Polymorphism in a Neotropical toad species: ontogenetic, populational and geographic approaches to chromatic variation in *Proceratophrys cristiceps* (Müller, 1883) (Amphibia, Anura, Odontophrynidae)

Kleber Silva Vieira[1,2,3], Erivanna Karlene Santos Oliveira[1],
Washington Luiz Silva Vieira[1] and Rômulo Romeu Nóbrega Alves[1,2]

[1] Laboratório de Ecofisiologia Animal do Departamento de Sistemática e Ecologia, Universidade Federal da Paraíba, João Pessoa, Paraíba, Brazil
[2] Departamento de Biologia, Universidade Estadual da Paraíba, Campina Grande, Paraíba, Brazil
[3] Programa de Desenvolvimento Científico e Tecnológico Regional (FAPESQPB), Universidade Estadual da Paraíba, Campina Grande, Paraíba, Brazil

ABSTRACT

Quantifying variability is important for understanding how evolution operates in polymorphic species such as those of the genus *Proceratophrys* Miranda-Ribeiro, 1920, which is widely distributed in South America. *P. cristiceps* distribution is limited to the Caatinga biome in Brazil. We examined its chromatic variation from a populational perspective, looking at different phenetic polymorphism levels and probable chromotypic association by applying statistical and GIS tools that could facilitate future taxonomic research regarding this and other species.
We characterized *P. cristiceps* colour patterns and re-evaluated its geographic variation, highlighting potential consequences for the taxonomy of the genus. Our results revealed six principle chromotypes whose frequencies varied among sex and ontogenetic classes. Phenotypic expression appeared to respect defined proportions and evidenced selective value for the species. We conclude that individual variation, together with typological traditionalism may overestimate the polymorphic magnitude at the population level and cause taxonomic inflation. Our data support the usefulness of *P. cristiceps* as a model for microevolutionary studies.

INTRODUCTION

Morphological variation plays a fundamental role in the evolution of species. Although not all characteristics are heritable, natural selection can potentially act on those that are transmissible to new generations (*Ridley, 2004*). Understanding how evolutionary mechanisms operate on populations through individual morphological variability has been

the main objective of large number of different studies ever since the times of Darwin (*Futuyma, 1987*; *Huxley, 1940*).

Such studies seek, in principle, to understand the origin of biodiversity, and how it can be accessed from the recognizable and comparable differences and similarities among organisms. Assessing morphological variation in an operationally adequate approach making use of different techniques, methods, or philosophies has proven to be a huge challenge by taxonomic, or even conservationist, criteria (*Coyne, Orr & Futuyma, 1988*; *Isaac, Mallet & Mace, 2004*; *Padial et al., 2010*; *Sokal, 1973*; *Zachos, 2016*), principally in polymorphic species such as those of the genus *Proceratophrys*.

The genus *Proceratophrys Miranda-Ribeiro, 1920*, comprises a group of South American amphibians popularly known as small ox-toads, or horned minor frogs. They are widely distributed across Brazil and also occur in Argentina and Paraguay (*Frost, 2021*; *Napoli et al., 2011*). The taxon was traditionally difficult to classify, as its species have been consistently confused with those of the genus *Ceratophrys* Wied-Neuwied, 1824, and often placed within the same genus (*Boulenger, 1882*; *Braun, 1973*; *Gravenhorst, 1829*; *Günther, 1873*; *Miranda-Ribeiro, 1920*, *1923*; *Müller, 1884*).

The genus *Proceratophrys* was originally described by *Miranda-Ribeiro (1920)* based on the presence of a "dilated post-tympanic bone, spiculated eyelid and the absence of a keratoid appendix" as diagnostic characteristics. The species included in the description were *P. appendiculata* (*Günther, 1873*); *P. boiei* (Wied-Neuwied, 1824); *P. cristiceps* (Müller, 1883) and *P. renalis* (*Miranda-Ribeiro, 1920*). Miranda-Ribeiro highlighted a number of morphological traits, some of which were morphometric and others chromatic.

The genus has been revised several times due to taxonomic ambiguities, and the validity of some species has been questioned (*Barrio & Barrio, 1993*; *Dias et al., 2013*; *Kwet & Faivovich, 2001*; *Lynch, 1971*). A total of 42 *Proceratophrys* species are currently recognized (*Frost, 2021*), distributed in different biomes and morphoclimactic dominions (such as the Amazon, Caatinga, Cerrado, Chaco, Atlantic Forest and Pampas) (*Barrio & Barrio, 1993*; *Giaretta, Bernarde & Kokubum, 2000*; *Izecksohn et al., 1998*; *Martins & Giaretta, 2011*). Although *Proceratophrys cristiceps* has been considerably discussed during the last decade, its proposed taxonomy still arouses doubts (*Cruz, Nunes & Juncá, 2012*; *Martins & Giaretta, 2013*), and the distribution suggested by *Junior et al. (2012)* and *Mângia et al. (2020)* contains somewhat dubious and questionable records—largely reflecting that some are syntopic with other species in the *goyana* group, or that they were encountered in unusual biome for that species.

Similar to some other anurans, *P. cristiceps* displays chromatic and morphometric polymorphism (*Vieira & Vieira, 2012*). At least two chromotypes have been described for this species (*Vieira et al., 2008*), which may reflect environmental fluctuations and/or genetic events on populations (*Dias & Gonçalves da Cruz, 1993*; *Smith & Skúlason, 1996*). This information has largely gone unnoticed in recent studies, but if extended to other species, it may partly explain the taxonomic inflation (*Aleixo, 2009*; *Alroy, 2003*; *Isaac, Mallet & Mace, 2004*; *Padial & De la Riva, 2006*) observed in the genus in recent decades, with high numbers of species being described in a short period of time in the absence of accurate taxonomic revisions (*Junior et al., 2012*).

Chromatic variability is common in anurans (*Hoffman & Blouin, 2000*; *Kakazu, Toledo & Haddad, 2010*), and facilitates their survival in areas with many predators (*Bourke, Busse & Bakker, 2011*). In such cases, chromatic polymorphism may provide a wide range of variation that enables, for example, avoidance of visual recognition by creating patterns that tend to match natural substrates in their environments (*Duellman & Trueb, 1994*; *Hoffman & Blouin, 2000*). At the taxonomic level, however, chromatic variation can generate confusion among taxonomists, although precise descriptions of external characteristics (such as intra- and interspecific colouration patterns), could potentially reduce or even resolve serious species identification or classification problems (*Grismer, Wong & Galina-Tessaro, 2002*).

The importance of polymorphism in certain organisms, which may include *P. cristiceps* (*Arruda, Costa & Recco-Pimentel, 2017*; *Arruda et al., 2012*), can reside in improving the adaptative capacities of individuals in relation to environment stress and/or predation (*Ridley, 2004*). Natural populations are constantly exposed to widely variable conditions, and regardless of the degree of accumulated or displayed differences among them, one limiting factor for individual survival will be morphophysiological adaptability (*Ricklefs, 2008*). The survival and adaptation of populations of organisms will depend on the maintenance of viable numbers of individuals on which evolutionary mechanisms can act and give rise to what we call biodiversity (*Badii et al., 2007*; *O'Neill, Beard & Pfrender, 2012*).

In light of the importance of populational polymorphism in taxonomic and evolutionary research, we have sought to precisely examine the chromatic variation in our model organism, *P. cristiceps*, and to determine the extent of its chromatic variability at both ontogenetic and population levels by searching for explanatory patterns (including morphometric) along defined geographic gradients that could corroborate or bring into question certain taxonomic proposals. We also attempt to heuristically explain the origin of the variability found, and produce information that could facilitate identifying the species and their congeners, and thus favour future studies of the ecology, biogeography and systematics of the genus—as well as of other species.

MATERIALS AND METHODS

Origin of the examined material

A total of 634 *Proceratophrys cristiceps* specimens from 37 localities were analyzed (Appendix). All the individuals were available in the Animal Ecophysiology Laboratory (*UFPB*) and the Herpetological Collection of the Universidade Federal da Paraíba (*CHUFPB*). The taxonomic identities of the samples were verified by consulting descriptions and diagnoses (*Cruz, Nunes & Juncá, 2012*; *Müller, 1884*). It was possible to identify excellent samples from different areas in northeastern Brazil in those collections, and their geographic information proved to be important for producing habitat suitability and spatial similarity maps for the study species.

Sexual and ontogenetic identifications, and stages of specimen development

The sexes of the preserved animals were identified by making an incision in the posterior ventrolateral portion of the abdomen with the aim of inspecting their sexual structures: ovaries or testicles (*Heyer, 2005*). The ontogenetic classification adopted herein for the metamorphosed animals follows *Izecksohn & Peixoto (1980, 1981)* and Mercadal de *Barrio & Barrio (1993)*. The individuals considered as juveniles were those with cloacal-rostrum lengths ≤25 mm; subadult lengths were from 26 to 35 mm, whereas adults had lengths ≥36 mm. The compatibility of those classes with the maturity of the individuals (animals potentially capable of competing for sexual partners) was tested using Pearson's Chi-square test of independence or association. We constructed two frequency tables, organized so that each cell represented a unique combination of specific values (cross tabulated), which allowed us to examine the frequencies of observation belonging to the determined categories in terms of more than one variable. Examinations of those frequencies allowed the identification of relationships (statistically significant or not) among the categories. The maturity of individuals was determined by their gonadosomatic index (the correlation between length and gonadal volume), oocyte type and the presence of developed and voluminous fatty bodies (*Costa et al., 1998*; *Duellman & Trueb, 1994*; *Noble, 1931*; *Tolosa et al., 2014*).

The aforementioned classification enabled us to identify operational ontogenetic units (OOUs) consistent with each suggested post-larval developmental phase (*Vieira & Vieira, 2012*), whose chromatic patterns were statistically consistent with respect to the analyzed frequencies (without distortions caused by small samples). In the case of the local populations studied (*sensu Mayr, 1977*), the ontogenetic categorization used herein expanded our understanding of variation in *P. cristiceps*, at both chromatic and morphometric levels.

Chromatic characterization

The chromatic characterization of both living and preserved *Proceratophrys cristiceps* specimens was performed based on the standardisation suggested by the colour catalogue for field herpetologists (*Köhler, 2012*) to decrease or avoid ambiguity issues in relation to the terminology and the description of the observed hues. The study of live animal colours was performed through *in loco* observations. The specimens preserved in alcohol (70° GL) were immersed in water to enhance the contrasts of their spots, stripes, and colouration under both natural and artificial light. That technique improved pattern identification as well as the descriptions and classifications of possible chromotypes.

The colours and dorsal spot patterns of *P. cristiceps* were recorded as digital images (DSC-H10 Sony, 8.1 Megapixels). All image captures were made at the same distance (25 cm) from the specimens with the camera lens in a horizontal position (using flash and a white background to highlight contrasts). We considered the numbers and sizes of the dark spots on the dorsal surface of the body of each specimen (*Rabbani, Zacharczenko & Green, 2015*). The dark spots were defined according to their contrast with the surrounding dorsal colour (Fig. S1B). The chromatic areas of the spots were calculated using ImageJ
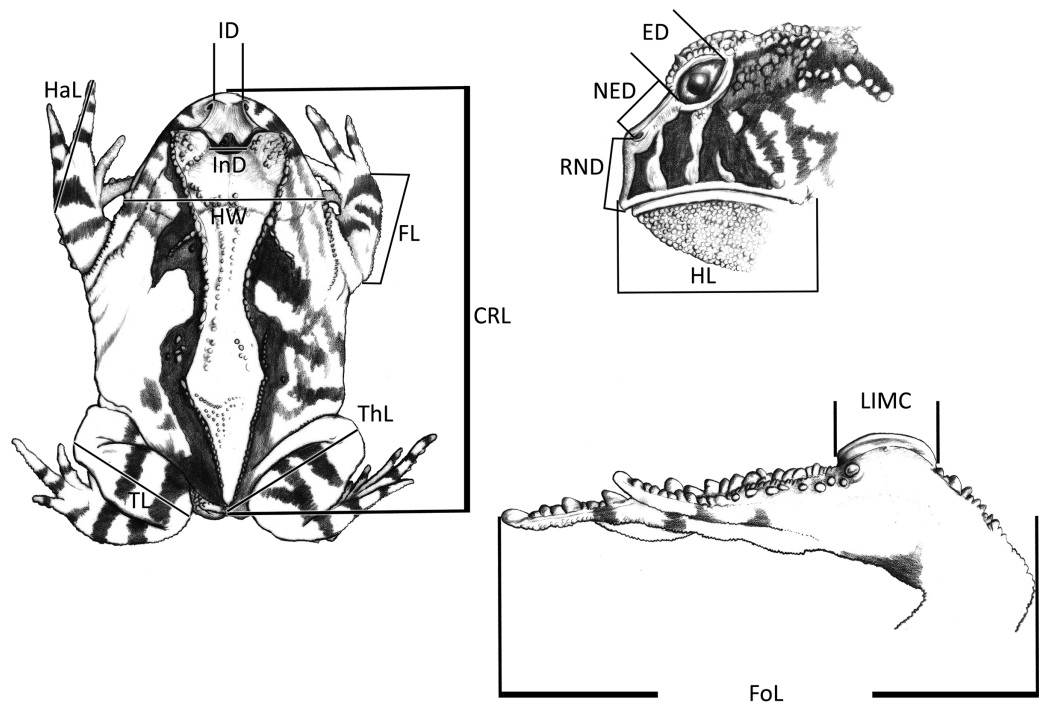
Figure 1 Measurements taken for the *Proceratophrys cristiceps* specimens (digital caliper/0.01 mm precision): CRL, Cloacal Rostrum Length; ED, Eye Diameter; FoL, Foot Length; FL, Forearm Length; HaL, Hand Length; HL, Head Length; HW, Head Width; IMCL, Internal Metatarsal Callus Length; ID, Internarinal Distance; InD, Interocular Distance; NED, Nostril Eye Distance; ThL, Thigh Length; TL, Tibial Length; RND, Rostrum Nostril Distance. More details in *Vieira & Vieira (2012)* and *Watters et al. (2016)*. Image credit: Kleber Vieira.   Full-size DOI: 10.7717/peerj.12879/fig-1

vol. 1.8.0 (*Rasband, 2018*). The images (.tiff) were processed, converted to 8-bit (grey value) files, and then quantified. The measurement interval was 0.1-infinity, which enabled calculating (in pixels/mm^2) even the smallest spots (by gradient), considering the total body area of each specimen (Fig. S1C). The reference scale used was 20 mm.

