoughby  
181 (1998) for estimation of the percent vegetation cover. We grouped the plants into eight life  
182 forms: graminoids, forbs, palm trees, mosses, lichens, vines, shrubs, and trees. We

183 divided vegetation into layers: 0–0.1 m, 0.1–1 m, 1–1.5 m, 1.5–3 m, 3–5 m, 5–12 m. We  
184 also considered the cover of other abiotic items such as leaf litter, bare soil and bare  
185 rocks. We estimated the vegetation cover of each of the quadrants where salamanders  
186 were surveyed, employing a set of 15 intercept points distributed in three parallel lines  
187 of five points separated by one meter of distance. At each point, we used a sampling  
188 bar of 1.5 m to register the contact of the life forms of each vegetation layer below 1.5  
189 m. This provided us with a 6.67% cover resolution by layer. We assessed the vegetation  
190 percentage cover of the vegetation layers above 1.5 m (mostly trees) using five  
191 intercept points: the corners of the quadrant and the central point of the third line. In this  
192 way, we reached a 20% cover resolution for upper layers.

193

### 194 **Statistical analysis**

195 We evaluated the association between the habitat structure and the natural history traits  
196 of *B. pandi* by multiple correlation analysis, with  $P < 0.05$  as the significance criterion.  
197 The following variables were considered: SVL (mm), weight (g), perch height (mm), leaf  
198 litter depth (mm), vegetation layers, vegetation life form, vegetation percentage cover,  
199 and altitude. Using the habitat variables, we performed a principal component analysis  
200 (PCA) to explore which of these variables presents greater variability between quadrats  
201 with the detection / non-detection of *B. pandi* and, therefore, which of these are  
202 responsible for the differences observed between them. The variable suitability for PCA  
203 analysis was tested performing a Kaiser-Meyer-Olkin test ( $KMO > 0.5$ ,  $P < 0.05$ ).  
204 Afterward, a quadratic discriminant analysis was performed to determine which of the  
205 habitat variables have the greatest discrimination capacity between quadrants where *B.*  
206 *pandi* was detected or non-detected.

207 We assessed the variability in the salamander abundance observed in the  
208 Supatá population through multiple regression analysis. First, we considered the  
209 following habitat structure variables at each sampling quadrant: leaf litter depth (mm),  
210 vegetation layers, vegetation life form, vegetation percentage cover, altitude,  
211 temperature, and relative humidity of the environment. All variables were Ln–  
212 transformed previously to perform the statistical analysis.

213 Second, we evaluated assumptions of normality, autocorrelation, and  
214 homoscedasticity using Kolmogorov–Smirnov’s test, Durbin–Watson test and Breusch–  
215 Pagan test, respectively. Given that the p–value of the Durbin–Watson test can easily  
216 be less than 0.05 when data size is very large, we used the Durbin–Watson statistic test  
217 (DW) as an autocorrelation criterion. According to Durbin and Waston (1950), a DW of  
218 less than 1 indicates a strong positive autocorrelation, a DW greater than 4 indicates a  
219 strong negative autocorrelation, values between 1 and 3 suggest a moderate  
220 autocorrelation, and a value close to 2 means that there is no autocorrelation.

221

222 Third, we tested for multicollinearity between the aforementioned variables using the  
223 variance inflation factor (VIF) with a threshold of 10. Fourth, we selected the “best”  
224 regression model employing the Akaike Information Criterion (AIC; Akaike, 1973),  
225 considering that models with  $\Delta$ AIC values of less than two are equally plausible (White  
226 & Burnham, 1999). Finally, we used the hierarchical partitioning method to evaluate the  
227 contribution of all the independent variables of the regression model (Chevan &  
228 Sutherland, 1991).

229

### 230 **Activity pattern and population structure**

231 We only assessed the activity pattern and the population structure of the *B. pandi*  
232 population at the Cuzcungos locality given the remarkable abundance observed (Table  
233 1). We estimated the activity pattern through Kernel Density Curves for each sampling  
234 occasion and explored the salamander abundance variability during their activity period  
235 by performing a nested ANOVA. Hence, the activity period of *B. pandi* was divided into  
236 nine-time intervals (from H1 = 18:30–19:30, H2 = 19:31–20:30, H3 =... until H9 =  
237 02:31–03:30), and each salamander sighting was allocated into its respective interval.  
238 The sampling occasion was employed as the primary factor, and the time intervals as  
239 the secondary factor nested in the primary factor. We evaluated assumptions of  
240 normality and homogeneity of variances using a Shapiro-Wilk test and Levene test,  
241 respectively. Additionally, we analyzed the variability in observed body size over the  
242 nine-time intervals through a non-parametric ANOVA using a Kruskal-Wallis test (KW)  
243 as a measure of the central tendency of the samples (Sokal & Rohlf, 1981).

244 We used SVL as a descriptive variable of the population structure of *B. pandi*.  
245 We compared the variability in population structure between sampling occasions  
246 through a Wilcoxon test, with the null hypothesis that the median of the population  
247 structure is the same across all sampling occasions. According to the categories  
248 proposed by Acosta-Galvis and Gutierrez-Lamus (2012) and Del Río-Gracia et al  
249 (2014), as well as the reproductive stage of the collected salamanders, the population  
250 was divided into body size classes as follows: neonates ( $\leq$  23 mm), juveniles (24–30  
251 mm), and adults ( $\geq$  30 mm).

252 All statistical analyses were performed using the software Rwizard 4.3  
253 (Guisande-González C, Vaamonde-Liste, A. Barreiro-Felpeto, 2014) and the following R  
254 packages: car (Fox & Weisberg, 2019), hier.part (Walsh & Macnally, 2015), lawstat  
255 (Hui, Gel, & Gastwirth, 2008), nortest (Gross & Ligges, 2015), overlap (Meredith &  
256 Ridout, 2017) stat (R Core Team, 2012) and usdm (Naimi, Hamm, Groen, Skidmore, &  
257 Toxopeus, 2014).

258

## 259 **Results**

### 260 **Geographic distribution**

261 We found a total of 34 individuals of *B. pandi* in three new localities, extending the  
262 geographical range of the species by 96.5 Km (airline) northwest from the type-locality,  
263 and 33.6 Km (airline) west from the northernmost locality in the municipality of Supatá  
264 (Fig. 1). These new localities belong to the municipalities of Guaduas and Villeta in the  
265 department of Cundinamarca. All the salamanders in the new localities were found at  
266 night, within the understory of oak groves dominated by ferns. The salamanders were  
267 sighted in different vertical strata ranging from leaf litter, where they remained hidden, to  
268 shrubby substrates up to 2.5 m. Also, two salamanders were found in ecotonal areas  
269 associated with sugar cane crops and rangeland areas for livestock.

270

### 271 **Associations of habitat structure and natural history traits**

272 We observed a significant association between habitat structure and natural history  
273 traits. Snout-vent length, tail length, and mass were significantly associated with all the  
274 habitat structure variables assessed, but less so with the vegetation layers. SVL, TL,  
275 and mass showed a negative correlation with leaf litter and altitude (Table 2). In  
276 contrast, SVL and mass showed a positive correlation with perch height and percent  
277 vegetation cover (Fig. 2). The square coefficients obtained indicate that the variability of  
278 SVL, TL, and mass correlated in 19.3%, 16.8%, and 18.6%, with habitat structure  
279 variables respectively.

280 Similarly, the habitat structure variables explain the detection or non-detection of  
281 *B. pandi* throughout the sampling quadrants. The first two components of the PCA  
282 analysis explained 62.41% of the variability observed. The habitat structure variability  
283 was clustered in two groups associated with the detection or non-detection of *B. pandi*  
284 (Fig. 3A). These groups were moderately overlapped in multivariate spaces, but they  
285 were strongly differentiated by altitude, leaf litter depth, and percent vegetation cover:  
286 the presence of *B. pandi* was positively correlated with quadrants highly structured and  
287 with a depth of leaf litter (Table 3). The quadratic discriminant analysis confirms that the  
288 detection or non-detection of *B. pandi* depends on habitat variables. The cross-  
289 validation percentage was 92.6%, indicating that the quadrants in which *B. pandi* was  
290 detected can be clearly distinguished by habitat variables such as vegetation layers,  
291 leaf litter and environmental mean temperature (Fig. 3B).

