Trabajo

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Color pattern polymorphism and chromatic aberrancies in *Physalaemus fernandezae* (Anura, Leptodactylidae)

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ABSTRACT
Skin coloration patterns in anurans could be markedly varied due to color pattern polymorphism and the occurrence of chromatic aberrancies. Knowledge about this variability allows to understand prey-predator relationships, contributes to the characterization of taxonomic groups, and may reflects genetic variability. We report herein a characterization of dorsal and ventral color pattern polymorphisms in the Neotropical frog *Physalaemus fernandezae*, and the finding of chromatic aberrancies in wild tadpoles. We assessed the sexual variation of four dorsal color morphs (Broadband, Reticulate, Vertebral Line, Uniform with Urostylar Line) and two ventral color morphs (Immaculate and Maculate). We found no association about the co-occurrence of different ventral and dorsal color patterns, also regardless sex, suggesting independent evolution forces influencing on them. In addition, we found geographic color pattern variation. A color aberrancy considered as leucism was observed in tadpoles, and is reported for the first time in *P. fernandezae*. We suggest that the patterns frequency distributions are suggestive of an apostatic selection mechanism for the color polymorphism in *P. fernandezae*.

Key words: Albinism; Color morphs; Crypsis; Leucism; Ventral; Whistling Dwarf Frog.

Introduction

Coloration and pattern variation in anurans are exhibited under two major and widespread phenomena, polymorphism and chromatic aberrancies. Polymorphism occurs when two or more discrete phenotypes coexist within the same population, and is usually studied regarding the dorsal and ventral skin external color variation (Hoffman and Blouin, 2000). Color patterns may also vary with sex, presumably as a result of sexual selection (Kramek and Stewart, 1980; Buchanan, 1994), or in the context of differences in microhabitat use, and cryptic behavior between males and females (Bourke *et al.*, 2011). Also, the occurrence of patterns can differ among geographic regions (Bonansea and Vaira, 2012), associated with the crypsis that substrates can provide (Rabbani *et al.*, 2015). Besides, color patterns may

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also vary seasonally within a population (Bourke *et al.*, 2011). The study of color pattern polymorphism in anurans is useful to understand for instance predator-prey relationships (Nevo, 1973; Bourne, 2001; Woolbright and Stewart, 2008; Toledo and Haddad, 2009), to solve taxonomic problems (Heyer, 1970; Nascimento *et al.*, 2005), and as a method for individual recognition in capture-recapture field studies (Doody, 1995).

The additional source of conspicuous skin color variation presented by anurans are "inherited chromatic aberrancies" (as referred by Borteiro et al., 2021), consisting of enhanced or diminished coloration, in this last case with partial or complete suppression of pigments like in leucism or albinism respectively (Rodrigues and Oliveira Filho, 2004; Salles *et al.*, 2013: Moraes and Kaefer, 2015; Brown *et al.*, 2020; Tavares-Pinheiro *et al.*, 2020; Hemnani *et al.*, 2021). Leucism affects the presence of chromatophores in the skin producing noticeable, almost total, depigmentation of the body that provides an overall whitish aspect without affecting the eyes (Henle *et al.*, 2017).

Both sources of color variation may have a different relationship with predation, and consequently survivorship. For instance, researchers usually assume that color pattern polymorphism in anurans has an adaptive cryptic function influenced by different factors such as predation, in the context of microhabitat use (Nevo, 1973). Visually oriented predators promote the differential selection of the color patterns and persistence of the rare phenotypes under a mechanism called apostatic selection (Clarke, 1969; Bond and Kamil, 2006). This hypothesis suggests that once predators identify a prey, predation pressure may be higher on the patterns most commonly discovered by them. Consequently, other patterns may increase in abundance and become more selected. This system of multiple color patterns could represent an evolutionary advantage, since the chance of detection by predators using a search image for a single morph are overall lower in polymorphic species (Bourne, 2001). Furthermore, there may be variation in the degree of crypsis among the pattern morphs that are under apostatic selection, keeping the less camouflaged morphs at lower frequencies (Bond and Kamil, 2006), that may also vary between populations (Crawford, 2000).

