Does *Pristimantis bogotensis* exhibit phenotypic plasticity? A study of color, pigmentation and dorsal design patterns.

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Abstract

Phenotypic plasticity has often been documented in amphibians, color and pattern variation are examples of variation in which phenotypic plasticity can play a vital role. Phenotypic plasticity was overlooked for several years, but recently has gained interest as its importance for understanding evolutionary processes has been elucidated. One great example of phenotypic plasticity is change in coloration; animal color patterns are also an excellent model system for studying evolution. Amphibians exhibit extraordinary color and color pattern variation which makes them an ideal group for studying pigmentation and evolution. *Pristimantis bogotensis* is an endemic species to Colombia and it presents a wide array of variations that had not been studied yet. The morphological variations and identification of phenotypic plasticity of *Pristimantis bogotensis* were studied via the descriptive and quantitative analysis of pigmentation, color, and design patterns of specimens present in a biological collection. Seventeen design patterns were identified and described, and their respective frequencies were calculated. A color palette was found for the sample, were 72 different colors were identified. Additionally, the percentage of melanin was calculated and a possible latitudinal cline in the degree of melanization of *P. bogotensis* was also evaluated. Finally, some suggestions for future research are made, proposing the need for an interdisciplinary and integrative approach for the study of coloration.

Key words: cryptic coloration, polymorphism, perceptual switching, image threshold.

Introduction

Phenotypic plasticity can be defined as the property of an organism (or genotype) to produce a range of different phenotypes in response to environmental variation (Scheiner, 1993; Schlichting & Smith, 2002; West-Eberhard, 2003). Nonetheless, there are several definitions that have been used to describe the term phenotypic plasticity (Woltereck, 1909; Bradshaw, 1965; Scheiner, 1993; Schlichting & Pigliucci, 1998; Pigliucci, 2001; Schlichting & Smith, 2002; West-Eberhard, 2003; Dewitt & Scheiner, 2004; Ananthakrishnan & Whitman, 2005; Freeman & Herron, 2007; Whitman & Agrawal, 2009).

Studies of phenotypic variation are exceptionally important for understanding the processes of evolution (Rosenblum, 2005). Organisms deal with prevailing environmental conditions by one of two approaches, by evolving new genetic adaptations or through phenotypic plasticity (PP) (Stanbrook *et al.*, 2021). PP is ubiquitous in nature and can affect morphological, physiological, behavioral aspects of an organism's phenotype as well as its life-history traits (Miner *et al*., 2005; Sommer, 2020). Different types of phenotypic plasticity (e.g. Opportunistic-switch plasticity, across-generational plasticity, coexisting morph plasticity) can contribute to adaptive evolution when populations are faced with new or altered environments (Ghalambor *et a*l., 2007; Sommer 2020). Although genetic and plastic traits contribute to adaptation in new environments, it is PP that allows species to persist in a wider range of conditions, facilitates occupation of novel habitats, and also provides a faster response to environmental changes (Agrawal, 2001).

One extraordinary example of PP in animals is the capacity for color change (Sköld et al., 2016). Change in coloration is associated with developmental PP and in many cases its adaptive (Stevens, 2016). This change can be either physiological or ontogenic depending on the type of stimuli the organism is reacting to, whether its long or short term (Stevens, 2016). PP can also be visualized through color change, this can include modifications that happen through physiological changes, long-term changes related to cellular distribution and morphology, pigment synthesis, and developmental changes (Umbers et al., 2014; Stuart-Fox & Moussalli, 2009). Animal color patterns are accessible for study and experimental manipulation, and consist of various components that have measurable interacting functions, thus making them an excellent model system for understanding evolution (Endler & Mappes, 2017). Animal coloration is important in various ecological contexts, including intra and interspecific communication, anti-predator defense, thermoregulation, mate choice, male-male competition, courtship, and other ecological interactions with direct impact on individual fitness (Rodríguez *et al*., 2020). Due to its wide-ranging functions, animal coloration is frequently characterized by phenotypic variation among and within species (Freeborn, 2021). In amphibians, PP often occurs to such an extent that it can be expected in almost every measurable trait (Urban *et al*., 2013; Relyea, 2001). There is a relationship between the variation of some morphological characteristics like pigmentation and the evolutionary processes that can occur in species, this translates to phenotypic diversity (Prieto *&* de Polanco, 2009). The majority of studies in camouflage and plasticity have focused on a small number of species that are able to change incredibly fast (Stevens, 2016).

Amphibians have a wide variety of colors in skin pigmentation (Alho *et al.,* 2010) and their unique colors and patterns can be used for individual recognition (Patel & Das, 2020). Two classes of pigment compounds melanins and carotenoids are responsible for much of the variation in animal coloration (Fox, 1976). Melanins are thought to be involved in a wide range of vital adaptive functions in animals including crypsis, thermoregulation, protection from UV radiation, signaling, and immune function (Jawor & Breitwisch, 2003; Griffith *et al*., 2006). Changes in the degree of melanins in a population could be driven either by environmental induction which is a plastic response or by selection acting upon heritable genetic variation, which would be an adaptation (Alho *et al.*, 2010). Intraspecific variation in melanism, can express itself as distinct color morphs (Alho *et al.*, 2010). It has been shown that melanism provides a thermally adaptive advantage and it also has a genetic base in several taxa (Vences *et al*., 2002), intraspecific pigmentation clines along altitudinal or latitudinal gradients would be expected in nature, since these correspond to ambient temperature.

Amphibians are a befitting group of organisms to study pigmentation and color pattern because of their extraordinary color variation between and within species (Hofmann & Blouin, 2000). Several studies have focused on how color is used in mate choice, anti-predator defense, and pollination, very few consider the use of patterns or textures (Endler, 2012; Rojas, 2016). The genus *Pristimantis* is particularly interesting for its distribution and its wide array of patterns and coloration variety (Goin, 1950; Goin 1960; Hoyos**,** 1991). This is one of the more complex anuran genera because of its richness in species number; in Colombia there have been reported 225 *Pristimantis* species and at least 78 of them are in some threat category (Acosta-Galvis, 2000). Despite the high diversity of species in the *Pristimantis* genus there continues to be a limited amount of information regarding its threats, distribution, ecology, and natural history, additionally, 39 species of this genus are reported in the IUCN with deficient data (Rincón *et al.*, 2014).