Analysis of interpopulation chromatic and morphometric variation

Morphological variation, as a continuous or discrete property, can generate mistakes when certain categories and explanatory variables are disregarded in comparative tests. It is therefore necessary to first verify the magnitude of any likely variation in recognized variables and/or factors to avoid fragile comparisons and mistaken conclusions regarding their simultaneous effects (*Zar, 2014*).

Morphometric variation, for example, originates either from sexual dimorphism or ontogeny but is often not considered when comparable categories are separately (or simultaneously) tested in recognized populations. With that in mind, we attempted to identify different forms of variability in our samples and test them within and among the chromatic observed categories.

The morphometric (Fig. 1; *Vieira & Vieira, 2012*) and chromatic variations observed in *Proceratophrys cristiceps* were tested using multiway ANOVA with unequal replications and the Kruskal–Wallis test, the latter being indicated for samples with unknown

distributions. Comparisons among frequency proportions were achieved through cross-tabulation and were carried out using Pearson's Chi-square tests. That representation was found to be very informative, enabling us to re-examine the data in a simplified manner (line plots).

Population analysis

The collection localities were accepted here as true populations for strictly operational reasons. This was done with the intention of producing sub-samples, presumably considered as distinct populations (following the traditional definition that they need to be contiguous, but situated in different territories) separated by geographical gaps of relative lengths (*Dobzhansky, 1970*; *Mayr, 1977*). The premise then was that the separation of samples by location would generate exclusive and independent populational sets (with no interbreeding or gene flow between them).

We therefore decided to identify presumed breeding cross sets to mitigate methodological eventualities, or the "*demes*" (*Gilmour & Gregor, 1939*; *Winsor, 2000*). According to our terminological redefinition (with strictly operational application), a *deme* would be any cluster of local populations closely related because of sharing at least one exclusive characteristic (*phenon*), without necessarily supporting any possible taxonomic distinction at the species level, but conferring a particular identity (as it is more frequent and statistically significant).

We subsequently excluded localities with only one collected specimen ($n = 6$) to access part of the variability of the presumed populations (the *phenons*) through certain attributes (see below). We established 15 individuals per location as the minimum sample size due to circumstantial and operational limitations. We considered here a statement of the central limit theorem (*Fischer, 2011*) where, if χ has well defined mean values and deviations, the mean terms will present an approximately normal distribution, even though the samples are not large. We also applied a distribution method with the sample replacement of random means for two elements in situations where the samples presented values less than those established (*Callegari-Jacques, 2004*; *Zar, 2014*). Thus, the possible averages of the individual samples were randomly obtained (two by two) and replaced in order to compose probable samples, until the established operational limit was reached. Finally, the distribution was ordered and the relative frequency of each element calculated, as well as its position in Z.

The graphical representation of the distances between the *demes* had a multiple comparison matrix of Z values derived from the Kruskal–Wallis test as support. Next, we applied three-dimensional ordination of the coordinates in Cartesian space (Multidimensional Scaling metric). The choice of the number of dimensions was determined by the traditional scree test (*Cattell, 1966*), establishing seven dimensions at the stress levels obtained to adapt the quadratic matrix in the representation space. Our intention was to identify geographical signals in the clusters (Euclidean distance) along the dimensional axes to later compare them to the diversity mapping of the phenetic traits of the sample populations, which were conducted at the regional level and arranged in a 0.78° raster cell (86.56 km × 86.58 km along the line of the equator). The geographical

similarity was calculated to compose a map based on the coefficients of variation of eight phenotypic traits (*Hijmans, Guarino & Mathur, 2012*; *Scheldeman & van Zonneveld, 2010*): chromatic (spot size; area occupied by the spot) and morphometric (CRL; HW; HL; FoL; TL and ThL. See Fig. 1).

Principal components analysis was an option regarding population variation in our model species and was used to determine a factor that could simply explain the probable variability found, based on the possible linear combination of our variables.

We confirmed the normality of the residuals (probability-probability plot) and the symmetry of the multivariate population distributions prior to the analyses (Figs. S2 and S3). For the latter, we calculated Mardia's multivariate skewness and kurtosis with tests based on Chi-square (skewness) and normal (kurtosis) distributions. All the tests were processed using the R v.3.5.0 basic package (*R Development Core Team, 2018*) and Past v.3.1.5 (*Hammer, 2016*) software.

In addition to the metric data, and to interpret probable variation among the *demes*, we collected information on several explanatory variables such as vegetation cover; climate, following the Köppen–Geiger classification (*Peel, Finlayson & McMahon, 2016*); altitude; rainfall; and temperature (min and max) of all of the locations where the specimens were collected. That information was obtained from the National Meteorological Institute (*INMET, 2020*) and from *Freemeteo (2019)*. Both provide regular climatological data (monthly and annual means) in an historical series from 1960 until the present, with a minimum radius of 2 km distance for each UTM (Universal Transverse Mercator) coordinate.

Environmental niche modelling

The potential distribution maps were generated with the intention of interpreting the distribution of *P. cristiceps* in terms of determined and defined predictor variables. We used two software programmes with the goal of mitigating any possible effects on the distributions of a species with restricted vagility caused by heuristic factors, such as variation in growth rates, the principle of exclusion or coexistence probabilities with predators, and dispersion limits—see the BAM scenario (*Soberón, 2007*; *Soberón & Nakamura, 2009*): the DIVA-GIS (*Hijmans et al., 2005*) and the MaxEnt (*Philips et al., 2017*; *Phillips, Dudik & Schapire, 2006*). We then estimated the proportional quantity of probable presence based on the real records of the sample through MaxEnt (*Soberón & Nakamura, 2009*), balancing the effects caused by the models generated in Maxent in terms of sensibility *vs.* specificity (*Jiménez-Valverde, 2012*) with the BIOCLIM (DIVA-GIS). This was because BIOCLIM is capable of correctly estimating the probabilities of A (regions where the fundamental or potential niches areas occurs) and G_0 (distribution area of the species where abiotic and biotic conditions are favourable and within reach of dispersing individuals) by including them in a relatively larger prediction compared to Maxent (*Qiao, Soberón & Peterson, 2015*).

Our predictions were generated through the information available in the WorldClim portal (Version 2.1), which were scenopoetic variables (temperatures and precipitation) with a range of annual means from 1970 to 2000 (*Fick & Hijmans, 2017*). All the maps

presented herein are at a resolution of 30 arc seconds (~1 km^2) in GCS WGS 1984 projections.

Checking the taxonomic functionality of phenetic characteristics

We analyzed the ambiguity and the frequencies of the diagnostic characteristics commonly used at the taxonomic level within the genus *Proceratophrys*. We tested the functionality of the information provided by the authors (see below) by comparing them to each other and with the phenotypic traits of our samples *P. cristiceps* individuals. We also checked the types of taxonomic features, and counted how many times they were applied by different authors (to different species). When one of those characteristics was recognized in our samples, or among the different authors, we could then verify the ambiguity of that phenetic trait. Our objective was to verify if identical diagnostic features could be found among distinct species (refutability principle). We constructed a matrix of meristic variables according to the frequency of the characteristics used. Next, we produced a set of common values from the available data based on six phenetic variables: colour; bone (considering the description of the head form); tissue (material: eye, eyelid, interdigital membrane, tympanum, tongue, vocal sac, warts, tubercles and nodules); measurements; sonogram and genetics (including karyotype).

We then generated a grouping in random blocks of partitioned density from the absolute values structured from *k* groups, so that the sets were brought together in a greater order of similarity (*Hartigan, 1975*). In this study we sought to identify significant patterns in the choice of specific features (by the authors) in descriptions and diagnoses that could define the underlying taxonomy. The studies consulted were *Gravenhorst (1829)*, *Günther (1873)*, *Müller (1884)*, *Miranda-Ribeiro (1937)*, *Lynch (1971)*, *Braun (1973)*, *Jim & Caramaschi (1980)*, *Izecksohn & Peixoto (1981)*, *Barrio & Barrio (1993)*, *Eterovick & Sazima (1998)*, *Giaretta, Bernarde & Kokubum (2000)*, *Gonçalves da Cruz, Prado & Izecksohn (2005)*, *Ávila, Kawashita-Ribeiro & Morais (2011)*, *Napoli et al. (2011)*, *Martins & Giaretta (2011)*, *Cruz, Nunes & Juncá (2012)*, *Junior et al. (2012)*, *Ávila, Pansonato & Strüssmann (2012)*, *Brandão et al. (2013)*, *Godinho et al. (2013)*, *Martins & Giaretta (2013)*, *Mângia et al. (2018)* and *Mângia et al. (2020)*. The sampling was performed in such a way as to unite all the information of the species in the controversial *cristiceps* group (*Dias, de Carvalho-e-Silva & de Carvalho-e-Silva, 2014*; *Giaretta, Bernarde & Kokubum, 2000*).

RESULTS

Chromatic analysis

Our observations indicated the existence of at least six main chromatic variations in the *Proceratophrys cristiceps* (Fig. 2):

Chromotype 1 (*n* = 93, 15%): brown bichromatic colouration in diverse hues (C22–C25) on a tawny olive and drab brown background (C17 and C19), whose spots or stripes, sometimes distributed in a well-defined direction, impede the recognition of a characteristic dorsal geometric figure—"arrowhead" (*Miranda-Ribeiro, 1937*). Conspicuous suborbital bands. Animals moderately melanised, and with two or more

Figure 2 Chromatic variation in *Proceratophrys cristiceps* individuals. The diversity found is characterised by the general colour pattern, saturation, and the distribution of dorsal patterns.

Full-size ⊡ DOI: 10.7717/peerj.12879/fig-2

interorbital stripes (often in contact, and with a lighter one in the middle). Generally occurring in leaf litter (98.48%);

Chromotype 2 ($n = 271$, 43%): similar to chromotype 1 in terms of having brown colouration and suborbital or interorbital bands (two bands, with one of being Y-shaped), however, there is a well-defined dorsal geometric figure laterally limited by dark bands (maroon—C38) in the orbit-cloaca direction. There are also lighter nuances on the flanks (salmon—C57 to C59) and on the limbs, stomach and snout (cyan white—C155). Usually occuring in leaf litter (97.02%) or gravel (2.98%);

Chromotype 3 ($n = 39$, 6%): with very clear brown-grey colouration, and slightly variegated (C256 to C259). Evident dorsal geometric figure and yellow-brown colouration (C84), distributed in the orbit-cloaca direction; limited by two bands (in opposing toothed arches) and lines of semi-parallel glandular nodules. Single interocular stripe and two well-defined suborbital stripes. May have discrete rusty tones (C253) in the supraocular portions and sides of the body. Generally occurring in earthy soil with sparse leaf litter (92.83%);

Chromotype 4 ($n = 58$, 9%): with evident trichromatic colouration, whose rusty red hue (C35 and C253) cover a large part of the body. Clear dorsal geometric figure with a pale-yellow colouration (C2 and C3), laterally limited by regular dark bands (C30) in an

orbit-cloaca direction. Suborbital stripes are not clearly evident; presence of only one interocular stripe. A pineal spot present. There are also white hues (C155 and C261) in the lateral portions of the body and limbs, similar to Chrom2. Generally inhabiting sandy soils (6.25%), grit or gravel (93.75%);

Chromotype 5 (n = 51, 8%): general colouration monochromatic as compared to the others chromotypes, generally with rusty red hues (C57 and C58) or yellow-brown characteristic (C17). Barely visible spots or streaks. Generally occurs in grit or gravel (93.30%);

Chromotype 6 (n = 122, 19%): general brown-grey colouration (C19) with diverse nuances, with evident yellow-brown spots (or lighter hues C12 and C111) distributed in characteristic areas: snout and suprascapula. The dorsal geometric figure is laterally outlined by spots in a toothed arch shape, although not well defined. Generally inhabiting earthy or sandy soils (81.26%) and even in leaf litter (18.74%).

The frequencies of these chromotypes did not indicate dimorphic variation in the species, demonstrating an almost identical distribution between males and females, except for Chrom5, whose frequency in males was similar to Chrom4 (Fig. 3). Furthermore, we observed a proportional expression of the six phenotypes for each relative frequency of *P. cristiceps* (≈14:43:6:9:8:20), which was also maintained internally among the samples and localities (Table S1), suggesting that these phenotypes may be governed much more by heritable factors than by environmental or epigenetic ones (apparently by Mendelian inheritance).

