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 flagship species on the Colombian conservation agenda. This species is endemic to the Andean cloud forests of the western slope of the Cordillera Oriental of Colombia, presenting a narrow elevational range only within the Cundinamarca department. At night, *B. pandi* can be seen perching on the upper side of leaves at heights ranging from ground level to 2.5 m. During the day, it can be found under leaf litter or covered objects . Few studies have provided relevant information that can help the Colombian government to formulate lines of action for the conservation of this speices; consequently, its threat assessments so far 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 analyses were employed to explore the correlation between habitat structure and natural history traits, abundance,and detection / not detection of *B. pandi.* We evaluated the *B. pandi* activity pattern through kernel density curves for each sampling occasion and explored the variability of salamander abundance 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 was observed. Multivariate analyses indicated that leaf litter depth, mean temperature, percent vegetation cover, and altitude were the habitat variables that together explained 60.3% of the *B. pandi* abundance variability, as well as the main determinants of its optimal habitat. *B. pandi* exhibits an activity pattern characterized by two main activity peaks, in which niche time-partitioning was observed. Across the surveyed area, we found a healthy, stable, highly dense population of *B. pandi* (>1300 individuals), with high transition rate between body size classes and a constant recruitment rate.

Discussion. Given the high habitat specificity of *B. pandi,* the species is highly vulnerable to local

changes. Thus, we recommend that *B. pandi* be 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^2) and the ongoing threats from agriculture, cattle ranching, logging, and urban development, which continue to reduce its suitable habitat.

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2   **endangered salamander *Bolitoglossa pandi***
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27

Abstract

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29

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31   most threatened amphibians in South America, as well as a flagship species on the
32   Colombian conservation agenda. This species is endemic to the Andean cloud forests
33   of the western slope of the Cordillera Oriental of Colombia, presenting a narrow
34   elevational range only within the Cundinamarca department. At night, *B. pandi* can be
35   seen perching on the upper side of leaves at heights ranging from ground level to 2.5 m.
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39   so far have been based on very limited information.
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41   Cundinamarca, Colombia, using two approaches: visual encounter surveys (Guaduas
42   and Villeta) and the basic sampling protocol for single-species occupancy modeling
43   (Supatá and Venecia). A multivariate analyses were employed to explore the correlation
44   between habitat structure and natural history traits, abundance, and detection / not
45   detection of *B. pandi.* We evaluated the *B. pandi* activity pattern through kernel density
46   curves for each sampling occasion and explored the variability of salamander
47   abundance during their activity period by performing a nested ANOVA.
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49   the most northwestern records known for the distribution of this species. A significant
50   correlation between body length, body mass, and habitat structure was observed.
51   Multivariate analyses indicated that leaf litter depth, mean temperature, percent
52   vegetation cover, and altitude were the habitat variables that together explained 60.3%
53   of the *B. pandi* abundance variability, as well as the main determinants of its optimal
54   habitat. *B. pandi* exhibits an activity pattern characterized by two main activity peaks, in
55   which niche time-partitioning was observed. Across the surveyed area, we found a
56   healthy, stable, highly dense population of *B. pandi* (>1300 individuals), with high
57   transition rate between body size classes and a constant recruitment rate.
58   **Discussion.** Given the high habitat specificity of *B. pandi,* the species is highly
59   vulnerable to local changes. Thus, we recommend that *B. pandi* be retained as
60   Endangered (EN) on the IUCN Red List, based on the IUCN Criterion B, given its
61   restricted extent of occurrence (ca. 2,500 km^2) and the ongoing threats from agriculture,
62   cattle ranching, logging, and urban development, which continue to reduce its suitable
63   habitat.

Introduction

Colombia is home to more than 850 species of amphibians, being mainly concentrated in the Andean Forest (Acosta-Galvis, 2020). Geographic distributions of fauna in this biome are often restricted and endemism is common (Lynch & Suárez-Mayorga, 2002). Andean forests are among the most threatened habitats in the country due to the drastic transformation of native vegetation by urban growth, mining, agriculture, and cattle ranching (Etter et al., 2018). As a consequence and given that most threatened Colombian amphibian species are concentrated between 1800-3600 m a.s.l., habitat loss is the main threat shared by most species.

Among threatened Colombian amphibians, salamanders of the genus *Bolitoglossa* are one of the flagship amphibian groups on the Colombian conservation agenda. This genus is the most diverse and geographically widespread lineage of plethodontid salamanders inhabiting the Western Hemisphere. Currently, *Bolitoglossa* comprises 134 species, 24 of which reside in Colombia across several types of tropical habitats (Wake, 2017; Acosta-Galvis, 2020; Frost. 2020). Many species of *Bolitoglossa* exhibit restricted geographic ranges [e.g. *Bolitoglossa capitana* (Brame & Wake, 1963), *B. hypacra* (Brame & Wake, 1962), *B. hiemalis* (Lynch, 2001), among others]. However, the high level of morphological crypsis and an incomplete understanding of the morphological variability among Andean *Bolitoglossa* species make suitable determinations regarding their distributional ranges hard to achieve (Acosta-Galvis & Gutiérrez-Lamus, 2012).

The ecology and life history of South American *Bolitoglossa* salamanders are poorly understood compared to their Central and North American congeners; less than 12% of *Bolitoglossa* species have been investigated with published information on their diet, reproduction, foraging activities, thermal ecology, demography, ecological interactions, microhabitat use, or habitat preferences (Houck, 1977; Jimenez, 1994; Bruce, 1997; Salgado-Aráuz, 2005; Anderson & Mathis, 2006; Cadenas et al., 2009; Ortega, Monares-Riaño & RamÍrez-Pinilla, 2009; Neckel-Oliveira et al., 2011; del Río-Gracía, Serrano-Cardozo & Ramírez-Pinilla, 2014; Cruz, Galindo & Bernal, 2016). Given this dearth of information, the conservation status of many of these species is Data Deficient (DD) or has been based on limited information.

According to the IUCN red list of threatened species, *Bolitoglossa pandi* (Brame & Wake, 1963) is Endangered B1ab (iii), based on its restricted geographical range. It was originally described based on a single specimen (holotype ZSZMH 2858, an adult female with SVL 50.4 mm, collected in 1913 by Wilhelm Frietsche) from the municipality of Pandi, Cundinamarca (exact locality within the municipality is unknown), in the cloud forests on the western slope of the Cordillera Oriental of Colombia. Subsequently, Hanken and Wake (1982) reported a second specimen 75km north airline from the type locality, in a bromeliad inside the primary cloud forest, near the municipality of Albán (Cundinamarca), at 2400 m a.s.l.

104   Acosta-Galvis and Rueda-Almonacid (2004) reported a third specimen (an adult
105   female with snout-vent length (SVL) 44.7 mm, collected by Franz Kaston, ICN 45500)
106   near the municipality of Pandi and described the associated habitat as relict wet areas
107   covered by lush trees and shrubs. Acosta-Galvis and Gutiérrez-Lamus, (2012) included
108   a new record for the Supatá region (adult male with SVL 37.63 mm, MUJ 7921)
109   representing the northernmost record of the species. Hence, the known distribution of *B.*
110   *pandi* includes four localities from the sub-Andean forests between 1300-2400 m a.s.l.,
111   throughout the western slopes of the Cordillera Oriental. Since its first extinction risk
112   assessment was performed 14 years ago, few studies have provided relevant
113   information for the formulation of strategies or an action plan for its conservation (del
114   Río-Gracía, Serrano-Cardozo, & Ramírez-Pinilla, 2014).
115   The aims in this study are: (1) to describe the geographic range extension of *B.*
116   *pandi* along the western slopes of the Cordillera Oriental; (2) to explore the relationship
117   between habitat structure and natural history traits of this species; (3) to describe its
118   activity pattern and population size-structure; (4) to expand the knowledge of the
119   variability of certain morphological characters and life-history traits of this poorly known
120   species; (5) and to provide a conservation status reassessment of *B. pandi*.
121
122   **Materials & Methods**
123   **Ethics statement**
124   Sex was not determined on living salamanders due to the high risk of injury to the
125   animal. Fieldwork was done under the scientific research non-commercial purpose
126   permit of collection of wild specimens of biological diversity issued by the National
127   University of Colombia (Research Project 38615), and the Colombian National
128   Environmental Licensing Authority (ANLA) by resolution No. 0255 of 14 March 2014.
129   This study was conducted following the Colombian animal welfare law and the collection
130   of wild specimens of the biological diversity acts (Ley 1774, 2016; Decreto 1376, 2013),
131   as well as considering the Universal Declaration on Animal Welfare (UDAW) endorsed
132   by Colombia in 2007.
133
134   **Study area**
135   We searched for salamanders at nine localities in four municipalities of Cundinamarca
136   located on the western slope of the Cordillera Oriental of Colombia: Guaduas, Supatá,
137   Venecia and Villeta (Fig. 1). Searches at each locality were carried out within an
138   altitudinal gradient ranging from 1638 to 2315 m a.s.l (Table 1). The sampled area
139   includes sub-Andean and Andean forests, as well as areas transformed by urban
140   growth, agriculture, and cattle ranching. The sampling area is characterized by a
141   bimodal climate [high dry season (from mid-December to mid-March); high rainy season
142   (from mid-March through June); low dry season (from July to mid-September), low rainy
143   season (from mid-September to mid-December)]. We monitored the environmental

144   temperature (ET) and relative humidity (RH) at sampling sites using Ebro® thermo-
145   hygrometers (model EBI 20-TH1).
146
147   **Sampling and data collection**
148   We conducted surveys for salamanders using two approaches. First, during the rainy
149   season in April–May 2013, we performed visual encounter surveys (VES; Crump &
150   Scott, 1994) in three localities associated with cloud forests throughout an altitudinal
151   gradient (1648-2002 m a.s.l) in the municipalities of Guaduas and Villeta (Table 1). Two
152   researchers surveyed day and night for five days, for a total of 100 h of sampling effort.
153   Second, we employed the basic sampling protocol described by Mackenzie et al.,
154   (2003) for single-species occupancy modeling in six localities, three in the municipality
155   of Supatá and three in Venecia. We randomly selected a total of 296 plots (5 m x 5 m),
156   which were located throughout an altitudinal gradient at each sampling locality (1600–
157   2300 m a.s.l), grouping the following vegetation covers: Andean forest fragments,
158   restored Andean riparian forest, pastures and roadsides (Table 1). During three
159   sampling occasions (September–October 2017, March 2018, and July 2018), each plot
160   was surveyed day and night for five consecutive days by ten researchers, resulting in a
161   total of 2100 h of sampling effort. During each survey, the detection / not detection of *B.*
162   *pandi* specimens were recorded. When a salamander was present, we measured its
163   perch height using a measuring tape (±0.1 cm). Once salamanders were caught, we
164   recorded their weight with a Pesola® dynamometer of 50g (±0.1 g) and took
165   photographs to measure their body size [SVL = snout-vent length (mm), TL = Tail length
166   (mm)] using the software Image–J v. 1.52 (Bourne, 2010). The sex of living
167   salamanders was not determined due to the high risk of injury to the animals. All
168   specimens of *B. pandi* were subsequently released near the quadrat where they were
169   sighted.
170   Based on 18 specimens collected, euthanized using 2% lidocaine, and fixed in
171   10% formalin (Chen & Combs, 2001), we described the morphological variability of *B.*
172   *pandi*. We made a small incision in the groin region to identify their sex and sexual
173   maturity through macroscopic observation of the gonads. All the morphological
174   terminology employed follows several contributions (Brame & Wake, 1962, Brame &
175   Wake, 1963; Acosta-Galvis & Restrepo, 2001; Lynch, 2001; Acosta-Galvis & Hoyos,
176   2006; Acosta-Galvis & Gutierrez-Lamus, 2012; Brcko, Hoogmoed & Neckel-Oliveira,
177   2013; Bingham et al., 2018). All specimens were deposited in the amphibian collection
178   at Instituto de Investigación de Recursos Biológicos Alexander von Humboldt, (IAvH-
179   Am), as well as in the amphibian collection at the Instituto de Ciencias Naturales de la
180   Universidad Nacional de Colombia (ICN).
181
182   **Habitat structure data collection**