Pristimantis bogotensis is a species belonging to the *Craugastoridae* family, distributed in Cundinamarca's moors and the south of Boyacá along 1750-3600 m.a.s.l. (Peters, 1863; Stebbins *et al.*, 1959; Lüddecke *et al*., 1997; Acosta-Galvis, 2000). Geological evidence for the nor-Andean region shows that the formation of the mountain ranges promoted speciation events in the *Pristimantis* genus, consequently this genus has a high diversity and endemism (Lynch & Duellman, 1997; Meza Joya & Torres, 2016; Acevedo *et al*., 2020). This species exhibits morphological variations that have not been examined. The aim of this research is to study the morphological variations of *Pristimantis bogotensis* via the analysis of pigmentation and color patterns of the dorsal spots of specimens present in a biological collection, for the possible identification of phenotypic plasticity in this species. The possible existence of a latitudinal cline in the degree of melanization in the Bogotá robber frog *P. bogotensis* will also be investigated.

Materials and methods

Specimens

For the examination of the *P. bogotensis* specimens present in the biological collection "Museo Javeriano de Historia Natural "Lorenzo Uribe, S.J." (MPUJ)" the data containing the catalog number, the collector label, latitude and longitude and the date of collection was downloaded from the GBIF (Global Biodiversity Information Facility) database. Each and every specimen was carefully examined making sure they belonged to the species *Pristimantis bogotensis* (Figure 1). For the identification of the design patterns and colors that the specimens presented, a collection of images was made, the frogs were placed under the stereoscope in a Petri dish and the images were taken. The frogs were also examined to know the developmental stage they were in (adult or juvenile), whether or not they were used for previous studies and if they were in good condition or missing any body part; these observations were documented in a table (Appendix 1).

Image collection

Each frog was photographed using a Nikon D5200 camera (effective pixel count of 24 megapixels and maximal resolution of 6000 x 4000 px) that was attached to a Nikon SMZ1270 Stereoscope. The frogs were placed one by one in Petri dishes and completely submerged in ethyl alcohol (70 %). In order to ensure that the specimens that were not “properly conserved” didn’t float in the alcohol, remained still, and were completely submerged, two pieces of modeling clay were placed on the rim of the Petri dish with a needle to hold the frog in place (Figure 1). The Petri dish was placed over a blue color background and under the stereoscope in order to take the photograph. To control for fluctuating light conditions two sources of light were used, two small white LED bulbs placed at the sides of the Petri dish illuminating the specimen from above, and a halogen incandescent lamp in the back illuminating the Petri dish (Figure 1).

Dorsal and ventral photographs for every specimen were taken with the blue color background; for those frogs that were bigger than the Stereoscope field of view, two ventral pictures were taken, dividing the specimen in top and bottom half. An additional picture of every frog was taken over a piece of graph paper.

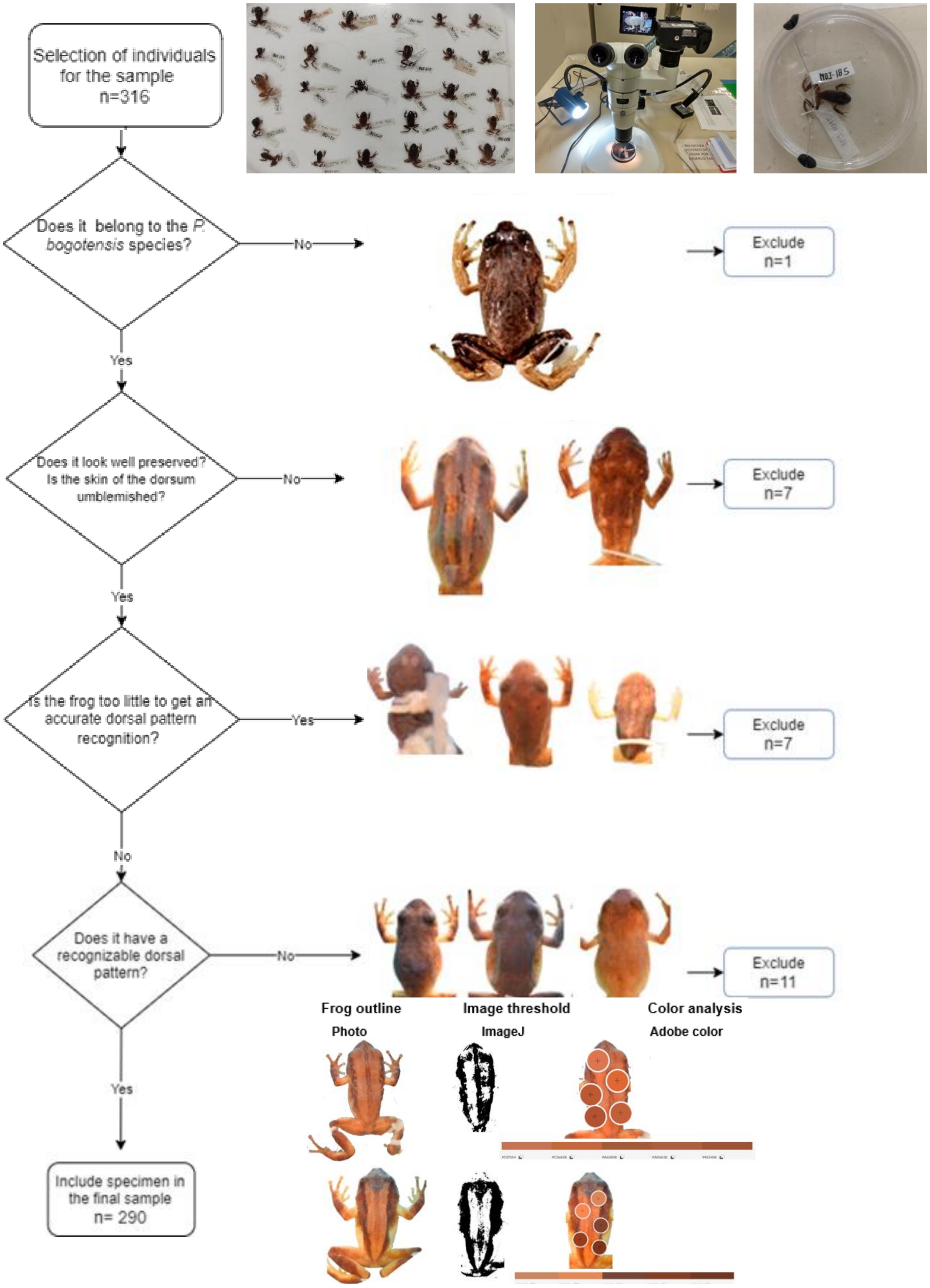

Figure 1: Process of selection of specimens from the original sample, dark pigmentation coverage and color analysis

Image analysis

A total of 1.680 photographs were taken and analyzed, of which, 1.327 were taken with the blue background and 353 with the graph paper. The blue background was removed from every picture with Photoshop (2020) so that only the outline of the frogs remained. With these images the measurement of the Snout-Vent length (SVL) was taken for every individual. Since the specimens were already dead, and thorough identification of the sex could only be done via dissection and taking into account that the frogs are part of a biological collection, this procedure was omitted and the sex of the individuals was not taken into account. The measurement of the Snout-Vent length (SVL) was taken using the ‘straight segment’ tool of ImageJ image analysis software, the scale was set using a known distance of 10 mm with the aid of the graph paper, a straight line was then traced form the tip of the snout to the posterior opening of the cloaca, thus obtaining the SVL. These results were registered in a table (Appendix 1).