The frequencies of Chrom5 were found to be higher in juveniles compared to sub-adults and adults when analyzing those same samples by ontogenetic class. We also verified the ontogenetic class frequencies for each sex—which demonstrated patterns with little difference from that of the species as a whole. Unlike females, the male chromotypic variations of Chrom3, Chrom4, and Chrom5 were significantly different, therefore moving away from the general species' pattern (Fig. 4).

The chromotypes also evidenced different frequencies in terms of maturity, with slightly lower frequency of Chrom4 and a higher frequency of Chrom5, mainly varying among mature individuals (Fig. 3). The variations revealed smaller numbers of adult Chrom4 individuals as compared to adult Chrom3 and Chrom5 individuals. Those differences were maintained in both males and females when analyzing the samples separately.

Another peculiarity of the studied specimens was their integumentary saturation (proportional quantity of dark in relation to light background). The Chrom5 individuals found studied here were less saturated than the others (Fig. 5), with a lower average size of the dorsal patches, and the area occupied by them (as well as their distribution) being reduced. Those variations, which characterised the form and extension of the dorsal designs, were significant and independent of sex, ontogenetic class and maturity, considering the species as a whole or internally among the samples (Figs. S4–S7).

Although the distribution of dorsal spots did not vary significantly between males and females, the average size (in mm^2) was greater in females; they were also more saturated than those of the males (Figs. S4–S7). The Chrom6 juvenile females (but not Chrom6
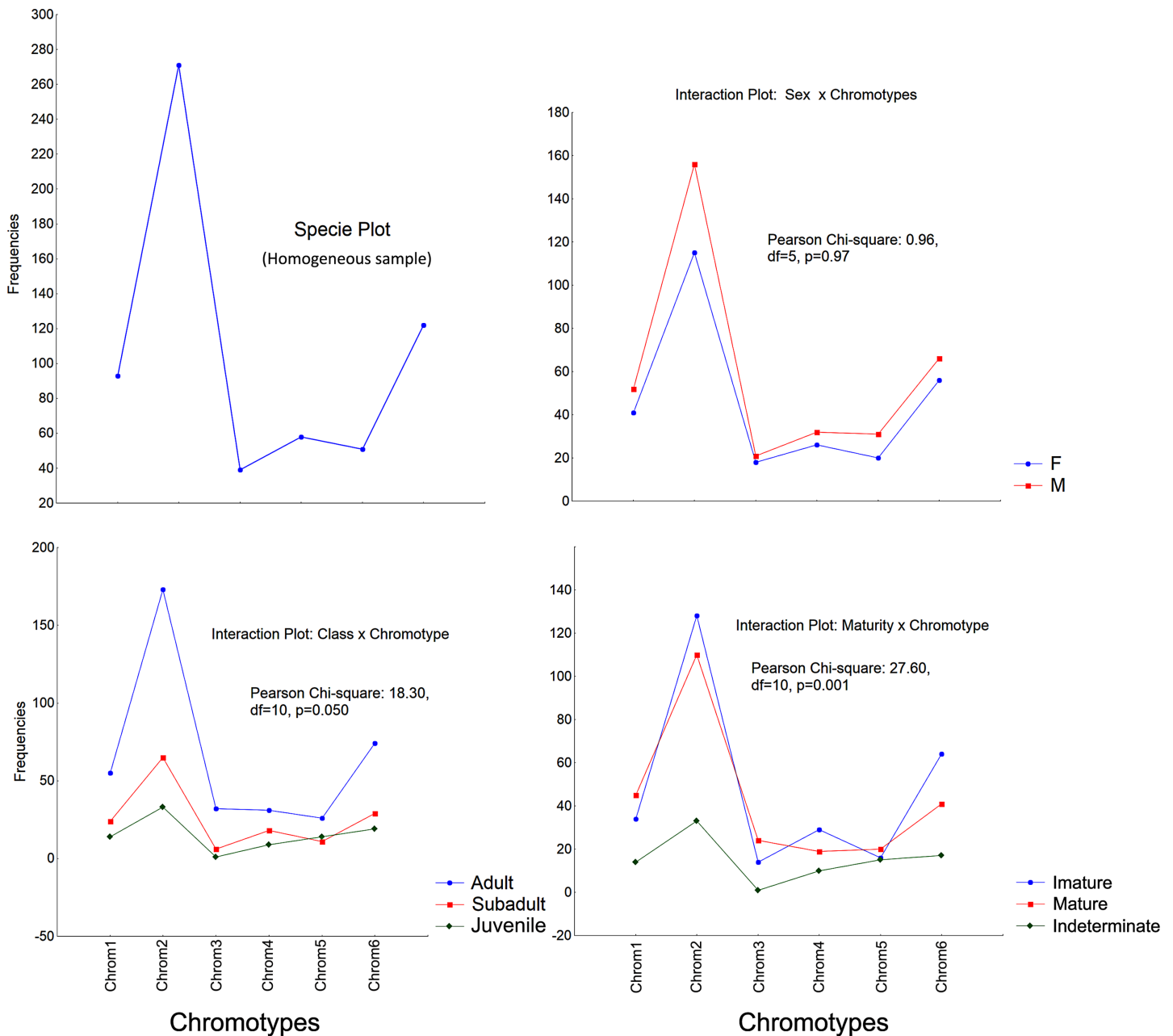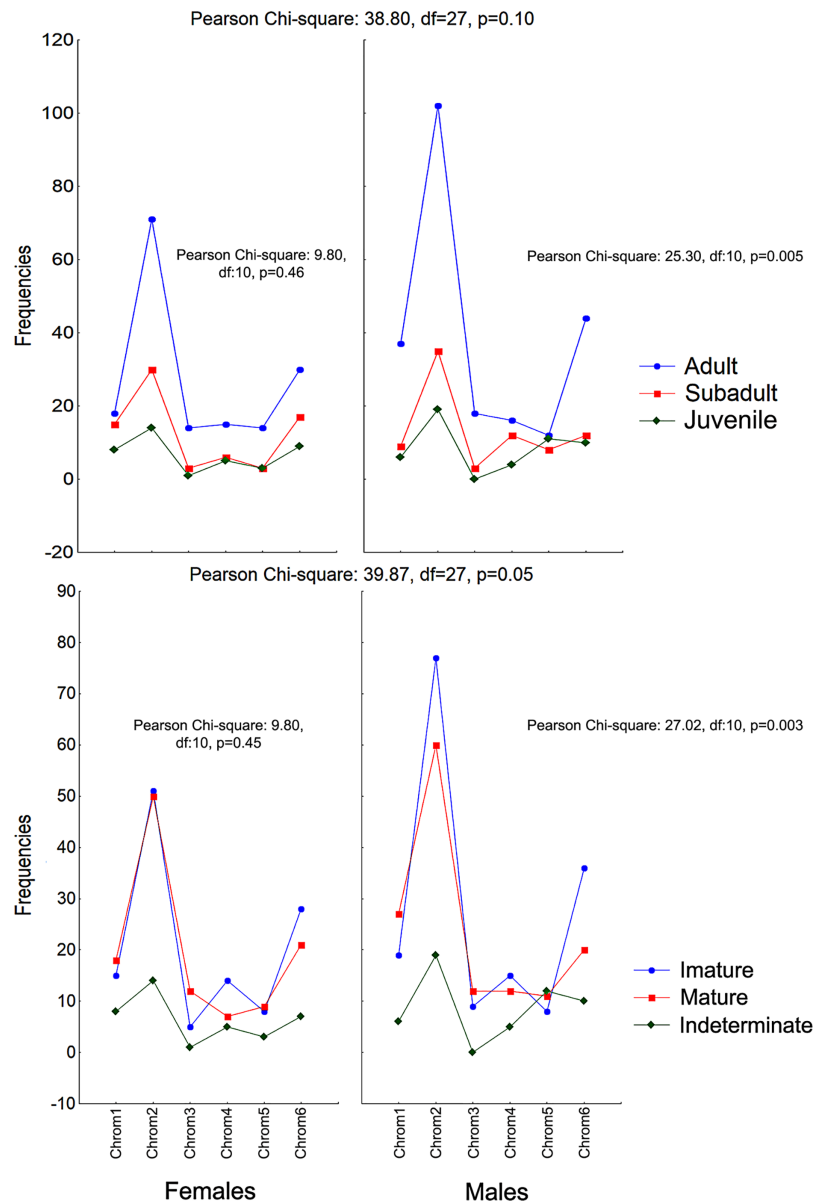
Figure 3 Chromotypes of *Proceratophrys cristiceps* with a distribution of their frequencies varying in terms of sex, maturity and ontogenetic development. Full-size 🖼 DOI: 10.7717/peerj.12879/fig-3

males) were very different from the other chromotypes, as their spots were observed to be larger.

Morphometric analysis and phenetic trait diversity

Males and females were morphometrically different in the general sample (except for ED, InD, FL and RND), but those variations were absent in juveniles and even in sub-adults (Figs. S8–S11). Males and females did not differ morphometrically in the permutations

Figure 4 Chromotypes of *Proceratophrys cristiceps* with the distributions of their frequencies varying between sexes according to their maturity and ontogenetic development (post-larval). The significant differences observed for the males suggest a curious and discreet effect of the factors acting on the sex variable. Full-size ⤢ DOI: 10.7717/peerj.12879/fig-4

performed in terms of chromotypes. Only adult males (Chrom6 and Chrom3) or mature males (Chrom3, Chrom1 and Chrom2) differed from each other in the internal analysis of the samples, with differences being observed in the cephalic region (RND, ED, HL and HW) and in relation to the internal metatarsal callus.

When examining the coordinate factors based on correlations, only Chrom5 and Deme5 were more concentrated in the superior portion of the second component (Fig. S12); the others were almost uniformly distributed in the Cartesian space, without any variable (active or supplementary, morphometric or chromatic) supporting the

Vieira et al. (2022), *PeerJ*, DOI 10.7717/peerj.12879

12/30
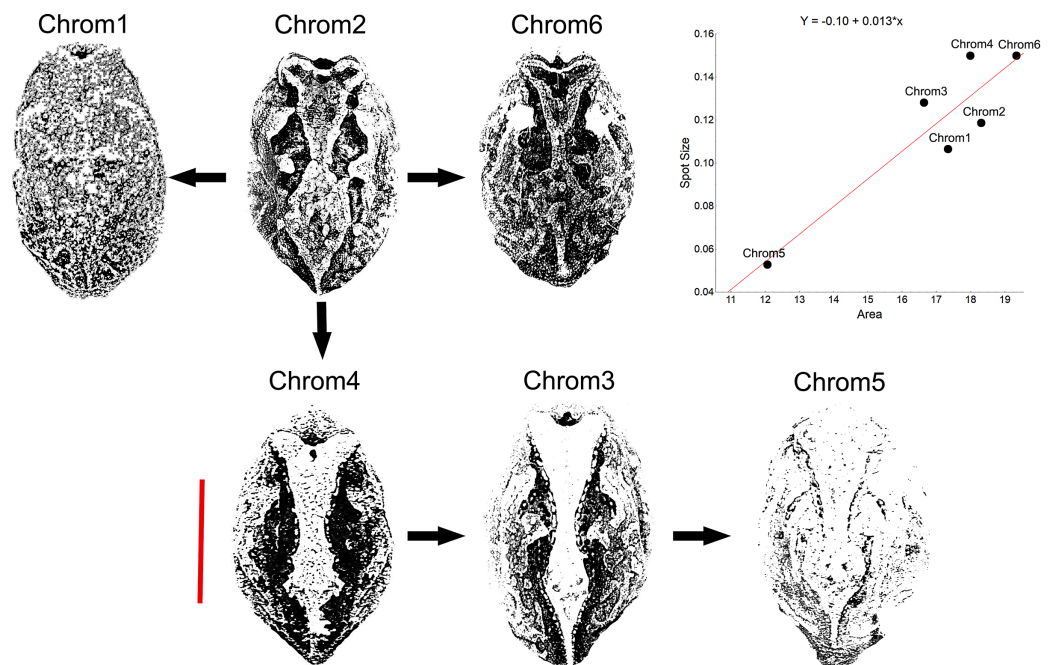
Figure 5 Saturation of *Proceratophrys cristiceps* chromotypes. The dorsal patterns are formed in accordance with the size of spots as well as their proximity to each other (distribution). The arrows represent derivation hypotheses, wherein Chrom2 is indicated as a basilar or heterozygous pattern (higher frequency, design complexity, and moderate saturation). Scatterplot graph for the mean saturation values (mm^2) highlighted. Bar: 25 mm.　　　　　Full-size ⬜ DOI: 10.7717/peerj.12879/fig-5

composition of the *demes*, and they were not easily explained by the environmental predictors. Geographically supported and consistent groups were produced, however, when the multidimensional scaling diagram was associated with the phenetic trait diversity mapping. The results indicated Almas and São Mamede; Serra Talhada and Caicó; Junco and Jaguaribe; Cabaceiras and São João do Cariri as markers of zones with shared phenons (Fig. 6), constituting a strong indicator of the occurrence of genetic flow between populations.

The phenetic trait diversity mapping indicated the existence of at least five *demes* in the *P. cristiceps* species (Fig. 7B) that were exclusively distributed in the Caatinga biome and transition phytophysiognomies, according to ecological niche modelling. The species is most likely found in predominantly arboreal-shrubby vegetation, under direct influence of precipitation and annual minimum temperatures (Fig. 7; Fig. S13).