292 Results of the multiple regression analysis showed that leaf litter depth,  
293 environmental mean temperature, percent vegetation cover, and altitude were the  
294 variables that contributed the most to explaining the observed variability in the  
295 abundance of *B. pandi* ( $R^2 = 60.32\%$ ,  $P < 0.001$ ). These habitat variables also composed  
296 the “best” fitted regression model (Table 4). However, this model showed a moderate  
297 autocorrelation ( $DW = 1.10$ ), signifying that the autocorrelation is very small and the  
298 variance explained by the habitat variables may be close to 60.32% (Fig. 4).

299

### 300 **Activity pattern**

301 *Bolitoglossa pandi* is completely nocturnal, its activity period extends throughout the  
302 night, beginning from 18:30 h until 05:00 h. The environmental temperature and relative  
303 humidity recorded during this activity period ranged from 12.6–25.6 °C ( $\bar{x}$  = 15.3), 73.8–  
304 98.8 RH ( $\bar{x}$  = 80.3). The activity peaks were all subject to environmental influences  
305 showing a significant association with the local weather conditions. The observed  
306 salamander abundance was strongly and positively correlated with the environmental  
307 temperature ( $R_{ET} = 0.251$ ,  $P < 0.001$ ), whereas the moderately and negatively were  
308 correlated with the relative humidity ( $R_{RH} = -0.174$ ;  $P = 0.017$ ). Despite the sampling  
309 occasion, we observed two main activity peaks, the first from 20:30 h to 21:30 h, and  
310 the second from 23:30 h to 00:30 h (Fig. 5). Results of the nested ANOVA indicated that  
311 salamander abundance between these peaks was significantly different ( $F_{9-259} = 4.57$ ,  $P$   
312  $< 0.001$ ; Table 5), being more abundant in the second activity peak during the second  
313 and third sampling occasions, and the opposite during the first sampling occasion (Fig.  
314 6A).

315 Likewise, the observed body length of the salamanders showed significant differences  
316 between the two activity peaks ( $K_{8df} = 99.70$ ,  $P < 0.001$ , Fig. 6B), suggesting niche  
317 partitioning between body size classes. Most of the salamanders observed during the  
318 first activity peak had SVL  $> 30$  mm (juveniles and adults), whereas the salamanders  
319 observed at the second activity peak had SVL  $< 27$  mm (juveniles and neonates).  
320 However, in contrast with the differences in observed abundance between activity  
321 peaks, the variation between salamander SVL were consistent across the three  
322 sampling occasions.

323

### 324 **Population structure**

325 A total of 1391 individuals of *B. pandi* were observed throughout the study in the Supatá  
326 population, exhibiting a population density ranging from 0.04– 1.44 individuals/m<sup>2</sup>. The  
327 population structure showed significant differences between sampling occasions,  
328 suggesting a high replacement rate between body size classes (Table 6). Regardless of  
329 the variability observed in the population structure of *B. pandi*, the population is mostly  
330 dominated by juveniles and neonates which represent between 34–64% of the  
331 individuals (Fig. 7). Neonates were observed across all sampling occasions, indicating  
332 constant recruitment. During the second sampling occasion, a notorious bias in the SVL  
333 median value toward small body length salamanders was observed, suggesting that the  
334 major recruitment peak occurs during March (Fig 7B). Adults were conspicuous  
335 throughout all sampling occasions, although predominantly in the first sampling  
336 occasion.

337

### 338 **Morphological variability**

339 *Bolitoglossa pandi* is a tiny species recognizable by SVL = 13.4–51 mm ( $n = 1034$ ),  
340 13.4–48mm adult males ( $n = 7$ ), and SVL 14–51 mm adult females ( $n = 8$ ). Extensively

341 webbed hands and feet with third toes and fingers triangular; ventral surfaces of digit  
342 tips without terminal flattened tubercles; snout short and rounded in the lateral profile;  
343 head length 4.9–10.1 mm; head width 5.1–7.9 (n =17); snout rounded in dorsal view  
344 irregular white spots, cream nasolabial grooves, and edges of the lips irregularly dark  
345 brown with irregular light spotting (Fig. 8A–B); protruding eyes on dorsal view, brown iris  
346 with black reticules (Fig. 8B); well-defined post-cephalic constriction; ventral surfaces  
347 (preserved); brown or dark grey with numerous tiny cream guanophores (Fig.  
348 8C); inverted bracket shaped scapular spots (Fig. 9F); males have white testes.

349 *Bolitoglossa pandi* can be distinguished from its Colombian congeners by having  
350 an extensive webbing of hands and feet (versus absence of extensive webbing of hands  
351 and feet in *B. adspersa*, *B. hiemalis*, *B. hypacra*, *B. palmata*, *B. ramosi*, *B. savagei*, *B.*  
352 *tamaense*, *B. tatamae*, *B. walkeri* and *B. valleculea*); also, It can be distinguished from  
353 species with extensive webbing as *Bolitoglossa lozanoi* and *B. nicefori*, in having more  
354 protruding eyes, and longer and triangular third finger. *B. pandi* also differs from *B.*  
355 *biseriata* and *B. silverstonei* by having a dark brown or dark grey ventral surface with  
356 irregular white dots (versus cream ventral surface with brown suffusions and pits in *B.*  
357 *biseriata* and *B. silverstonei*), brown-reddish iris with black reticules (versus golden with  
358 brown pits in *B. biseriata* and *B. silverstonei*), and white testes in adult males (versus  
359 black mesorchium in testes of adult males of *B. biseriata*). It also differs from *B. medemi*  
360 by the absence of digital depressions in digital tips on the fingers and toes (present in *B.*  
361 *medemi*). *B. pandi* has an upper lip with irregular light spotting (versus uniform in *B.*  
362 *guaneae*). *B. pandi* can be differentiated from *B. altamazonica* and *B. leandrae*  
363 by having extensive interdigital webbing with a longer and triangular third finger  
364 (complete webbing and tips rounded in *B. altamazonica* and *B. leandrae*). *B. pandi* is  
365 morphologically very similar to *B. phalarosoma*, but it differs by having a dark brown or  
366 dark grey ventral surface with diffuse white pits and some white blotches (versus usually  
367 light brown ventral surfaces, with some irregular cream spots in *B. phalarosoma*),  
368 plantar and palmar regions in ventral dark brown or dark grey view (versus cream in *B.*  
369 *phalarosoma*). *B. pandi* also differs from *B. capitana* by having smaller adult size with a  
370 longer and triangular third finger (versus rounded third finger in *B. capitana*) and less  
371 head width.

372

### 373 **Color variability**

374 *Bolitoglossa pandi* exhibited a wide polychromatic variability ranging from a uniform  
375 dorsal color pattern of different shades of light to a dark brown one (Fig. 9A); one  
376 reddish-brown pattern that can have diffused grey or dark blotches scarcely  
377 distinguishable to the dorsolateral region (Fig. 9B), or an ochre pattern, with some  
378 diffuse irregular dark brown, yellow or cream spots (Fig. 9C–E). Some specimens  
379 exhibit an exceptionally diffuse band in the paravertebral region covering almost the  
380 entire dorsal surface or including a dark brown inverted triangle shape in the interorbital

381 region (Fig. 9 F–G). The caudal region is highly variable, ranging from uniform reddish-  
382 brown or completely ochre segments, with very small scattered white pits (Fig. 9 A,B, D)  
383 to irregular cream, yellow or orange patches (Fig. 9C, D), and very small or irregular  
384 longitudinal black spots (Fig. 9F–G). The distal end of the tail becomes uniform light  
385 brown and has cream blotches towards the proximal region in some individuals (Fig.  
386 9B). The cephalic region is dark brown with some white spots with irregular pits up to  
387 the supralabial region in lateral view (Figure 8B); a loreal region with ochre patches and  
388 light brown iris with black reticles; nasolabial projections are creams; the ventrolateral  
389 surface is dark brown. Ventral surfaces are dark brown with some cream and white  
390 blotches with scattered pits; the mental and gular surfaces are uniformly dark brown or  
391 dark grey with some irregular white circular spots bordering the maxillary region. In adult  
392 males the mental gland is light brown; the palmar and plantar surfaces are always dark  
393 brown or dark grey.