The general dorsal patterns present in the sample were identified with an overview of the images taken. For this identification of the dorsal patterns both pigmentation and color were taken into account to get a precise recognition of the patterns. Then, each individual was classified into its corresponding dorsal pattern. Throughout the process, some specimens were excluded from the sample because they did not fulfill some of the requirements that were needed to identify dorsal patterns and/ or because they were in bad condition (Figure 1). The final sample size was 290 frogs.

Color analysis

For the color analysis, the images were examined with Adobe Color (2021) in order to extract the color themes. Each image was uploaded to Abode Color and 5 spots on the dorsal region of the frog were randomly selected to extract the colors from each of the spots and to create a color palette for every specimen in the sample (Figure 1). The identifier numbers for each of the 5 selected colors present in the individual were registered in a table (Appendix 1). The Adobe Color codification was then contrasted with the color catalogue for field biologists (Köhler, 2012). Köhler's catalogue (2012) allows for an accurate description of coloration in organisms and a close approximation of the colors that can be present in anurans. Given that the color palettes from Adobe Color and the catalogue for field biologists (Köhler, 2012) are not the same, the color values needed to be compared, so for every color in the palette from Adobe the closest resembling color from Köhler's catalogue was selected, this was done with the 290 frogs.

Pigment coverage

For the analysis of dark pigment coverage of each individual photographed, the graphic editor ImageJ was used. The images were segmented into darker-lighter regions by means of thresholding. The images were first turned into an 8-bit format and the threshold was set with the ‘Threshold’ function, which segments images based on pixel values. The most suitable thresholding algorithm was selected by viewing the performance of all of the algorithms in two different pictures, the algorithm selected was the ‘Percentile’. Manual alteration of the thresholding level was avoided for the objectivity of the analysis. Then, the polygon selection tool was used to select the area that was going to be measured, the dorsum and the head. With this tool, the frogs’ dorsal area was circumscribed excluding the front and hind legs. The area of pigments was calculated with the ‘Analyze Particles’ command and a percentage of the area (within the previously selected area) that contains darker or lighter pigments was obtained for every image (Figure 1).

Geographic and statistical analysis

The elevation from the places where the specimens were collected was reviewed in the original data downloaded from the GBIF database. Taking into account the patterns the individuals presented and the elevation, maps at different altitudes were made with the QGIS software (Quantum Geographic Information System 3.16.1), department layers, and vector layers of contour lines were also used. Contour layers every 100 m were obtained from the web page IGAC (Instituto Geográfico Agustín Codazzi).

To analyze if melanism in *P. bogotensis* increases as a function of altitude, a Spearman rank correlation test was performed using both Excel and PAST (Paleontological statistics software). To model the relationship between pigment coverage and body size a linear regression was done.

Results and discussion

Design patterns

Anuran polymorphisms manifest as variation in dorsal patterns or in body colors (Hoffman & Blouin, 2000), within the sample of *P. bogotensis* there was evidence for both color and dorsal pattern polymorphism. 17 different phenotypes were identified*;* a description of the patterns is also provided (Figure 2).

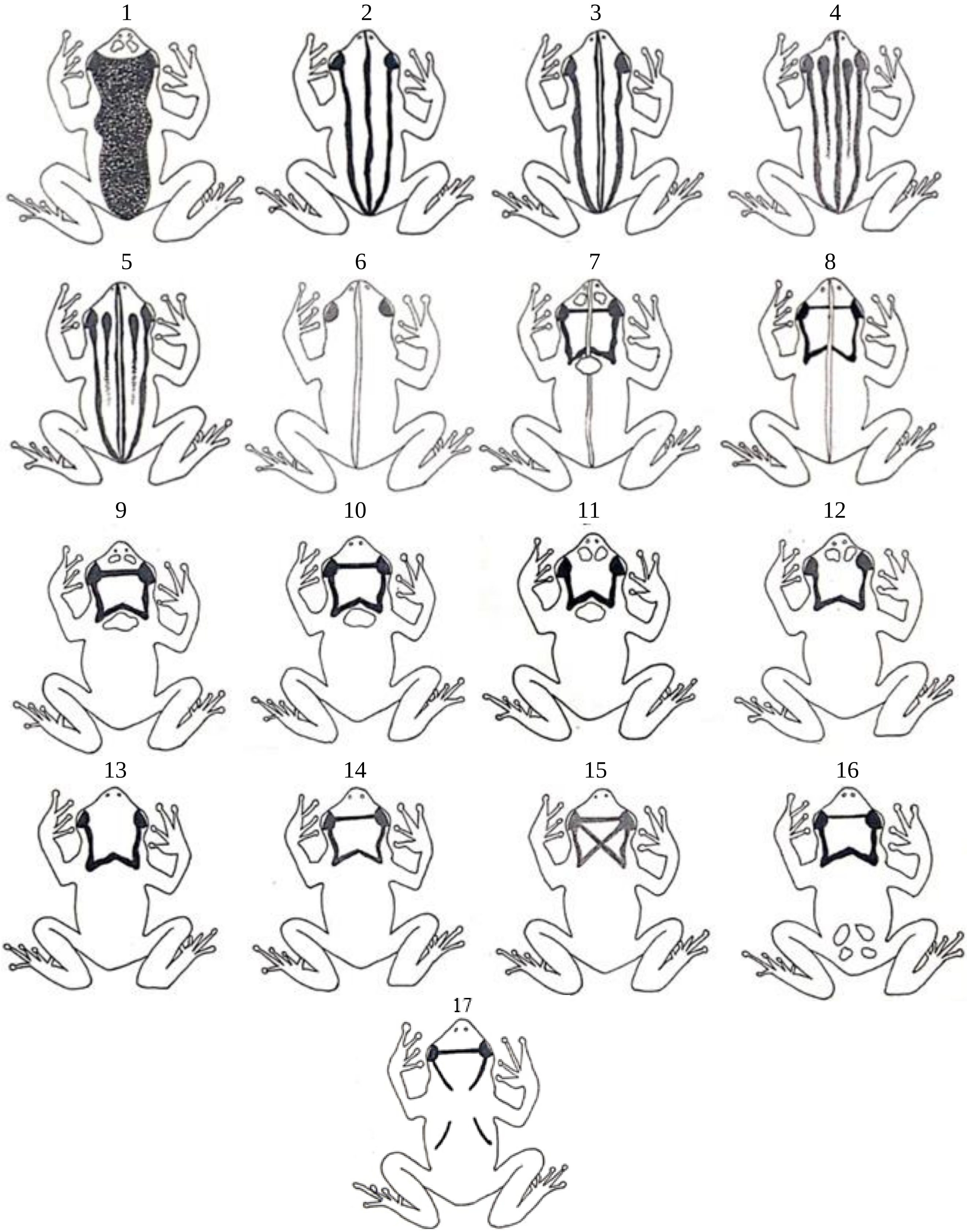

Figure 2: Diagrammatic sketch of the dorsal design Patterns

Description of the patterns and their frequency:

1: This pattern starts in between the eyes and runs along the mid-section of the body extending all the way to the cloaca. The pattern is darker in coloration than the rest of the body and the dorsal sides are usually white or cream color. White spots along the body can be also present. Frequency: 8

2: Three conspicuous stripes, one mid-stripe that originates in the front part of the snout and two dorsal ones that originate in the back part of the eyes. The stripes are darker in coloration than the rest of the body and extend to the cloacal slit. Frequency: 39

3: Three conspicuous stripes, as previously described, but in this case, there is a white or cream color midsagittal stripe in the middle of the darker mid-stripe. Frequency: 5

4: Three conspicuous stripes and two additional stripes that are located at both sides of the mid-stripe. The additional two stripes are lighter in coloration, as they run along the body of the frog the coloration starts to fade and only reaches half of the body. Frequency: 21

5: Three conspicuous stripes and two lighter and shorter stripes as described before, a white midsagittal stripe is present right in the middle of the body. The white stripe originates in the front part of the snout and extends to the cloacal slit. Frequency: 11

6: A midsagittal thin white line that runs along the body. Frequency: 9

7: A W pattern with an interocular line, two white spots in the nostril area, another white spot below the W and a midsagittal thin white line that disrupts the dark patterns. Frequency: 8

8: A dark W pattern with an interocular band and a midsagittal white line that disrupts the W, and the interocular line. This white line originates in the front part of the snout and extends to the cloacal slit. Frequency: 31

9: A W pattern usually dark brown and two white spots in the nostril area, another white spot is present located right below the W pattern. Frequency: 9
10: A dark brown W pattern with an interocular band similar if not identical in coloration to the W pattern and a white or cream spot below the W pattern. Frequency: 7

11: A W pattern with an interocular band a white or cream spot below the W and two white spots in the nostril area. Frequency: 9

12: A W pattern that is dark brown and two white or cream-colored spots present in the nostril area. Frequency: 2

13: A W pattern that has a dark brown coloration. Frequency: 41

14: A W pattern with a straight interocular band that is also dark brown in coloration. Frequency: 68

15: A W pattern with the interocular band and two converging lines in the form of an X that originate in the back part of the eyes and extend to the outer peaks of the W pattern. All of the components of this pattern are dark brown or reddish-brown. Frequency: 9

16: A W pattern with a thin interocular line and four dark spots in the posterior part of the body.
Frequency: 11

17: A dark interocular band, with two other lines that do not connect and originate in the back part of the eyes going inwards, two other short dark lines are present in the middle of the body going outwards. Frequency: 1

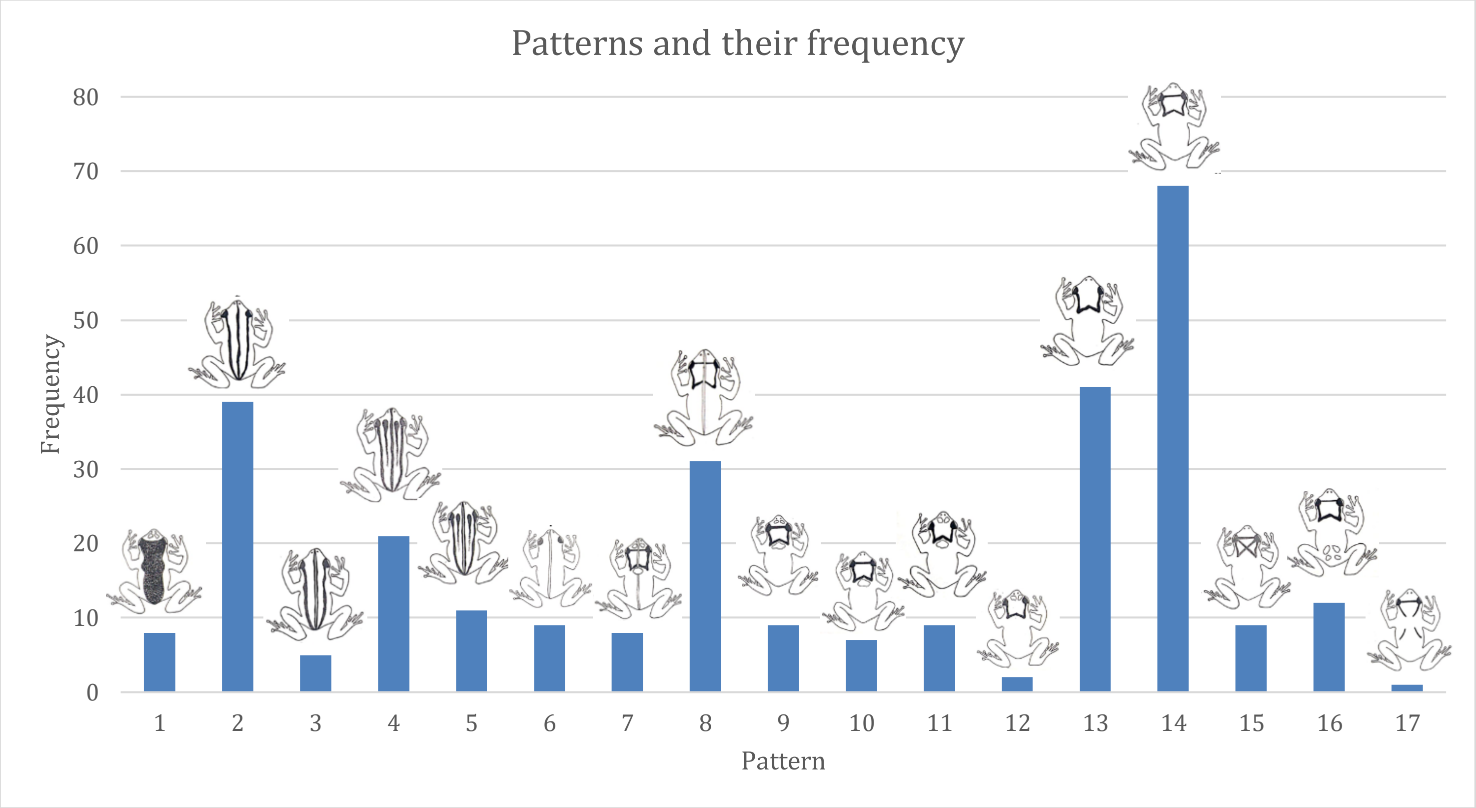

Figure 3: Histogram of the dorsal patterns and their frequency in the sample.