183   We used the Point Intercept Method described by Elzinga, Salzer, & Willoughby (1998)
184   to estimate the percent vegetation cover. We grouped plants into eight life forms:
185   graminoids, forbs, palm trees, mosses, lichens, vines, shrubs, and trees. We divided
186   vegetation into layers: 0–0.1 m, 0.1–1 m, 1–1.5 m, 1.5–3 m, 3–5 m, and 5–12 m. We
187   also considered ground characteristics such as leaf litter, bare soil and bare rocks. We
188   estimated the vegetation cover of each of the plots where salamanders were surveyed,
189   employing a set of 15 intercept points distributed in three parallel lines of five points
190   separated by one meter of distance. At each point, we used a sampling bar of 1.5 m to
191   register the contact of the life forms of each vegetation layer below 1.5 m. This provided
192   us with a 6.67% cover resolution by layer. We assessed the percentage cover of the
193   vegetation layers above 1.5 m (mostly trees) using five intercept points: the corners of
194   the quadrant and the central point of the third line. In this way, we reached a 20% cover
195   resolution for upper layers.
196
197   **Statistical analysis**
198   We evaluated the association between habitat structure and the natural history traits of
199   *B. pandi* by multiple correlation analysis, with P < 0.05 as the significance level. The
200   following variables were considered: SVL (mm), weight (g), perch height (mm), leaf litter
201   depth (mm), vegetation layers, vegetation life form, percent vegetation cover and
202   elevation (meters above sea level). Using the habitat variables, we performed a
203   principal component analysis (PCA) to explore which of these variables presented
204   greater variability between plots with regards the detection / not detection of *B. pandi*
205   and, therefore, which of these could explain the observed differences between plots.
206   The variable suitability for PCA analysis was tested performing a Kaiser-Meyer-Olkin
207   test (KMO > 0.5, P < 0.05). Afterward, a quadratic discriminant analysis was performed
208   to determine which of the habitat variables had the greatest discrimination capacity
209   between plots where *B. pandi* was detected or not detected.
210   We assessed the variability in salamander abundance observed in the Supatá
211   population through multiple regression analysis. First, we considered the following
212   habitat structure variables at each sampling plot: leaf litter depth (mm), vegetation
213   layers, vegetation life forms, percent vegetation cover, elevation (meters above sea
214   level), temperature (°C) and environment relative humidity. All variables were Ln–
215   transformed prior to perform the statistical analysis.
216   Second, we evaluated assumptions of normality, autocorrelation, and
217   homoscedasticity using Kolmogorov–Smirnov's test, Durbin–Watson test and Breusch–
218   Pagan test, respectively. Given that the p–value of the Durbin–Watson test can easily
219   be less than 0.05 when data size is very large, we used the Durbin–Watson statistic test
220   (DW) as an autocorrelation criterion. According to Durbin & Waston (1950), a DW of
221   less than 1 indicates a strong positive autocorrelation, a DW greater than 4 indicates a

222 strong negative autocorrelation, values between 1 and 3 suggest a moderate
223 autocorrelation, and a value close to 2 means that there is no autocorrelation.
224
225 Third, we tested for multicollinearity between the variables using the variance inflation
226 factor (VIF) with a threshold of 10. Fourth, we selected the "best" regression model
227 employing the Akaike Information Criterion (AIC; Akaike, 1973), considering that models
228 with ∆AIC values of less than two are equally plausible (White & Burnham, 1999).
229 Finally, we used the hierarchical partitioning method to evaluate the contribution of all
230 the independent variables of the regression model (Chevan & Sutherland, 1991).
231

232 **Activity pattern and population size-structure**
233 We only assessed the activity pattern and the population size-structure of the *B. pandi*
234 population at the Cuzcungos locality given the remarkable abundance observed (Table
235 1). We estimated the activity pattern through Kernel Density Curves for each sampling
236 occasion and explored the salamander abundance variability during their activity period
237 by performing a nested ANOVA. Hence, the activity period of *B. pandi* was divided into
238 nine time-intervals (from H1 = 18:30–19:30, H2 = 19:31–20:30, H3 =… until H9 =
239 02:31–03:30), and each salamander sighting was allocated into its respective interval.
240 The sampling occasion was used as the primary factor, and the time intervals as the
241 secondary factor nested in the primary factor. We evaluated assumptions of normality
242 and homogeneity of variances using a Shapiro-Wilk test and Levene test, respectively.
243 Additionally, we analyzed the variability in observed body size over the nine time-
244 intervals through a non-parametric ANOVA using a Kruskal-Wallis test (KW) as a
245 measure of the central tendency of the samples (Sokal & Rohlf, 1981).
246   We used SVL as a descriptive variable of the population structure of *B. pandi*.
247 We compared the variability in population structure among sampling occasions through
248 a Wilcoxon test, with the null hypothesis being that population median structure was the
249 same across all sampling occasions. According to the categories proposed by Acosta-
250 Galvis & Gutierrez-Lamus (2012) and del Río-Gracía, Serrano-Cardozo & Ramírez-
251 Pinilla (2014), as well as the reproductive stage of the collected salamanders, the
252 population was divided into body size classes as follows: neonates (≤ 23 mm), juveniles
253 (24–30 mm), and adults (≥ 30 mm).
254   All statistical analyses were performed using the software Rwizard 4.3 (Guisande
255 et al., 2014) and the following R packages: car (Fox & Weisberg, 2019), hier.part (Nally
256 & Walsh, 2004), lawstat (Hui, Gel, & Gastwirth, 2008), nortest (Gross & Ligges, 2015),
257 overlap (Ridout & Linkie, 2009) stat (Bolar, 2019) and usdm (Naimi et al., 2014).
258
259 **Results**
260 **Geographic distribution**

261 We found a total of 34 *B. pandi* individuals at 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. Additionally, two salamanders were found in ecotonal
269 areas 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 morphological
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 were correlated with habitat structure variables,(19.3%, 16.8% and
279 18.6% respectively).
280   Similarly, the habitat structure variables were significantly associated with the
281 detection or not detection of *B. pandi* throughout the sampling plots. The first two
282 components of the PCA analysis retrieved 62.4% of the variability observed. The habitat
283 structure variability was clustered in two groups associated with the detection or not
284 detection of *B. pandi* (Fig. 3A). These groups were moderately overlapped in
285 multivariate space, but they were differentiated by elevation, leaf litter depth, and
286 percent vegetation cover. The presence of *B. pandi* was positively correlated with highly
287 structured plots and with deep leaf litter (Table 3). The quadratic discriminant analysis
288 confirms that the detection or not detection of *B. pandi* depends on habitat variables.
289 The cross-validation percentage was 92.6%, indicating that the quadrants in which *B.*
290 *pandi* was detected can be clearly distinguished by habitat variables such as vegetation
291 layers, leaf litter depth and mean environmental temperature (Fig. 3B).
292   Results of the multiple regression analysis showed that leaf litter depth, mean
293 environmental temperature, percent vegetation cover, and elevation were the variables
294 that contributed the most to explaining the observed variability in the abundance of *B.*
295 *pandi* (R^2 = 60.3%, P< 0.001). These habitat variables also composed the "best" fitted
296 regression model (Table 4). However, this model showed a moderate autocorrelation
297 (DW = 1.10), which means that the variance explained by the habitat variables may be
298 close to 60.3%.
299
300 **Activity pattern**

301   *Bolitoglossa pandi* is completely nocturnal; its activity period extends throughout the
302   night, from 18:30 h until 05:00 h. The environmental temperature recorded during this
303   activity period ranged from 12.6–25.6 °C ($\square$ =15.3), and relative humidity ranged from
304   73.8–98.8 ($\square$ = 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 it was moderately and negatively
308   correlated with relative humidity (R_{RH} = -0.174; P = 0.017). Across sampling occasions,
309   we observed two main activity peaks, the first from 20:30 h to 21:30 h, and the second
310   from 23:30 h to 00:30 h (Fig. 4). Results of the nested ANOVA indicated that
311   salamander abundance between these peaks was significantly different ($F_{9\text{-}259}$ = 4.57, P
312   < 0.001; Table 5), being more abundant in the second activity peak during the second
313   and third sampling occasions, with the opposite pattern during the first sampling
314   occasion (Fig. 5A).
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. 5B), suggesting niche time-
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 all 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 size-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/m². The
327   population size-structure showed significant differences between sampling occasions,
328   suggesting a high transition 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. 6). Neonates were observed across all sampling occasions, indicating
332   constant recruitment. During the second sampling occasion, a bias in the SVL median
333   value towards smaller individuals was observed, suggesting that the major recruitment
334   peak occurs during March (Fig 6B). Adults were conspicuous throughout all sampling
335   occasions, especially so in the first sampling occasion.
336
337   **Morphological variability**
338   *Bolitoglossa pandi* is a tiny species, with SVL = 13.4–51 mm (N= 1034), 34.4–48mm
339   adult males (n =7), and SVL 36.6–51 mm adult females (N = 8). Extensively webbed
340   hands and feet with third toes and triangular fingers; ventral surfaces of digit tips without