394

### 395 Discussion

396 The new localities added to *Bolitoglossa pandi* distribution and the detailed examination  
397 of the Supatá population have allowed not only the expansion of knowledge about the  
398 distribution and natural history traits of this species but also the reassessment of its  
399 conservation status and the validation of its characters for the taxonomic identification  
400 among its congeners. The apparent local endemism of *B. pandi* is the result of a  
401 general misunderstanding due to the examination of restricted samples, insufficient  
402 sampling effort, lack of comparisons with the relevant type material, and the incomplete  
403 knowledge of the variability of certain morphological characters of *Bolitoglossa*  
404 salamanders (Wake, 2017).

405 Since Brame and Wake's (1963a) original description of *B. pandi*, several  
406 characters such as dorsal surfaces color (Figure 9) have had conflicting or ambiguous  
407 diagnostic characters (Acosta-Galvis and Gutiérrez-Lamus, 2012). However, our  
408 findings significantly increased the understanding of the morphological variability of *B.*  
409 *pandi*. The chromatic variations reported here contrast with the original description  
410 (Brame and Wake, 1963b) because these have not been described in life (and the  
411 holotype is poorly preserved), and evidence of a broad intrapopulational  
412 polychromatism was not available due to restricted sampling. Our findings allow  
413 clarification of taxonomical misidentifications in the literature. For example, Acosta-  
414 Galvis and Rueda-Almonacid (2004), during the first threat assessment of *B. pandi*,  
415 included erroneously a picture of *B. walker* as the species' portrait. The color and  
416 morphological description provided here comes from living specimens obtained in the  
417 municipalities of Guaduas, Supatá, and Villeta.

418 Neotropical salamanders have been considered inconspicuous amphibians of the  
419 Andean Forest (Brame and Wake, 1963; Gibbons, 1983; Barrio-Amoros and Fuentes,  
420 1999; Acosta-Galvis and Gutiérrez-Lamus, 2012). Nevertheless, our results challenge

421 this general assumption and provide support to previous studies relating specific  
422 environmental conditions with high abundance and density of Andean salamander  
423 populations (Cadenas, Pérez-Sánchez, Villa, & De Ascensão, 2009; Ortega et al., 2009;  
424 Gutiérrez-Lamus, Lynch, & Martínez-Villate, 2011; del Río-García et al., 2014). Altitude  
425 (1700-2000 m. a.s.l), leaf litter depth (> 6 cm), vegetation layers (> 5 vegetation strata),  
426 and environmental mean temperature (16–19 °C) arise as the “best” predictor variables  
427 of presence, abundance and population density of *B. pandi*. Given the *B. pandi*'s  
428 arboreal habits, diet preferences, as well as thermoregulation behavior of  
429 Bolitoglossines (del Río-García et al., 2014; Cruz et al 2016; Mendieta Donaire et al.,  
430 2019);), the selection of these predictor variables by the regression models was  
431 expected.

432 Likewise, highly homogenous sampling quadrants in which pastures, graminoids,  
433 bare soil, and rocks were dominant, as a result of human activities, showed the lowest  
434 or no detection grade of *B. pandi*. Conversely, sampling quadrants in which *B. pandi* was  
435 present were positively correlated with highly structured habitats and deep leaf litter,  
436 characteristics associated with relicts of conserved Andean forest or highly restored  
437 Andean riparian forest (Rangel-Ch, Lowy, & Aguilar, 1997; Alvear, Betancur, & Franco-  
438 Rosselli, 2010; Quintero Vallejo, Benavides, Moreno, & Gonzalez-Caro, 2017). This  
439 result indicates that *B. pandi* requires Andean forests in a good conservation state and  
440 a narrow range of environmental conditions. This strong link between *B. pandi* and a  
441 narrow range of environmental conditions has been observed in other Andean  
442 salamanders such as *B. adspersa*, *B. altamazonica*, *B. nicefori*, and *B. orestes*  
443 (Valdivieso & Tamsitt, 1965; Cadenas et al., 2009; Gutiérrez-Lamus et al., 2011;  
444 Leenders & Watkins-Colwell, 2013). The detection and high local abundance of these  
445 species are strongly correlated with the same habitat variables that promoting the  
446 detection and abundance of *B. pandi*. Thus, the deep dependency of a narrow  
447 environmental range of conditions makes the Andean Bolitoglossines highly vulnerable  
448 to local changes in habitat variables.

449 Our study provided evidence for the first time of intraspecific niche partitioning on  
450 Bolitoglossine salamanders. Niche partitioning has been plenty reported on populations  
451 of Nearctic salamander species (Jaeger & Gergits, 1979; Wicknick, 1995; Arif, Adams,  
452 & Wicknick, 2007; Jaeger, Gollman, Anthony, Gabor, & Kohn, 2016). Evidence of  
453 Nearctic salamander's niche intraspecific partitioning has been explained as a life  
454 history strategy to maximize foraging success, predator avoidance, and mating success  
455 (Jaeger & Gergits, 1979; Holomuzki, 1986; Cloyed & Eason, 2017). Nevertheless, our  
456 data are insufficient to infer any fundamental ecological trade-off between body size and  
457 microhabitat use, activity and vulnerability to predation, or activity and foraging success.  
458 Future experimental studies must be addressed to understand how niche intraspecific  
459 partitioning is acting through environmental heterogeneity in the ecology and evolution

460 of the South American Bolitoglossines. Also, future conservation efforts must consider  
461 this life history strategy into conservation interventions.

462

### 463 **Conclusions**

464 Our result agrees with the general pattern observed in other amphibian taxa in which  
465 habitat disturbance driven by human activities has deleterious effects on presence or  
466 population densities given their high habitat specificity (Lips, 1998; Collins & Storfer,  
467 2003). Habitat loss continues protruding as the central threat for *B. pandi*. This fact has  
468 significant importance in conservation issues because the Andean forest is one of the  
469 most threatened ecosystems by human activities in Colombia (Etter et al., 2018).

470 Therefore, despite that we directly observed a healthy and stable salamander  
471 population that showed high population density, high replacement rate between body  
472 size classes and constant recruitment in the municipalities of Supatá, Guaduas, and  
473 Villeta, we recommend that *B. pandi* is retained as Endangered (EN) on the IUCN Red  
474 List based on the IUCN Criterion B, given its restricted extent of occurrence (ca. 2,500  
475 km<sup>2</sup>), as well as the ongoing habitat loss within its range due to agriculture, cattle  
476 ranching, logging, and urban development that constantly reduce its suitable habitat.