On the other hand, the inherited chromatic aberrancies causing depigmentation are presumably associated to enhanced predation by visually oriented predators (Sazima and Pombal, 1986). Consequently, depigmentation aberrancies would be more frequent in animals with less dependence on camouflage to survive, such as fossorial species (Sazima and Di-Bernardo, 1991). In addition, chromatic aberrancies such as leucism or albinism can be associated to deleterious traits that correlate with lower survivorship (Maneyro and Achaval, 2004; Browder, 2005), tadpole scoliosis (Sanabria *et al.*, 2010), diminished tadpole developmental rate and growth, influencing the size at metamorphosis (Childs, 1953; Sazima, 1974).

High intraspecific color pattern variation was recognized by several authors for the Neotropical frog genus *Physalaemus* (Barrio, 1965; Gallardo, 1965; Cei, 1980; Yanosky *et al.*, 1997; Nascimento *et al.*, 2005). For example, this is noticeable in the southernmost distributed species of the genus, *P. fernandezae* that inhabits the grassland plains of southern Uruguay and the Buenos Aires province in central-eastern Argentina (Barrio, 1964; Barrio, 1965; Gallardo, 1965; Núñez *et al.,* 2004).

Our exploratory research studied both color pattern polymorphism in *P. fernandezae* and chromatic aberrancies. The main goals were: 1) to identify the occurrence of different dorsal and ventral skin color patterns, 2) assess the relative frequency of each pattern in both sexes, 3) explore their geographic variation, 4) report for first time in this species a chromatic aberrancy, and finally 5) interpret the results in the context evolutionary processes.

Materials and methods

We examined the skin color pattern of 520 specimens of *P. fernandezae* from 24 localities throughout its whole geographic distribution. The sample included 202 specimens housed at the herpetological collection of Museo Argentino de Ciencias Naturales "Bernardino Rivadavia" Buenos Aires, Argentina (MACN and CENAI), 91 from Museo de La Plata, Argentina (MLP), 101 from Universidad Nacional del Nordeste, Corrientes, Argentina (UNNEC), and 126 live individuals observed (but not collected) during surveys held throughout localities in Buenos Aires Province, Argentina: Punta Indio, 24 July 2005 (n = 28); Punta Lara, 28 July 2005 (n = 76); La Balandra, 23 June 2006 (n = 22). We determined sex of individuals according to presence/absence of external nuptial features, consisting the sample of 340 males and 180 females (Barrio, 1964; 1965).

The observation of adults of *P. fernandezae* in life allowed the recognition of four dorsal color patterns (Fig. 1), that were subsequently recognized in specimens housed in collections. The coloration of some of the latter was deteriorated and only in a few of them cases the patterns not were possible to identify. Color patterns considered were the following. *Broadband* (B): characterized by a broad midline dorsal band of irregular edges, extending from the tip of the snout to the vent, usually covering the upper eyelid. The band is clearer than the rest of the spots that complete the dorsal pattern. Many of the spots flank the dorsal band for almost its whole extension, but are absent near the vent. *Reticulate* (R): dorsum dominated by several irregular dark spots that form a reticulate pattern, without a noticeable longitudinal line or band. This pattern fades towards its edges. *Vertebral Line* (VL): characterized by a narrow and well-defined median stripe from the snout to the vent, lighter than the spotted background and dorsal stains to which it markedly contrasts. The dorsal stripe is flanked by dark longitudinal spots with clear centers, which are also fairly noticeable against the light background. *Uniform with Urostylar Line* (UUL): uniformly dark dorsum that sometimes present a few small clear spots, usually including a short and clear line over the urostyle.

The ventral region of the body is far more homogenous than the dorsal color patterns. All the observed variation can be arranged into two phenotypes (Fig. 1). *Immaculate* (I): The belly is brownish, and spots are absent. *Maculate* (M): The belly is covered by small connected clear spots that contrast over a dark background.