DISCUSSION

The probable meaning of variation in *P. cristiceps*

Species are a multidimensional phenomenon (*Harold, 2002*; *Zachos, 2016*), and studies of variation in organisms can provide essential information for the field of experimental taxonomy (*Sneath & Sokal, 1973*; *Sokal & Rohlf, 1995*), and consequently for systematics, biogeography and ecology. Taxonomic characteristics (defining or diagnostic) must
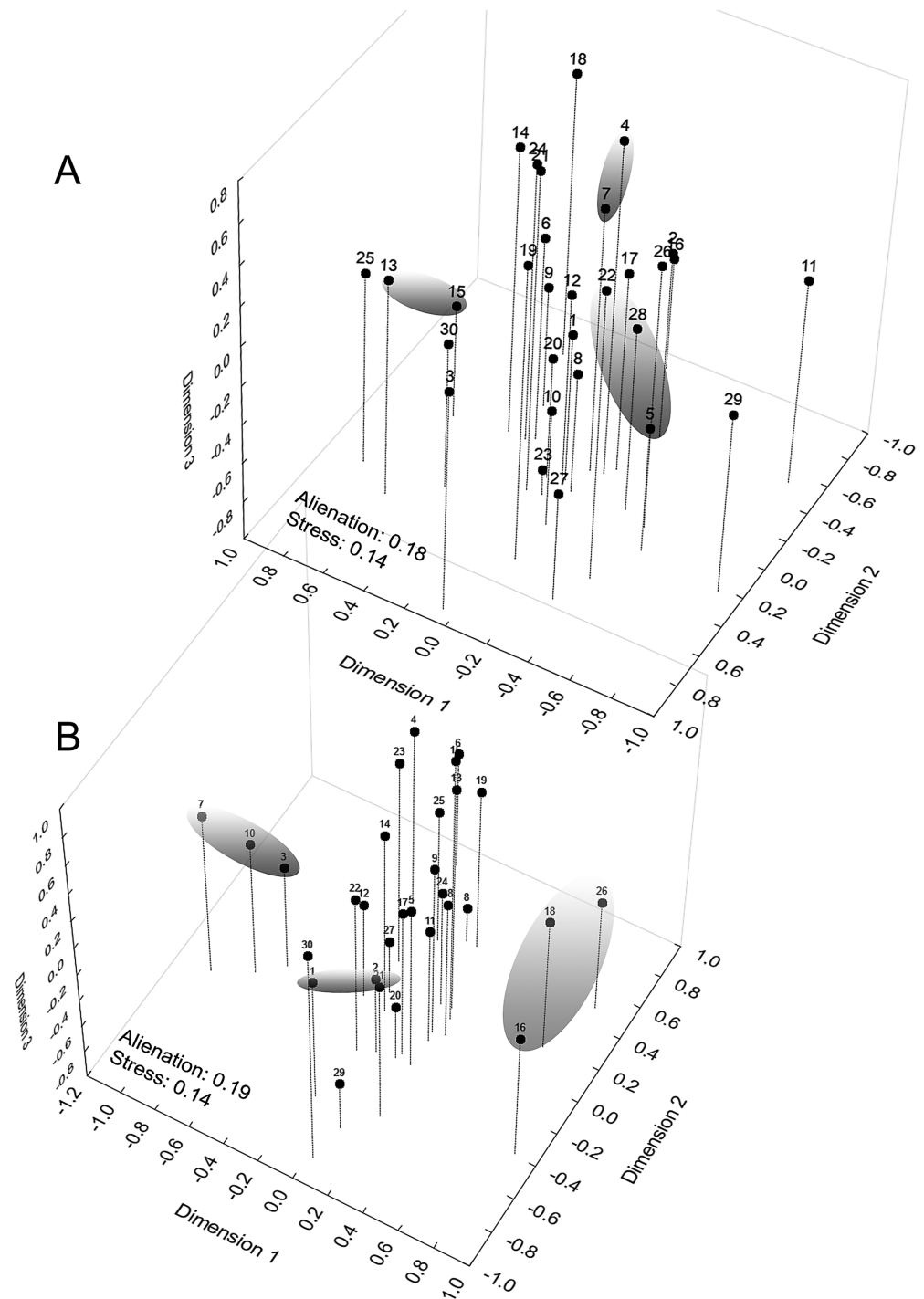
Figure 6 Mapping of *demes* obtained by multidimensional scaling using *Z* value similarity of the relative Kruskal–Wallis scores. Start config.: Guttman-Lingoes. Area occupied by dorsal spots (A) and Mean size of dorsal spots (B). 1. Almas; 2. Arcoverde; 3. Boa Vista; 4. Cabaceiras; 5. Caicó; 6. Caracol; 7. São João do Cariri; 8. Serra das Confusões; 9. São José dos Cordeiros; 10. Crato; 11. Desterro; 12. Exú; 13. Jaguaribe; 14. João Câmara; 15. Junco; 16. Nascente; 17. Paulo Afonso; 18. Patos; 19. Pedra da Boca; 20. Quixadá; 21. São Mamede; 22. Serra Talhada; 23. Trindade; 24. Ubajara; 25. Várzea da Conceição; 26. Buíque; 27. Macaíba; 28. Santana dos Matos; 29. Serra de São Bento; 30. Santa Quitéria.

Full-size 🖼 DOI: 10.7717/peerj.12879/fig-6
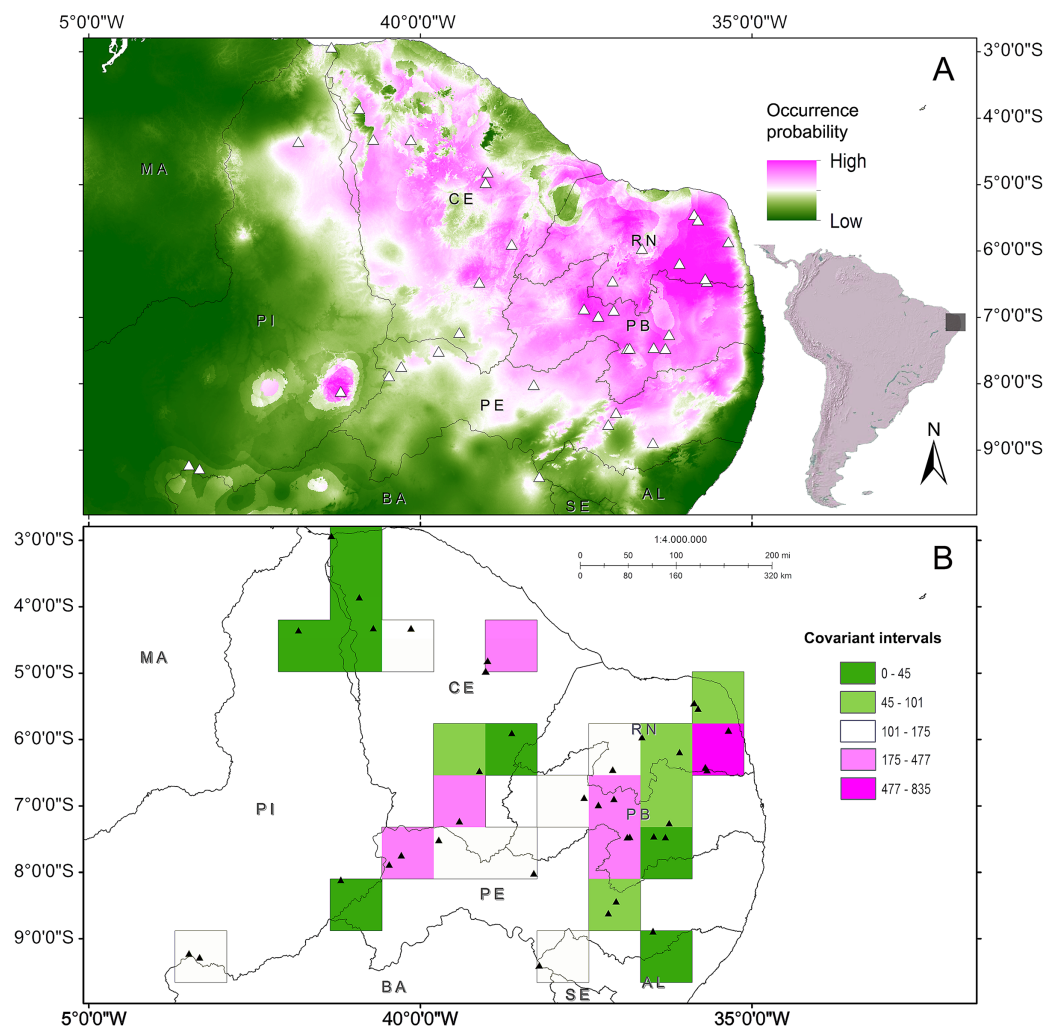
Figure 7 Distribution of *Proceratophrys cristiceps* within the Caatinga biome and in transition areas. According to the results of environmental niche modelling (ENMs) (A) and the mapping of their *demes* (B) based on the geographic similarity of the covariance of eight phenotypic traits (chromatic and morphometric). Full-size ⬙ DOI: 10.7717/peerj.12879/fig-7

therefore be thoroughly discriminated and understood, especially with respect to probable intraspecific variation.

In order to be able to deal with this probable variation, categories must be defined that are equivalent in experimentally comparable ways, so that any possible effects of simultaneous interaction between factors in terms of specific variables can be identified. Thus, it is not difficult to perceive that variation can be expressed as altered phenotypes that, within morphological limits, can determine the different forms that we normally identify when dealing with individual and populations (*Nicoglou, 2015*). With this in mind, our results indicated two clear levels of variation in *P. cristiceps*: morphometric and chromatic, with both having apparent and substantial adaptive value.

The chromatic variation observed in *Proceratophrys cristiceps* presumably has selective value, as the differential frequencies of the chromotypes suggest certain advantages to
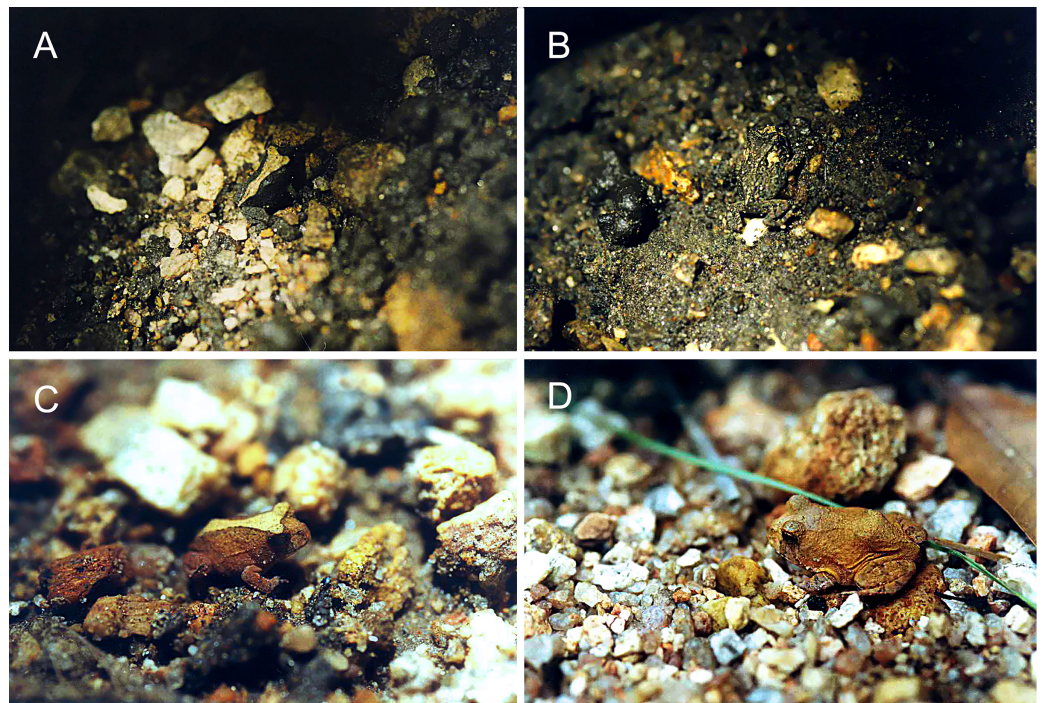
Figure 8 **Juveniles of *Proceratophrys cristiceps* observed in the Pedra da Boca State Park.** (A) Chrom3; (B) Chrom6; (C) Chrom4 and (D) Chrom5. The contrasting colourations in relation to the soil types suggests reinforced adaptability in individual survival abilities (crypsis and disruptive colouration). Photo credit: Kleber S. Vieira. Full-size ◩ DOI: 10.7717/peerj.12879/fig-8

individual survival. The distribution of animals on different soil types seems to play a predominant role in the observed bias of chromotypic frequencies throughout post-larval development, and may indicate some type of frequency-dependent selection (*Bond, 2007*). In this case, the numbers of less saturated animals decreases as maturity or adulthood is reached, suggesting that certain phenotypes may be reinforced by local edaphic conditions (Fig. 8; Fig. S14), and that crypsis may have an important role (*Bonte & Maelfait, 2004*; *Endler, 1981*; *Moreno-Rueda, 2020*; *Rabbani, Zacharczenko & Green, 2015*). The contrasting colours and spots create disruptive patterns that could function, when combined with general colouration and saturation, as a highly effective strategy against predators (*Cuthill et al., 2005*). Together, the two mechanisms (disruptive colour and crypsis) may at least partially explain the observed variation in their frequencies, especially among juveniles, although they cannot explain the relative sample proportionality.