477

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495

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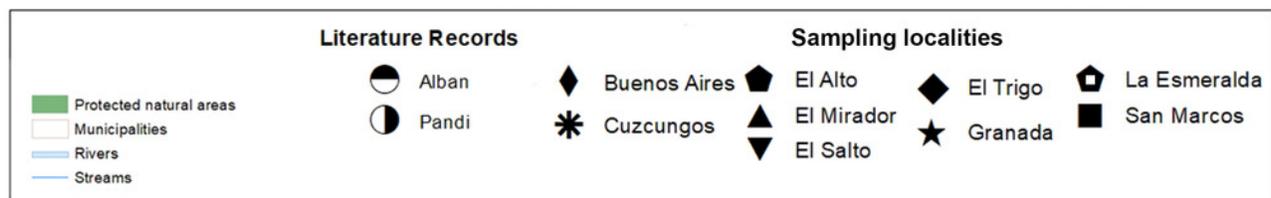
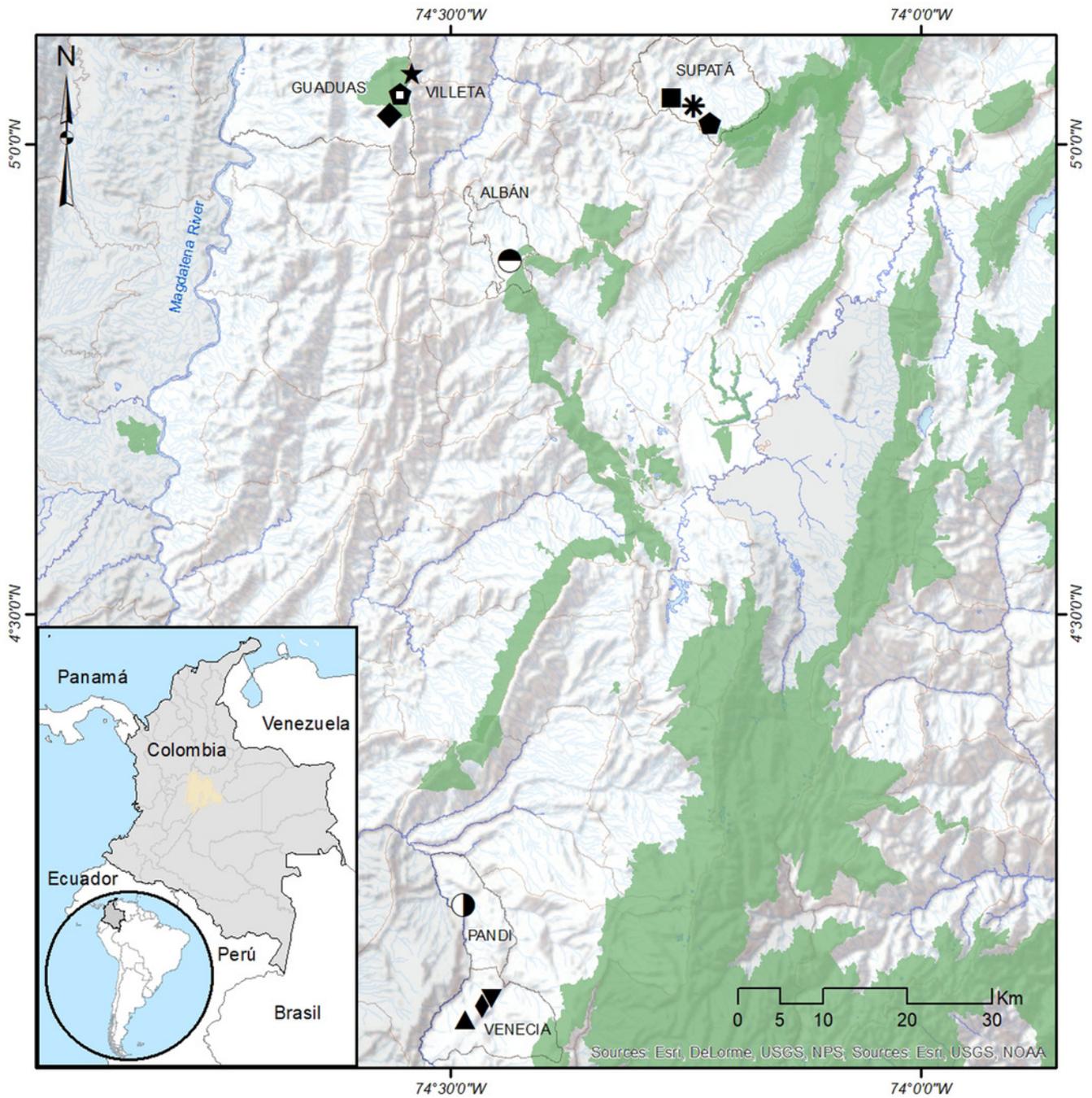
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659

# Figure 1

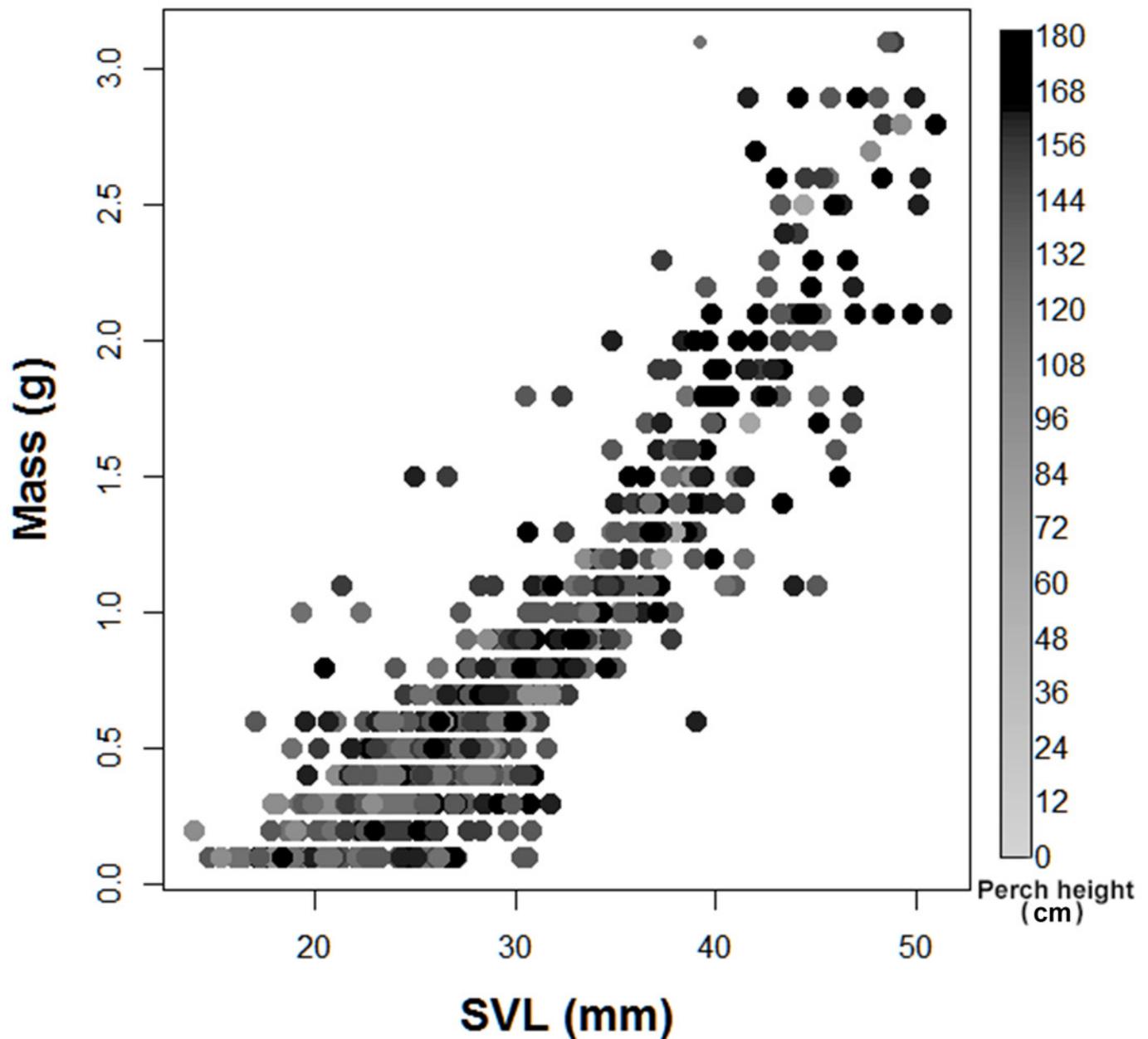
Distribution range of the poorly-known salamander *Bolitoglossa pandi*

New Northwestern records for the known distribution of *Bolitoglossa pandi* (IAvH-Am 10303-4, IAvH-Am 10305-8). Pentagon with a white square inside: La Esmeralda (municipality of Villeta), black star: Granada (municipality of Villeta), broad diamond: El Trigo (municipality of Guaduas). Sampled localities in the municipality of Supatá. Black square: San Marcos, asterisk: Cuzcungos Natural Reserve, solid pentagon: "El Alto" Vereda Monterey. Sampled localities in the municipality of Venecia. Narrow diamond: Buenos Aires, invert triangle: El Alto de la Chorrera, triangle: El Mirador.