These 17 dorsal design patterns did not have a homogenous distribution within the sample studied (Figure 3). These frequencies show that there are some predominant patterns and some other patterns that have a very low frequency in the sample, which is the case for pattern number 17 that is present only in one specimen in the sample. Throughout the sample the predominant patterns were: 14, 13, 2, 8 and 4. The prevailing pattern above all was the one that presents a W shape with an interocular band. The design patterns also differ in coloration within the specimens. The mean of the SVL measurements was calculated for adult and juvenile specimens, for adults the mean was 53.348 mm and for juveniles was 37.208 mm.

Coloration

Frogs exhibit a wide array of color and pattern polymorphisms including variation in background color and the presence or absence of stripes and spots (Hoffman & Blouin, 2000). Color variation can involve the entire body or just parts of the dorsum; in anurans, polymorphism for red, green, or brown/ gray dorsal color is a common motif (Hoffman & Blouin, 2000). Colors of *P. bogotensis* in life vary, the predominant colors are brown, and reddish-brown in the dorsal region, specimens can also have a light or dark brown color with a cream color in the dorsolateral region (Hoyos, 1991). The loreal region is usually brown and some of the frogs present small grey-blue spots in the back and light blue or violet tones in the axillary region (Hoyos, 1991). It has been reported that in alcohol the dark colors such as brown, dark brown and reddish brown don't really change from what can be seen when the specimens are alive (Hoyos, 1991).

The colors found in the specimens from the biological collection were also variable (Figure 4), the predominant colors being brown, reddish-brown and orange, a cream color was usually present along the sides of the frog. Flesh, and salmon colors were also present as well as gray, blue-gray and lavender tones. The colors found for every specimen were documented (Figure 4), where each number represents one of the colors in the catalogue for field biologists (Köhler, 2012). There is a high variety of colors, and it is important to take into account that the 5 spots on the dorsum of the frogs were randomly selected. The color palette and its frequencies in the sample (Figure 4) reveal that most of the colors present in the frogs are earth tones that emulate natural colors found in moss, leaf litter, soil, trees and rocks; these types of colors are consistent with the frogs' surroundings which helps them camouflage in their habitat. This type of camouflage is called cryptic coloration.

Although the specimens that were used here were already dead they were well-preserved and the colors found are consistent with previous descriptions of coloration of the *P. bogotensis* species; which indicates that working with conserved specimens in order to analyze color and pigmentation is possible and suitable. These results open up several possibilities for taxonomic and color-related research using individuals present in biological collections.

Light Buff Color 2 f: 5	Pale Pinkish Buff Color 3 f: 2	Burnt Orange Color 10 f: 1	Cream Color Color 12 f: 1	Tawny Olive Color 17 f: 6	Clay Color Color 18 f: 5	Drab Color 19 f: 9	Mars Brown Color 25 f: 5	Hazel Color 26 f: 35
Walnut Brown Color 27 f: 22	Kingfisher Rufous Color 28 f: 14	Robin Rufous Color 29 f: 28	Chestnut Color 30 f: 31	Cinnamon-Rufous Color 31 f: 33	Raw Sienna Color 32 f: 1	Mahogany Red Color 34 f: 44	Ferruginous Color 35 f: 90	Brick Red Color 36 f: 59
Burnt Sienna Color 38 f: 79	Warm Sepia Color 40 f: 14	Sayal Brown Color 41 f: 2	Dark Drab Color 45 f:1	Burn Omber Color 48 f: 14	Cinnamon-Drab Color 50 f: 34	Cream White Color 52 f: 4	Pale Cinnamon Color 55 f: 5	Orange-Rufous Color 56 f: 17
Flesh Ocher Color 57 f: 61	Salmon Color Color 58 f: 30	Dark Salmon Color Color 59 f: 9	Tawny Color 60 f:4	Dark Carmine Color 61 f: 26	Crimson Color 62 f: 1	Poppy Red Color 63 f: 1	Peach Red Color 70 f: 3	Light Pratt's Rufous Color 71 f: 2
Pratt's Rufous Color 72 f: 12	Chamois Color 84 f: 1	Blue Black Color 187 f: 2	Indigo Color 190 f:1	Dark Blue Gray Color 194 f: 2	Lavender Color 202 f: 2	Dark Lavender Color 203 f: 29	Pale Mauve Color 204 f: 1	Vinaceous Pink Color 245 f: 106
Light Russet Vinaceous Color 246 f: 77	Deep Vinaceous Color 248 f: 70	Flesh Color Color 249 f: 53	Light Flesh Color Color 250 f:18	Salmon Color Color 251 f: 33	Dark Salmon Color Color 252 f: 16	Pink Flesh Color Color 253 f: 6	Beige Color 254 f: 11	Cinnamon Color 255 f: 15
Drab-Gray Color 256 f: 3	Medium Fawn Color Color 257 f: 1	Fawn Color Color 258 f: 5	Cinnamon Drab Color 259 f:29	True Cinnamon Color 260 f: 34	Ground Cinnamon Color 270 f: 3	Sepia Color 279 f: 6	Raw Umber Color 280 f: 21	Fuscous Color 283 f: 2
Dark Grayish Brown Color 284 f: 1	Dusky Brown Color 285 f: 9	Brownish Olive Color 292 f: 1	Plumbeous Color 295 f:1	Pale Neutral Gray Color 296 f: 1	Light Neutral Gray Color 297 f: 15	Medium Neutral Gray Color 298 f:37	Dark Neutral Gray Color 299 f: 67	Jet Black Color 300 f: 1

Figure 4: Color palette found in the specimens, using Köhler'**s** color codes; and their frequency (f).

The mode of inheritance for these color polymorphisms has been investigated in few species and decisively demonstrated in only two species *Discoglossus pictus* (Lantz, 1947) and *Rana pipiens* (Volpe, 1961; Anderson & Volpe 1958). Some studies involving single generation crosses have suggested that background colors are genetically determined in some species (Summers *et al.,* 2004), however, in other species its linked with aspects of environmental variation (Wente & Phillips, 2005). Additionally, some species that present color patterns, such as stripes or melanistic patterns exhibit simple Mendelian inheritance, while patterns such as spots present in other species are partly determined by environmental factors (Summers *et al.,* 2004; Goin, 1960). The occurrence of color polymorphism in natural populations can result from biased mutations, trade-offs and pleiotropy, gene flow, spatially and temporally fluctuating selection, and negative frequency-dependent selection that can counter the loss of variation by genetic drift (Gray & McKinnon, 2006; McKinnon & Pierotti, 2010). Moreover, phenotypic and developmental plasticity can allow the maintenance of polymorphism, if they don't imply a significant cost (Valverde & Schielzeth, 2015), which can be especially advantageous in variable environments.