As the chromatic expressivity (the observed percentage of a given phenotype) found in *Proceratophrys cristiceps* was not exclusive to specific samples, but was maintained even within and among categories (Table S1), phenotypic divergence due to local effects (polyphenism) can be easily discarded as an alternative explanation for the patterns identified. We therefore deduce that the observed chromatic polymorphism is grounded in a strong genetic basis (*White & Kemp, 2016*) and is reflected in the differential abundance and almost invariability of the poly- or dichromatic chromotypes identified (*Mângia et al., 2020*; *Nunes et al., 2015*; *Vieira et al., 2008*).
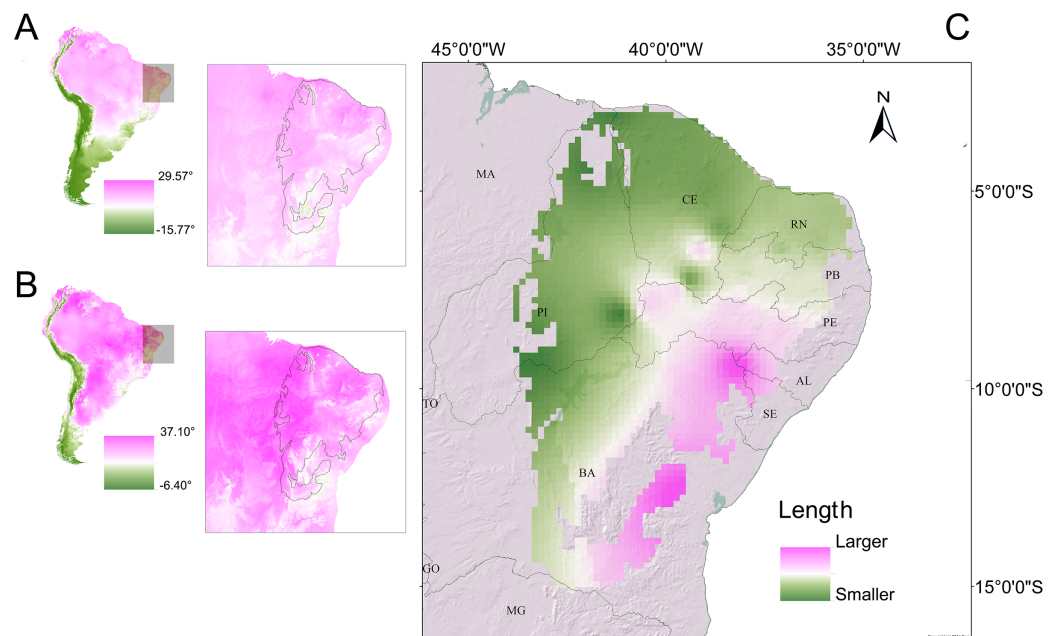
Figure 9 Morphometric gradients (cline and isophenes) observed in the distributions of the *Proceratophrys cristiceps* populations analysed. The interpolation of the length values (cloacal rostrum distance) indicated that smaller individuals are found in the north-western region of the Caatinga (C), where temperatures are higher. Maps of South America showing the average annual temperature (A) and maximum temperature of the hottest month (B) for the years 1970–2000. The outlined area indicates the extent of the Caatinga biome. Climate data source: *Fick & Hijmans (2020)*.

Full-size ◧ DOI: 10.7717/peerj.12879/fig-9

Another important factor in relation to the biogeographic aspect of our results was the existence of *demes* (understood herein as conglomerate populations) that were morphometrically smaller (on the average) in the north-western (hotter and drier) regions of the Caatinga. The most likely explanation for that observation would involve temperature-associated effects (Fig. 9). That explanation appears plausible when considering the determinants of potential distributions (Fig. S13), with the mean annual minimum temperature and the precipitation of the last quarter of the year significantly contributing to the habitat suitability model.

While this cline effect appears to point to Bergmann's rule (*Bergmann, 1848*; *Blackburn, Gaston & Loder, 1999*; *Salewski & Watt, 2017*), there is no clear concordance with anurans, where phenotypic plasticity controlled by genes may be involved (*Ashton, 2002*; *Berven, 1982a, 1982b*) and would favour adaptative strategies to avoid thermoregulatory imbalances and hydric stress, with geographic selection gradients (*Endler, 1977*; *Stebbins & Cohen, 1995*), in turn, conferring a low metabolic energy cost to the animals (*Bernardo, 1994*).

At a more restricted level, the morphometric variation observed in *Proceratophrys cristiceps* is partly a consequence of sexual dimorphism, ontogenetic effects (*Vieira & Vieira, 2012*), and cranial morphological alterations in response to adaptations to available food resources (*Atencia, Solano & Liria, 2020*; *Brito et al., 2012*; *Emerson, 1985*). The observed metric variations are negligible when compared to the chromotypes, either

between sexes or among the developmental categories (maturity and ontogenesis as described herein). Thus, although chromotypic variation is evident and quite informative in *P. cristiceps*, it could be deceptive and lead to serious taxonomic problems if misinterpreted and examined in isolation. Thus, the evolutionary implication of variation (whether chromatic or morphometric) is difficult to approach experimentally, and taxonomic studies often view operational morphological units (OMUs) as different sub-species or even species. There are also underlying factual (and experimental) requirements necessary to explain the morphological divergence and the alleged taxonomic diversity (*Van Holstein & Foley, 2020*), where taxonomic richness is clearly correlated with rates of intraspecific population divergence.

Taxonomic implications of variation in *P. cristiceps*

There have been significant increases in the numbers of species descriptions in the genus *Proceratophrys* over the past 20 years. The taxonomic inflation rate between 2011 and 2021 was 45% (Fig. S15A), with the *cristiceps* group (with reduced eyelid appendages), which inhabits open and dry environments in the Cerrado and Caatinga (*Dias, de Carvalho-e-Silva & de Carvalho-e-Silva, 2014*) reaching 12% (Fig. S15B). Although the taxonomy of *Proceratophrys cristiceps* (and other species of the genus) has been studied and debated for decades (*Barrio & Barrio, 1993*; *Cruz, Nunes & Juncá, 2012*; *Lynch, 1971*; *Mângia et al., 2020*), it is difficult to determine if this increase in group diversity reflects true species diversity or only a typification of the intraspecific variability already observed (*Junior et al., 2012*).

When revisiting the original species descriptions, it could be seen that not only body coloration, but also the amount, sizes and appearances of nodules and tubercles are among the most common diagnostic (or defining) characteristics for all species in the *cristiceps* group (and others groups as well) (Fig. 10)—suggesting that the species were defined based on traits evidencing significant phenotypic plasticity.

Our observations, for example, indicated that nodules (including warts and tubercles) are extremely variable in terms of numbers, shapes and distributions, either isolated or regionally, on the same individual or among specimens (Figs. S16 and S17). Some animals have large and round nodules; distributed regularly or irregularly; with glandular appearances and salient; or smaller and more conical, or even flat—but they serve little purpose as defining or diagnostic characteristics of the chromotypes. In addition to the nodules, the shape of the snout, when viewed laterally or dorsally, was equally variable, due not only to allometric factors (*Vieira & Vieira, 2012*), but also in terms of the position of the specimens in the viewing plane. The difficulties encountered while using this information has also been discussed by other taxonomists (*Brandão et al., 2013*).

Another common characteristic used in descriptions of these species are the rows of opposite oculum-dorsal nodules and their associated spots and stripes. Those rows appear to be important in forming the arrowhead shape of the dorsal design (*Miranda-Ribeiro, 1937*). This shape becomes much less distinct, however, when those rows are discontinuous and dissolve into patterns of irregular spots and bands (very variable among individuals) that interconnect at various points, especially in the middle dorsal portion
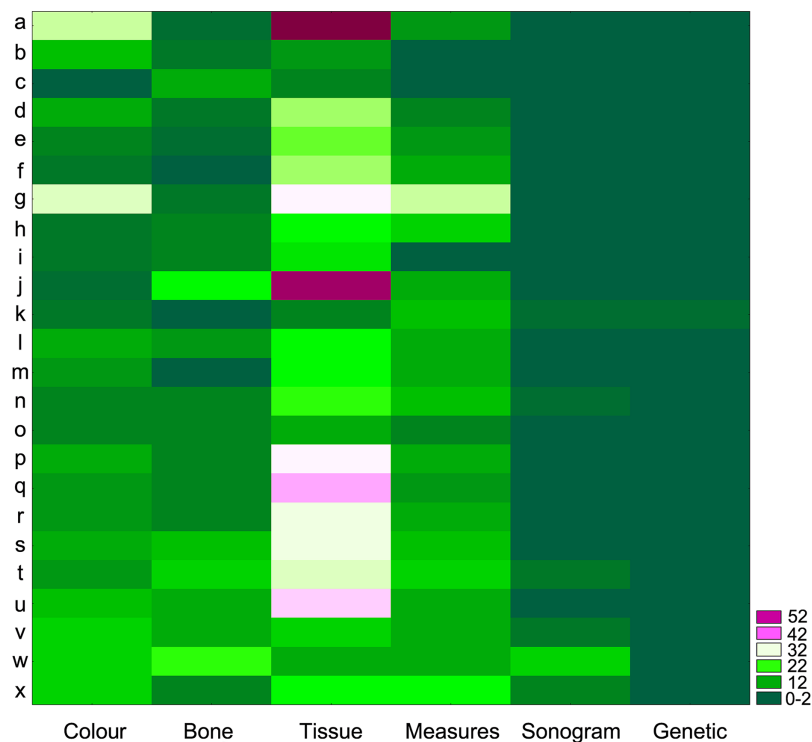
Figure 10 Authors grouped based on the identification of regions with high densities of similar values (Two-Way Joining). Clusters generated through the diagnostic use of identical phenetic traits. The highlighted blocks in light colours reflect greater sets of tissue characteristics (mainly nodules, warts and tubercles) used in the descriptions of the species of the genus *Proceratophrys*. Threshold Computed: 5.46 (St. Dv./2). Number of Blocks: 44. Total Sample Mean: 9.65. Standard Deviation: 10.92. The score on the right is the number of groups by the number of k-observations. The data indicate that certain categories of phenetic traits have been used uncritically (reflecting taxonomic traditionalism), which has led to a dependence on variable features. (a) *Gravenhorst (1829)*; (b) *Miranda-Ribeiro (1937)*; (c) *Lynch (1971)*; (d) *Jim & Caramaschi (1980)*; (e) *Eterovick & Sazima (1998)*; (f) *Ávila, Kawashita-Ribeiro & Morais (2011)*; (g) *Napoli et al. (2011)*; (h) *Günther (1873)*; (i) *Müller (1884)*; (j) *Cruz, Nunes & Juncá (2012)*; (k) *Mângia et al. (2020)*; (l) *Braun (1973)*; (m) *Izecksohn & Peixoto (1981)*; (n) *Mângia et al. (2018)*; (o) *Barrio & Barrio (1993)*; (p) *Caramaschi (1996)*; (q) *Giaretta, Bernarde & Kokubum (2000)*; (r) *Junior et al. (2012)*; (s) *Brandão et al. (2013)*; (t) *Martins & Giaretta (2013)*; (u) *Gonçalves da Cruz, Prado & Izecksohn (2005)*; (v) *Godinho et al. (2013)*; (w) *Martins & Giaretta (2011)*; (x) *Ávila, Pansonato & Strüssmann (2012)*. Full-size ⬚ DOI: 10.7717/peerj.12879/fig-10

(Chrom1). The nodules in those discontinuities can spread in the suprascapular direction and the flanks of the animal, forming sinuous (or bifurcated) designs, with the larger branch sometimes expanding to the sacral area. This is usually evident in Chrom5 individuals.

We assume that specialists have been constrained by a typological traditionalism (see Fig. 10) that seems to interfere with their perception and forces them to choose more traditionally used morphological traits, while ignoring their evident plasticity or ambiguity. The consequence of acting in that matter (*i.e.*, disregarding probable variation) is that species descriptions may not be sustainable in reality (*Dobzhansky, 1970*; *Mayr, 1996*).

By reviewing the descriptions of the species of genus *Proceratophrys* and comparing the information provided by the authors with each other and with the characteristics of the

individuals in our samples, and then testing the probable ambiguity of the proposed diagnostic traits, it became evident that some species described in recent decades are not actually morphologically different from *P. cristiceps* or *P. goyana*, or even among themselves—as, for example, *P. carranca* (*Godinho et al., 2013*), *P. branti* (*Brandão et al., 2013*), *P. huntingtoni* (*Ávila, Pansonato & Strüssmann, 2012*) and *P. dibernardoi* (*Brandão et al., 2013*). Similarly, the same chromatic varieties observed in *P. cristiceps* may be equally recognisable in their congeners (*Ávila, Kawashita-Ribeiro & Morais, 2011*; *Brandão et al., 2013*; *Junior et al., 2012*; *Martins & Giaretta, 2013*)—This leads us to the conclusion that those presumed diagnostic patterns are, to a greater or lesser extent, common to the genus as a whole.