## Figure 2

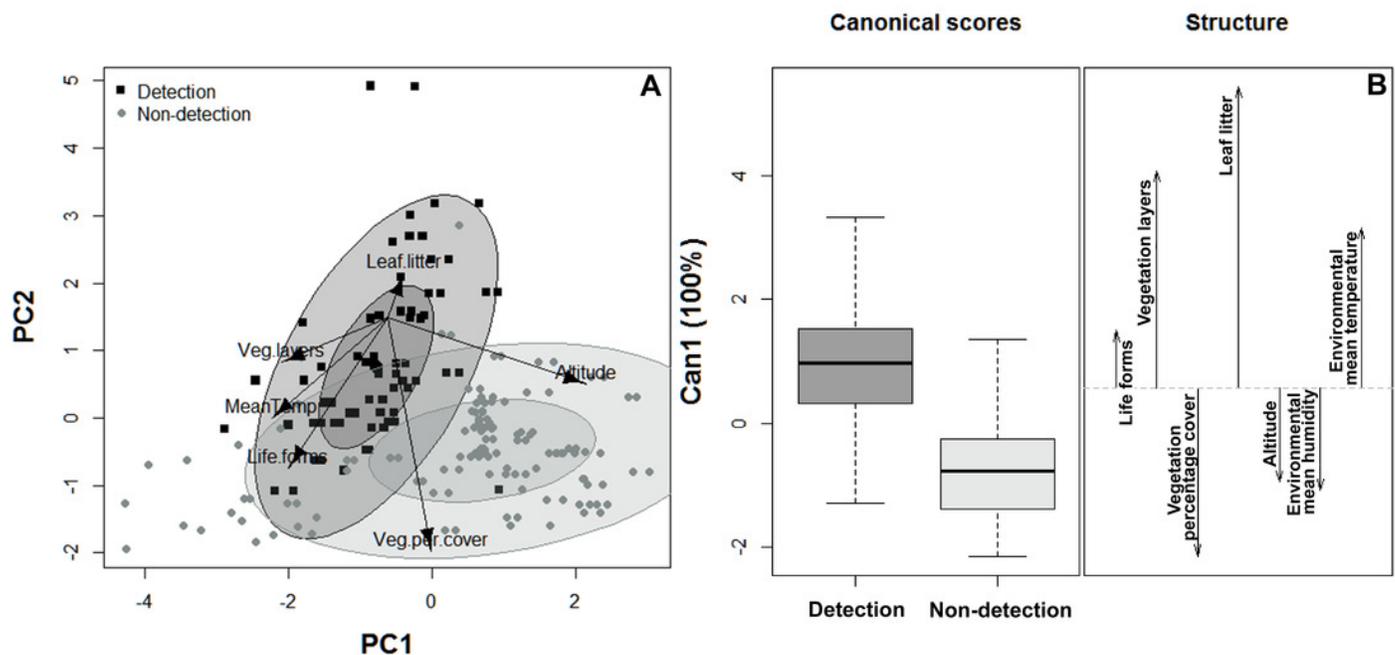
Scatterplot depicting the positive correlation between body length, weight and perch high



## Figure 3

### Main drivers of the *Bolitoglossa pandi* optimal habitat

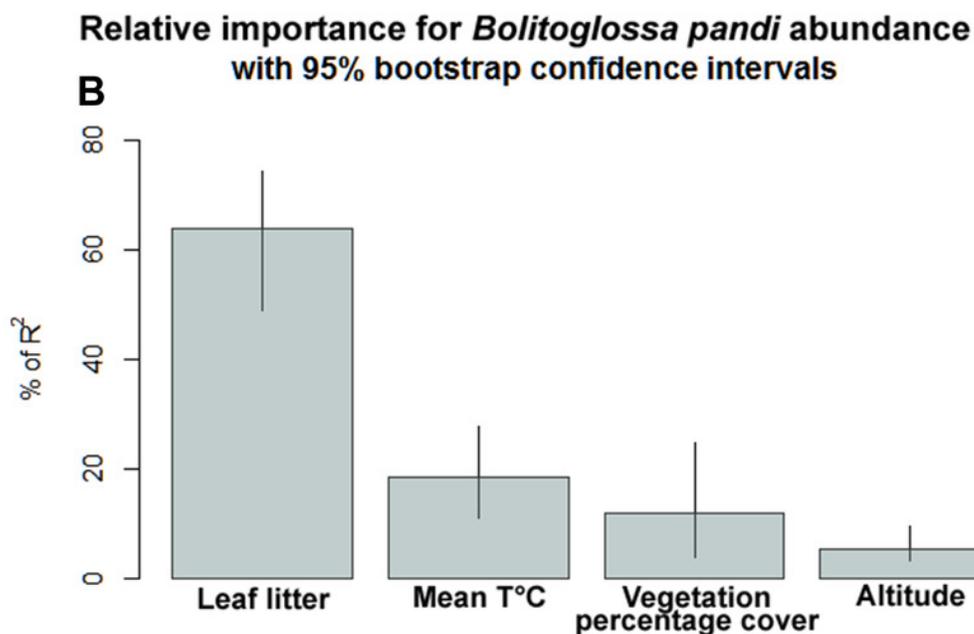
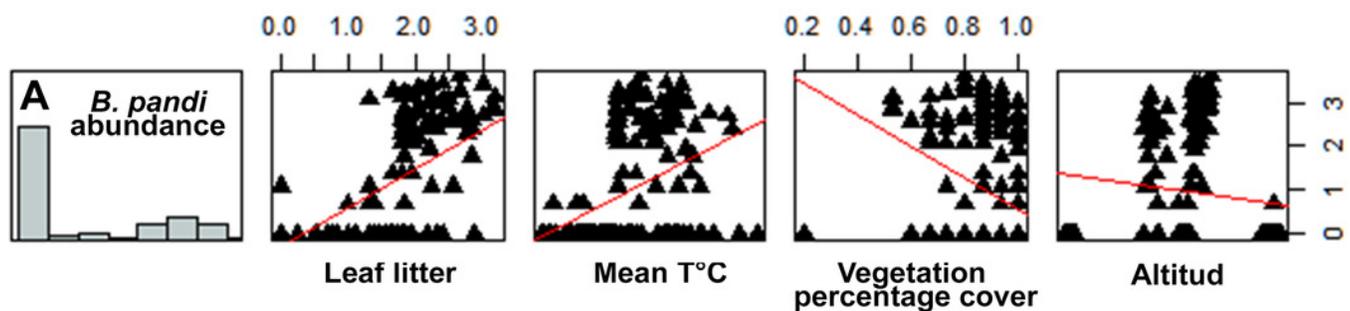
(A) Principal component analysis showing the observed variability in habitat structure attributes between quadrats with detection /non-detection of *Bolitoglossa pandi*. PC1 = First principal component (38.8%). PC2 = Second principal component (23.6%). Veg.layers= Vegetation layers. Veg.per.cover = Vegetation percentage cover. Mean Temp = Environmental mean temperature. Black squares = detection of *Bolitoglossa pandi*. Grey dots = Non-detection of *Bolitoglossa pandi*. The inner ellipse represents 0.5 of significance; the outer ellipse represents 0.95 of significance. (B) Quadratic discriminant analysis depicting the habitat variables that have the greatest discrimination capacity between quadrants where *Bolitoglossa pandi* was detected or non-detected. The length of the vector denotes the discrimination capacity of each habitat variable.



## Figure 4

The “best” multiple regression model that relates *Bolitoglossa pandi* abundance and habitat structure variables

(A) Plots depicting linear regressions between *B. pandi* and habitat structure variables assessed. (B) Relative contributions of the variables that compose the “best” regression model.