Many organisms have evolved different adaptations that reduce the risk of being eaten by predators, common among these are color patterns that help to camouflage the animal (Woolbright & Stewart 2008).
One example of such concealing coloration can be found on many terrestrial anurans with characteristic dull browns, grays, and blacks (Duellman & Trueb, 1986). Cryptically colored anurans are often polymorphic, exhibiting a variety of distinctive patterns (Woolbright & Stewart 2008). Several studies have shown that color and design patterns can reduce the risk of predation either because they disrupt the outline of the animal, or because they help the animal match its background (Stevens & Cuthill,2006). Therefore, cryptic coloration reduces the prey's vulnerability to visually searching predators (Edmunds, 1974). Polymorphism can also serve a protective function if predators that forage in polymorphic populations suffer from reduced efficiency, as a result of longer prey information processing time (Punzalan *et al.,* 2005).

Irregular markings and stripes present in many amphibians are thought to function as disruptive coloration because they break the body image and confuse predators (Duellman & Trueb, 1986). *Pristimantis bogotensis* is a species that can be found under rotting trunks or under rocks during the day and at night can be found between leaf litter or hidden in moss, and its cryptic coloration and design patterns helps them disrupt their outline and blend into its surroundings avoiding predators. This disruptive coloration breaks the appearance of body form, and camouflage can be enhanced by having high-contrast lines or blotches on the edges of colored patterns (Duellman & Trueb, 1986; Toledo & Haddad, 2009).

It has been suggested that the coexistence within a population of two or more phenotypic variants may contribute to reduce predation pressure and enhance survival in polymorphic prey populations (Glanville & Allen, 1997). Such protective function of color polymorphism may arise if predators are conservative in their choice and search of prey (Wennersten & Anders, 2009), and feed disproportionately on prey similar to well-known items or if they search for one type of prey at a time (Croze, 1970; Allen, 1984). More specifically, if predators prey disproportionately on the most common phenotypes, this will confer cryptic polymorphic species with an advantage over monomorphic species (Clarke, 1962; Bond, 2007; Rojas, 2016). Consequently, if predators select in favor of abundant color patterns or phenotypes this will drive their numbers down, while rare ones will be overlooked and allowed to increase, this in turn could stabilize the numbers of an array of alternative color phenotypes; this mechanism is called apostatic selection (Clarke, 1962).

Several studies have shown that behavioral processes at the level of individual predators are responsible for generating apostatic selection (Endler, 1988; Bond, 1983; Dukas & Kamil, 2001). Search image formation is one of the most widely investigated mechanisms (Punzalan *et al.*, 2005; Dawkins 1971, Bond,1983), and it is defined as a perceptual change in the ability of a predator to detect familiar cryptic prey (Dawkins, 1971; Punzalan *et al.*, 2005). Tinbergen (1960) compared the number of larvae captured by great tits (*Parus major*) and their relative abundance and saw that while rare prey were generally avoided or left unnoticed the birds had captured disproportionately more of the common prey. He proposed, this was due to the result of perceptual processes occurring in the predator, referred to as ‘adopting a search image’, through which prior experience with a prey-type facilitates the detection of that specific prey-type in later encounters (Punzalan *et al.*, 2005). The adoption of a searching image can provide predators with an improved ability to detect cryptic prey items, as follows, prey that are detected more often or more recently will be found more readily than those with which the predator has had less experience (Bond, 2007).

Although color pattern polymorphisms among cryptically color anurans are fairly common, the specific mechanisms that allow for their maintenance still remain unclear. Extending Timberg’s model of perceptual switching, Clarke (1962) argued that perceptual switching could account for the maintenance of color polymorphism in cryptic prey species (Bond, 2007). Hoffman and Blouin (2000) argue, that predation could be the selective pressure that contributes to color pattern diversity, and state there is evidence that variation in color patterns can be inherited (Rojas, 2016; Wente & Phillips, 2005). Hochkirch *et al.,* (2008) studied the influence of substrate color on various aspects of the coloration of two grasshopper species and found that color polymorphisms may not only be maintained by natural selection acting on color patterns, but also by phenotypic plasticity, which enables organisms to adjust to the environmental conditions experienced during ontogeny. For *Pristimantis bogotensis* the developmental stage data was analyzed and there were no indications of a relationship between the developmental stage the organisms were in and the patterns they presented.

Adaptive evolution requires traits with heritable variation (Rosemblum,2005). Many ecologically relevant characters have a simple genetic basis, nonetheless, environmental variation can affect phenotypes in a number of complex ways (Schlichting & Pigliucci, 1998). Often, studies in natural selection assume the traits of interest are genetically based overlooking the potential importance of phenotypic plasticity (Rosenblum, 2005). Some authors use the term ‘‘polyphenism’’ to describe environmentally determined variability and to highlight the difference to purely genetic cues (Hochkirch *et al.,* 2008), however, it is worth noting that any physiological response to environmental cues is highly likely also genetically determined (Suzuki & Nijhout. 2008; Hochkirch *et al.,* 2008), and, consequently phenotypic plasticity is and should be regarded as a complement rather than a contrast to genetic determination. When it comes to the production of a phenotype, genes and environment are inextricably linked (Crispo, 2007). Although the genetic basis of coloration and the modes of inheritance for the color and pattern polymorphisms in *Pristimantis bogotensis* have not been studied yet, taking into account the results of this study it is reasonable to suppose that the coloration and the patterns described here have both a genetic and an environmental component, where phenotypic plasticity plays an important role in the determination of the color and pattern diversity present in *P. bogotensis*.

The color and pattern polymorphisms found here in the *Pristimantis bogotensis* species need to be further studied taking into consideration the genetic makeup of the specimens to determine if these phenotypic variations are the result of phenotypic plasticity. Furthermore, it is important to point out that this study was done using the human visual system; coupling coloration and pattern studies with various predator visual systems can provide a deeper understanding of how these colors and patterns are perceived by their predators, thus gaining insight into the predator-prey relationship. This in turn, will help us gain a better understanding of the adaptive functions of color and pattern variations in species.