To analyze the occurrence of color pattern polymorphism we previously tested the independence between ventral and dorsal patterns using a 2 x 4 Chi-Square contingency tables according Zar (1999). The relationship between the color patterns and sex was tested using Generalized Linear Models, setting each multinomial distribution for the "color pattern" variable setting a logit link function. Finally, we explored the influence of geographic location using the frequency distribution of patterns, only for those localities for which all patterns described for *P. fernandezae* were represented. Analyses were made with the software STATISTICA 7.0.

In addition to the observations made in adults, we recorded anomalous coloration in some tadpoles (Stages 31 – 36; Gosner, 1960) collected on 8 September 2009 at the Punta Lara Natural Reserve (34º 47' 56.6" S 58º 00'43.2" W - Buenos Aires, Argentina) during amphibian surveys made on a vertebrate reserve inventory (Roesler and Agostini, 2012). These tadpoles (n = 10) were raised to reach metamorphosis in captivity and deposited in the UNNEC collection (Appendix I). All froglets were raised in the same aquarium, and provided with fresh leaf litter on which they fed arthropods.

Results

The Chi-Square analysis showed independence between the dorsal and ventral patterns ($\chi2 = 3.73$; df = 3; $p = 0.292$). Therefore, further analyses considered each pattern separately. The dorsal color pattern of eleven female specimens housed at collections, were not possible to identify due to their conservation condition. The frequencies of the different dorsal patterns discriminated by sex were as follows: B = 213 (134 ♂, 79 ♀), R = 186 (121 ♂, 65 ♀), VL = 86 (65 ♂, 21 ♀), and UUL = 24 (20 ♂, 4 ♀) (Fig.

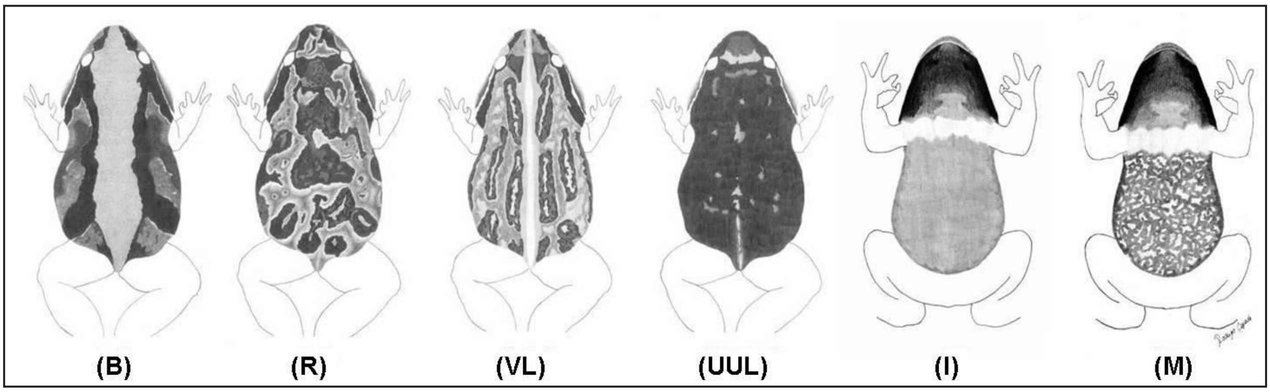

Figure 1. Dorsal and ventral color patterns in *Physalaemus fernandezae*. Dorsal patterns: broadband (B), reticulate (R), vertebral line (VL), uniform with urostylar line (UUL). Ventral patterns: immaculate (I), maculate (M).

2). They frequencies did not significantly differ in male-female comparisons, W = 5.480, df = 3, p = 0.1390. The corresponding frequencies of ventral patterns were: M = 396 (265 ♂, 127 ♀), I = 124 (78 ♂, 46 ♀) (Fig. 2). As occurred with dorsal patterns, the frequencies did not significantly differ between sexes, W= 3.566, df = 1, p = 0.058).