341 terminal flattened tubercles; snout short and rounded in the lateral profile; head length
342 4.9–10.1 mm; head width 5.1–7.9 (N =17); snout rounded in dorsal view, irregular white
343 spots, cream-colored nasolabial grooves, and edges of the lips irregularly dark brown
344 with irregular light spotting (Fig. 7A–B); protruding eyes on dorsal view, brown iris with
345 black reticules (Fig. 7B); well-defined post-cephalic constriction; ventral surfaces
346 (preserved) brown or dark grey with numerous tiny cream guanophores (Fig. 7C-
347 D);inverted bracket shaped scapular spots (Fig. 7J); males have white testes.
348   *Bolitoglossa pandi* can be distinguished from its Colombian congeners by the
349 extensive webbing of its hands and feet (versus webbing of hands and feet reduced or
350 absent in *B. adspersa, B. hiemalis, B. hypacra, B. palmata, B. ramosi, B. savagei, B.*
351 *tamaense, B. tatamae, B. walkeri* and *B. vallecula*). Furthermore, it can be distinguished
352 from species with extensive webbing (such as *Bolitoglossa lozanoi* and *B. nicefori*) for
353 having more protruding eyes, and a longer and triangular third finger. *B. pandi* also
354 differs from *B. biseriata* and *B. silverstonei* by having a dark brown or dark grey ventral
355 surface with irregular white dots (versus cream ventral surface with brown suffusions
356 and pits in *B. biseriata* and *B. silverstonei*), brown-reddish iris with black reticles (versus
357 golden with brown pits in *B. biseriata* and *B. silverstonei*), and white testes in adult
358 males (versus black mesorchium in testes of adult males of *B. biseriata*). It also differs
359 from *B. medemi* by the absence of digital depressions in the digital tips on the fingers
360 and toes (present in *B. medemi*). *B. pandi* also presents an upper lip with irregular light
361 spotting (versus uniform in *B. guaneae*). *B. pandi* can be differentiated from *B.*
362 *altamazonica* and *B. leandrae* by having extensive interdigital webbing with a longer
363 and triangular third finger (complete webbing and tips rounded in *B. altamazonica* and
364 *B. leandrae*). *B. pandi* is morphologically very similar to *B. phalarosoma,* but it differs by
365 having a dark brown or dark grey ventral surface with diffuse white pits and some white
366 blotches (versus usually light brown ventral surfaces, with some irregular cream spots in
367 *B. phalarosoma*), plantar and palmar regions in ventral dark brown or dark grey view
368 (versus cream in *B. phalarosoma*). *B. pandi* also differs from *B. capitana* by having
369 smaller adult size with a longer and triangular third finger (versus rounded third finger in
370 *B. capitana*) and shorter head.
371

Color variability
373 *Bolitoglossa pandi* exhibited a wide variation in color, ranging from a uniform dorsal
374 color pattern of different shades of light to dark brown(Fig. 7E); one reddish-brown
375 pattern that can have diffuse grey or scarcely distinguishable dark blotches that extend
376 to the dorsolateral region (Fig. 7F), or an ochre pattern, with some diffuse irregular dark
377 brown, yellow or cream spots (Fig. 7G–I). Some specimens exhibit a very diffuse band
378 in the paravertebral region covering almost the entire dorsal surface or including a dark
379 brown inverted triangle shape in the interorbital region (Fig. 7J–K). The caudal region is
380 highly variable, ranging from uniform reddish-brown or completely ochre segments, with

381   very small scattered white pits (Fig. 7D–F) to irregular cream, yellow or orange patches
382   (Fig. 7I), and very small or irregular longitudinal black spots (Fig. 7I–K). The distal end
383   of the tail becomes uniform light brown and has cream blotches towards the proximal
384   region in some individuals (Fig. 7H). The cephalic region is dark brown with some white
385   spots with irregular pits up to the supralabial region in lateral view (Figure 7B); a loreal
386   region with ochre patches and light brown iris with black reticles; nasolabial projections
387   are cream; the ventrolateral surface is dark brown. Ventral surfaces are dark brown with
388   some cream and white blotches with scattered pits; the mental and gular surfaces are
389   uniformly dark brown or dark grey with some irregular white circular spots bordering the
390   maxillary region. In adult males the submental gland is light brown; the palmar and
391   plantar surfaces are always dark brown or dark grey.
392

393   **Discussion**
394   The new localities added to the known range of *Bolitoglossa pandi* and the detailed
395   examination of the Supatá population have allowed not only the expansion of
396   knowledge about the distribution and natural history traits of this species but also the
397   reassessment of its conservation status and the validation of its characters for
398   taxonomic identification vis-a-vis its congeners. Despite enormous efforts done by
399   herpetologists who sought to characterize and describe the Andean amphibians in the
400   second half of the 20th century and the beginning of the 21st (Ruiz-Carranza, Ardila-
401   Robayo & Lynch, 1996; Lynch, Ruiz-Carranza & Ardila-Robayo,1997; Lynch 1999;
402   Arroyo, Jerez, & Ramires-Pinilla, 2003; Acosta-Galvis, 2015; Acosta-Galvis et al.,
403   2020), large areas of the western slope of the Cordillera Oriental of Colombia still lack
404   intensive sampling. Therefore, the known distribution of *B. pandi* as well as other
405   Andean amphibians is still fragmentary. We expect local herpetologists interested in
406   Andean herpetofauna to be motivated to invest important fieldwork efforts to fill the gaps
407   over the known distribution records of *B. pandi* pointed out in this study.
408   Since Brame & Wake's (1963) original description of *B. pandi,* several characters
409   such as dorsal surfaces color (Figure 7) have had conflicting or ambiguous diagnostic
410   characters (Acosta-Galvis and Gutiérrez-Lamus, 2012). However, our findings
411   increased the understanding of the morphological variability of *B. pandi*. Based on living
412   specimens obtained in the municipalities of Guaduas, Supatá, and Villeta, we add new
413   data to the original description (Brame and Wake, 1963) because these had not been
414   described in life (and the holotype is poorly preserved), and evidence of a broad
415   intrapopulation color variation was not available due to restricted sampling. Our findings
416   allow clarification of taxonomical misidentifications in the literature. For example,
417   Acosta-Galvis & Rueda-Almonacid (2004), during the first threat assessment of *B pandi,*
418   included erroneously a picture of *B. walkeri* as the species' portrait.
419   Neotropical salamanders have been considered amphibians with secretive habits
420   and low encounter rate into the Andean Forest (Brame & Wake, 1963; Gibbons, 1983;

421   Barrio-Amoros & Fuentes, 1999; Acosta-Galvis & Gutiérrez-Lamus, 2012).
422   Nevertheless, our results challenge this general assumption and provide support for
423   previous studies which related specific environmental conditions with high abundance
424   and density of Andean salamander populations (Houck, 1977; Jimenez, 1994; Salgado-
425   Aráuz, 2005; Cadenas et al., 2009; Ortega, Monares-Riaño & Ramírez-Pinilla, 2009;
426   Gutiérrez-Lamus, Lynch & Martínez-Villate, 2011; Neckel-Oliveira et al., 2011; del Río-
427   Gracía, Serrano-Cardozo & Ramírez-Pinilla, 2014; Cruz, Galindo & Bernal, 2016).
428   *Bolitoglossa pandi* follows the common pattern observed in upland tropical
429   salamanders, having a narrow elevational range (1700-2000 m. a.s.l), in which
430   elevation, leaf litter depth (> 6 cm), vegetation layers (> 5 vegetation strata), and
431   environmental mean temperature (16–19 °C) were the main predictor variables for their
432   presence, abundance and population density (Wake & Lynch, 1976; Gutiérrez-Lamus,
433   Lynch & Martínez-Villate, 2011; Cruz, Galindo & Bernal, 2016; Donaire et al., 2019).
434   Likewise, observed patterns of habitat use by B. pandi agree with those previously
435   reported by del Río-Gracía, Serrano-Cardozo & Ramírez-Pinilla(2014) in which the
436   salamanders exhibiting both arboreal and terrestrial habits, perching on the upper side
437   of leaves (at heights between 2.5 and 250 cm above the forest floor), showing a positive
438   correlation between perch height, SVL and mass. However, opposite or absent
439   correlations between perch height and salamander body size has been reported for *B.*
440   *paraensis* and *B. nicefori*, respectively, suggesting that climatic variability or elevational
441   range distribution could determine microhabitat use (Ortega, Monares-Riaño &
442   Ramírez-Pinilla, 2009; Simões-Correa & Chagas-Rodrigues, 2017). This and many
443   more questions concerning microhabitat use of South American *Bolitoglossa*
444   salamanders remain open, highlighting the fact that the state of knowledge on their
445   ecology and natural history still presents many gaps.
446   The occurrence of individuals of *Bolitoglossa pandi* was strongly related with
447   habitat structure. Sampling plots in which *B. pandi* was present were positively
448   correlated with highly structured habitats and deep leaf litter, characteristics traditionally
449   associated with suitable habitats for amphibians because they provide food (leaf litter
450   arthropods), shelter, nesting sites, and microclimate stability by retaining moisture
451   through the soil interface after rainfall events (Heatwole, 1962; Jaeger, 1980; Harvey-
452   Pough et al., 1987; Vitt & Caldwell, 2001). Also, the leaf litter depth was significant
453   correlated with percent vegetation cover and vegetation layers, that agrees with the
454   pattern previously reported by de Maynadier & Houlahan (2008) in which the
455   composition and structure of the leaf litter and local tree canopy is significantly related in
456   tropical forests.
457   Conversely, highly homogenous sampling plots in which were dominant
458   pastures, graminoids, bare soil, and rocks, showed the lowest or no detection grade of
459   *B. pandi*. Therefore, this result showed that habitat degradation due local human
460   activities such logging, and cattle ranching have direct negative effects in the quality of

461   leaf litter general habitat structure of *B. pandi* (Vitt & Caldwell, 2001). The detection and
462   high local abundance of *B. pandi* was strongly correlated with the same habitat
463   variables associated with the detection of other Andean salamanders such as *B.*
464   *adspersa, B. altamazonica, B. nicefori,* and *B. orestes,* and *B. ramosi* (Valdivieso &
465   Tamsitt, 1965; Wake & Lynch, 1976; Cadenas et al., 2009; Gutiérrez-Lamus, Lynch &
466   Martínez-Villate, 2011; Leenders & Watkins-Colwell, 2013; Galindo, Cruz & Bernal,
467   2018). Thus, the strong dependency on a narrow environmental range of conditions
468   makes the Andean Bolitoglossines highly vulnerable to local changes by human
469   activities affecting these habitat variables.
470   Usually, activity in Bolitoglossines has been associated with variation in relative
471   humidity or climatic conditions, as well as breeding season (Vial, 1968; Wake & Lynch,
472   1976; Ortega, Monares-Riaño & Ramírez-Pinilla, 2009; Simões-Correa & Chagas-
473   Rodrigues, 2017). Activity of *Bolitoglossa pandi* follows this general pattern but with
474   certain deviations compared to some of its congeners. For example, the number of *B.*
475   *pandi* active individuals was strongly and positively correlated with the environmental
476   temperature, whereas it was moderately and negatively correlated with the relative
477   humidity [versus a positive correlation between active individuals and relative humidity
478   observed in *B. mombachoensis, B. paraensis,* and *B. subpalmata* (Vial, 1968; Salgado-
479   Aráuz, 2005; Simões-Correa & Chagas-Rodrigues, 2017)]. This correlation was
480   consistent across all sampling occasions, suggesting independence of climatic
481   variability. However, future studies should be carried out to clarify which environment
482   variables can determine the general activity pattern in the tropical *Bolitoglossa* species.
483   Niche partitioning has been reported for many populations of Nearctic and
484   Neotropical salamander species (Jaeger & Gergits, 1979; Wicknick, 1995; Arif, Adams,
485   & Wicknick, 2007; Jaeger et al,. 2016).Intraspecific niche partitioning in salamanders
486   has been explained as a life history strategy to maximize foraging success, predator
487   avoidance, and mating success (Jaeger & Gergits, 1979; Holomuzki, 1986; Cloyed &
488   Eason, 2017). Particularly, intraspecific niche partitioning due to ontogenetic shifts or
489   sexual microhabitat selection has been documented for some *Bolitoglossa* species [e.g.
490   *B. dofleini* and *B. nicefori* (Raffaëlli, 2007; Ortega, Monares-Riaño & Ramírez-Pinilla,
491   2009). Previously, del Río-Gracía, Serrano-Cardozo & Ramírez-Pinilla (2014) explored
492   whether intraspecific niche partitioning due to ontogenetic shifts or sexual microhabitat
493   selection was present in *B. pandi* populations at the municipality of Supatá. However,
494   these authors did not find significant differences in microhabitat use between sexes or
495   body size classes. Meanwhile, our study showed that body size classes of the *B. pandi*
496   population from the Cuzcungos locality showed significant differences between the
497   activity peaks, indicating that the use of resources available did not overlap in the time-
498   axis of the *B. pandi* niche. This is the first documented evidence of intraspecific niche
499   time-partitioning in Bolitoglossine salamanders. Thus, our results characterize an
500   entirely new aspect of the ecology of *B. pandi* and an important addition to our