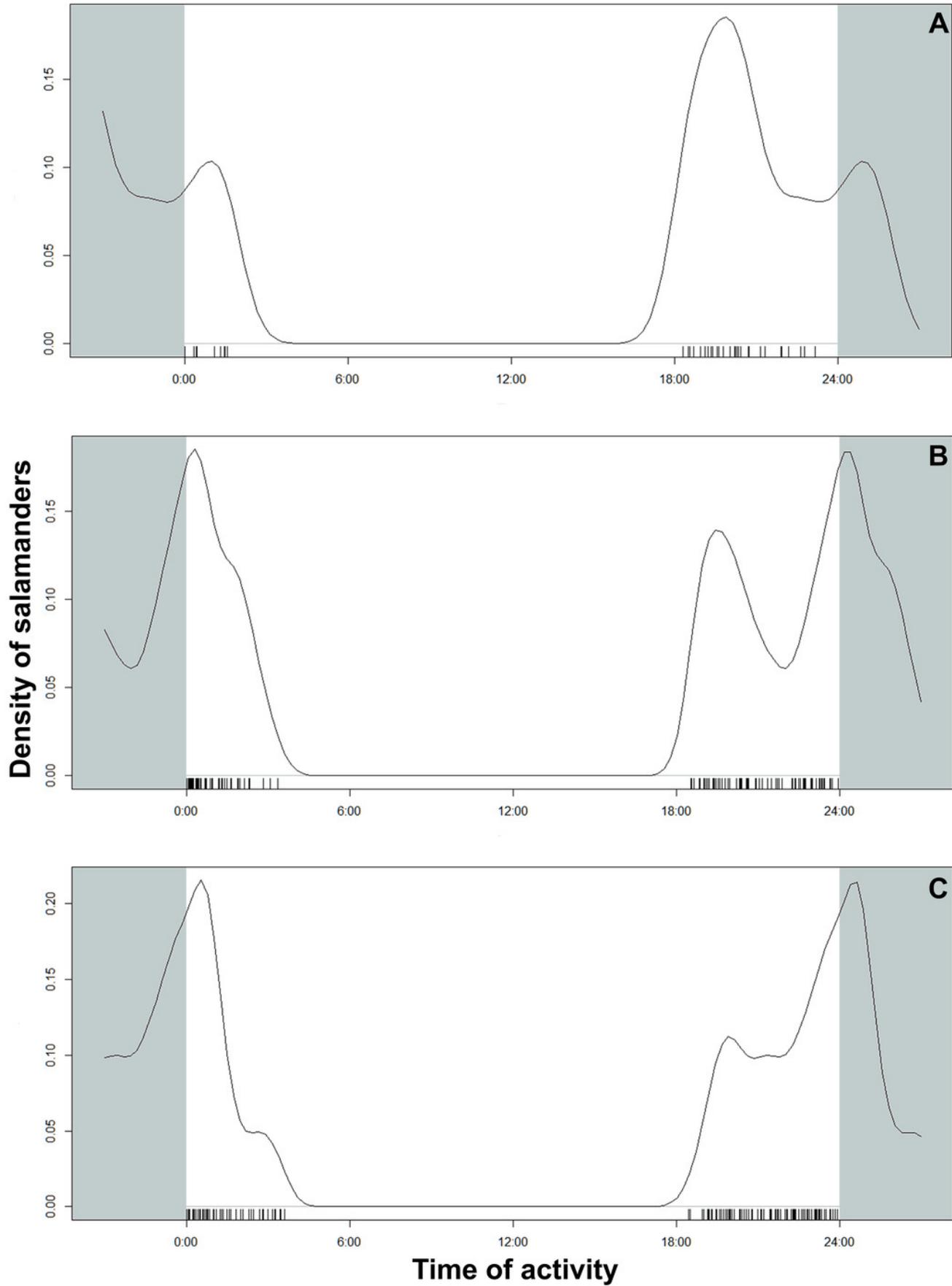


$R^2 = 60.32\%$ , metrics are normalized to sum 100%.

## Figure 5

Circular kernel density models showing overall daily activity patterns of *Bolitoglossa pandi* during each sampling occasion.

The small vertical bars on the bottom depicts the independent detections observed during day and night for five consecutive days by ten researchers. (A) First sampling occasion, September–October 2017. (B) Second sampling occasion, March 2018. (C) Third sampling occasion, July 2018.

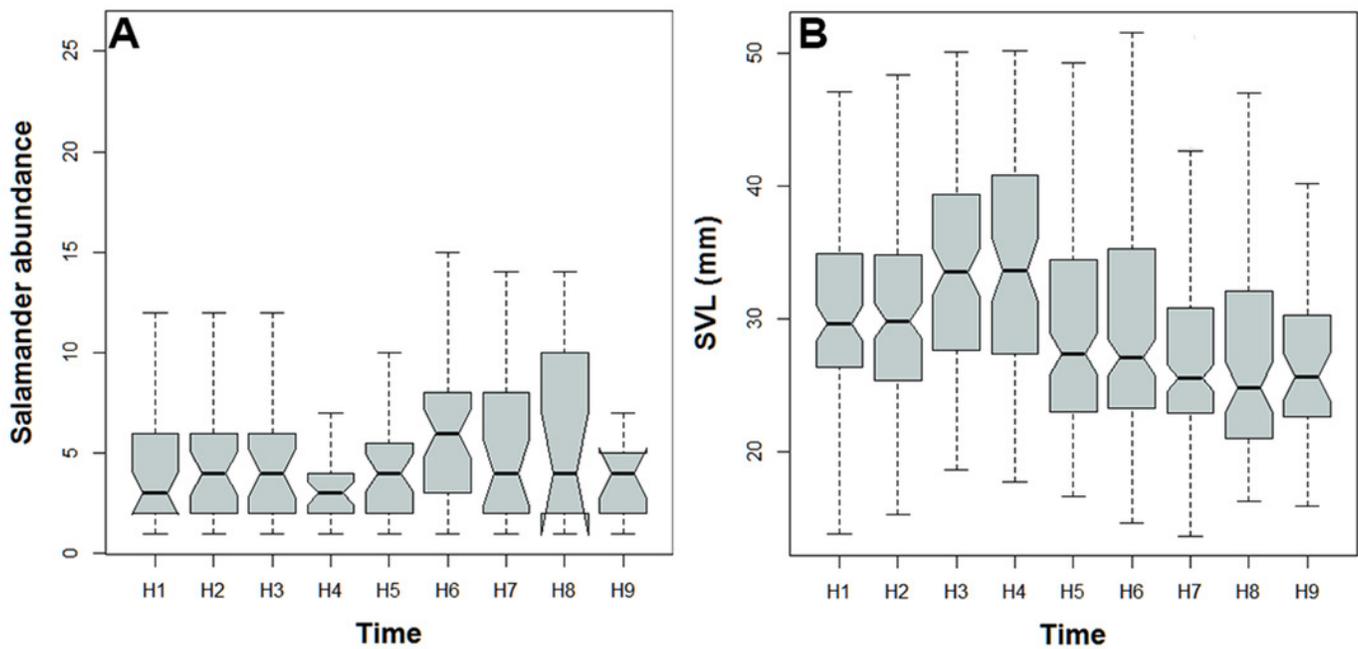


## Figure 6

Abundance and body size variability during the activity of *Bolitoglossa pandi*.

(A) Nested ANOVA depicting the abundance variability among the activity peaks observed.