The identification of species as being distinct in recent decades often presupposed the hypothesis of sympatric speciation in the absence of an evident vicariant element (*Godinho et al., 2013*; *Mângia et al., 2018*; *Martins & Giaretta, 2013*). This has been the case with taxa (cryptic) that share many similarities, but whose distinctions (mostly linked to colour, warts or tubercles, or sometimes by acoustic [not immune to variability] and genetic analysis) can be ambiguous and conceptually confusing. Additionally, those distinctions have not even been tested under any experimental model of diversification dynamics (*Ajmal Ali et al., 2014*; *Annibale et al., 2020*; *Schindel & Miller, 2005*; *Van Holstein & Foley, 2020*), where patterns of trait richness are equivalent to the rates of intraspecific population divergence (and would thus reinforce the divergence hypotheses). This is mainly the case for species of the *P. goyana* and *P. cristiceps* groups (*Martins & Giaretta, 2011*); but why not then for the *P. biggibosa*, *P. boei* and *P. appendiculata* groups, whose taxonomic histories depend on variable phenetic traits, while evidence of pre- or post-zygotic barriers or their biogeographies continue to be elusive?

We, therefore, suggest that future studies using traditional characteristics be based on preliminarily sampling and statistical testing to determine whether they are truly diagnostic. Likewise, we cannot discount the hypothesis of taxonomic inflation in the genus *Proceratophrys*, especially the *cristiceps* group, due to poorly interpreted population peculiarities emerging from microevolutionary processes (*Amaro et al., 2012*; *Mângia et al., 2020*) instead of a taxonomic quality, due to the simple and unfortunate confusion of methods and concepts.

Finally, we conclude that individual variation, together with typological traditionalism, may overestimate the polymorphic magnitude of variation at the population level and be the cause of taxonomic inflation in many anuran species. Our data also support the usefulness of *P. cristiceps* as a model for microevolutionary studies.

APPENDIX

Specimens examined

BAHIA| Paulo Afonso (−9.401130556°S.; −38.20623333°W): UFPB12112, UFPB12114, UFPB12115, UFPB12116, UFPB12118, UFPB12124. Santa Terezinha (−12.771725°S; −39.52416389°W): CHUFPB24169. CEARÁ| Crato (−7.229958333°S; −39.41229722°W): CHUFPB19690, CHUFPB20690. Ipu (−4.321944444°S; −40.71083333°W): UFPB6127. Jaguaribe (−5.901030556°S; −38.62215°W): CHUFPB19946, CHUFPB20656,

CHUFPB20657, CHUFPB20675, CHUFPB20940, CHUFPB21058, CHUFPB22183, CHUFPB22188, CHUFPB22195, CHUFPB22233. Junco (−4.814325°S; −38.98613889°W): UFPB10033, UFPB10034, UFPB10035, UFPB10036, UFPB10037. Quixadá (−4.972555556°S; −39.01541389°W): CHUFPB19935, CHUFPB22177, CHUFPB22191. Santa Quitéria (−4.324272222°S; −40.14281111°W): UFPB10752, UFPB10759, UFPB10760. Ubajara (−8.616025°S; −37.16555833°W): CHUFPB19726, CHUFPB19729, CHUFPB19886, CHUFPB19925, CHUFPB19969, CHUFPB20654, CHUFPB20662, CHUFPB20671, CHUFPB20680, CHUFPB20681, CHUFPB20683, CHUFPB20792, CHUFPB20818, CHUFPB20820, CHUFPB20821, CHUFPB20822, CHUFPB20827, CHUFPB20830, CHUFPB20854, CHUFPB20876, CHUFPB20894, CHUFPB20896, CHUFPB20921, CHUFPB20930, CHUFPB20933, CHUFPB20938, CHUFPB20939, CHUFPB20943, CHUFPB20946, CHUFPB21056, CHUFPB21347, CHUFPB21349, CHUFPB21351, CHUFPB21355, CHUFPB22178, CHUFPB22179, CHUFPB22187, CHUFPB22190, CHUFPB22194, CHUFPB22201, CHUFPB22205, CHUFPB22217, CHUFPB22222, CHUFPB22225. PARAÍBA| Boa Vista (−7.260538889°S; −36.24889444°W): UFPB1571, UFPB1572, UFPB1573, UFPB1574, UFPB1575, UFPB1576, UFPB1577, UFPB1579, UFPB1580, UFPB1581. Cabaceiras (−7.469663889°S; −36.30575833°W): UFPB11266, UFPB11267, UFPB11268, UFPB11269, UFPB11270, UFPB11271, UFPB11272, UFPB11273, UFPB11274, UFPB11275, UFPB11276, UFPB6691, UFPB6692, UFPB6693, UFPB6694. Desterro (−6.875280556°S; −37.53213333°W): UFPB1582, UFPB1583, UFPB1584, UFPB1585, UFPB1586. Fazenda Almas (−7.470833333°S; −36.88083333°W): FA01, FA44, FA45, FA46, FA149, FA154, FA158, FA159, UFPB4267, UFPB4270, WLSV1308, WLSV1346, WLSV1349, WLSV1463, WLSV1470, WLSV1472, WLSV1474, WLSV1475, WLSV1476, WLSV1477, WLSV1485, WLSV1487, WLSV1488, WLSV1497, WLSV1505, WLSV1566, WLSV1567, WLSV1572, WLSV2021, WLSV2026, WLSV2042, WLSV2131, WLSV2170, WLSV2252, WLSV2259, WLSV2260, WLSV2339, WLSV2340, WLSV2341, WLSV2388, WLSV2391, WLSV2935, WLSV3007, WLSV3016, WLSV3017A, WLSV3018, WLSV3019, WLSV3031, WLSV3032, WLSV3303, WLSV3304, WLSV3305, WLSV3318, WLSV3319, WLSV3320, WLSV3321, WLSV3990, WLSV4057, WLSV4063, WLSV4091, WLSV4093, WLSV4095, WLSV4207, WLSV4208, WLSV4209, WLSV4237, WLSV4335, WLSV4365, WLSV4375, WLSV4388, WLSV4397, WLSV4398, WLSV4399, WLSV4411, WLSV4492, WLSV4493, WLSV4494, WLSV4515, WLSV4529, WLSV4530, WLSV4533, WLSV4604, WLSV4646, WLSV4647, WLSV4765, WLSV4766, WLSV4767, WLSV4768, WLSV4769, WLSV4770, WLSV4771, WLSV4772, WLSV4773, WLSV4774, WLSV4775, WLSV4776, WLSV4777, WLSV4778, WLSV4779, WLSV4780, WLSV4789, WLSV4791, WLSV813, WLSV814, Y039. Patos (−6.986025°S; −37.31695278°W): KSV041, KSV053, KSV055, KSV079, KSV113, KSV196, KSV232, KSV233, KSV237, KSV246, KSV247, KSV248, KSV251, KSV266, KSV278, KSV313, KSV319, KSV320, KSV321, KSV322, KSV325, KSV326, KSV327, KSV328, KSV330, KSV346. Pedra da Boca (−6.459583333°S; −35.67788333°W): KSV02, UFPB8423, UFPB8424, UFPB8425, UFPB8426, UFPB8427, UFPB8428, UFPB8429, UFPB8430, UFPB8431, UFPB8432, UFPB8433, UFPB8434, UFPB8435, UFPB8436, UFPB8437, UFPB8438, UFPB8439, UFPB8440, UFPB8441, UFPB8442, UFPB8443, UFPB8444,

UFPB8445, UFPB8446, UFPB8447, UFPB8448, UFPB8449, UFPB8450, UFPB8451, UFPB8452, UFPB8453, UFPB8454, UFPB8455, UFPB8456, UFPB8457, UFPB8458, UFPB8459, UFPB8460, UFPB8461, UFPB8462, UFPB8463, UFPB8464, UFPB8465, UFPB8466, UFPB8467, UFPB8468, UFPB8470, UFPB8471, UFPB8472, UFPB8473, UFPB8474, UFPB8475, UFPB8476, UFPB8477, UFPB8478, UFPB8479, UFPB8480, UFPB8481, UFPB8482, UFPB8483, UFPB8484, UFPB8485, UFPB8486, UFPB8487, UFPB8488, UFPB8489, UFPB8490, UFPB8491, UFPB8492, YL005, YL013, YL101, YL117, YL135, YL144, YL173, YL238, YL280, YL283, YL293, YL325, YL348. São João do Cariri (−7.45825°S; −36.48094444°W): WLSV001, WLSV002, WLSV173, WLSV209, WLSV244, WLSV245, WLSV258, WLSV596, WLSV884, WLSV885, WLSV886, WLSV899, WLSV900, WLSV901, WLSV902, WLSV903, WLSV904, WLSV904, WLSV905, WLSV906, WLSV965, WLSV966, WLSV967. São José dos Cordeiros (−7.4675°S; −36.84327778°W): UFPB11253, UFPB11254, UFPB11255, UFPB11256, UFPB11257, UFPB11258, UFPB11259, UFPB11260, UFPB11261, UFPB11262, UFPB11263, UFPB11264, UFPB11265, UFPB5866. São Mamede (−6.893888889°S; −37.08300833°W): UFPB11686, UFPB11687. PERNAMBUCO| Arcoverde (−8.437488889°S; −37.04850556°W): UFPB9678, UFPB9679, UFPB9680, UFPB9681, UFPB9682, UFPB9683, UFPB9684, UFPB9685, UFPB9686, UFPB9687, UFPB9688, UFPB9689, UFPB9690, UFPB9691, UFPB9692, UFPB9693, UFPB9694, UFPB9695, UFPB9696, UFPB9697, UFPB9698, UFPB9699, UFPB9701. Bezerros: UFPB7098. Buíque (−8.616025°S; −37.16555833°W): CHUFPB19895, CHUFPB19903, CHUFPB19908, CHUFPB19920, CHUFPB19921, CHUFPB19977, CHUFPB19978, CHUFPB20672, CHUFPB20830, CHUFPB20833, CHUFPB20855, CHUFPB20868, CHUFPB20884, CHUFPB20924, CHUFPB21057, CHUFPB22174, CHUFPB22175. Exú (−7.511944444°S; −39.72388889°W): UFPB7208, UFPB7209, UFPB7210, UFPB7211, UFPB7212, UFPB7213, UFPB7214, UFPB7216, UFPB7217. Nascente (−7.883244444°S; −40.47074167°W): UFPB9670, UFPB9671. Serra Talhada (−8.014947222°S; −38.28990833°W): UFPB9655, UFPB9656, UFPB9657, UFPB9658, UFPB9659. Trindade (−7.741983333°S; −40.288475°W): UFPB9672, UFPB9673, UFPB9674, UFPB9676, UFPB9677, UFPB974. Várzea da Conceição (−6.472177778°S; −39.11150278°W): UFPB9661, UFPB9662, UFPB9666, UFPB9668, UFPB9664, UFPB9667, UFPB9665, UFPB9663. PIAUÍ| Cajueiro (−2.932194444°S; −41.34146389°W): UFPB7086. Caracol (−9.281308333°S; −43.32954722°W): GGS2-01, GGS2-02, GGS2-03, GGS2-04, GGS2-05, GGS2-06, GGS2-07. Paulistana (−8.115602778°S; −41.20048889°): UFPB9669. Piripiri (−4.354030556°S; −41.83985556°W): UFPB10339. Serra das Confusões (−9.223002778°S; −43.48978333°W): GGS560, GGS608, GGS656, GGS657, GGS658, GGS673, GGS674, CHUFPB19973, CHUFPB19986, CHUFPB20878, CHUFPB22176, CHUFPB22193, CHUFPB22215, CHUFPB22219, CHUFPB22221, CHUFPB22227. RIO GRANDE DO NORTE| Caicó (−6.454016667°S; −37.10038889°W): UFPB14903, UFPB14904, UFPB14905, UFPB14906. João Câmara (−5.449719444°S; −35.87196389°W): GGS01, GGS02, GGS03, GGS04, GGS05, GGS06, GGS07, GGS08, GGS09, GGS10, GGS11, GGS12,

GGS13, GGS14, GGS15, GGS16, GGS17, GGS18, GGS19, GGS20, GGS21, GGS22, GGS23, GGS24, GGS25, GGS26, GGS27, GGS28, GGS29, GGS30, GGS31, GGS100, GGS101, GGS102, GGS103, GGS104, GGS105, GGS106, GGS107, GGS108, GGS109, GGS110, GGS111, GGS112, GGS113, GGS114, GGS115, GGS116, GGS117, GGS118, GGS119, GGS120, GGS121, GGS122, CHUFPB19900, CHUFPB19984, CHUFPB20872, CHUFPB21300, CHUFPB21844, CHUFPB21860, CHUFPB21884, CHUFPB22224, CHUFPB23174. Macaíba (−5.862877778°S; −35.35527222°W): CHUFPB19847, CHUFPB19948, CHUFPB19949, CHUFPB19953, CHUFPB19961, CHUFPB19966, CHUFPB19972, CHUFPB19974, CHUFPB19976, CHUFPB19980, CHUFPB19995, CHUFPB20679, CHUFPB20682, CHUFPB20684, CHUFPB20790, CHUFPB20802, CHUFPB20834, CHUFPB20842, CHUFPB20848, CHUFPB20858, CHUFPB20864, CHUFPB20866, CHUFPB20869, CHUFPB20874, CHUFPB20883, CHUFPB20900, CHUFPB20903, CHUFPB21063, CHUFPB21348. Santa Cruz (−6.189175°S; −36.09248333°W): CHUFPB21054. Santana dos Matos (−5.964152778°S; −36.65888056°W): CHUFPB19938, CHUFPB20660, CHUFPB20840, CHUFPB20857, CHUFPB20890, CHUFPB20897, CHUFPB20928. Serra de São Bento (−6.418805556°S; −35.704275°W): CHUFPB22200, CHUFPB22203. TOCANTINS| Aliança (−11.37906111°S; −48.92268333°W): UFPB1588.