501 understanding of the natural history of South American Bolitoglossines. Nevertheless,
502 our data are insufficient to infer any fundamental ecological trade-off between body size
503 and microhabitat use, activity and vulnerability to predation, or activity and foraging
504 success. Future experimental studies will be needed to understand how intraspecific
505 niche time-partitioning is acting through environmental heterogeneity in the ecology and
506 evolution of the South American Bolitoglossines. Also, future conservation efforts must
507 consider this life history strategy in order to carry out effective interventions.
508
509 **Conclusions**
510   The significant association between habitat structure and morphological traits
511 exhibited by *Bolitoglossa pandi* follows the general adaptive radiation pattern of
512 neotropical salamanders, correlating the diversity of forms with habitats variability (Darda
513 & Wake, 2015). Habitats with high percent of vegetation cover and leaf litter depth provide
514 the suitable environmental conditions for this species and define the sets of predicting
515 variables associated with occupation of *B. pandi* in forests across the Andes. Given the
516 narrow elevational range of this species, local changes of these habitat variables along
517 small areas could lead to the local extinction of their populations. Based on the results of
518 this study, we recommend conducting occupation models and extinction rate estimations
519 in order to generate predictive scenarios for the conservation and management of *B.*
520 *pandi*.
521   Our result agrees with the general pattern observed in other amphibian taxa in
522 which habitat disturbance driven by human activities has deleterious effects on their
523 presence or population densities given their high habitat specificity (Lips, 1998; Collins
524 & Storfer, 2003). Habitat loss continues to stand out as the main threat for *B. pandi*.
525 This fact has significant importance in conservation issues because the Andean forest is
526 one of the most threatened ecosystems by human activities in Colombia (Etter et al.,
527 2018). Therefore, despite our observations of a healthy and stable salamander
528 population that showed high population density and constant recruitment in the
529 municipalities of Supatá, Guaduas, and Villeta, we recommend that *B. pandi* be retained
530 as Endangered (EN) on the IUCN Red List based on the IUCN Criterion B, given its
531 restricted extent of occurrence (ca. 2,500 km^2), as well as the ongoing habitat loss
532 within its range due to agriculture, cattle ranching, logging, and urban development.
533   Finally, Colombian herpetologists must encourage private and public research
534 centers, universities, conservation agencies and industries to support fieldwork that
535 seeks to increase the knowledge on amphibian diversity, filling the distribution gaps of
536 the Andean species (particularly salamanders) and to perform ecology and natural
537 history studies (Ospina-Sarria & Angarita-Sierra, 2020). The latter is essential to
538 develop successful conservation and management strategies and environmental
539 regulations in a megadiverse country as Colombia (Bury, 2006). An example is this
540 study, wherein, we characterized several important aspects of population biology and

541 ecology of the endangered species *B. pandi*. By filling gaps in our knowledge of this
542 species, we were able to describe in detail the habitat requirements for its conservation
543 and provide an update of its conservation status.
544

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565

References

567 Acosta-Galvis AR, Gutiérrez-Lamus DL. 2012. A new species of salamander (Bolitoglossa:
568   Plethodontidae) from the Cordillera Oriental of the Colombian Andes. *Papéis Avulsos de*
569   *Zoologia* 52(18): 201–218 DOI: 10.1590/s0031-10492012001800001.
570 Acosta-Galvis AR, Restrepo AE. 2001. Una nueva especie de Bolitoglossa (Caudata:
571   Plethodontidae) de las selvas del Magdalena Medio en Colombia. *Caldasia* 23(2):467-
572   473.
573 Acosta-Galvis AR, Rueda-Almonacid JV. 2004. Salamandra de Pandi Bolitoglossa pandi. In:
574   Rueda-Almonacid JV, Lynch DL, Amezquita A, eds. *Libro rojo de anfibios de Colombia*.
575   Bogotá D.C: Panamericana,206–209.
576 Acosta-Galvis AR. 2015. Una nueva especie del género Pristimantis (Anura: Craugastoridae) del
577   complejo de páramos Merchán-Iguaque (Boyacá, Colombia). *Biota Colombiana* 16 (2): 107-
578   127.
579 Acosta-Galvis   AR.   2020.   Lista   de   los   Anfibios   de   Colombia.   Available   at
580   https://www.batrachia.com (accessed 21 May 2020).
581 Acosta-Galvis, AR, Hoyos JM. 2006. A new species of salamander (Caudata: Plethodontidae:
582   Bolitoglossa) from the subandean forest Western Cordillera of Colombia. *Herpetologica*
583   62(3):302-308.

584 Akaike H. 1973. Information theory and an extension of the maximum likelihood principle.
585   In: Petrovand BN, Caski F. Proceeding of the Second   International   Symposium   on
586   Information Theory. Budapest: Akademiai Kiado, 1–451.
587 Anderson MT, Mathis A. 2006. Diets of Two Sympatric Neotropical Salamanders, Bolitoglossa
588   mexicana and B. rufescens, with Notes on Reproduction for B. rufescens. *Journal of*
589   *Herpetology* 33(4):601-602 DOI:10.2307/1565576.
590 Arif S, Adams DC, Wicknick JA. 2007. Bioclimatic modeling, morphology, and behavior reveal
591   alternative mechanisms regulating the distributions of two parapatric salamander species.
592   *Evolutionary Ecology Research* 9(5): 843–854.
593 Arroyo S, Jerez A, Ramírez-Pinilla MP. 2003. Anuros de un bosque de niebla de la Cordillera
594   Oriental de Colombia*. Caldasia*. 25:153-167.
595 Barrio-Amorós CL, Fuentes-Ramos O. 1999. Bolitoglossa spongai a new species of salamander
596   (Caudata: Plethodontidae) of Venezuelan Andes, with comments on the genus in Venezuela.
597   *Acta Biologica Venezuelica* 19(4): 9-19.
598 Bingham RE, Papenfuss TJ, Lindstrand L, Wake DB. 2018. Phylogeography and Species
599   Boundaries In the Hydromantes shastae Complex, With Description of Two New Species
600   (Amphibia; Caudata; Plethodontidae). *Bulletin of the Museum of Comparative Zoology*
601   *161*(10):403–427 DOI:10.3099/mcz42.1
602 Bolar K. 2019. STAT: Interactive Document for Working with Basic Statistical Analysis. R package
603   version 0.1.0.
604 Bourne R. 2010. ImageJ. In: *Fundamentals of Digital Imaging in Medicine*. London: Springer-
605   Verlag.
606 Brame AH, Wake DB. 1962. A new plethodontid salamander (genus Bolitoglossa) from Venezuela
607   with redescription of the Ecuadorian B. palmata (Werner). *Copeia* 1962(1): 170–177.
608 Brame AH, Wake DB. 1963. Contributions to the Salamanders of South America. *Contributions*
609   *in Science. Natural History Museum of Los Angeles County* 69:1–72.
610 Brcko IC, Hoogmoed MS, Neckel-Oliveira S. 2013. Taxonomy and distribution of the salamander
611   genus Bolitoglossa Duméril, Bibron & Duméril, 1854 (Amphibia, Caudata, Plethodontidae)
612   in Brazilian Amazonia. *Zootaxa* 3686(4):401–431 DOI:10.11646/zootaxa.3686.4.1
613 Bruce RC. 1997. Life History Attributes of the Salamander Bolitoglossa colonnea. *Journal of*
614   *Herpetology* 31(4):592–594 DOI: 10.2307/1565618.
615 Bury RB. 2006. Natural history, field ecology, conservation biology and wildlife management: time
616   to connect the dots. *Herpetological Conservation and Biology* 1(1):56-61.
617 Cadenas DA, Pérez-Sánchez AJ, Villa P, De Ascenção AA. 2009. Relative Abundance Habitat
618   Use and Diet of Bolitoglossa orestes (Urodela: Plethodontidae) in a Venezuelan Andean
619   Cloud Forest. *ECOTRÓPICOS* 22(2):99–109.
620 Chen M, Combs C. 2001. An alternative killing agent for amphibians. *Herpetological Review*
621   32(2):93–94.
622 Chevan, A, Sutherland M. 1991. Hierarchical partitioning. *The American Statistician* 45(2): 90-96
623   DOI: 10.1080/00031305.1991.10475776.
624 Cloyed CS, Eason PK. 2017. Niche partitioning and the role of intraspecific niche variation in
625   structuring a guild of generalist anurans. *Royal Society Open Science*: 4(3): 170060
626   DOI:10.1098/rsos.170060.
627 Collins J, Storfer A. 2003. Global amphibian declines: sorting the hypotheses. *Diversity and*
628   *Distributions* 9(2003):89–98.
629 Crump ML, Scott NJ. 1994. Visual encounters surveys. In: Heyer W, Donelly M, MacDiarmind R,
630   Hayeck L, Foster M, eds. *Measuring and monitoring biological diversity, standard methods*
631   *for amphibians*. Washington: Smithsonian Institution Press,354-352.
632 Cruz EX, Galindo CA, Bernal MH. 2016. Dependencia térmica de la salamandra endémica de
633   Colombia Bolitoglossa ramosi (Caudata, Plethodontidae). *Iheringia. Série Zoologia* 106 DOI:

634   10.1590/1678-4766e2016018.
635 Darda DM, Wake DB. 2015. OsteologicalVariation among Extreme Morphological Forms in the
636   Mexican Salamander Genus Chiropterotriton (Amphibia: Plethodontidae): Morphological
637   Evolution and Homoplasy. *PLoS ONE* 10(6): e0127248 DOI: 10.1371/journal.pone.0127248.
638 de Maynadier PG, Houlahan JE. 2008. 13 Conserving Vernal Pool Amphibians in Managed
639   Forests. In: Calhoun AJK & deMaynadier PG, eds. *Science and Conservation of Vernal*
640   *Pools in Northeastern North America: Ecology and Conservation of Seasonal Wetlands in*
641   *Northeastern North America*. New York: CRC Press, 253-280.
642 del Río-Gracía JS, Serrano-Cardozo VH, Ramírez-Pinilla MP. 2014. Diet and Microhabitat Use of
643   Bolitoglossa cf. pandi (Caudata: Plethodontidae) from the Cordillera Oriental of Colombia.
644   *South American Journal of Herpetology* 9(1):52–61 DOI: 10.2994/sajh-d-13-00031.1.
645 Donaire RA, Pallais, JM, Chung J, Rizo, Cobos MA, De los Santos Rosales M, Jun J,
646   Casco-Robles MM. 2019. Constricted spatiotemporal foraging of the regenerating
647   salamander, Bolitoglossa mombachoensis. *Ecosphere*10(10) DOI: 10.1002/ecs2.2897.
648 Durbin J, Watson GS. 1950. Testing for serial correlation in least squares regression: I. *Biometrika*
649   37(3/4): 409-428 DOI: 10.1093/biomet/37.3-4.409.
650 Elzinga CL, Salzer DW, Willoughby JW. 1998. *Measuring and monitoring plant populations*.
651   Denver: U.S. Dept. of the Interior, Bureau of Land Management.
652 Etter A, Andrade Á, Saavedra K, Cortés J. 2018. Actualización de la Lista Roja de los
653   Ecosistemas Terrestres de Colombia. In: Moreno LA, Rueda C, Andrade GI, eds.
654   *Biodiversidad 2017: Estado y tendencias de la biodiversidad continental de Colombia*.
655   Bogotá D.C: Instituto de Investigación de Recursos Biológicos Alexander von Humboldt, 19.
656 Fox J, Weisberg S. 2019. An R Companion to Applied Regression, Third edition. Thousand Oaks
657   CA: Sage.
658 Frost DR. 2020. Amphibian Species of the World: an Online Reference. Version 6.1. Electronic
659   Database. Available at https://amphibiansoftheworld.amnh.org/index.php (accessed 28
660   May 2020).
661 Galindo CA, Cruz EX, Bernal MH. 2018. Evaluation of the combined temperature and relative
662   humidity preferences of the Colombian terrestrial salamander Bolitoglossa ramosi
663   (Amphibia: Plethodontidae). *Canadian Journal of Zoology* 96(11):1230-1235
664   DOI:10.1139/cjz-2017-0330.
665 Gibbons, W. 2013. *Their blood runs cold: Adventures with reptiles and amphibians*. University of
666   Alabama Press.
667 Gross J, Ligges U. 2015. nortest: Tests for Normality. R package version 1.4.
668 Guisande C, Heine J, González-DaCosta J, García-Roselló E. 2014. RWizard Software. Vigo:
669   University of Vigo.
670 Gutiérrez-Lamus DL, Lynch JD, Martínez-Villate GC. 2011. Population estimators and adult sex
671   ratio for a population of Bolitoglossa altamazonica (Caudata: Plethodontidae). *Basic and*
672   *Applied Herpetology* 25(2011):43–54 DOI: 10.11160/bah.11008.
673 Hanken J, Wake DB. 1982. Genetic differentiation among plethodontid salamanders (genus
674   Bolitoglossa) in Central and South America: implications for the South American invasion.
675   *Herpetologica* 32(2): 272–287.
676 Harvey-Pough F, Smith EM, Rhodes DH, Collazo A. 1987. The abundance of salamanders in
677   forest stands with different histories of disturbance. *Forest Ecology and Management*
678   20(1):1-9 DOI: 10.1016/0378-1127(87)90146-0.
679 Heatwole H. 1962. Environmental Factors Influencing Local Distribution and Activity of the
680   Salamander, Plethodon Cinereus. *Ecology*: 43(3): 460-472 DOI:10.2307/1933374.
681 Holomuzki JR. 1986. Predator Avoidance and Diel Patterns of Microhabitat Use by Larval Tiger

682   Salamanders. *Ecology* 67(3):737–748.
683 Houck L. 1977. Reproductive biology of a Neotropical Salamander, Bolitoglossa rostrata. *Copeia*
684   1977(1):70–83.
685 Hui W, Gel YR, Gastwirth JL. 2008. Lawstat: An R package for law, public policy, and biostatistics.
686   *Journal of Statistical Software* 28(3) DOI: 10.18637/jss.v028.i03.
687 Jaeger RG. 1980. Microhabitats of a Terrestrial Forest Salamander. *Copeia* 1980(2): 265-268
688   DOI: 10.2307/1444003
689 Jaeger RG, Gergits WF. 1979. Intra- and interspecific communication in salamanders through
690   chemical signals on the substrate. *Animal Behaviour* 27(PART 1):150–156 DOI
691   https:10.1016/0003-3472(79)90134-9.
692 Jaeger RG, Gollman B, Anthony DD, Gabor CR, Kohn N. 2016. *Behavioral Ecology of the Eastern*
693   *Red-backed Salamander: 50 Years of Research*. New York: Oxford University Press.
694 Jimenez CE. 1994. Utilization of Puya dasylirioides (Bromeliaceae: Pitcairnoideae) as foraging
695   site by Bolitoglossa subpalmata (Plethodontidae: Bolitoglossini). *Revista De Biologia*
696   *Tropical* 42(3): 703–710.
697 Leenders TAAM, Watkins-Colwell GJ. 2013. Morphological and behavioral adaptations in
698   Bolitoglossa colonnea (Caudata, Plethodontidae) in relation to habitat use and daily activity
699   cycle. *Phyllomedusa: Journal of Herpetology* 2(2):101-104 DOI:10.11606/issn.2316-
700   9079.v2i2p101-104.
701 Lips. 1998. Decline of a tropical amphibian fauna. *Conservation Biology* 12(1):106–117.
702 Lynch JD, Ruiz-Carranza PM, Ardila-Robayo MC.1997. Biogeographic patterns of Colombian
703   frogs and toads. *Revista de la Academia Colombiana de Ciencias Exactas, Físicas y*
704   *Naturales* 21(80): 237–248.
705 Lynch JD, Suárez-Mayorga AM. 2002. Análisis Biogeográfico de los Anfibios Paramunos.
706   *Caldasia* 24(2): 471-480.
707 Lynch JD. 1999. Ranas pequeñas, la geometría de evolución y la especiación en los Andes
708   colombianos. *Revista de la Academia Colombiana de Ciencias Exactas, Físicas y Naturales*
709   23 (86): 143-159.
710 Lynch JD. 2001. A small amphibian fauna from a previously unexplored páramo of the Cordillera
711   Occidental in western Colombia. *Journal of Herpetology* 35(2):226–231.
712 Mackenzie DI, Nichols JD, Hines JE, Knutson MG, Franklin AB. 2003. Estimating site occupancy,
713   colonization, and local extinction when a species is detected imperfectly. *Ecology*
714   84(8):2200-2207.
715 Naimi B, Hamm Na, Groen TA, Skidmore AK, Toxopeus AG. 2014. "Where is positional
716   uncertainty a problem for species distribution modelling." Ecography 37(2) : 191-203.
717   DOI: 10.1111/j.1600-0587.2013.00205.x.
718 Nally RM, Walsh CJ. 2004. "Hierarchical partitioning public-domain software." Biodiversity and
719   Conservation 13: 659-660 DOI: 10.1023/B:BIOC.0000009515.11717.0b.
720 Neckel-Oliveira S, Sarmento JFM, Galatti U, Suárez P, Lima C, Lima AA, Fáveri SB. 2011.
721   Reproductive Traits of the Brazilian Salamander Bolitoglossa paraensis (Urodela:
722   Plethodontidae). *Copeia* 2011(3):457–462 DOI: 10.1643/ce-08-219
723 Ortega JE, Monares-Riaño JM, Ramírez-Pinilla MP. 2009. Reproductive Activity, Diet, and
724   Microhabitat Use in Bolitoglossa nicefori (Caudata: Plethodontidae). *Journal of Herpetology*
725   43(1):1–10 DOI: 10.1670/07-250r2.1.
726 Ospina-Sarria, JJ, Angarita-Sierra T. 2020. A New Species of Pristimantis (Anura:
727   Strabomantidae) from the Eastern Slope of the Cordillera Oriental, Arauca, Colombia.
728   *Herpetologica* 76(1): 83–92.
729 R Core Team. (2012). R: A language and environment for statistical computing. Viena: R
730   Foundation for Statistical Computing. Retrieved from http://www.r-project.org/*
731 Raffaëlli, J. 2007. *Les Urodèles du Monde*. Condé-sur-Noireau: Penclen Édition.

732   Ridout M, Linkie M. 2009. "Estimating overlap of daily activity patterns from camera trap
733   data." Journal of Agricultural, Biological, and Environmental Statistics 14(3) : 322-337 DOI:
734   10.1198/jabes.2009.08038.
735   Ruiz-Carranza PM, Ardila-Robayo MC, Lynch JD. 1996. Lista actualizada de la fauna de amphibia
736   de Colombia. *Revista de la Academia Colombiana de Ciencias Exactas, Físicas y Naturales*
737   20 (77): 365-415.
738   Salgado-Aráuz H. 2005. Caracterización de la población y del hábitat de la salamandra endémica
739   (Bolitoglossa mombachoensis) en la Reserva Natural Volcán Mombacho. *Encuentro*
740   37(73):77–86.
741   Simões-Correa F, Chagas-Rodriguez L. 2017. On the distribution of Neotropical climbing
742   salamanders (Bolitoglossa paraensis) in a forest fragment of the eastern Amazon.
743   *Salamandra* 53(3): 445-450.
744   Sokal R, Rohlf R.1981. *Biometry: The principles and practice of statistics in biological research*
745   (First edition). San Francisco: W.H. Freeman.
746   Valdivieso D, Tamsitt JR. 1965. Reproduction in a Neotropical Salamander, Bolitoglossa
747   adspersa (Peters). *Herpetologica* 21(3):228–236.
748   Vial JL. 1968. The Ecology of the Tropical Salamander, Bolitoglossa subpalmata, in Costa Rica.
749   *Revista de Biologia Tropical*:15(1): 13-115.
750   Vitt LJ, Caldwell JP. 2001. The effects of logging on reptiles and amphibians of tropical forests.
751   In: Fimbel RA, Grajal A, Robinson JG, eds. *The Cutting Edge: Conserving Wildlife in Logged*
752   *Tropical Forests*. New York: Columbia University Press, 239–259.
753   Wake DB, Lynch JF. 1976. The distribution, ecology, and evolutionary history of plethodontid
754   salamanders in tropical America. *Bulletin of the Natural History Museum of Los Angeles*
755   *County* 25: 1–65.
756   Wake DB. 2017. Persistent Plethodontid Themes: Species, Phylogenies, and Biogeography.
757   *Herpetologica* 73(3):242-251 DOI: 10.1655/HERPETOLOGICA-D-16-00065.1
758   White G, Burnham K. 1999. Program MARK: survival estimation from 594 populations of marked
759   animals. *Bird Study* 4(1):S120–S139 DOI: 10.1080/00063659909477239.
760   Wicknick JA. 1995. *Interspecific competition and territoriality between a widespread species of*
761   *salamander and a species with a limited range*. University of Southwestern Louisiana.
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Figure 1