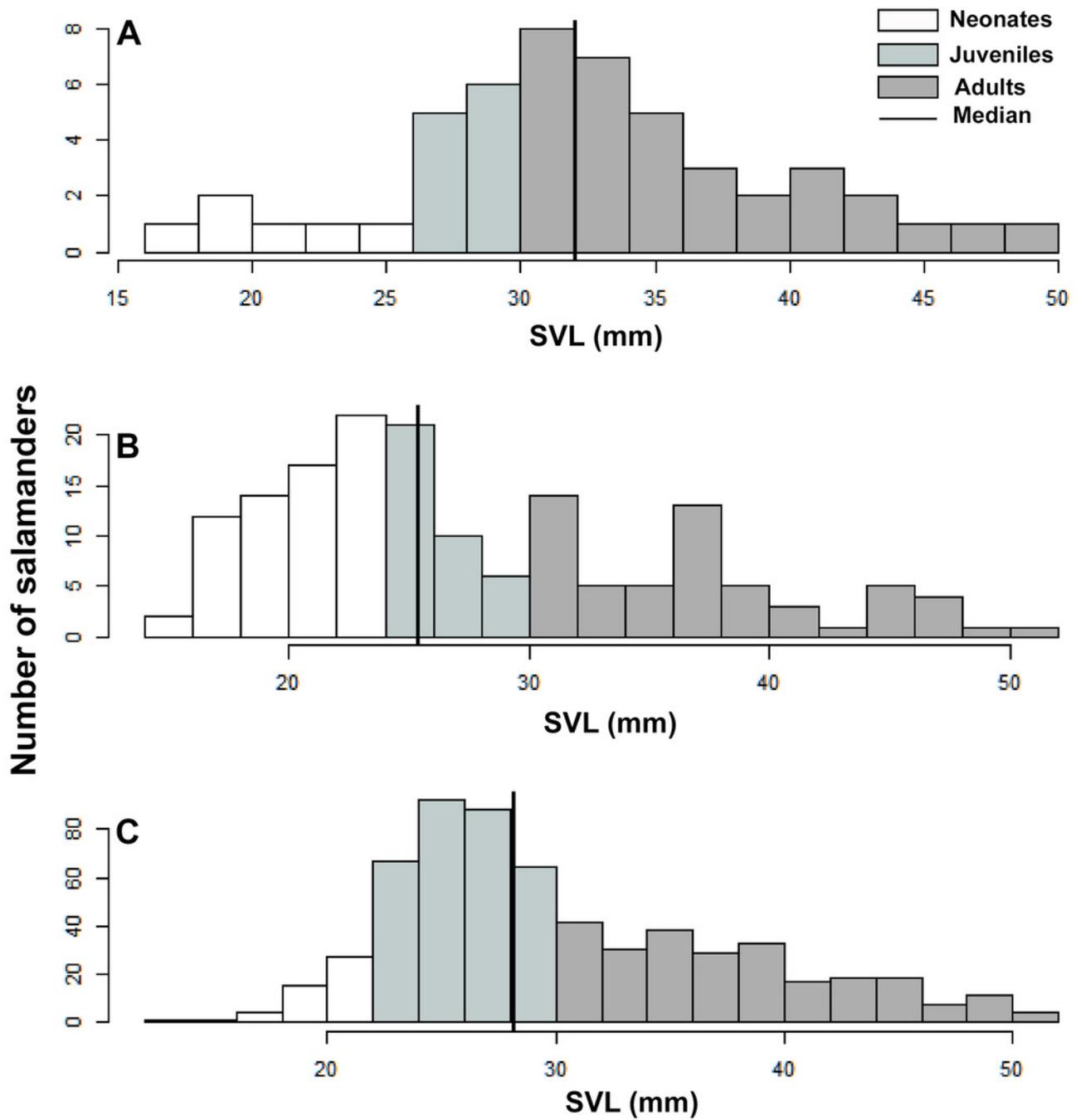
(B) Kruskal-Wallis test depicting niche partitioning between the body size classes



## Figure 7

Population structure of the *Bolitoglossa pandi* population at the Cuzcungos locality across the sampling period.

(A) First sampling occasion, September–October 2017. (B) Second sampling occasion, March 2018. (C) Third sampling occasion, July 2018.



## Figure 8

Color variability on ventral surfaces in preserved specimens of *Bolitoglossa pandi*.

(A) ventral surface of the head (ICN 45000; Pandi, Cundinamarca). (B) Lateral view of the lip of the edge showing the color pattern irregularly dark brown with irregular light spotting (ICN 58501, in life. Supatá, Cundinamarca). (C) ventral surfaces uniformly dark brown or dark grey with some irregular white circular spots (ICN 45000; Pandi, Cundinamarca. ICN 58502, Supatá, Cundinamarca).

**A**



**B**



**C**



**5 mm**

## Figure 9

Polychromatic variability observed in living specimens of *Bolitoglossa pandi* obtained in the municipalities of Guaduas, Supatá, and Villeta.

(A) uniform dorsal color pattern. (B) reddish-brown dorsal color that can have diffused grey or dark blotches scarcely distinguishable to the dorsolateral. (C-E) dorsal surfaces exhibiting ochre pattern, with some diffuse irregular dark brown, yellow or cream spots. (F-G) Diffuse dorsolateral band in the paravertebral surfaces covering almost the entire dorsal surface or including a dark brown inverted triangle shape in the interorbital region.



**Table 1** (on next page)

Sampling localities on the western slope of the Cordillera Oriental of Colombia.

VES: Visual encounter surveys (Crump and Scott 1994). QRS: Quadrats. N: Number of quadrants randomly selected.

| Municipalities<br>Vereda/locality                 | Latitude North   | Longitude West | Altitude (m)<br>a.s.l | Sampling<br>protocol | <i>B. pandi</i><br>specimens<br>observed | <i>B. pandi</i><br>specimens<br>collected |
|---|------------------|----------------|-----------------------|----------------------|--|---|
| <b>Supatá</b>                                     |                  |                |                       |                      |  |   |
| Vereda Las Lajas/Monterey                         | From 5° 2' 18.5" | 74° 14' 6.6"   | 2119–2315             | QRS (N = 45)         | 1  | 0   |
|   | To 5° 1' 59.9"   | 74° 14' 3.5"   |                       |                      |  |   |
| Vereda Las Lajas/<br>Cuzcungos Natural<br>Reserve | From 5° 2' 27.7" | 74° 14' 32.2"  | 1931–2016             | QRS (N = 43)         | 1390                                     | 12  |
|   | To 5° 2' 27.2"   | 74° 14' 27.9"  |                       |                      |  |   |
| Vereda San Marcos                                 | From 5° 2' 58.9" | 74° 15' 53.7"  | 1743–1773             | QRS (N = 28)         | 0  | 0   |
|   | To 5° 2' 58.3"   | 74° 15' 54.9"  |                       |                      |  |   |
| <b>Villeta</b>                                    |                  |                |                       |                      |  |   |
| Vereda La Esmeralda                               | 5° 3' 17.9"      | 74° 32' 49.1"  | 1996                  | VES                  | 15                                       | 6   |
| El Tigre site                                     | 5° 2' 9.2"       | 74° 33' 48.8"  | 1650                  | VES                  | 12                                       | 0   |
| <b>Guaduas</b>                                    |                  |                |                       |                      |  |   |
| Vereda Granada                                    | 5° 4' 1.49"      | 74° 32' 59.6   | 1816                  | VES                  | 7  | 0   |
| <b>Venecia</b>                                    |                  |                |                       |                      |  |   |
| Vereda Buenos Aires                               | From 4° 4' 57.9" | 74° 27' 57.7"  | 1809–2128             | QRS (N = 90)         | 0  | 0   |
|   | To 4° 4' 44.1"   | 74° 27' 33.3"  |                       |                      |  |   |
| Vereda El Diamante/El<br>salto de la Chorrera     | From 4° 5' 24.6" | 74° 27' 25.5"  | 1637–1735             | QRS (N = 36)         | 0  | 0   |
|   | To 4° 5' 21.0"   | 74° 27' 33.2"  |                       |                      |  |   |
| Vereda El Alto/ Road<br>Venecia- Cabrera          | From 4° 4' 5.2"  | 74° 29' 6.9"   | 2034–2114             | QRS (N = 54)         | 0  | 0   |
|   | To 4° 4' 11.1"   | 74° 28' 54.8"  |                       |                      |  |   |

**Table 2** (on next page)

Multiple correlation analysis.