Our data included only 6 localities with representatives of all dorsal patterns, and for which the number of specimens on each sample was ≥ 28. The frequency distribution varied among localities, and the R pattern was the most frequent in four of them, whereas the B pattern was the most common in two (Fig. 3).

Regarding ventral patterns, only one of them was recorded in 9 of the 24 localities. In the remaining, the "Maculate" pattern was more frequent than "Immaculate", in a mean proportion of 15.2 (Fig. 2).

Three of the tadpoles raised in the laboratory were leucistic, two lacked body pigmentation except for the eyes and in the remaining one only the legs were unpigmented (Fig. 4). One of the tadpoles lacking body pigmentation died before reaching metamorphosis, while the others completed it successfully along with normal tadpoles. The two leucistic froglets (Fig. 4) died five days after reaching the metamorphosis, while normal individuals were kept for three months until euthanized, during which they grew normally.

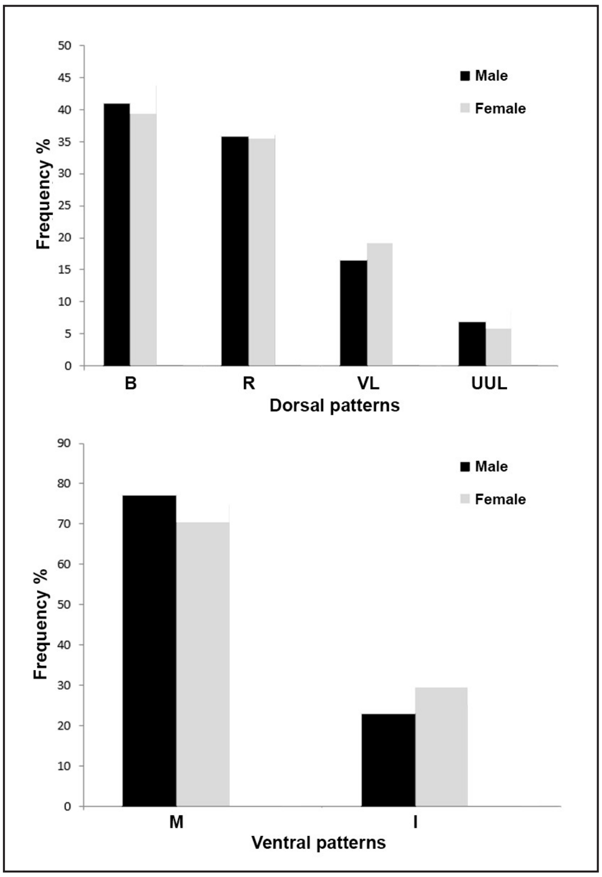

Figure 2. Frequency distribution of dorsal and ventral color patterns in *Physalaemus fernandezae*. Dorsal patterns: broadband (B), reticulate (R), vertebral line (VL), uniform with urostylar line (UUL). Ventral patterns: immaculate (I), maculate (M).

Discussion

The occurrence of color pattern polymorphism was not indicated for *P. fernandezae* prior to this work. Nascimento *et al.* (2005) reported two patterns ("pattern 4" and "pattern 7", see their Fig. 4-D and 4-G) to define the *P. henselii* morphological species group, which included the species *P. fernandezae*, *P. henselii*, and *P. riograndensis* (but see Tomatis *et al.*, 2009; Lourenço *et al.*, 2015). The authors did not indicate which pattern was present in each species. The "pattern 4" quoted by Nascimento *et al.* (2005) is presumably similar to our R pattern, although the specimens we analyzed did not exhibited the "Ω" shaped stain described by them. The varied color patterns exhibited by *P. fernandezae* seem to be distinctive within the genus. From the color pattern drawings presented by Nascimento *et al.*, (2005) we can recognize the main features of two described by us in *P. fernandezae*: the urostylar line of the pattern UUL is present in "pattern 8" of these authors, whereas the vertebral line determining VL is correspondingly present in "pattern 1" (see their Figs. 4-H and 4-A respectively). Remarkably, the B pattern defined herein for *P. fernandezae* seems to be exclusive of this species within the genus. The remaining patterns of *P. fernandezae* are widespread in anurans from several families worldwide, and may be of limited taxonomic value.