ACKNOWLEDGEMENTS

ADDITIONAL INFORMATION AND DECLARATIONS

Funding

Grant Disclosures

Competing Interests

The authors declare that they have no competing interests.

Author Contributions

- Kleber Silva Vieira conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.
- Erivanna Karlene Santos Oliveira performed the experiments, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.
- Washington Luiz Silva Vieira performed the experiments, analyzed the data, authored or reviewed drafts of the paper, and approved the final draft.
- Rômulo Romeu Nóbrega Alves analyzed the data, authored or reviewed drafts of the paper, and approved the final draft.

Data Availability

The following information was supplied regarding data availability:

The raw data and other data are available in the Supplemental Files.

Supplemental Information

Supplemental information for this article can be found online at http://dx.doi.org/10.7717/peerj.12879#supplemental-information.

REFERENCES

Ajmal Ali M, Gyulai G, Hidvégi N, Kerti B, Al Hemaid FM, Pandey AK, Lee J. 2014. The changing epitome of species identification—DNA barcoding. *Saudi Journal of Biological Sciences* **21**:204–231 DOI 10.1016/j.sjbs.2014.03.003.

Aleixo A. 2009. Conceitos de Espécie e suas Implicações para a Conservação. *Megadiversidade* **5**:87–95.

Alroy J. 2003. Taxonomic inflation and body mass distributions in North American fossil mammals. *Journal of Mammalogy* **84**:431–443 DOI 10.1644/1545-1542(2003)084<0431:TIABMD>2.0.CO;2.

Amaro RC, Rodrigues MT, Yonenaga-Yassuda Y, Carnaval AC. 2012. Demographic processes in the montane Atlantic rainforest: molecular and cytogenetic evidence from the endemic frog *Proceratophrys boiei*. *Molecular Phylogenetics and Evolution* **62**:880–888 DOI 10.1016/j.ympev.2011.11.004.

Annibale FS, de Sousa VTT, da Silva FR, Murphy CG. 2020. Geographic variation in the acoustic signals of *Dendropsophus nanus* (Boulenger 1889) (Anura: Hylidae). *Herpetologica* **76**:267–277 DOI 10.1655/Herpetologica-D-19-00046.1.

Arruda MP, Costa WP, Recco-Pimentel SM. 2017. Genetic diversity of Morato's Digger Toad, *Proceratophrys moratoi*: spatial structure, gene flow, effective size and the need for differential management strategies of populations. *Genetics and Molecular Biology* **40(2)**:502–514 DOI 10.1590/1678-4685-gmb-2016-0025.

Arruda MP, Costa WP, Silva CC, Pimentel SMR. 2012. Development of 22 polymorphic microsatellite loci for the critically endangered Morato's Digger Toad, *Proceratophrys moratoi*. *International Journal of Molecular Sciences* **13(12)**:12259–12267 DOI 10.3390/ijms131012259.

Ashton KG. 2002. Do amphibians follow Bergmann's rule? *Canadian Journal of Zoology* **80(4)**:708–716 DOI 10.1139/z02-049.

Atencia P, Solano L, Liria J. 2020. Morphometric diferentiation and diet of *Engystomops pustulosus* (Amphibia: Leptodactylidade) in three populations from Colombia. *Russian Journal of Herpetology* **27(3)**:156–164 DOI 10.30906/1026-2296-2020-27-3-156-164.

Ávila RW, Kawashita-Ribeiro RA, Morais DH. 2011. A new species of *Proceratophrys* (Anura: Cycloramphidae) from western Brazil. *Zootaxa* **2890(1)**:20–28 DOI 10.11646/zootaxa.2890.1.2.

Ávila RW, Pansonato A, Strüssmann C. 2012. A new species of *Proceratophrys* (Anura: Cycloramphidae) from Midwestern Brazil. *Journal of Herpetology* **46**:466–472 DOI 10.2307/23327161.

Badii MH, Landeros J, Foroughbakhch R, Abreu JL. 2007. Biodiversidad, evolución, extinción y sustentabilidad *Daena*. *International Journal of Good Conscience* **2**:229–247.

Barrio ITM, Barrio A. 1993. Una Nueva Especie de *Proceratophrys* (Leptodactylidae) del Nordeste de Argentina. *Amphibia-Reptilia* **14**:13–18 DOI 10.1163/156853893X00156.

Bergmann C. 1848. *Über die Verhältnisse der Wärmeökonomie der Thiere zu ihrer Größe.* Göttingen: bei Vandenhoeck und Ruprecht.

Bernardo J. 1994. Experimental analysis of allocation in two divergent, natural salamander populations. *American Naturalist* **143**:14–38 DOI 10.1086/285594.

Berven KA. 1982a. The genetic basis of altitudinal variation in the wood frog *Rana Sylvatica*. I. An experimental analysis of life history traits. *Evolution* **36(5)**:962–983 DOI 10.1111/j.1558-5646.1982.tb05466.x.

Berven KA. 1982b. The genetic basis of altitudinal variation in the wood frog *Rana sylvatica* II. An experimental analysis of larval development. *Oecologia* **52(3)**:360–369 DOI 10.1007/BF00367960.

Blackburn TM, Gaston KJ, Loder N. 1999. Geographic gradients in body size: a clarification of Bergmann's rule. *Diversity and Distributions* **5(4)**:165–174 DOI 10.1046/j.1472-4642.1999.00046.x.

Bond AB. 2007. The evolution of color polymorphism: cryptitiy, searching images, and apostatic selection. *Annual Review of Ecology, Evolution, and Systematics* **38(1)**:489–514 DOI 10.1146/annurev.ecolsys.38.091206.095728.

Bonte D, Maelfait J-P. 2004. Colour variation and crypsis in relation to habitat selection in the males of the crab spider *Xysticus sabulosus* (Hahn, 1832) (Araneae: Thomisidae). *Belgian Journal of Zoology* **134**:3–7.

Boulenger GA. 1882. *Catalogue of the Batrachia Salientia S. Ecaudata.* London: Printed by order of the Trustees.

Bourke J, Busse KC, Bakker TM. 2011. Sex differences in polymorphic body coloration and dorsal pattern in Darwin's frogs (*Rhinoderma darwinii*). *Herpetological Journal* **21**:227–234.

Brandão RA, Caramaschi U, Vaz-Silva W, Campos LA. 2013. Three new species of *Proceratophrys* Miranda-Ribeiro 1920 from Brazilian Cerrado (Anura, Odontophrynidae). *Zootaxa* **3750(4)**:321–347 DOI 10.11646/zootaxa.3750.4.2.

Braun PC. 1973. Nova Espécie do Gênero *Proceratophrys* Miranda-Ribeiro, 1920 do Estado do Rio Grande do Sul, Brasil (Anura, Ceratophrynidae). *Iheringia, Série Zoologia* **43**:91–99.

Brito L, Telles F, Roberto I, Ribeiro S, Cascon P. 2012. Different foraging strategies within congenerics? The diet of *Proceratophrys cristiceps* (Müller, 1883) from a Dry Forest in Northeast Brazil. *Herpetology Notes* **5**:85–89.

Callegari-Jacques SM. 2004. *Bioestatística-Princípios e Aplicações.* São Paulo: Artmed Editora S.A.

Caramaschi U. 1996. Nova Espécie de *Odontophrynus* Reinhardt & Lütken, 1862 do Brasil Central (Amphibia, Anura, Leptodactylidae). *Boletim do Museu Nacional* **367**:1–8.

Cattell RB. 1966. The scree test for the number of factors. *Multivariate Behavioral Research* **1(2)**:245–276 DOI 10.1207/s15327906mbr0102_10.

Costa C, Lima SL, Andrade DR, Agostinho CÂ. 1998. Caracterização Morfológica dos Estádios de Desenvolvimento do Aparelho Reprodutor Feminino da Rã-touro, *Rana catesbeiana*, no Sistema Anfigranja de Criação Intensiva. *Revista Brasileira de Zootecnia* **27**:642–650.

Coyne JA, Orr HA, Futuyma DJ. 1988. Do we need a new species concept? *Systematic Zoology* **37(2)**:190–200 DOI 10.2307/2992276.

Cruz CAG, Nunes I, Juncá FA. 2012. Redescription of *Proceratophrys cristiceps* (Müller, 1883) (Amphibia, Anura, Odontophrynidae), with description of two new species without eyelid appendages from Northeastern Brazil. *South American Journal of Herpetology* **7(2)**:110–122 DOI 10.2994/057.007.0201.

Cuthill IC, Stevens M, Sheppard J, Maddocks T, Párraga CA, Troscianko TS. 2005. Disruptive coloration and background pattern matching. *Nature* **434**:72–74 DOI 10.1038/nature03312.

Dias PHdS, Amaro RC, de Carvalho-e-Silva AMPT, Rodrigues MT. 2013. Two new species of *Proceratophrys* Miranda-Ribeiro, 1920 (Anura; Odontophrynidae) from the Atlantic Forest, with taxonomic remarks on the genus. *Zootaxa* **3682(2)**:277–304 DOI 10.11646/zootaxa.3682.2.5.

Dias PHdS, de Carvalho-e-Silva AMPT, de Carvalho-e-Silva SP. 2014. The tadpole of *Proceratophrys izecksohni* (Amphibia: Anura: Odontophrynidae). *Zoologia* **31(2)**:181–194 DOI 10.1590/S1984-46702014000200010.

Dias AG, Gonçalves da Cruz CA. 1993. Análise das Divergências Morfológicas de *Hyla bipunctata* Spix em duas Populações do Rio de Janeiro e Espírito Santo, Brasil (Amphibia, Anura, Hylidae). *Revista Brasileira de Zoologia* **10(3)**:439–441 DOI 10.1590/S0101-81751993000300010.

Dobzhansky T. 1970. *Genética do Processo Evolutivo*. São Paulo: Editora Polígono S.A.

Duellman WE, Trueb L. 1994. *Biology of amphibians*. Baltimore and London: The Johns Hopkins University Press.

Emerson SB. 1985. Skull shape in frogs: correlations with diet. *Herpetologica* **41**:177–188.

Endler JA. 1977. *Geographic variation, speciation, and clines*. Princeton: Princeton University Press.

Endler JA. 1981. An overview of the relationships between mimicry and crypsis. *Biological Journal of the Linnean Society* **16(1)**:25–31 DOI 10.1111/j.1095-8312.1981.tb01840.x.

Eterovick PC, Sazima I. 1998. New species of *Proceratophrys* (Anura: Leptodactylidae) from Southeastern Brazil. *Copeia* **1(1)**:159–164 DOI 10.2307/1447712.

Fick S, Hijmans R. 2017. WorldClim 2: new 1-km spatial resolution climate surfaces for global land areas. *International Journal of Climatology* **37(12)**:4302–4315 DOI 10.1002/joc.5086.

Fick S, Hijmans R. 2020. WorldClim version 2.1 climate data for 1970-2000. *Available at https://worldclim.org/data/worldclim21.html*.

Fischer H. 2011. *A history of the central limit theorem: from classical to modern probability theory*. New York, Dordrecht, Heidelberg, London: Springer.

Freemeteo. 2019. Freemeteo.com. *Available at http://freemeteo.com/default.asp?pid=116&la=18* (accessed 26 November 2020).

Frost DR. 2021. Amphibian species of the world: an online reference. *Available at http://research.amnh.org/vz/herpetology/amphibia/* (accessed 11 January 2021).

Futuyma DJ. 1987. On the role of species in anagenesis. *American Naturalist* **130(3)**:465–473 DOI 10.1086/284724.

Giaretta AA, Bernarde PS, Kokubum MNdC. 2000. A new species of *Proceratophrys* (Anura: Leptodactylidae) from the Amazon rain forest. *Journal of Herpetology* **34(2)**:173–178 DOI 10.2307/1565412.

Gilmour J, Gregor J. 1939. Demes: a suggested new terminology. *Nature* **144**:333 DOI 10.1038/144333a0.

Godinho LB, Moura MR, Lacerda JVA, Feio RN. 2013. A new species of *Proceratophrys* (Anura: Odontophrynidae) from the Middle São Francisco River, Southeastern Brazil. *Salamandra* **49**:63–73.

Gonçalves da Cruz CA, Prado GM, Izecksohn E. 2005. Nova Espécie de *Proceratophrys* Miranda-Ribeiro, 1920 do Sudeste do Brasil (Amphibia, Anura, Leptodactylidae). *Archivos do Museu Nacional* **63**:289–295.

Gravenhorst JLC. 1829. *Deliciae Musei Zoologici Vratislaviensis—Fasciculus primus—Continens Chelonios et Batrachia.* Lipsiae: Leopold Voss.

Grismer LL, Wong H, Galina-Tessaro P. 2002. Geographic variation and taxonomy of the sand snakes, *Chilomeniscus* (Squamata: Colubridae). *Herpetologica* **58**:18–31 DOI 10.1655/0018-0831(2002)058[0018:GVATOT]2.0.CO;2.

Günther A. 1873. XLVII—Contribution to our knowledge of *Ceratophys* and *Megalophrys*. *Annals and Magazine of Natural History* **4**:416–419 DOI 10.1080/00222937308696844.

Hammer Ø. 2016. *Paleontological statistics version 3.14—reference manual.* Oslo: Natural History Museum/University of Oslo.

Harold AS. 2002. Species concepts and phylogenetic theory: a debate. *Copeia* **2**:543–545 DOI 10.1643/0045-8511(2002)002[0543:]2.0.CO;2.

Hartigan JA. 1975. *Clustering algorithms.* New York/London/Sydney/Toronto: John Wiley & Sons.