Bold values denote statistical significance at the  $p < 0.05$  level.

| Habitat structure variable     | SVL (mm) | TL (mm) | Mass (g) | Perch height (mm) | Life forms | Percentage of vegetation cover | Vegetation layers | Leaf litter | Altitude (m) a.s.l |
|--------------------------------|----------|---------|----------|-------------------|------------|--------------------------------|-------------------|-------------|--------------------|
| SVL (mm)                       | *****    | 0.890   | 0.901    | 0.316             | 0.216      | 0.287                          | 0.006             | -0.306      | -0.155             |
| TL (mm)                        | <0.001   | *****   | 0.820    | 0.281             | 0.241      | 0.262                          | -0.037            | -0.306      | -0.126             |
| Mass (g)                       | <0.001   | <0.001  | *****    | 0.288             | 0.194      | 0.271                          | 0.018             | -0.266      | -0.134             |
| Perch height (mm)              | <0.001   | <0.001  | <0.001   | *****             | 0.084      | 0.141                          | 0.029             | -0.058      | 0.004              |
| Life forms                     | <0.001   | <0.001  | <0.001   | 0.026             | *****      | 0.490                          | -0.039            | -0.448      | -0.043             |
| Percentage of vegetation cover | <0.001   | <0.001  | <0.001   | <0.001            | <0.001     | *****                          | 0.063             | -0.396      | -0.152             |
| Vegetation layers              | 0.883    | 0.322   | 0.627    | 0.445             | 0.300      | 0.096                          | *****             | 0.203       | 0.133              |
| Leaf litter                    | <0.001   | <0.001  | <0.001   | 0.125             | <0.001     | <0.001                         | <0.001            | *****       | 0.092              |
| Altitude (m) a.s.l             | <0.001   | 0.001   | <0.001   | 0.906             | 0.260      | <0.001                         | <0.001            | 0.015       | *****              |

**Table 3** (on next page)

Principal component correlation matrix.

KMO: Kaiser-Meyer-Olkin test. BST: Kaiser-Meyer-Olkin test

| Habitat structure variables    | Life forms | Vegetation percentage cover | Vegetation layers | Leaf litter | Altitude (m) a.s.l | Environmental mean temp °C | KMO | BST     |
|--------------------------------|------------|-----------------------------|-------------------|-------------|--------------------|----------------------------|-----|---------|
| Life forms                     | ****       | 0.481                       | 0.263             | 0.002       | -0.384             | 0.368                      |     |         |
| Vegetation layers              | <0.001     | ****                        | -0.078            | 0.340       | -0.364             | 0.410                      |     |         |
| Percentage of vegetation cover | <0.001     | 0.285                       | ****              | -0.274      | 0.045              | -0.061                     |     |         |
| Leaf litter                    | 0.979      | <0.001                      | <0.001            | ****        | 0.149              | 0.060                      | 0.6 | <0.0001 |
| Altitude (m) a.s.l             | <0.001     | <0.001                      | 0.541             | 0.040       | ****               | -0.629                     |     |         |
| Environmental mean temp °C     | <0.001     | <0.001                      | 0.401             | 0.409       | <0.001             | ****                       |     |         |

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**Table 4**(on next page)

Multiple regression models.

Akaike Information Criterion (AIC) employed to select the 'best model' that relates the fluctuation in habitat structure variables and the abundance of *Bolitoglossa pandi*.

Dependent variable: Ln-transformed abundance of *B. pandi* (Lnn). Independent variables: Veg.per.cover = Vegetation percentage cover. Ln-transformedMean Temp = Environmental mean temperature. LnMeanH = Ln-transformed relative humidity. Lnlife forms = Ln-transformed number of vegetation life form Veg.layers = Vegetation layers. Lnleaf litter= leaf litter depth. Nor.test = Kolmogorov-Smirnov's test for normality, Hom.test = Breusch-Pagan test for homoscedasticity, and Aut.test = Durbin-Watson test for autocorrelation. Values shown are standard error (SE) and *t* test-value. Bold values denote statistical significance at the  $p < 0.05$  level.

| Multiple regressions models   | AIC    | $\Delta$ AIC | Norm. test | Hom. test | DW      |                    |
|---|--------|--------------|------------|-----------|---------|--------------------|
| Lnn~Lnleaf litter+LnMean temp+Veg.per.cover+Altitude  | -73.64 | 0.0          |            |           |         |                    |
| Lnn~Lnleaf litter+LnMean temp+Veg.per.cover+Altitude+Lnlife forms                           | -73.08 | -0.56        |            |           |         |                    |
| Lnn~Lnleaf litter+LnMean temp+Veg.per.cover+Altitude+LnMeanHr                               | -71.92 | -1.72        | 0.45       | 0.06      | 1.10    |                    |
| LnAbundance~Lnleaf litter+LnMeantemp+Veg.leyers+Veg.per.cover+Altitude+LnMeanH+Lnlife forms | -70.04 | -3.60        |            |           |         |                    |
| The "best" multiple regression model  |        |              | Estimate   | SE        | t-value | P(> t )            |
| Intercept   |        |              | 3.33       | 1.93      | 2.79    | <b>0.006</b>       |
| Ln leaf litter  |        |              | 0.88       | 0.071     | 12.34   | <<br><b>0.0001</b> |
| Ln Mean temp  |        |              | 0.69       | 0.167     | 4.12    | <<br><b>0.0001</b> |
| Vegetation percentage cover   |        |              | -1.87      | 0.50      | -3.73   | < <b>0.001</b>     |
| Altitude  |        |              | -0.001     | 0.0004    | -2.46   | <b>0.014</b>       |
| <b>F= 69.18, df= 4-182, P &lt; 0.0001</b>   |        |              |            |           |         |                    |

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**Table 5** (on next page)

Nested ANOVA results.

Values shown are mean standard error (SE) and *t* test-value. Bold values denote statistical significance at the  $p < 0.05$  level.

| Time interval         | Salamander observed                  | Estimate | SE     | t value | P(> t )      | Normality test | Levene test |
|-----------------------|--------------------------------------|----------|--------|---------|--------------|----------------|-------------|
| H1<br>(18:30 a 19:30) | Max= 16, Min= 1<br>□ = 4.16, N= 154  | -0.0257  | 0.0234 | -1.085  | 0.279        |                |             |
| H2<br>(19:31 a 20:30) | Max= 20, Min= 1<br>□ = 4.91, N= 172  | 0.010    | 0.022  | 0.461   | 0.645        |                |             |
| H3<br>(20:31 a 21:30) | Max= 12, Min= 1<br>□ = 4.73, N= 123  | 0.013    | 0.023  | 0.525   | 0.599        |                |             |
| H4<br>(21:31 a 22:30) | Max= 8, Min= 1<br>□ = 3.46, N= 97    | -0.047   | 0.023  | -2.037  | <b>0.042</b> |                |             |
| H5<br>(22:31 a 23:30) | Max= 16, Min= 1<br>□ = 4.393, N= 158 | -0.006   | 0.020  | -0.309  | 0.757        | P = 0.08       | P = 0.2     |
| H6<br>(23:31 a 00:30) | Max= 20, Min= 1<br>□ = 6.05, N= 260  | 0.049    | 0.020  | 2.493   | <b>0.013</b> |                |             |
| H7<br>(00:31 a 01:30) | Max= 18, Min= 1<br>□ = 4.73, N= 196  | 0.040    | 0.022  | 1.834   | 0.067        |                |             |
| H8<br>(01:31 a 02:30) | Max= 26, Min= 1<br>□ = 6.47, N= 116  | 0.003    | 0.030  | 0.100   | 0.920        |                |             |
| H9<br>(02:31 a 03:30) | Max= 26, Min= 1<br>□ = 4.43, N= 62   | 0.003    | 0.030  | 0.100   | 0.920        |                |             |

**Table 6** (on next page)

Wilcoxon test results.

N= number of salamanders observed. Bold values denote statistical significance at the  $p < 0.05$  level.

| <b>Sampling occasion</b> | <b>Occasion 1</b>             | <b>Occasion 2</b>              | <b>Median</b> | <b>Neonates</b> | <b>Juveniles</b> | <b>Adults</b> |
|--------------------------|-------------------------------|--------------------------------|---------------|-----------------|------------------|---------------|
| Occasion 1               | –                             | –                              | 32.05         | N = 4 (8%)      | N= 13 (26%)      | N = 33 (66%)  |
| Occasion 2               | W= 11825,<br>P < <b>0.001</b> | –                              | 25.40         | N = 55 (34%)    | N= 49 (30%)      | N = 57 (36%)  |
| Occasion 3               | W= 22590,<br>P = <b>0.006</b> | W = 84198,<br>P < <b>0.001</b> | 28.20         | N = 76 (13%)    | N= 277 (46%)     | N = 252 (42%) |

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