Reasons for the patterns

Environmental conditions are known to affect melanin density at several temporal scales (Rosenblum, 2005). Physiological or rapid color change can occur in response to short-term stimuli, this can happen when existing melanin becomes aggregated or dispersed (Rosemblum, 2005), so the animal will be lighter or darker in coloration. Ontogenic color change can occur in response to long-term environmental stimuli as melanin increases or decreases (Rosemblum, 2005). It has been shown that in some amphibians the degree of melanism can increase as a function of latitude or altitude (Alho *et al.*, 2010; Riobó *et al.*, 2000). Other studies show that in at least some anurans melanism increases with their development (Riobó *et al.*, 2000). In this research the developmental stage, whether the frogs were adults or juveniles was evaluated, this and the Snout vent length (SVL) allow for an approximation regarding the development of the individuals. To understand the correlation of the portion of dark pigment coverage with body size (SVL), the data was analyzed with a linear regression $y= -0.012x+ 60.78$ $R^2 =0.0133$. According to this results, there is no association between the variables, the percentage of body pigmentation is not related to body size for the sample.

Furthermore, the resulting values of the Spearman rank correlation test were (r_s: -0.06943518 pValue: 0.238495817) this suggest that there is a very weak correlation between the degree of melanism and altitude for this species. It is important to note, that dark pigmentation was measured for every frog and the amount of dark pigments vary considerably between individuals. Taking into account the thermally adaptive hypothesis that states that melanins provide a wide range of vital adaptive function including thermoregulation, a pigmentation cline along a latitudinal or altitudinal gradient would have been expected, however there are few cases in which this has been reported in amphibians (*Rana temporaria*), this types of clines are seldom reported in other taxa than insects (Alho, 2010). Furthermore, both the adaptive nature and the genetic basis of melanism clines remain to be investigated.

Altitude is inversely proportional to its temperature, and a main characteristic of the tropics is that altitude is influenced by temperature in a different way that in other areas around the world. As proposed by Janzen (1967), organisms inhabiting tropical areas have a lower range of tolerance when it comes to environmental conditions than those species present in temperate areas; because tropical areas experience relatively uniform climatic conditions. This promotes the specialization of species to a narrow set of climatic conditions. Since tropical species would adapt to a small set of climatic condition, Jansen (1967), proposed they would exhibit smaller elevation ranges and that species turnover along elevational gradients would be higher in the tropics (Page & Shanker, 2020).

The moors are a type of habitat that present extreme environmental conditions such as low temperature, high relative humidity, low atmospheric pressure, abrupt temperature changes, among others (Saenz, 2001); these allow for the formation of regions with microclimates. Frogs inhabiting this ecosystem can be subject to unique and specific conditions that may not be found anywhere else. The general geographical distribution of patterns is represented on the map (Figure 6), this figure shows that there is no agglomeration of individuals based on their dorsal design patterns instead, there is a mixture of patterns. Although most of the patterns are clustered together in a common area, some of them can be found further apart, which is the case for pattern number 14, which was located in the northeastern region, in the department of Boyacá. The maps showing the patterns present at different altitudinal scales (Figure 6a) and the histogram (Figure 6) reinforce the previous statement that there is no agglomeration by patterns and indicate that there is an increase in frequency of *Pristimantis bogotensis* between 2900 and 3200 m.

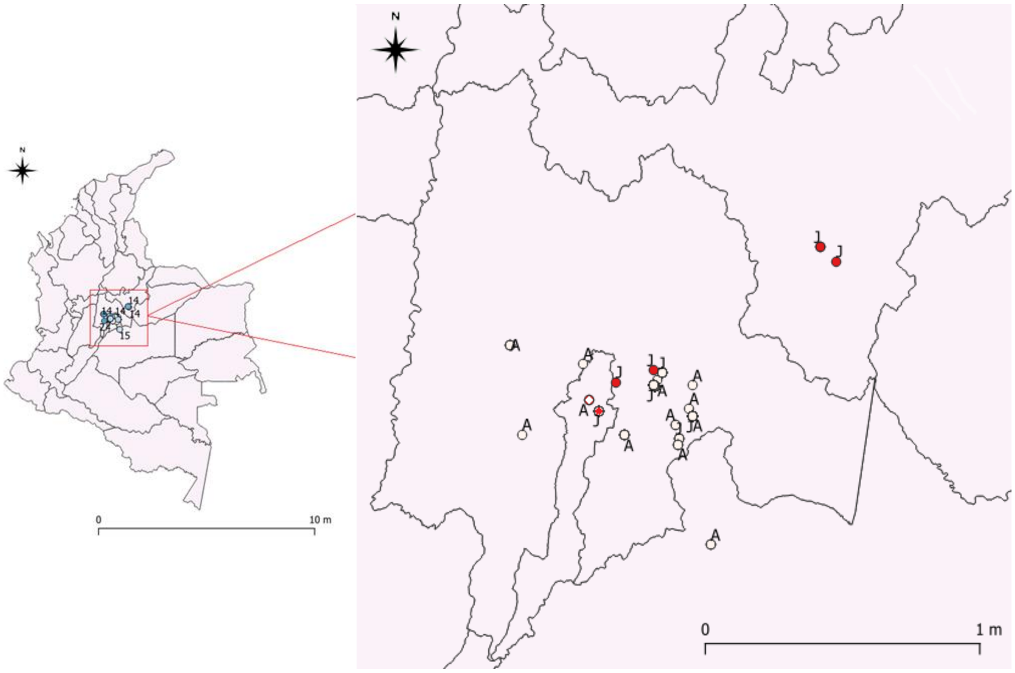

Figure 5: Geographical distribution of individuals by developmental stage, juveniles are represented by a red dot and a J (Juvenile) and adults with a white dot and an A (Adult).