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

New Northwestern records for the known distribution of *Bolitoglossa pandi* (IAvH-Am 10303-103058). Pentagon with a white square inside: La Esmeralda (municipality of Villeta), black star: Granada (municipality of Guaduas), 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 salto de la Chorrera, triangle: El Mirador. Background map was retrieved from the Esri open database accessing the following sources: DeLorme, USDS, NPS; USGS, NOAA.
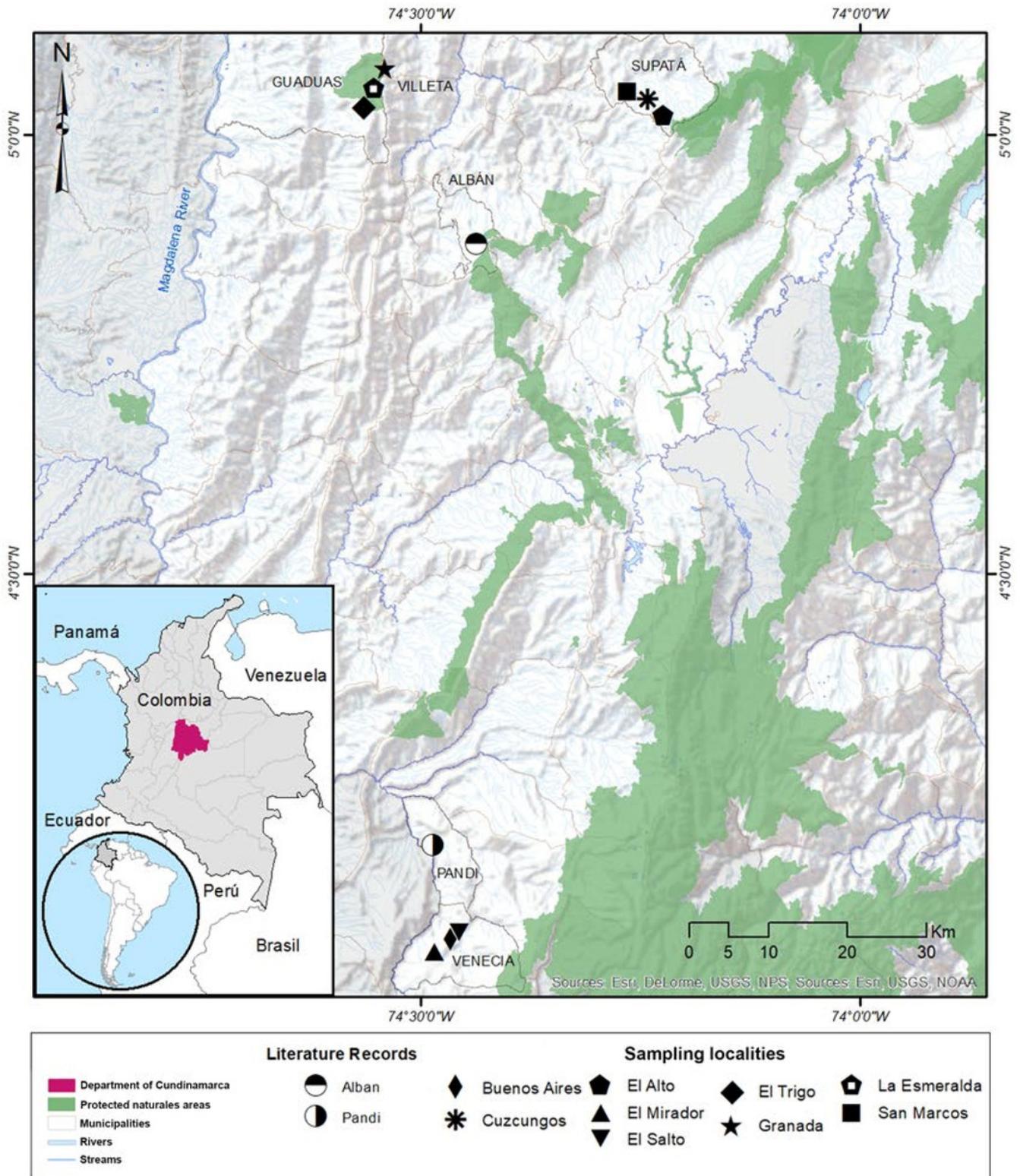
Figure 2

Scatterplot depicting the associations of habitat structure and natural history traits.

(A) Positive correlation between body length and perch high. (B) Positive correlation between weight and perch high.
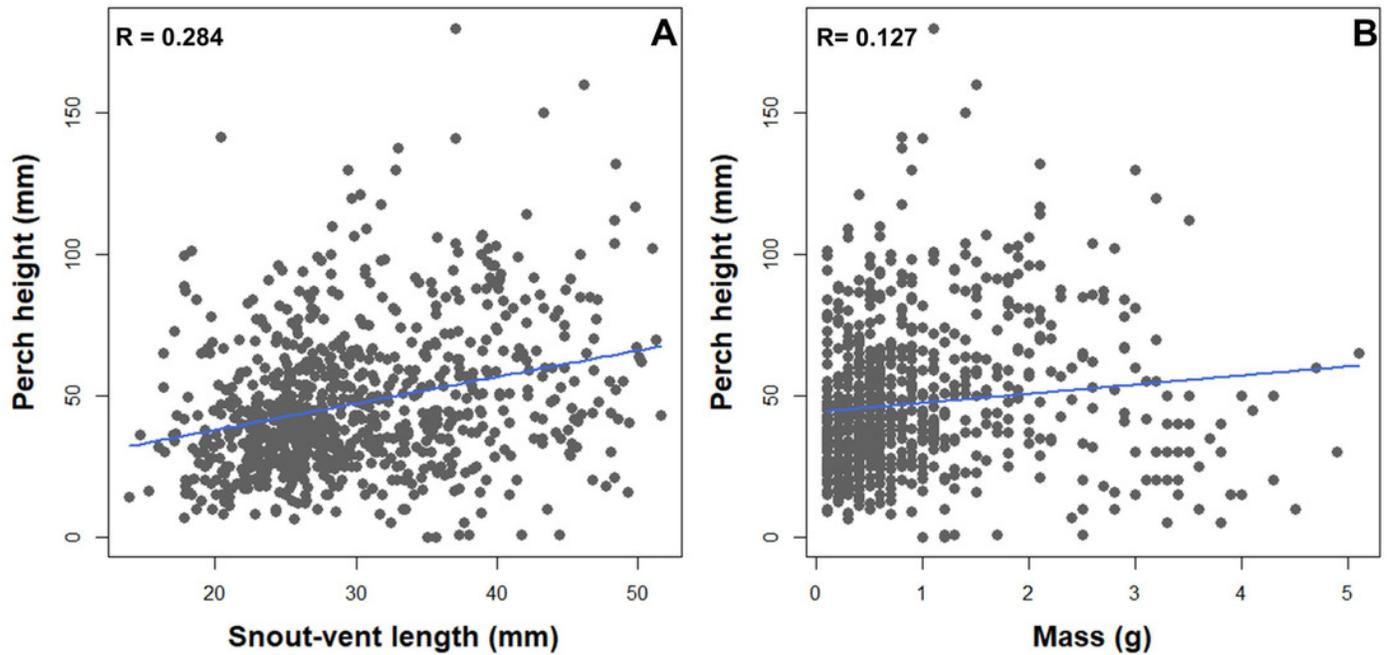
Figure 3

Habitat suitability of *Bolitoglossa pandi*

(A) Principal component analysis showing the observed variability in habitat structure attributes between plots with detection / not detection of *Bolitoglossa pandi*. PC1 = First principal component (38.8%). PC2 = Second principal component (23.6%). Black squares = detection of *Bolitoglossa pandi*. Grey dots = Not detection of *Bolitoglossa pandi*. Inner ellipse represents 0.5 of significance; outer ellipse represents 0.95 of significance. Quadratic discriminant analysis: (B) Boxplot depicting the variance of the canonical scores between the plots where *B. pandi* was detected / not detected. (C) Habitat variables that have greatest discrimination capacity between plots where *B. pandi* was detected / not detected. The length of the vector denotes the discrimination capacity of each habitat variable.
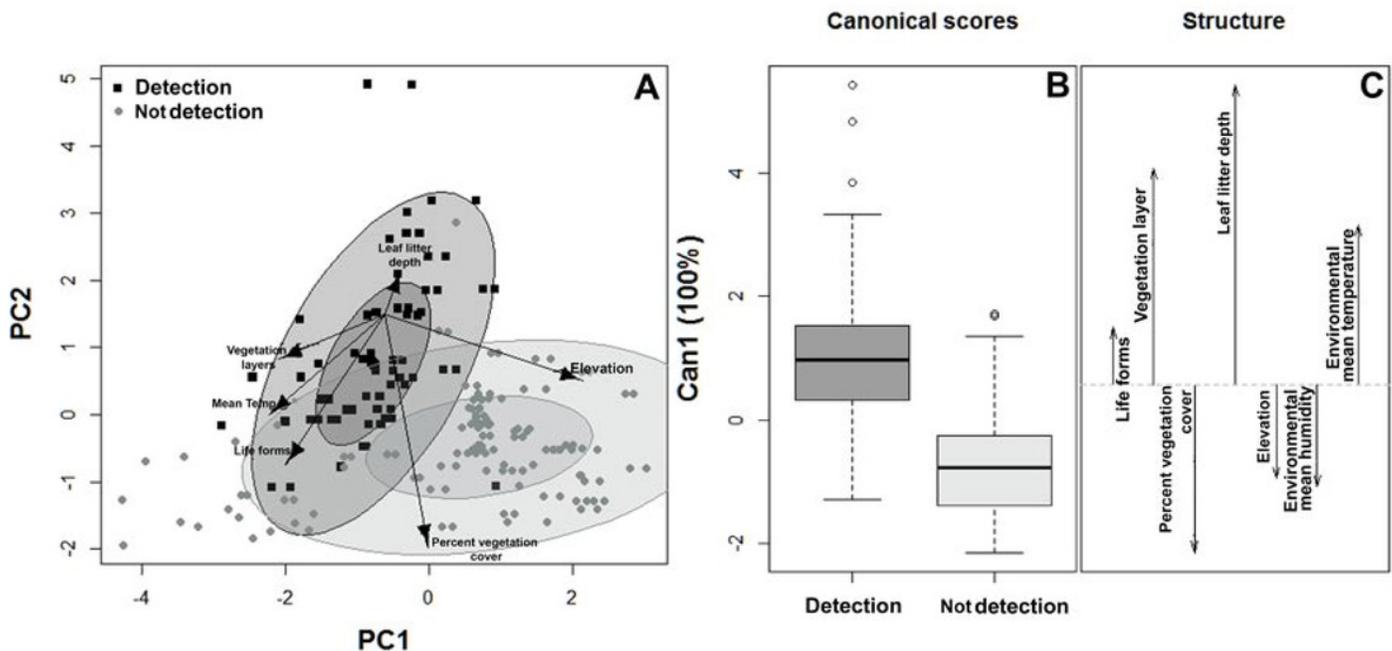
Figure 4

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

The small vertical bars on bottom depicts the independent detections observed 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.