The dorsal patterns in *P. fernandezae* show staggered frequency distributions across populations, with one pattern clearly dominant, some others similarly represented, and a pattern barely present. This would account for a pattern polymorphism in *P. fernandezae*, related to an adaptive cryptic function. For instance, the vertebral line pattern could disrupt body shape among vegetation, hindering predators' visual recognition (Nevo, 1973). Visually oriented predators promote the persistence of varied patterns and also of the rare morph types under a mechanism called apostatic selection (Clarke, 1969; Bond and Kamil, 2006), such as the UUL pattern in

P. fernandezae. Apostatic selection usually explains the color pattern polymorphism found in several anuran species (Milstead *et al.*, 1974; De la Riva, 1977; Crawford, 2000; Bourne, 2001). Bond and Kamil (2006) concluded that this polymorphism is influenced by a complex interaction between habitat structure and predator recognition. Polymorphism in *P. fernandezae* could play a cryptic function or camouflage against visually oriented predators like it was proposed in several anuran species (Nevo, 1973; Bond and Kamil, 2006; Bourke *et al.*, 2011).

The geographic variation of the polymorphism present in *P. fernandezae* could be favored by the characteristics of the microhabitat occupied (e.g. substrate) and predation, promoting the dominance of one pattern (Bourke *et al.*, 2011). On the other hand, the geographic variation could be attributable to the load of color patterns present during founder effects, and the random incidence throughout the process of apostatic selection.

Polymorphism in ventral color patterns has been studied in some leptodactylid frogs (Heyer, 1970; Duellman and Morales, 1990; Nascimento *et al.*, 2005). Although these studies linked the ventral patterns to geographic distribution in a taxonomic framework, they did not explain the ventral pattern variation as an adaptive function. As terrestrial anurans have their bellies in contact with the ground, the adaptive cryptic function is plausible for species that call on the water surface, where the ventral skin is exposed to underwater predators. We are unaware of the adaptive function of the ventral pattern of *P. fernandezae*, but our analysis showed a clear independence between ventral and dorsal variation, suggesting that they could be subject to different selective pressures. The differences in color pattern exhibition between sexes were associated to differential microhabitat use in some polymorphic anuran species (see Bourke *et al.*, 2011). We are unaware of differences in habitat use between sexes in *P. fernandezae*, but if being the case it seems not to influence dorsal and ventral pattern polymorphism.

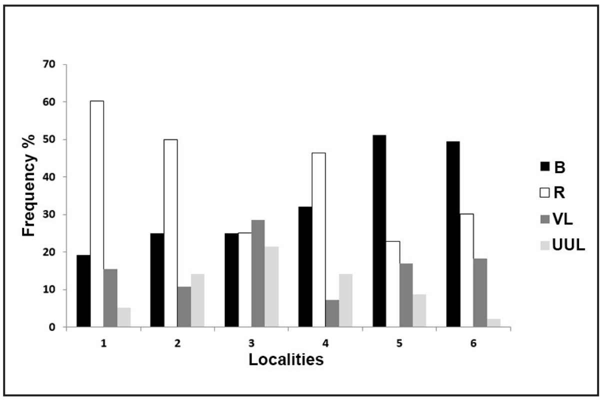

Figure 3. Geographic variation of frequency distribution of dorsal and ventral color patterns in *Physalaemus fernandezae*. Localities: 1 (D´Orbigny), 2 (General Lavalle), 3 (Santiago Vásquez), 4 (Punta Indio), 5 (Punta Lara), and 6 (La Balandra). References: broadband (B), reticulate (R), vertebral line (VL), uniform with urostylar line (UUL), immaculate (I), and maculate (M).