Heyer WR. 2005. Variation and taxonomic clarification of the large species of the *Leptodactylus pentadactylus* species group (Amphibia: Leptodactylidae) from middle America, northern South America, and Amazonia. *Arquivos de Zoologia* **37(3)**:269–348 DOI 10.11606/issn.2176-7793.v37i3p269-348.

Hijmans RJ, Guarino L, Jarvis A, O'Brien R, Mathur P, Bussink C, Cruz M, Barrantes I, Rojas E. 2005. *DIVA-Gis version 7.5.* Berkeley: Bioversity International, the International Potato Center, the International Rice Research Institute, the University of California-Berkeley Museum of Vertebrate Zoology, and others, 11.

Hijmans R, Guarino L, Mathur P. 2012. *DIVA-GIS version 7.5.* Berkeley: LizardTech, Inc., and/or the University of California.

Hoffman EA, Blouin MS. 2000. A review of colour and pattern polymorphisms in anurans. *Biological Journal of the Linnean Society* **70(4)**:633–665 DOI 10.1006/bijl.1999.0421.

Huxley JS. 1940. *The new sistematics.* London: Oxford University Press.

INMET. 2020. Instituto Nacional de Meteorologia. *Available at* http://www.inmet.gov.br/portal/ (accessed 07 April 2020).

Isaac NJB, Mallet J, Mace GM. 2004. Taxonomic inflation: its influence on macroecology and conservation. *Trends in Ecology and Evolution* **19(9)**:464–469 DOI 10.1016/j.tree.2004.06.004.

Izecksohn E, Cruz Gd, Alberto C, Peixoto OL. 1998. Sobre *Proceratophrys appendiculata* e Algumas Espécies Afins (Amphibia; Anura; Leptodactylidae). *Revista Universidade Rural Série Ciências da Vida* **20**:37–54.

Izecksohn E, Peixoto OL. 1980. Sobre a utilização do nome *Stombus precrenulatus* Miranda-Ribeiro, 1937 e a validez da espécie (Amphibia, Anura, Leptodactylidae). *Revista Brasileira de Biologia* **40**:605–609.

Izecksohn E, Peixoto OL. 1981. Nova espécie de *Proceratophrys* da Hiléia bahiana, Brasil (Amphibia, Anura, Leptodactylidae). *Revista Brasileira de Biologia* **41**:19–24.

Jim J, Caramaschi U. 1980. Uma Nova Espécie de *Odontophrynus* da Região de Botucatu, São Paulo, Brasil (Amphibia, Anura). *Revista Brasileira de Biologia* **40**:357–360.

Jiménez-Valverde A. 2012. Insights into the area under the receiver operating characteristic curve (AUC) as a discrimination measure in species distribution modelling. *Global Ecology and Biogeography* **21(4)**:498–507 DOI 10.1111/j.1466-8238.2011.00683.x.

Junior MT, Amaro RC, Recoder RS, Dal Cechio F, Rodrigues MT. 2012. A new dwarf species of *Proceratophrys* Miranda-Ribeiro, 1920 (Anura, Cycloramphidae) from the Highlands of Chapada Diamantina, Bahia. *Brazil Zootaxa* **3551(1)**:25–42 DOI 10.11646/zootaxa.3551.1.2.

Kakazu S, Toledo LF, Haddad CFB. 2010. Color polymorphism in *Leptodactylus fuscus* (Anura, Leptodactylidae): a defensive strategy against predators? *Herpetology Notes* **3**:69–72.

Köhler G. 2012. *Color catalogue for field biologists*. Offenbach: Herpeton.

Kwet A, Faivovich J. 2001. *Proceratophrys bigibbosa* species group (Anura: Leptodactylidae), with description of a new species. *Copeia* **1**:203–215 DOI 10.1643/0045-8511(2001)001[0203:PBSGAL]2.0.CO;2.

Lynch JD. 1971. Evolutionary relationships, osteology, and zoogeography of leptodactyloid frogs. *Miscellaneous Publication* **53**:1–238.

Martins LB, Giaretta AA. 2011. A new species of *Proceratophrys* Miranda-Ribeiro (Amphibia: Anura: Cycloramphidae) from Central Brazil. *Zootaxa* **2880(1)**:41–50 DOI 10.11646/zootaxa.2880.1.4.

Martins LB, Giaretta AA. 2013. Morphological and acoustic characterization of *Proceratophrys goyana* (Lissamphibia: Anura: Odontophrynidae), with the description of a sympatric and related new species. *Zootaxa* **4(4)**:301–320 DOI 10.11646/zootaxa.3750.4.1.

Mayr E. 1977. *Populações, espécies e evolução*. São Paulo: Editora da Universidade de São Paulo.

Mayr E. 1996. What is a species, and what is not? *Philosophy of Science* **63(2)**:262–277.

Miranda-Ribeiro Ad. 1920. Algumas Considerações sobre o Gênero *Ceratophrys* e suas Espécies. *Revista do Museu Paulista Tomo* **XII**:288–313.

Miranda-Ribeiro Ad. 1923. Observações Sobre Algumas Phases Evolutivas de Ceratophrys e Stombus. *Archivos do Museu Nacional* **XXIV**:200–207.

Miranda-Ribeiro Ad. 1937. Espécies Novas do Gênero "Stombus" da Série de Appendices Oculares Reduzidos. *O Campo* **24**.

Moreno-Rueda G. 2020. The evolution of crypsis when pigmentation is physiologically costly. *Animal Biodiversity and Conservation* **43**:89–96 DOI 10.32800/abc.2020.43.0089.

Mângia S, Koroiva R, Nunes PMS, Roberto IJ, Ávila RW, Sant'Anna C, Santana DJ, Garda AA. 2018. A new species of *Proceratophrys* (Amphibia: Anura: Odontophrynidae) from the Araripe Plateau, Ceará State, Northeastern Brazil. *Herpetologica* **74(3)**:255–268 DOI 10.1655/Herpetologica-D-16-00084.1.

Mângia S, Oliveira EF, Santana DJ, Koroiva R, Paiva F, Garda AA. 2020. Revising the taxonomy of *Proceratophrys* Miranda-Ribeiro, 1920 (Anura: Odontophrynidae) from the Brazilian semiarid Caatinga: morphology, calls and molecules support a single widespread species. *Journal of Zoological Systematics and Evolutionary Research* **58(4)**:1151–1172 DOI 10.1111/jzs.12365.

Müller F. 1884. Katalog der Typusexemplare in der Amphibiensammlung des Naturhistorischen Museums zu Basel. *Separatabdruck aus den Verhandlungen der Naturforschenden Gesellschaft in Basel Band* **LVII**:8–9 DOI 10.5962/bhl.part.2387.

Napoli MF, Cruz CAG, de Abreu RO, Del-Grande ML. 2011. A new species of *Proceratophrys* Miranda-Ribeiro (Amphibia: Anura: Cycloramphidae) from the Chapada Diamantina, State of Bahia, Northeastern Brazil. *Zootaxa* **3133(1)**:37–49 DOI 10.11646/zootaxa.3133.1.2.

Nicoglou A. 2015. Phenotypic plasticity: from microevolution to macroevolution. In: Heams T, Huneman P, Lecointre G, Silberstein M, eds. *Handbook of Evolutionary Thinking in the Sciences*. London: Springer, 8–42.

Noble GK. 1931. *The biology of amphibians*. New York and London: McGraw-Hill Book Company, Inc.

Nunes I, Loebmann D, Cruz CAG, Haddad CFB. 2015. Advertisement call, colour variation, natural history, and geographic distribution of *Proceratophrys caramaschii* (Anura: Odontophrynidae). *Salamandra* **51**:103–110.

O'Neill EM, Beard KH, Pfrender ME. 2012. Cast adrift on an island: introduced populations experience an altered balance between selection and drift. *Biology Letters* **8(5)**:890–893 DOI 10.1098/rsbl.2012.0312.

Padial JM, De la Riva I. 2006. Taxonomic inflation and the stability of species lists: the perils of ostrich's behavior. *Systematic Biology* **55(5)**:859–867 DOI 10.1080/1063515060081588.

Padial JM, Miralles A, De la Riva I, Vences M. 2010. The integrative future of taxonomy. *Frontiers in Zoology* **7(1)**:16 DOI 10.1186/1742-9994-7-16.

Peel MC, Finlayson BL, McMahon TA. 2016. *Climatic classification Köppen–Geiger*. Melbourne: University of Melbourne, 7.

Philips S, Anderson RP, Dudík M, Schapire RE, Blair ME. 2017. Opening the black box: an open-source release of Maxent. *Ecography* **40(7)**:887–893 DOI 10.1111/ecog.03049.

Phillips S, Dudik M, Schapire R. 2006. *Maximum entropy modeling of species geographic distributions*. MaxEnt Version 3.3.3e. Princeton: AT&T Labs-Research, Princeton University, and the Center for Biodiversity and Conservation, American Museum of Natural History.

Qiao H, Soberón J, Peterson AT. 2015. No silver bullets in correlative ecological niche modelling: insights from testing among many potential algorithms for niche estimation. *Methods in Ecology and Evolution* **6(10)**:1126–1136 DOI 10.1111/2041-210X.12397.

R Development Core Team. 2018. *R: a language and environment for statistical computing*. Vienna: The R Foundation for Statistical Computing. *Available at* http://www.R-project.org/.

Rabbani M, Zacharczenko B, Green DM. 2015. Color pattern variation in a cryptic amphibian, *Anaxyrus fowleri*. *Journal of Herpetology* **49(4)**:649–654 DOI 10.1670/14-114.

Rasband W. 2018. *ImageJ*. 1.52a edition. Bethesda: National Institutes of Health.

Ricklefs RE. 2008. *The economy of nature*. New York: W. H. Freeman and Company.

Ridley M. 2004. *Evolution*. Oxford: Blackwell Publishing.

Salewski V, Watt C. 2017. Bergmann's rule: a biophysiological rule examined in birds. *Oikos* **126(2)**:309 DOI 10.1111/oik.03698.

Scheldeman X, van Zonneveld M. 2010. *Training manual on spatial analysis of plant diversity and distribution*. Rome: Bioversity International.

Schindel DE, Miller SE. 2005. DNA barcoding a useful tool for taxonomists. *Nature* **435**:17 DOI 10.1038/435017b.

Smith TB, Skúlason S. 1996. Evolutionary significance of resource polymorphism in fishes, amphibians, and birds. *Annual Review of Ecology and Systematics* **27(1)**:111–133 DOI 10.1146/annurev.ecolsys.27.1.111.

Sneath PHA, Sokal RR. 1973. *Numerical taxonomy—the principles and practice of numerical classification*. San Francisco: W. H. Freeman and Company.

Soberón J. 2007. Grinnellian and Eltonian niches and geographic distributions of species. *Ecology Letters* **10(12)**:1115–1123 DOI 10.1111/j.1461-0248.2007.01107.x.

Soberón J, Nakamura M. 2009. Niches and distributional areas: concepts, methods, and assumptions. *Proceedings of the National Academy of Sciences of The United States of America* **106**:19644–19650 DOI 10.1073/pnas.0901637106.

Sokal RR. 1973. The species problem reconsidered. *Systematic Zoology* **22(4)**:360–374 DOI 10.2307/2412944.

Sokal RR, Rohlf FJ. 1995. *Biometry: principles and practice of statistics in biological research*. New York: W. H. Freeman and Company.

Stebbins RC, Cohen NW. 1995. *A natural history of amphibians*. Priceton: Princeton University Press.

Tolosa Y, Molina-Zuluaga C, Restrepo A, Daza JM. 2014. Sexual maturity and sexual dimorphism in a population of the rocket-frog *Colostethus* aff. *fraterdanieli* (Anura: Dendrobatidae) on the Northeastern Cordillera Central of Colombia. *Actualidades Biológicas* **37**:287–294.

Van Holstein L, Foley RA. 2020. Terrestrial habitats decouple the relationship between species and subspecies diversification in mammals. *Proceedings of the Royal Society B* **287**:20192702 DOI 10.1098/rspb.2019.2702.

Vieira KS, Arzabe C, Hernández MIM, Vieira WLS. 2008. An examination of morphometric variations in a neotropical toad population (*Proceratophrys cristiceps*, Amphibia, Anura, Cycloramphidae). *PLOS ONE* **3(12)**:e3934 DOI 10.1371/journal.pone.0003934.

Vieira KS, Vieira WLd. 2012. Morphometric analyses and ontogenetic variation in a neotropical toad species (Amphibia, Anura, Cycloramphidae). *Herpetological Review* **43**:564–568.

Watters JL, Cummings ST, Flanagan RL, Siler CD. 2016. Review of morphometric measurements used in anuran species descriptions and recommendations for a standardized approach. *Zootaxa* **4072(4)**:477–495 DOI 10.11646/zootaxa.4072.4.6.

White TE, Kemp DJ. 2016. Colour polymorphism. *Current Biology* **26(13)**:R515–R522 DOI 10.1016/j.cub.2016.03.017.

Winsor MP. 2000. Species, demes, and the omega taxonomy: gilmour and the new systematics. *Biology and Philosophy* **15(3)**:349–388 DOI 10.1023/A:1006774217770.

Zachos FE. 2016. *Species concept in biology: historical development, theoretical foundations and practical relevance*. Cham: Springer.

Zar JH. 2014. *Biostatistical analysis*. Singapore: Pearson Education, Inc.

Vieira et al. (2022), *PeerJ*, DOI 10.7717/peerj.12879

30/30