Density of salamanders

Time of activity
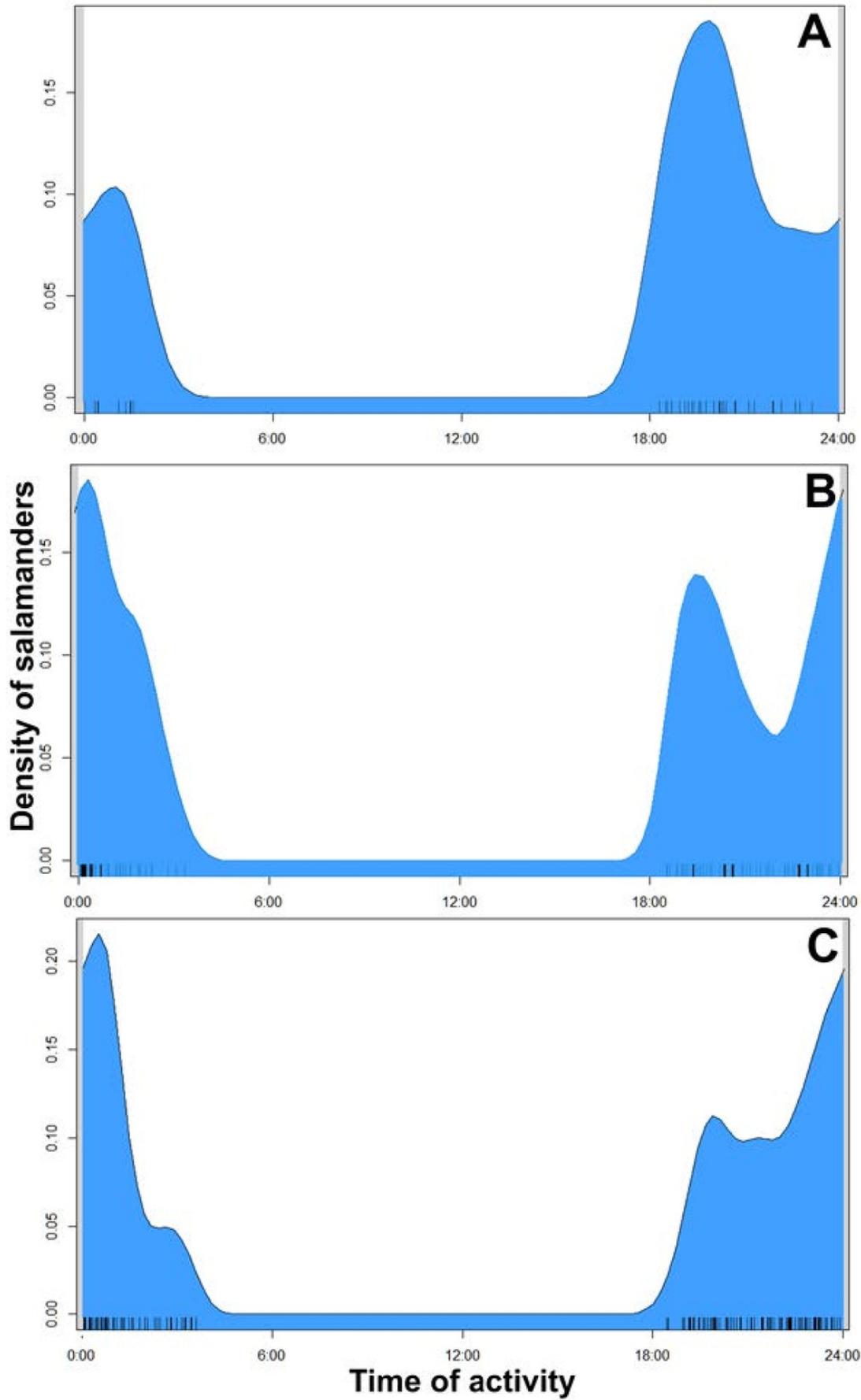
Figure 5

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

(A) Nested ANOVA depicting the abundance variability among activity peaks observed. (B)
Kruskal-Wallis test depicting niche partitioning between body size classes.
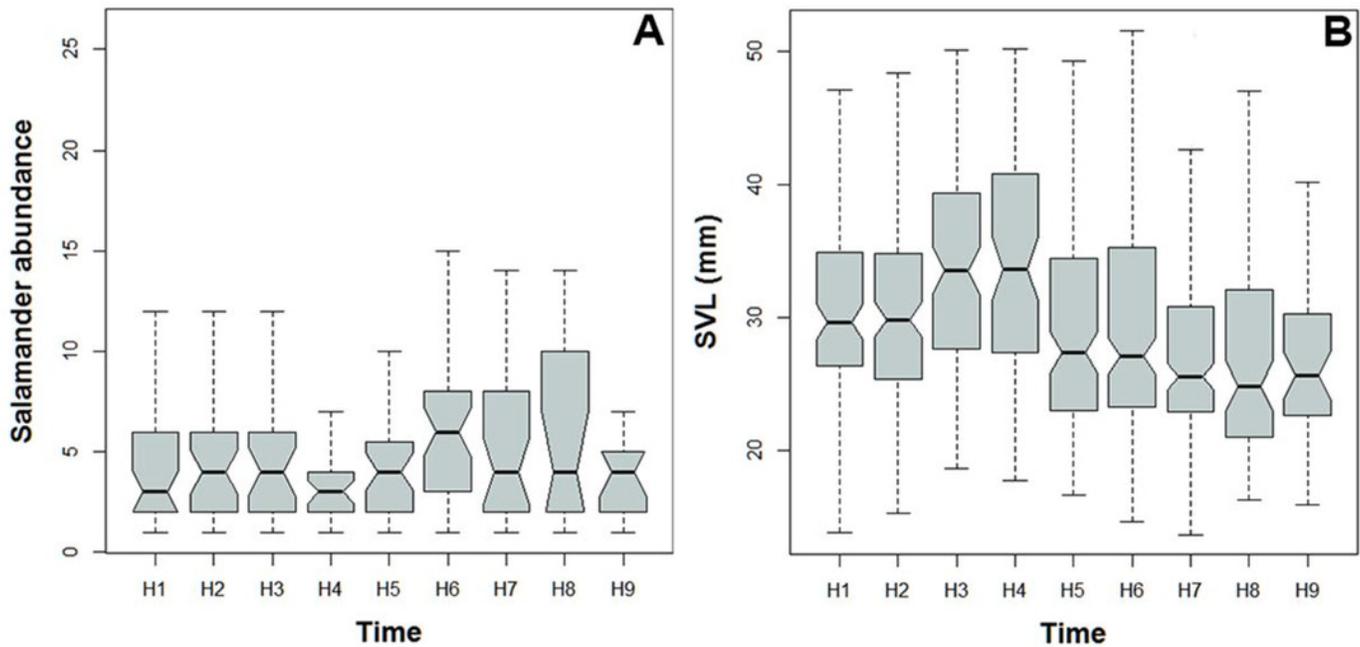
Figure 6

Population size-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.
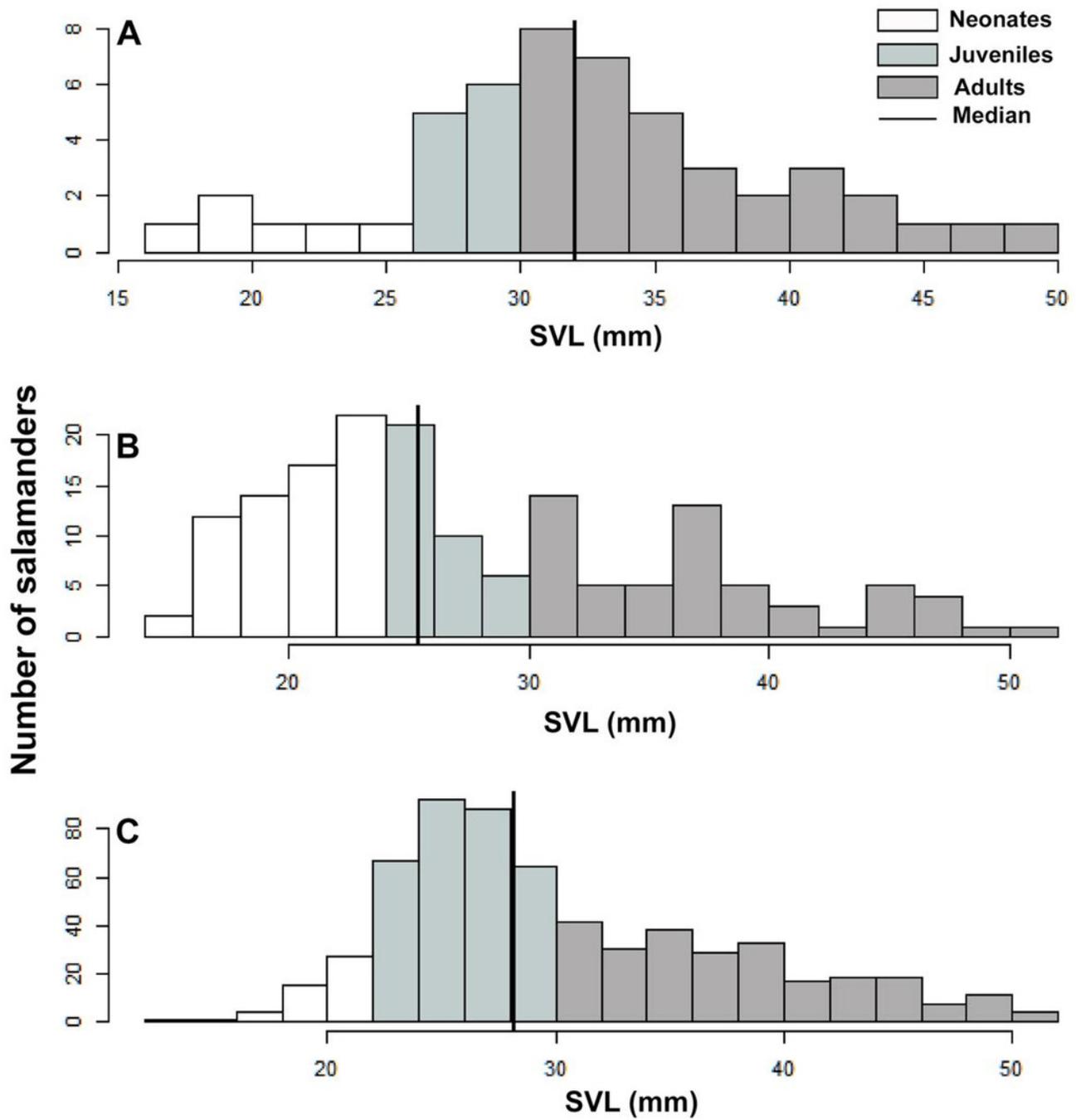
Figure 7

Color variability observed in living and preserved specimen of *Bolitoglossa pandi*.