Physalaemus fernandezae is not a strictly nocturnal species (Barrio, 1964; 1965; pers. obs. of authors), and its polymorphism suggests a possible camouflage adaptive function. The chromatic aberrancy cases reported here for larvae could not be confidently attributed to the nocturnal or fossorial behavior hypotheses (Sazima and Di-Bernardo, 1991). Adult specimens, neither those in collections nor the several ones observed by us in nature exhibited chromatic aberrancies, accounting for the likely negative effects of conspicuous hypopigmentation on survival.

In conclusion, *P. fernandezae* is a polymorphic species that shows a highly variable skin regarding both color and pattern, which may include the spontaneous occurrence of aberrant phenotypes like leucism. The frequency distributions of patterns suggest a maintenance mechanism by apostatic selection, although specific studies are needed to test this hypothesis. The similarity of the observed polymorphism with that of representatives of other anuran families (i.e. Hoffman and Blouin, 2000) adds to a large amount of evidence in regard to convergent color pattern evolution in anurans, notwithstanding an old phylogenetic signal (Nevo, 1973; Crawford 2000).

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Figure 4. Leucism in *Physalaemus fernandezae*: tadpole (A) and metamorphic (B) with lack of pigmentation in body, and metamorphic (C) with lack of pigmentations in legs.

Buenos Aires, Argentina (Exp. N° 2578-2294/06).

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Appendix I - Material Examined

Physalaemus fernandezae specimens from the herpetology collections of Museo Argentino de Ciencias Naturales Bernardino Rivadavia (MACN), Centro Nacional de Investigaciones Iológicas, Buenos Aires, Argentina (CENAI, collection now transferred to MACN), Museo de La Plata (MLP), La Plata, Buenos Aires, Argentina, and Universidad Nacional del Nordeste (UNNEC), Corrientes, Argentina.

ARGENTINA: Provincia de Buenos Aires: Ayacucho: MACN 2167; Balcarce: MACN 11012 to 11014, MACN 24771, MACN 24772; Cacharí: MACN 29534 to 29536; Benito Juárez: MACN 23510 to 23515; Cristiano Muerto: CENAI 130-3, CENAI 135-3 to 135-5, CENAI 135-7, CENAI 135-9, CENAI 135-10; D'Orbygny: MACN 11245, MACN 15979, MACN 15992, MACN 16010 to 16015, MACN 21091, MACN 21724, MACN 21725, MACN 21843 to 21851, MACN 21857 to 21863, MACN 30117 to 30123; Dolores: MACN 29509 to 29516; General Lavalle: MACN 29609 to 29611, MACN 29643 to 29653, MACN 30241 to 30245; General Alvear: MACN 21665 to 21672; General Madariaga: MACN 21134 to 21154, CENAI 129, CENAI 1348, CENAI 1353; Ingeniero Maschwitz: CENAI 124-1, CENAI 124-3 to 124-8, CENAI 3441, CENAI 3442; La Balandra: UNNEC 11668 to 11705, 22 unvouchered specimens; Magdalena: MACN 25287 to 25291; Mar Chiquita: MACN 21101 to 21127; Pereyra Iraola: CENAI 128-1, CENAI 128-3, CENAI 128-5, CENAI 128-6; Pirovano: MACN 28267, MACN 28268; Punta Indio: UNNEC 11706 to 11721, 28 unvouchered specimens; Punta Lara: MLP 5264 to 5354, UNNEC 11619 to 11667, 76 unvouchered specimens; Roque Pérez: MACN 20725; San Miguel del Monte: MACN 16468, MACN 36460; Tandil: MACN 21675 to 21680; Olivos: MACN 32022; Zelaya: MACN 6985. **URUGUAY: Departamento de Montevideo:** Santiago Vázquez:

CENAI 122-1, CENAI 122-2, CENAI 122-6 to CENAI 122-9, CENAI 122-11 to CENAI 122-14, CENAI 122-16, CENAI 122-18 to CENAI 122-22, CENAI 122-24, CENAI 122-25, CENAI 122-28, CENAI 122-31 to CENAI 122-35, CENAI 122-37, CENAI 122-40, CENAI 122-42, CENAI 122-43.