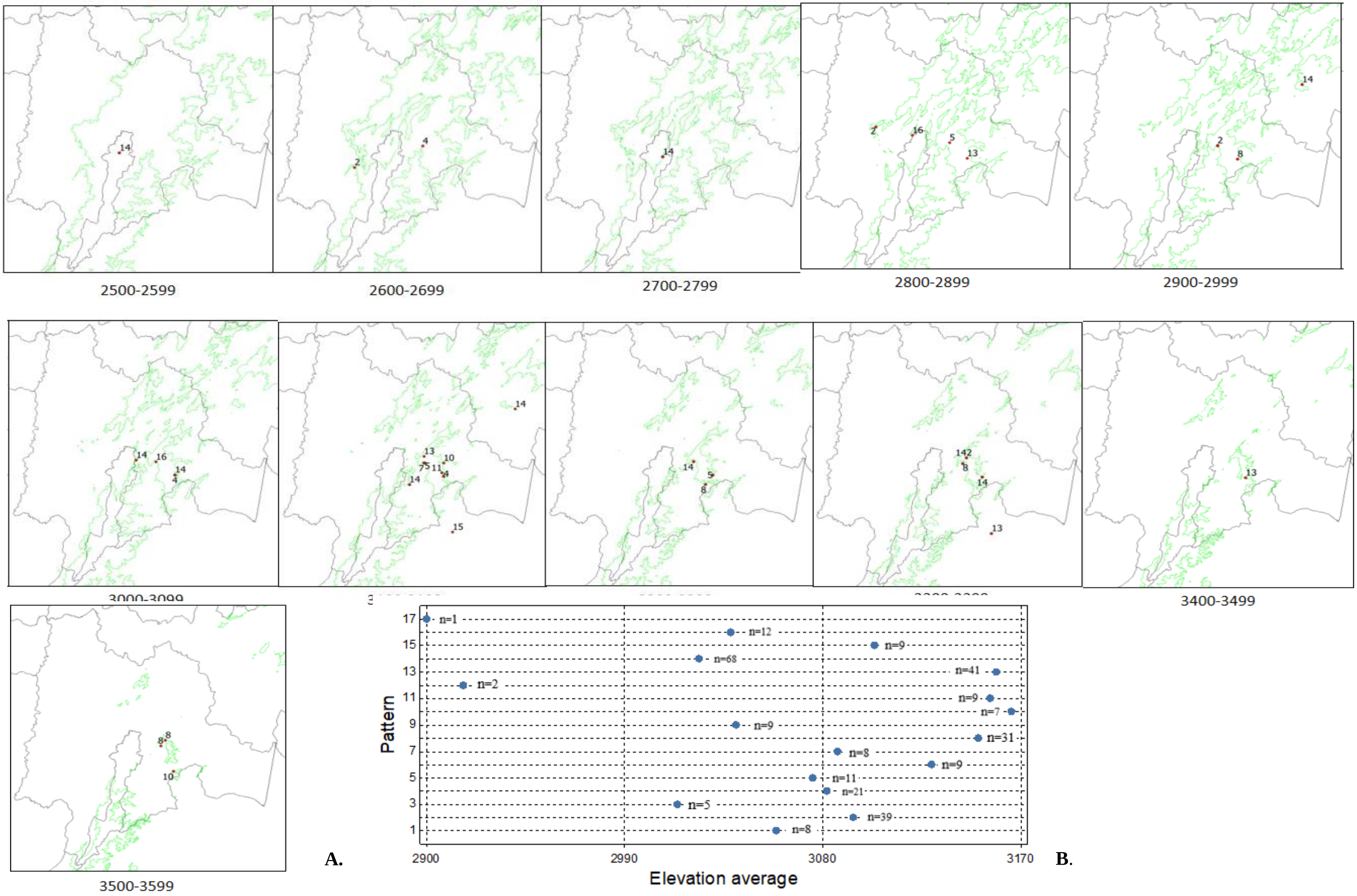
2500-2599
2600-2699
2700-2799
2800-2899
2900-2999
3000-3099
3400-3499
3500-3599
A.
Pattern
Elevation average
2900
2990
3080
3170
1
3
5
7
9
11
13
15
17
n=1
n=12
n=9
n=68
n=41
n=2
n=9
n=7
n=9
n=31
n=8
n=9
n=11
n=21
n=5
n=39
n=8
B.

Figure 6: Design patterns a) shown in a map with different contour lines and b) its elevation average.

The study of the molecular and genetic basis of pigmentation in amphibians has only recently received some attention, and it has been shown that changes in gene regulation during development could influence the coloration of organisms (Gompel *et al.*, 2005; Hochkirch *et al.*,2008). If the activation of such regulatory genes is affected by environmental conditions, color polymorphism might be a consequence of environmental heterogeneity rather than a consequence of genetic variability (Hochkirch *et al.*,2008). In amphibians integumentary coloration is produced by three types of chromatophore cells: melanophores, xanthophores, and iridiophores (Rodríguez *et al.*, 2020; Bagnara *et al.*,1968). Melanophores synthesize brown/black melanin pigment, xanthophores express yellow/red carotenoid pigments and iridiophores produce crystals that contribute to structural coloration (Rodríguez *et al.*, 2020). Although the genetic basis of pigmentation for *Pristimantis bogotensis* were not evaluated here, the amount of melanin as a percentage for every individual was found, this was taken into account and evaluated for a possible altitudinal pigmentation cline in the sample. The amount of melanin present in the individuals was also evaluated against the developmental stage they were in; the results show that the amount of melanin was not dependent on the developmental stage for this species.

Although the individuals are not grouped together by design patterns, there are patterns that appear more often at certain altitudes. The elevation average for the 17 patterns was plotted (Figure 6b), only two of the pattern's elevation average was below 2990 m, number 17 that is represented only by one individual in the sample, and number 12. The rest present an elevation average ranging from 3100-3160 m. It is highly likely that this trend in pattern frequency is due to the particular conditions of the habitat this individuals are in, as temperature and humitidy change along altitudinal gradients; it is important to take into account that amphibians are exceptionally sensitive to environmental change.

Even though an altitudinal trend in the degree of melanization was not found, it is important to thoroughly research the intraspecific variation of dark pigmentation found here between the individuals to clarify the possible adaptive value of this coloration. It is also crucial to study the genetic basis for the color polymorphism in *Pristimantis bogotensis* and investigate if this color variation is mediated by natural selection, sexual selection, genetic drift, or by a combination of these factors.

Considerations for future research

This work underlines the importance and value of biological collections and illustrates the possibility of examining coloration in well-preserved organisms. It also advocates for the proper conservation treatments as well as a complete, systematic, and consistent record of the information of each collected specimen and its observations. In this research color and pattern polymorphisms were found, future investigations on this pattern and color polymorphism will provide essential data for understanding the evolution and maintenance of color polymorphism as well as its ecological, evolutionary basis, and adaptive function.

Color polymorphic species can be considered important model systems (Endler & Mapes, 2012) with which researchers can infer and understand the connections between evolutionary processes. Color patterns are part of a network where various factors are connected and interacting simultaneously, this includes the environment, the signalers, the receivers as well as their behavior. Taking this into account, and taking into consideration that organisms are integrated complex phenotypes, an integrative approach is needed to understand phenotypic plasticity and the role it plays in color and pattern variation. Consequently, further research should address these types of questions with aspects relating to genetics, molecular biology, epigenetics, ethology, population ecology, and cognitive psychology, among others. Furthermore, methods that allow for the quantification of plasticity and plasticity experiments conducted under circumstances that are similar to those that organisms may encounter in the wild are needed; in addition to experiments allowing the manipulation of developmental plasticity. There is also the need for a consensus within the scientific community regarding the definition of phenotypic plasticity (Bradshaw, 1965; Scheiner, 1993; Pigliucci, 2001**;** Schlichting & Smith, 2002; Freeman & Herron, 2007; Whitman & Agrawal, 2009).

Thanks to the advances in technology there are now several tools available for identifying, measuring, and quantifying colors and pigments in nature that boost studies in this field, but there are still big gaps in frog coloration research. There's still much to do in this new era of color science that with an interdisciplinary and integrative approach holds enormous promise.

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