(A) ventral surface of the head (ICN 45000; Pandi, Cundinamarca). (B) Lateral view of the edges lip 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). (D) Uniform dorsal color pattern. (E) dorsal color reddish brown that can have diffuse grey or dark blotches scarcely distinguishable to the dorsolateral. (F-H) dorsal surfaces exhibiting ochre pattern, with some diffuse irregular dark brown, yellow or cream spots. (I-J) dorsolateral surfaces diffuse band in the paravertebral region covering almost the entire dorsal surface or includes a dark brown inverted triangle shape in the interorbital region. Pictures by: Teddy Angarita-Sierra.
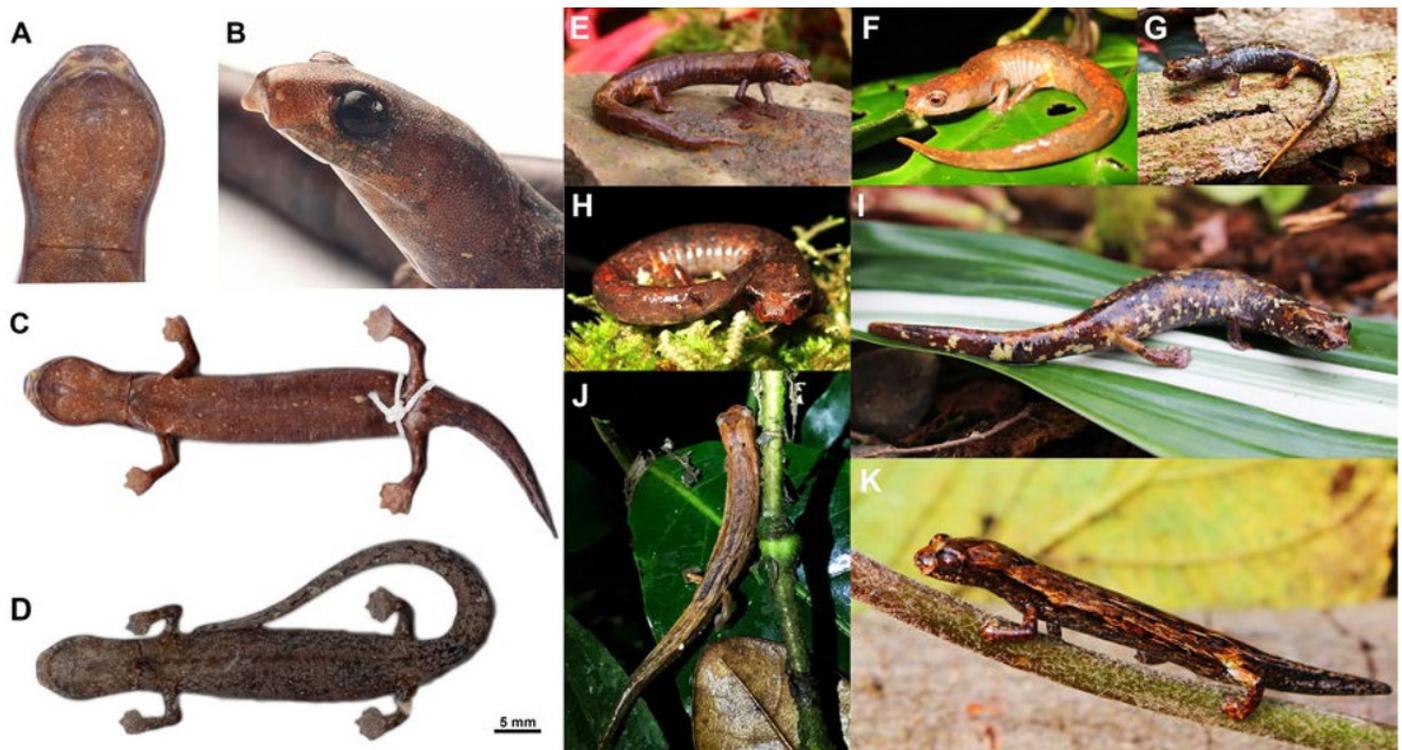
Table 1(on next page)

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

VES: Visual encounter surveys (Crump & Scott, 1994). P: Plots. N: Number of plots randomly selected.

Municipalities Vereda/locality	Latitude North	Longitude West	Elevation (m) a.s.l	Sampling protocol	*B. pandi* specimens observed	*B. pandi* specimens collected
Supatá						
Vereda Las Lajas/Monterey	From 5º 2' 18.5" To 5º 1' 59.9"	74º 14' 6.6" 74º 14' 3.5"	2119–2315	P (N = 45)	1	0
Vereda Las Lajas/ Cuzcungos Natural Reserve	From 5º 2' 27.7" To 5º 2' 27.2"	74º 14' 32.2" 74º 14' 27.9"	1931–2016	P (N = 43)	1391	12
Vereda San Marcos	From 5º 2' 58.9" To 5º 2' 58.3"	74º 15' 53.7" 74º 15' 54.9"	1743–1773	P (N = 28)	0	0
Villeta						
Vereda La Esmeralda	5º 3' 17.9"	74º 32' 49.1"	1996	VES	10	2
Guaduas						
Vereda Granada	5º 4' 1.49"	74º 32' 59.6	1816	VES	16	4
El Trigo site	5º 2' 9.2"	74º 33' 48.8"	1650	VES	8	0
Venecia						
Vereda Buenos Aires	From 4º 4' 57.9" To 4º 4' 44.1"	74º 27' 57.7" 74º 27' 33.3"	1809–2128	P (N = 90)	0	0
Vereda El Diamante/El salto de la Chorrera	From 4º 5' 24.6" To 4º 5' 21.0"	74º 27' 25.5" 74º 27' 33.2"	1637–1735	P (N = 36)	0	0
Vereda El Alto/ Road Venecia- Cabrera	From 4º 4' 5.2" To 4º 4' 11.1"	74º 29' 6.9" 74º 28' 54.8"	2034–2114	P (N = 54)	0	0

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Table 2(on next page)

Multiple correlation analysis

Upper diagonal part contains correlation coefficient estimates. Lower diagonal part contains corresponding p-values. 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	Percent vegetation cover r	Vegetation layers	Leaf litter depth	Elevation (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
Percent 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 depth	**<0.001**	**<0.001**	**<0.001**	0.125	**<0.001**	**<0.001**	**<0.001**	*****	0.092
Elevation (m) a.s.l	**<0.001**	**0.001**	**<0.001**	0.906	0.260	**<0.001**	**<0.001**	**0.015**	*****

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Table 3(on next page)

Principal component correlation matrix

Upper diagonal part contains correlation coefficient estimates. Lower diagonal part contains corresponding p-values. Bold values denote statistical significance at the p < 0.05 level. KMO: Kaiser-Meyer-Olkin test. BST: Kaiser-Meyer-Olkin test.

Habitat structure variables	Life forms	Vegetation Layer	Percent vegetation cover	Leaf litter depth	Elevation (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		
Percent vegetation cover r	**<0.001**	0.285	*****	-0.274	0.045	-0.061		
Leaf litter depth	0.979	**<0.001**	**<0.001**	*****	0.149	0.060	0.6	**<0.001**
Elevation (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: Per.veg.cov = Percent vegetation cover. Ln–transformedMean Temp = Environmental mean temperature. LnMeanH = Ln–transformed relative humidity. Lnlife forms = Ln–transformed number of vegetation life form. Veg.leyers = 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	ΔAIC	Nor.test	Hom.test	DW
Lnn~Lnleaf litter+LnMean temp+Per.veg.cov+Elevation	−73.64	0.0			
Lnn~Lnleaf litter+LnMean temp+Per.veg.cov+Elevation +Lnlife forms	−73.08	−0.56			
Lnn~Lnleaf litter+LnMean temp+Per.veg.cov+Elevation +LnMeanHr	−71.92	−1.72	0.45	0.06	1.10
LnAbundance~Lnleaflitter+LnMeantemp+Veg.leyers+ Per.veg.cov+Elevation+LnMeanH+Lnlife forms	−70.04	−3.60			

The "best" multiple regression model	Relative importance for *B. pandi* abundance	Estimate	SE	*t*-value	P(>|*t*|)
Intercept		3.33	1.93	2.79	**0.006**
Ln leaf litter	64%	0.88	0.071	12.34	**< 0.001**
Ln Mean temp	18.5%	0.69	0.167	4.12	**< 0.001**
Percent vegetation cover	12%	−1.87	0.50	-3.73	**< 0.001**
Elevation (m) a.s.l.	5.5%	−0.001	0.0004	−2.46	**0.014**

F= 69.18, df= 4–182, P < 0.0001

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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 (18:30 a 19:30)	Max= 16, Min= 1 $\overline{x}$= 4.16, N= 154	-0.0257	0.0234	-1.085	0.279		
H2 (19:31 a 20:30)	Max= 20, Min= 1 $\overline{x}$= 4.91, N= 172	0.010	0.022	0.461	0.645		
H3 (20:31 a21:30)	Max= 12, Min= 1 $\overline{x}$= 4.73, N= 123	0.013	0.023	0.525	0.599		
H4 (21:31 a 22:30)	Max= 8, Min= 1 $\overline{x}$= 3.46, N= 97	-0.047	0.023	-2.037	**0.042**		
H5 (22:31 a 23:30)	Max= 16, Min= 1 $\overline{x}$= 4.393, N= 158	-0.006	0.020	-0.309	0.757	P = 0.08	P = 0.2
H6 (23:31 a 00:30)	Max= 20, Min= 1 $\overline{x}$= 6.05, N= 260	0.049	0.020	2.493	**0.013**		
H7 (00:31 a 01:30)	Max= 18, Min= 1 $\overline{x}$= 4.73, N= 196	0.040	0.022	1.834	0.067		
H8 (01:31 a 02:30)	Max= 26, Min= 1 $\overline{x}$= 6.47, N= 116	0.003	0.030	0.100	0.920		
H9 (02:31 a 03:30)	Max= 26, Min= 1 $\overline{x}$= 4.43, N= 62	0.003	0.030	0.100	0.920		

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Table 6(on next page)

Wilcoxon test results

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

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

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